Perspectives in Physiology
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Lawrence D. Longo

The Rise of Fetal and Neonatal Physiology

Basic Science to Clinical Care

Second Edition

Springer

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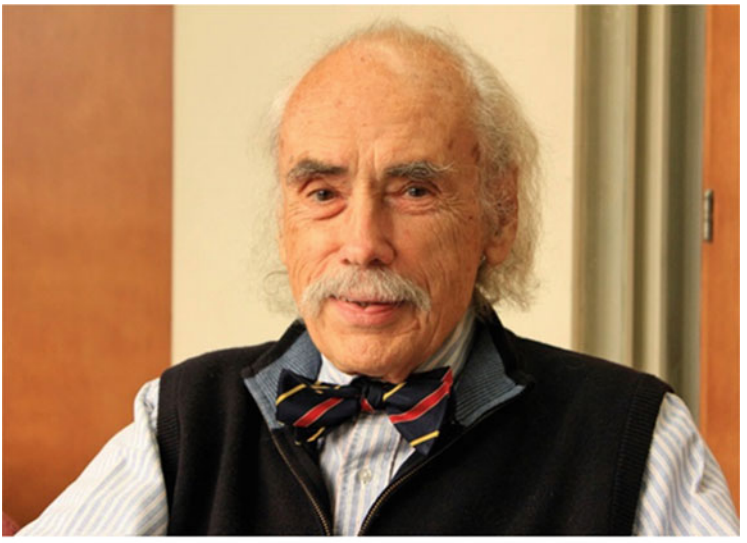

Lawrence D. Longo (1926-2016)

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This work was completed by Steven M. Yellon, Ravi Goyal, Ciprian P. Gheorghe, Justo Alonso, and Michael A. Kirby

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Foreword by Kent L.R. Thornburg

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Foreword to the Second Edition

When you ask a successful scientist about their training, he or she will inevitably refer to one or more heroes who have changed the course of their careers. In every case, the hero being revered will have been a brilliant scientist and marvelous mentor. In addition, he or she will have had a gift for writing, caring, nurturing, and teaching academic survival skills. Thus, for those of us who are among the privileged who pursue the secrets of the natural world, we have our heroes to thank. Hero reproduction, it appears, is the mechanism by which cutting-edge science is passed from one generation to the next.

In my case, I was molded by professors who encouraged me, beginning as an undergraduate. Those who influenced me most included Drs. Elver Voth, Howard Hilleman, Job Faber, and James Metcalfe. However, not all of my heroes were overseeing my education. I came to know and appreciate others at national scientific meetings and at different universities during my travels. These included Geoffrey Dawes, Robert Boyd, and David Barker. In addition, there were scientific contemporaries from universities around the world with whom I "grew up" in the field and for whom I have great admiration to this day.

I am particularly pleased to honor one of my heroes in this foreword, the late Lawrence D. Longo (1926–2016). Dr. Longo had the personal appearance, not so far from the Albert Einstein look, and the intense personality of a natural born leader. Not only have I admired him for decades because he paid so much attention to me when I was a junior faculty member, but more because I discovered that he was a source of encouragement to a host of people in the field of human development both on a personal level and as a cheerleader for the Society for Reproductive Investigation.

Over the course of Dr. Longo's long career, he saw dramatic changes in the field he loved most—pregnancy and fetal development. As for all scientists in this highly important field, his lineage began decades ago with greats such as Joseph Barcroft (1872–1947) at Cambridge University, Donald Barron (1905–1993) at Yale University, Geoffrey Dawes (1918–1996) at Oxford University, Geoffrey Thorburn (1930–1996) at Monash University in Melbourne Australia, Elizabeth Ramsey

(1906–1993) at the Carnegie Institution in Washington, and Jeffrey Robinson (professor emeritus) at the University of Adelaide, Australia. It was the powerful contributions to the field of pregnancy and fetal development of these leaders and their colleagues that led Dr. Longo to enshrine a few of them in this volume and by so doing remind young scientists of their roots.

By the mid-twentieth century, the field of fetal development was warming up. The pioneering work of these aforementioned forefathers and mothers set the field aflame and the fire spread across the globe, especially across North America, Australia, and New Zealand. Longo himself was swept up in the quest to understand the mysteries of the invisible, and mostly inaccessible, fetus. Once it was discovered in the 1960s that the sheep fetus could be studied chronically in its natural habitat, it became the model of choice for dozens of groups worldwide. The resulting information rush brought a thorough description of fetal hemodynamics and metabolism, placental blood flow, brain development, endocrine regulation, and pulmonary maturation. We now take for granted our understanding that the hemoglobin of the fetus binds oxygen more tightly than does its mother's, that blood flows through the heart muscle of the fetus at twice the rate found in the adult heart, and that the fetus must drink its amniotic fluid and practice breathing before it is born. From all these marvelous discoveries, modern obstetrical medicine owes a great debt to fetal physiologists who discovered the intricacies of development and which now provide the foundation for the practice of clinical fetal medicine.

New technologies appeared during the 1970s and 1980s that allowed fetal measurements that were not previously thought possible. These included measurement of fetal blood flow with radiolabeled microspheres, miniature Doppler flow sensors, Doppler ultrasound, and electromagnetic and transit time flow sensors in addition to implantable electrodes to measure electrical activity in the brain and striated and smooth muscle. Many discoveries in the fetus, like surfactant therapy from Dr. Mont Liggins' laboratory in Auckland, changed clinical practice for women and their fetuses forever. Toward the end of the 1980s, after hundreds of papers had been written demonstrating the homeostatic mechanisms ensuring fetal survival in the womb, scientists began to wonder what new frontiers would be needed to provide better clinical applications of the knowledge gained over the previous 30 years. Then, without warning, the landscape changed dramatically.

In 1989, Professor David J.P. Barker from the University of Southampton published data showing an inverse relationship between mortality from ischemic heart disease and birthweight among 15,000 men and women in Hertfordshire, UK. Many fetal scientists, including me, were skeptical, lacking an obvious biological explanation. However, Barker was undeterred. He embraced the world of basic science and sought out fetal physiologists to find answers. Soon, a new field of so-called fetal programming, now officially called the developmental origins of health and disease (DOHaD), was born. Suddenly, experts on pregnancy and fetal development were uncovering mechanisms explaining the very core of human existence, the early life origins of chronic disease. To this day, fetal biologists and pregnancy experts sprinkled across the Western world are making headway in

understanding how developmental plasticity in early life leads to vulnerability for disease in adulthood. Until their deaths, Drs. Longo and Barker were among them.

With a sudden link to human disease, no longer would fetal biologists be content to describe obscure facts regarding physiological development. The quest had changed. Now the question for all developmental biologists became: what are the mechanisms though which environmental stressors influence reproduction and postnatal development and lead to vulnerability for adult-onset disease? This change in mind-set came at a time when the prevalences of obesity, diabetes, and uncontrolled hypertension were increasing year after year as they are today. The rapid epidemic of chronic disease in the USA over the past 20 years cannot be explained by changes in DNA sequence. Rather, based on recent evidence from fetal biologists, one can fairly argue that the recent unprecedented increases in chronic diseases are rooted in responses to environmental challenges during early development. Thus, current students of development have a new mandate linked directly to human health.

Dr. Longo was clever of mind; he intuitively understood the importance of the developmental origins of disease as a game changer for the field of fetal physiology. Over his career, he gained expertise on one topic after the next as he followed his interests. His focus moved from pregnancy to placental function to fetal cardiovascular function and finally to fetal brain development. In every case, he and his colleagues made highly significant discoveries.

After becoming the director of the Center for Perinatal Biology (1973–2012), he recruited a strong team of young scientists who carried the field forward with enthusiasm. Dr. Longo and collaborators began to study the adaptations made by the vasculature of the fetal brain under conditions of hypobaric hypoxia when pregnant ewes were housed at 12,000 feet at the White Mountain Research Station in California. Their findings led to concerns about how fetuses deprived of oxygen might suffer later as adults, a field of study for which the Loma Linda team is deservedly held in high esteem to this day.

During his long tenure at Loma Linda University, Dr. Longo became an important leader for the entire field because somehow he was able to "adopt" scientists from around the country as part of the Loma Linda family. An invitation to visit the Loma Linda laboratories meant an invitation to join a new family of investigators. Thus, scientists, invited from around the world, came to Loma Linda to join in the quest for answers to the most difficult problems in human pregnancy and late life diseases of offspring. While the contributions made by Dr. Longo and colleagues will be long remembered through their hundreds of significant contributions to the literature, Dr. Longo himself has contributed much more to science than an impressive list of published papers. He modeled for young men and women how to become a scientist of stature and integrity. It is that very contribution to the lives of others that will continue, like heritable DNA replication, to be passed on from this generation to the next.

What makes a person a highly effective mentor? Reliving Dr. Longo's success may go some way toward giving an answer.

Dr. Longo had vision. Visionary people often have trouble keeping their feet on the ground. They sometimes become hopeless utopians. However, Dr. Larry Longo's vision was practical. He envisioned building a world-class group of fetal investigators and along with his talented colleague, Gordon Power, he saw his vision come to fruition.

Dr. Longo had courage. Young people may not know that a great deal of courage is required to build an organization from scratch. Why, because if talent and resources are not forthcoming, the enterprise fails. Overcoming the risk of failure with courage is key to success. Dr. Longo charged into an area of biology, the developing brain, in which he had not previously worked. His early discoveries were highly complex and difficult to explain. Nevertheless, those novel findings now shed new light on the regulation of the vascular elements in the brain in response to hypoxia and apply to millions of people who were deprived of oxygen before birth.

Dr. Longo had a warm sense of humor. No one who knew him will forget his hearty laugh. One time when I visited Loma Linda, I showed our new function curves from the right and left ventricles of the fetal heart. I remarked that Ray Gilbert, one of Dr. Longo's esteemed colleagues, was indeed the father of the fetal function curve. Larry Longo was tickled by the comment and he asked, if Ray was the father, who was the mother. I said I guessed I was. He could hardly stop laughing. In spite of the fact that he was not above being angry over an inept comment by a grant reviewer, or worse, a grant application rejection, he was always able to cool down and resume his true nature as a kind and gentle person who cared about Loma Linda University, his scientific colleagues, and a host of people across the country who knew him as a friend.

When you read this volume, I suggest that you see it through the eyes of Lawrence Longo who was fascinated by history and who knew more about the historical roots of fetal biology than any other person alive today.

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Foreword to the First Edition

The 1960s and 1970s were wonderful times to be doing research in fetal physiology. To be in Oxford during that period was to be in one of the great centers of research activity. I had the good fortune to be at the right place at the right time. Oxford was our Camelot. Colleagues from around the world spoke enviously of one having a "Been to Oxford" (BtO) degree. Geoffrey S. Dawes (1918–1996), Director of the Nuffield Institute for Medical Research in Oxford, was undoubtedly the father figure (some said godfather figure) of fetal physiology in his day. His contributions were of such significance that his position as a giant in the field was unassailable. He had built upon the foundations laid by Barcroft, Eastman, Barron, and those others who had preceded him to define a new field of investigation.

Why was Oxford so special? Partly, it was the lure of that venerable city, and the Oxford "way" of doing things. Partly, it was the coming together of a remarkable group of outstanding and enthusiastic young physiologists working with some extraordinary senior leaders and visitors. There was a critical mass of colleagues and supportive technical staff, and a buzz around Geoffrey's Nuffield Institute. Time was immaterial, there always was someone working at a new problem or ready to share a new finding. Crucial was the realization that the fetus as not just a little adult, but was a distinct, viable entity, with a separate and often quite different physiology. It was clear that everything that we knew about adult physiology did not necessarily apply to the fetus, and needed to be rediscovered or at least reexamined. Every day seemingly brought another discovery, another surprise. The institute was adjacent to the University of Oxford's John Radcliffe Hospital and its Nuffield Departments of Obstetrics and Gynecology and of Pediatrics. The leaders of those departments were sympathetic towards research, and it was quickly clear that this new science had immediate application to clinical practice in obstetrics and pediatrics. The proximity of the institute to the clinical wards, to enthusiastic young physicians, and our attendance at clinical rounds facilitated this process. We were doing translational research and knowledge exchange before it became fashionable, without even the need to give it a fancy name.

Without question, the simultaneous and coincidental development of an array of new techniques was crucial to the opening of this new field. There was a rapid advance from the study of the sheep fetus in short-term acute experiments, *ex utero,* to extraordinary studies with the in vivo chronically catheterized fetal lamb. In 1969, Geoffrey Thorburn had published with John Bassett the rise in plasma cortisol concentrations that preceded the onset of birth in chronically catheterized fetal sheep, and later had helped establish that procedure in Oxford. I shall always remember that great physiologist and chronicler of the placenta, Emmanuel Ciprian Amoroso, returning to the ARC Institute of Animal Physiology at Babraham, Cambridge, from a trip to Australia. I was a graduate student at that time, and Amo lost no chance to sing the praises of Geoff Thorburn and this remarkable advance. Little did I know that in 4 years I would be working with him and I could not have guessed at the influence that he would have on my own career. But, let us get back to Oxford. Mention also must be made of Derek Wyatt, an outstanding physicist and member of the institute, who was crucial in developing many of the flow probes that would be used in these new "fetal" preparations. The new technique of radioimmunoassay had just been developed to the point that we could measure multiple hormones in very small samples of fetal blood. We had techniques for recording electrical signals from the fetal brain in utero, we could measure blood flows and distribution, and we could ask the "undisturbed fetus" questions of critical clinical importance: "how are you? how are your blood gases? what are your glucose levels?" We could measure fetal responses to perturbations, infusions of hormones and drugs, follow physiologic changes through the birth process and into the newborn period. I remember well the time that we were making the first measurements of the rise in Prostaglandin E2 in the fetal circulation before birth. I proudly showed the print out from the scintillation counter to Geoffrey Dawes. He was unimpressed. I had not explained that as the counts went down, the concentration was going up. But then he asked whether this might have anything to do with the decline in fetal breathing movements that occurs at that time, and suddenly I had his full attention, and a year's worth of suggested experiments!

Our scientific advances were helped by good-natured fellowship and by competition. There was great collegiality and daily debate at morning coffee and afternoon tea held around the round table in the lobby of the institute (see my letter to LDL in Chap. 20). Here Geoffrey Dawes was masterful and a wonderful stimulator of new ideas. There was also great debate at the White Hart Pub at lunch time and after work in the evening. It is strange that today we have to force these interactions with scheduled meetings. But, there was also competition. In the early 1970s there were three related Medical Research Council (MRC) program grants at Oxford, led by Geoffrey Dawes (GSD), Geoffrey Thorburn (the big G) and Alexander Cuthbert Turnbull (later Sir Alexander; 1925–1990), respectively, dealing with parturition and fetal physiologic changes near birth. The competition in research was and is healthy. It helps to drive us forward. It also infuses an

environment with measureable energy that leads to pride and excitement, and eventually to a legacy of accomplishment. It helps create leadership and lifelong friendships and networks. It was an environment that brought Oxford together at international meetings, particularly in exchanges with other major centers that were emerging at that time. Importantly locally amongst these was the excellent group in Cambridge of Robert Comline, Marion Sliver, successors of Sir Joseph Barcroft in the perinatal research field, with the young Peter Nathanielsz and Abigail Fowden (although, as a Cambridge graduate, I was just a little uneasy with the light blue–dark blue conflict). The University of Oxford Nuffield Department of Obstetrics and Gynaecology photographs from 1974 to 1975 are so revealing of the environment at that time. Virtually every one of the junior staff members and trainees in those pictures went on to hold a major chair or a directorship later in their career, but as colleagues they have remained in touch with each other, bonded by the Oxford experience.

The public environment was also "right" for doing fetal research. In the United Kingdom, the MRC was enthusiastically supportive through staff appointments and research grants; in the USA, fetal physiology was gaining momentum at the National Institutes of Health. The media, the general public, and some key politicians wanted to know about life and development in the womb. Preterm birth occurred in one in ten pregnancies. At a time when we were just starting to learn about the regulation of lung surfactant, the public wanted to know how to prevent preterm birth and how to look after the premature baby. The landmark study of "Mont" Liggins and Ross Howie on the use of glucocorticoids to prevent respiratory distress syndrome was published in 1971. It resulted directly from studies of cortisol infusion into fetal sheep, the perceptive insight of Mont Liggins, and minimal bureaucracy in moving basic research into a clinical trial. Mothers wanted to know how the environment might affect their baby. Research offered answers. The magic and mystique of the environment inside the womb became mainstream reading. A black box was opening quickly with new information based on excellent science. Politicians and funding agencies listened carefully and were extremely supportive.

But research happens in cycles. Often these last only 5–10 years. An area becomes topical, a new approach offers a major advance and folk jump onto the bandwagon, until the research becomes routine. For a short time, grant funding committees and study sections look favorably on something that is cutting edge. It may be a new field of research (such as fetal physiology) a new topic (prostaglandins, insulin-like growth factors), a fascinating and important discovery (surfactant), or the emergence of a new technique (the chronic fetal sheep preparation and assay of hormone receptors). Fetal physiology seemed to happen in a way and at a time that allowed a convergence of these different factors and approaches. The field opened up technically, the translation (such as in fetal

monitoring, stimulation of lung surfactant, or surfactant therapy), was obvious in obstetric and neonatal practice, and that fuelled public interest and media support. Because there was a whole field of fetal physiology to be discovered, the wave lasted a bit longer than many. In many ways this was good. But I also believe that fetal physiologists lost the game or only recognized it when it was too late for them, to the developmental biologists, initially, and then to mice. We missed the fiscal reality of switching from sheep to mice and we were slow to appreciate the power of mutating a gene and the ability to knock in and knockout genes. Of course, we soon became useful in helping to interpret phenotypes (and there was some very naïve phenotypic interpretation of some early mouse knockouts), but the agenda had changed, and a new generation of investigator was driving it.

The field of fetal physiology continues today, of course; that is the nature of scientific waves. But, I think this is what Geoffrey Dawes meant by his oft (mis) quoted comment about the major questions in fetal physiology being answered by the time of his retirement. Most major questions had been addressed at a physiologic level, and needed new genetic approaches to achieve further mechanistic advance. Geoffrey also saw the emerging importance of understanding the fetal origins of adult disease, and championed the meeting in Italy in 1989 where David J.P. Barker presented his very early information to a group of fetal physiologists. If there was an obvious way forward for fetal physiology, it was through understanding the developmental origins of disease. This needed a physiologic approach but had to be coupled with application of different 'omic techniques and epigenetics. But, many Ob-Gyn departments had missed an opportunity to emerge as the hotbeds of university and hospital research, as departments of developmental reproductive biology, combining integrative physiology with cellular and molecular mechanisms of development.

Interestingly, the antivivisection movement was also a factor in the decline of fetal physiology as we knew it. It easily generated more adverse press against research with sheep and subhuman primates, than it did against research on mice. New rules for the conduct of research, new animal requirements, and spiraling costs have driven many classic fetal physiologists out of the animal house. Ironically, the antivivisection movement and fiscal reality actually forced some sheep fetal physiologists toward developmental biology, to develop new models and gain familiarity with new molecular approaches. The new models of translational research that link basic science to population biology and the health care systems will allow fetal physiologists to flourish, and I am optimistic that we are still training a cadre of needed and worthy successors.

Geoffrey Dawes became Director of the Nuffield Institute at age 30 in 1948; 5 years before Watson and Crick published the structure of DNA. At the time, he had only eight publications to his name. This volume portrays him accurately. He was an astute and critical investigator, maintaining the highest standards of scholarship and expecting others to reach those same values. He was sometimes

antagonistic. He was harshly critical when he felt it justified, and he could be polarizing. But if one matched his standard, worked hard and thoughtfully, one gained his respect. You had won a friend, not just in science but for life. But if you had come up through one of the other "schools," it would always be that much harder, and to my mind Geoffrey never accepted you in quite the same way. Over the years he developed a special affinity with Canada, and it has saddened me that our great Canadian Universities did not recognize Geoffrey appropriately for that.

There was also a very compassionate side to Geoffrey Dawes that often went unrecognized. I saw him go to extraordinary lengths to help a colleague with a medical problem, or to assist a student in financial difficulty. He was kind and thoughtful towards the institute staff, knew their names, and quietly would offer his advice or assistance if he thought that it would be helpful. His trainees became his extended family. He followed their progress with great interest and enthusiasm. He shared in the excitement of their discoveries and would enjoy the intellectual discourse with them, clearly proud as they established their independence and faculty appointments. He was, of course devoted to his wife, Margaret. In this book Christopher Redman describes Geoffrey coming into his office, discussing a new finding, and leaving with the comment, "Isn't this fun." That vignette captures the essential Geoffrey Dawes, enthused about good science, just like a small boy.

Finally, I must say a brief word about the author of this book, Lawrence D. Longo. Larry is one of my heroes. He was there, seemingly at the beginning and is still going strong! This volume chronicles the foundations built by the great historic leaders, it tells how they laid the building blocks, and with Dawes and others created a new field of investigation. It tells of the science and of the scientific societies that underpin the discipline. Geoffrey was not there at the beginning of the Society for Gynecologic Investigation in 1953, but he was clearly aware of the advances being made by colleagues across the Atlantic. This volume is a scholarly account of the development of the new field of fetal physiology and the translation of its research to help mothers and babies. It is also a story of relationships, sometimes fuelled by competitiveness, often fuelled by collegiality. It is a story with many subtexts driven by the desire to acquire new knowledge. There was healthy competition, between Cambridge, Oxford, San Francisco, New Haven, Boston, and others as they emerged as leaders and were then linked by new partnerships and the next generation of scientists. There may be other descriptions of the growth of this field, but it is unlikely that there will be any that captures the spirit, the excitement, and the hope for mothers and their children as effectively as Longo has given here. In this volume he has ensured his place as the great chronicler of a generation of investigators, and of a new approach to science. We are fortunate to have a colleague of his intellect and modesty, of such insight into the accomplishment of others. This is the story about how a field of research unfolds. But any new

field needs its champion. For many, Geoffrey S. Dawes was that champion; a man of formidable intellect, a great experimentalist, commanding yet compassionate, the leader of his time.

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Preface to the Second Edition

Dr. Longo had sent copies of the first edition of his book on the history of fetal physiology to friends, former postdoctoral fellows, and colleagues around the world. From my conversation with Jimin Suh, the Center's Program Manager who worked closely with Dr. Longo on the first edition, many who read his book, based upon their expertise and insights from research activities, sent thank you notes with praise and comments. Some sent personal recollections of a notable historical figure featured in the book. In addition to comments, other perspectives were provided and wishes that expressed more information would have complemented certain topics. The accumulation of feedback from respected sources and the recognition that important advances in the field had been made since the first edition was published, based upon his proclivity for nocturnal readings, motivated Dr. Longo to make plans for a 2nd edition. Esteemed colleagues with unique expertise were invited to make contributions to this volume with a goal of providing an even more "comprehensive" review of the history and future of the fields of fetal and neonatal physiology. This project added to his many other responsibilities, foremost as principal investigator for an NIH Program Project grant for over 20 years, and an R01 that was approaching 45 years of continuous funding. Though shy of attention, he personally knew almost all of the pioneering modern contributors to development of this field of study during the last half of the twentieth century. His personal contributions were the writing of more than 350 papers, actually with a mechanical pencil; most were peer reviewed, with dozens of chapters and over 20 books. The impact of his efforts on society was enhanced by his writing several United States Surgeon Generals' Reports to Congress (1979–1981) about the Health Effects of Smoking on Pregnancy and Infants, his membership on many NIH Center for Scientific Research grant review panels, and international recognition for publications on the history of medicine. His illustrious career came as a true Clinician scientist based upon a balance of responsibilities for patient care, call schedules, surgery as a practicing Obstetrician, many academic responsibilities with scientific societies, and multiple research grants to support laboratory operations and trainees, often at the sacrifice of

personal time with family. Despite his seniority, he worked at a seemingly indefatigable pace to build a world-class perinatal research center in the School of Medicine at Loma Linda University, as well as helped to create other centers of research excellence as part of promoted infrastructure improvements that are regarded by peer reviewers as outstanding. Amidst all these efforts, no one knew that his work to revise this book would be the last major project of his career. Even when hospitalized in intensive care, revisions were provided and sections of text edited. His efforts are well represented by Lord Byron (1788–1824) in the quote from *Childe Harold's Pilgrimage, Canto 4 [5 issues, page*. 71] (1818), "But I have lived, and not lived in vain. My mind may lose its force, my blood its fire and my frame perish even in conquering pain, but there is that within me which shall tire torture and time, and breathe when I expire." These words and the desire to honor Dr. Longo inspired several past trainees to help complete his final opus. Contributors to this volume were, for Chaps. 12 and 15, Dr. Ravi Goyal, M.D., Ph.D. (Associate Professor, Basic Sciences, LLUSM; postdoctoral fellow with Dr. Longo 2007–2009 and successor principal investigator of several projects); for Chap. 14, Professor Justo Alonso, M.D. (Professor and Chairman, Department of Obstetrics and Gynecology, University of Uruguay (Montevideo) School of Medicine, Fogarty fellow with Dr. Longo 1988); and for Chaps. 11 and 12, Ciprian Gheorghe, M.D., Ph.D. (doctoral student with Dr. Longo 2000–2006). Dr. Michael A. Kirby, Ph.D. (member of the Center since 1986 and Professor of Human Anatomy and Pediatrics, LLUSM) contributed, as Editor, an inestimable time to proof- and fact-check the entire document. My contribution to this book was to serve as Senior Editor, tasked to oversee interactions with the publisher (Spinger) and the American Physiological Society, to maintain a uniformity of voice in writing style, as well as organize and complete unfinished chapters to as close as possible to Dr. Longo's standards. This impossible task was made easier by what seemed like near daily interactions with him from my first day at Loma Linda University, May 1, 1985, for my first and only academic job. This opportunity was, in a large part, due to his efforts. Over many years, our common interests in science and life, intense professional collaborations in research projects and grants, as well as a personal friendship stood the test of time. I am especially grateful for the help of Jimin Suh upon whom Dr. Longo relied during his work to prepare this revised edition. I also wish to acknowledge the exceptional competencies of Charlotte Marshall, who stepped into this role as Editor's assistant to locate sources and references and follow through with due diligence for preparation of this manuscript.

Accordingly, this revision and expansion is dedicated to the memory of Lawrence D. Longo, M.D. (1926–2016). Collectively, our efforts honor his memory as an inspirational and exemplary leader (Director of the Center for Perinatal Biology 1972–2012), mentor, inquisitive polymath, and at times quixotic motivator to push boundaries of understanding. His efforts provided a sustaining contribution to the development of each of our professional perspectives and those of many more trainees and colleagues to pursue important clinically relevant questions. His values, sense of wonder, persistent questions, and thoughtfulness, which encouraged critical thinking and problem solving in a supportive environment, were to the

professional and personal benefit of all who knew him and for a greater good. For Dr. Longo his lifelong quest, included in the Epilogue to the first edition of this book (p. 487), could be summarized as to advance in practical and theoretical ways the understanding of fetal and neonatal physiology so as to lessen the gap between fundamental and clinical sciences and reduce perinatal morbidity and mortality. He appreciated that a vast *terra incognito* of unknowingness remains, as evidenced by the recalcitrance of current preterm birth rates and the epigenetics of fetal origins of adult diseases. Clearly, the task of basic and clinician scientists is to stand on the shoulders of pioneers in this field, brought to current attention by this treatise, to generate replicable data that promotes useful knowledge of the mechanism through which species reproduce with successful pregnancy and the natural process of birth. The critical value of this understanding must be widely communicated as having enormous benefit to confront challenges to the health and well-being of present and future generations, as well as surmount the impact of hypercompetition for dwindling resources that creates risk for academic life as we know it. As Dr. Longo so often said, "Our task therefore is clear: it is to Persevere!"

August 2017 Steven M. Yellon

Preface to the First Edition

> History is always best written generations after the event, when cloud, fact and memory have all fused into what can be accepted as truth, whether it be so or not" (Theodore Harold White 1961, p. 188)

It was in the autumn of 2008 that Charles Evans Wood, of the University of Florida, Gainesville, chairman of the program committee of the Fetal and Neonatal Physiological Society, invited me to present the Geoffrey S. Dawes Memorial Lecture at the 2009 meeting of that society. This lecture, initiated in 1998 to honor Geoffrey Sharman Dawes (1918–1996), traditionally has been presented by an established investigator who reviews some aspect of his or her studies during the previous several decades. Rather than follow that formula, however, I developed a different plan. As one interested in the history of ideas and the evolution of medicine, for some time I had thought that it would be of value to document some of the major issues and events associated with the genesis and development of the rather specialized field of fetal and neonatal physiology. This was, in part, because of its relatively brief history of less than a century, but also because of its enormous contributions to understanding functional physiologic principles, and because of its concentration on a vital and yet often overlooked aspect of biomedical science that has made a profound impact on clinical medicine.

Among the seminal figures and major forces in developing the field of fetal and neonatal physiology was Geoffrey S. Dawes of the Nuffield Institute for Medical Research, University of Oxford. Trained as a physician, he dedicated his career to understanding the physiologic and pharmacologic basis of important clinical problems relating to the developing organism. In a sense, this monograph could be viewed as a case study of the role of an individual scientist, and many of the individuals he trained, in fostering, advancing, and shaping a given field of research. Dawes' scientific career covered a period of 55 years (1941–1996), during which time he published over 220 papers. Included among these were a number of highly cited scientific contributions, major reviews, introductions to symposia, and chapters in books. As noted in the Foreword by John Richard George Challis,

critically, Oxford's Nuffield Institute served as a seedbed and salutary environment for the education and training of a generation of bright young scientists.

Several individuals have asked why I would undertake such a formidable endeavor. Actually, about a decade ago I had commenced working on an article reviewing some of Geoffrey Dawes' many contributions to life. We had been good friends, meeting once or twice a year in Oxford, at international meetings, or my home base in Loma Linda, and I had high regard for his work. Within several years the project had expanded beyond a mere review. Then, following the 2009 Dawes Lecture I realized that to place it all into perspective the enterprise would require at least a small monograph. Several other reasons are relevant.

I probably am one of the last people alive who knew most of the leading figures (with the exception of Huggett and Sir Joseph Barcroft) and lesser lights who contributed to the evolution of ideas, methodologies, and the synthesis of the problems and issues of developmental physiology. In addition, I have participated with many of these notables in relatively small seminars, large conferences, National Institutes of Health (NIH)-supported study sections, and various brainstorming sessions to identify some of the vital issues and challenges that lie ahead.

Also over the years, I have conducted active correspondence with these individuals on a regular basis, and have had the pleasure of having a large number serve as visiting scientists and seminar speakers at our Center for Perinatal Biology. Almost without exception, these discussions have been an enriching experience. Remembrance of many of their comments, experiences, frustrations, and insights, can perhaps provide a thoughtful background for the vicissitudes in science, and the life of the mind.

In addition, I elected to survey this field in an effort to assist young investigators, both basic scientists in physiology as well as clinical researchers in perinatology—fetal and neonatal clinical medicine—to gain an appreciation of their heritage and what has gone before. With today's World Wide Web, information technology, and nanosecond communication, it is sobering to acknowledge that for many young investigators, that which is more than a few years old is *terra incognito*. In general, we live in an ahistorical age. Life is for Now—the Present. For most of the biomedical literature, reference citations in MEDLINE and PubMed go back only to the late 1940s. Thus for practical purposes, contributions before that time simply do not exist. Our perpetual, annihilating present tends to sever our kinship with the past. A sense of our history and tradition, however, argues for the continuity of thought, experience, and feeling that accompanies the journey across the gulf of time. Without our hieroglyphic scribbling, we lose not only the heritage of the past but also our perspective and outlook, and our sense of who and where we are. With the arrangement as presented, readers will have the ability to review quickly the background of a given problem in a single chapter or subchapter. In addition to the story itself, perhaps of greatest value will be the accompanying references (each of which I have perused myself, many in great detail), which they may read and evaluate for themselves. In the present essay, I have attempted to present some of the epistemology of the threads of scientific thought in the context of their times. Nonetheless, we cannot ignore the words of Theodore Harold White (1915–1986)

that opened this Preface, and found in his *Making of the President* (White 1961). As the Harvard pediatrician Clement Andrew Smith (1901–1988) observed regarding this aspect of developmental physiology, growth in knowledge increases desire to understand its special fields, and "this is particularly true of those periods during which life is more dynamic. In no other brief span of existence can such profound alterations and adjustments be studied . . ." (Smith 1945, p. 3).

Nonetheless, several caveats are in order. Although I have attempted to be reasonably complete in considering the experimental studies of various investigators, rather than exhaustive detail, my goal has been to stress the significance of their contributions. Because of the many subjects encompassed by this field of research, and its complexity and progress, the present essay makes no attempt to survey the topic either *in extenso* or to the present day. Rather, it focuses on the role of some individual scientists and those in their circle. Also because of the extent and vastness to which this field has expanded, I have limited the review chiefly to the second half of the twentieth century, considering issues that came to the fore during that time. One might ask, where does history end, and contemporary physiology commence. As can be appreciated, no history of a given field of discipline can be completely current and up-to-date. With each new day and passing week and month, the advances move the frontiers and expand the horizons. With that in mind, for the most part the present survey concludes about the time of Geoffrey Dawes' death in the mid-1990s.

As a corollary, so that this synthesis may be of value to investigators and others with interest in this facet of science, the general bibliography is rather extensive, and that for Dawes includes every paper of which I am aware he wrote (abstracts are not included). The bibliography also includes a number of review articles and volumes that the interested reader may consult to pursue a given topic in depth. Although the over 2,000 references given may appear somewhat exhaustive, it constitutes only a tithe of those papers published in the field during the period of this survey. As such, I trust that these may be of value as a "taking-off" point for one who wishes to explore the topic in greater depth. Importantly, rather than being viewed as an encyclopedic list of names, dates, and isolated facts, I trust that these would help to place the rise of fetal and neonatal physiology in its proper context. In the paragraph that contains the opening quotation of Theodore H. White, he notes, "What can be reconstructed now out of the contemporary recall of those present must be seen as a fog-shrouded range of facts in which occasionally one peak or another appears at a given hour of the day, but whose connection to the next peak of facts is obscured by the clouds in between" (White 1961, p. 188). Or as Napoleon Bonaparte (1769–1821) is alleged to have stated, "What then is ... the truth of history? A fable agreed upon." A work in progress, history is best served by constant reanalysis and rewriting, as opposed to a museum-quality sculpture in resplendent marble.

An additional caveat is in order. For the most part, investigators in this field worked in what Thomas Samuel Kuhn (1922–1996) referred to as canonical "normal science," or "current paradigm." That is, their studies were conducted within a relatively restricted "model" or "system" with an accepted body of

concepts, techniques, and methodologies that guided their thinking and worked to determine the problems to be explored (Kuhn 1962). Several discoveries of what might be regarded as "revolutionary science" or "paradigm shifts" occurred during this period, such as that of the role of the fetal hypothalamic-pituitary-adrenal axis in the initiation of labor, and the role of pulmonary surfactant in respiration. However, despite a number of breakthrough advances, these were not typical of the period as a whole.

That being said, a number of exclusions and gaps will be evident to the reader versed in this discipline. From the standpoint of contemporary biomedical science, for the most part, much of what is reviewed is general organ physiology, with little consideration of advances in cellular and molecular biology. In fact, some would regard this era as "nineteenth-century" descriptive science, phenomenology, or worse. Nonetheless, it is important to recall that our present understanding is based on previous description of fundamental facts and advances. In the words of Sir Isaac Newton (1642–1727) and those before, "If I have been able to see farther than others, it was because I stood on the shoulders of giants" (Merton 1965).

As is well known, "Clio's many mansions" of history may be considered from a number of standpoints: macro-, micro-, global, national, regional, local, social, cultural, political, economic, biographical, and others. For the most part, the present essay is a combination of technological science and internal history. It also includes a fair bit of biography. I would like to think that not inappropriate, for as Ralph Waldo Emerson, (1803–1881) observed, "All history becomes subjective . . . there is properly no History, only Biography" (Emerson 1883, p. 5). As one who has spent almost five decades as a laborer in this field, as noted an advantage in this approach is that with the exception of the very earliest workers, I knew each of the contributors and many were dear friends. Thus, without sounding self-serving, I would like to think that I have more than superficial insight into the developments and issues involved. A limitation, of course, is that in this presentation only a cursory attempt is made to include a number of related social, cultural, political, and economic aspects. In part, the constraints of scholarly research, but also the limitations in publication, require focus of narrative. Although considering chiefly internal events and the "foreground," I have attempted to place the work within the context of its times. In this regard, I deliberately reject the concept of "continuity" in the development of this field of research. Also, the present essay makes no attempt to resolve certain battles of priority of particular innovation, or to impose "progressive" or teleological schemes on this record. A "Whig" view of historical progressivism (Butterfield 1965), this is not.

In preparing this work it has been inspiring to recall the fine, dedicated individuals and the accomplishments of those who have labored so diligently to develop this field of research—to glimpse the greatness of some of the early achievements that we now take for granted. Rather than being the definitive history, however, I trust that it will be viewed as one perspective of fetal and neonatal physiology, albeit one that is rather personal.

In closing, I am particularly grateful to a number of colleagues, many of whom worked at Oxford's Nuffield Institute, who shared stories, anecdotes, and

impressions of their work and interactions with other colleagues. Importantly, I am in great debt to Jimin Suh who worked indefatigably in helping to locate obscure references and other sources, and to prepare this manuscript in its present form. She is absolutely the finest associate for whom one could wish.

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Contents

Chapter 1
Introduction

> *Some Divines count* Adam 30. *yeares old at his creation, because they suppose him created in the perfect age and stature of man; and surely we are all out of the computation of our age, and every man is some moneths elder than hee bethinks him; for we live, move, have a being, and are subject to the actions of the elements, and the malice of diseases, in that other world, the truest Microcosm, the wombe of our mother. . .. In that obscure World. . ., our time is short, computed by the Moone; yet longer than the days of many creatures that behold the Sunne, our selves being not yet without life, sense, and reason; though for the manifestation of its actions, it awaits the opportunity of objects; and seemes to live there but in its roote and soule of vegetation: entring afterwards upon the scene of the world, wee arise up and become another creature. . ..*
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> (Sir Thomas Browne 1642, 1964, p. 38)

In his "Thoughts on the evolution of a scientific problem," Sir Cyril Norman Hinshelwood (1897–1967), Dr. Lee's Professor of Chemistry and Fellow of Exeter College, Oxford, in this 1953 Presidential Address to the Science Masters' Association of Oxford University, observed "the scientific aspiration towards the understanding of Nature represents one of the great movements of the human mind. . .." (Hinshelwood 1954, p. 300). Hinshelwood wisely noted, "Science is not the dryly syllogistic handling of obvious facts. It is an imaginative adventure of the mind seeking truth in a world of mystery." He continued, ". . . and, as it happens, one of the most important steps is almost always that made by people who have the vision to realize that certain phenomena raise questions of unusual interest. And it may be that the first tentative answers to these questions go further along the road than the latter amendments simply because they provide the motive and occasion for the key discoveries" (Hinshelwood 1954, pp. 300–301). A decade later in his 1965 Presidential Address to the British Association for the Advancement of Science, Hinshelwood noted that "at all the boundaries of science we come up against what are probably the inherent limitations of human understanding. At the edge of biology we meet the chasm between what science describes and what the mind

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experiences…" (Hinshelwood 1965, p. 355). George Santayana (1863–1952) observed, "Science is nothing but developed perception, integrated intent, common sense rounded out and minutely articulated" (Santayana 1906, p. 307), and Sir Winston Leonard Spencer Churchill (1874–1965) is alleged to have echoed a somewhat similar theme in a lighter manner, "Science is no more than organized curiosity" (Priestley 1957, p. 148).

Science also has been defined as the observation, identification, description, experimental investigation, and theoretical explanation of natural phenomena. As an intellectual exercise, it continues to expand, both in breadth of inquiry and in the depth to which questions are explored. Science extends from the outer limits of cosmology to the molecular, atomic, and subatomic basis of existence. The Latin word *scientia* is derived from *sciens* the past participle of *scire*, to know. As stressed by George Alfred Leon Sarton (1884–1956) of the Johns Hopkins University, science is the only field of intellectual activity that is progressive (Sarton 1927–1948, 1937, 1952). He later reemphasized this view:

> The history of science may be defined as the story of the gradual unveiling of objective truth and of the conquest of matter by mind, it describes the age long and endless struggle for freedom of thought … .. The history of science is one of the essential parts of the spiritual history of mankind, the other main parts being the history of art and of religion. It is not more important or more enlightening than these other parts, but it differs from them in that the development of knowledge is truly cumulative and progressive … if we would explain the progress of mankind, the history of science should be the very axis of our explanation.
> (Sarton 1957, pp. 1–2)

Midway between the extremes of the infinitesimally minute subnuclear particle to that of the infinitesimally expansive cosmos is the human being, *Homo sapiens*, that sentient creature that observes, contemplates, questions, and wonders. As the study of vital life processes and functions, the discipline of physiology (from the Greek *physis* "nature" or "origin" and *logia* "the word" or "study of") lies at the core of an integrated understanding of biological function. Unique among the biomedical sciences, physiology is the study of the dynamics of life, describing the vital functions of living organisms, their tissues, and cells. As a consummate example of reductionism, the science of physiology includes integrative function of the whole body and its system organs, cells, and molecules. That is, critical to a reductionist approach is that of integration of the sum of the parts into a greater, global view of the body. Claude Bernard (1813–1878), the great nineteenth-century Parisian biologist-physiologist, was an articulate proponent of this concept. Among his contributions, he asserted the integral importance of the *milieu intérieur* [internal environment] and the role of physiologic functions in maintaining the constancy of organs and their constituents (Bernard 1878). In his *The wisdom of the body*, Walter Bradford Cannon (1871–1945) championed the view of homeostasis of the *milieu intérieur* (Cannon 1932).

From the standpoint of ontogeny, one can inquire into the critical features of development and their functional capabilities that give rise to the individual being who can utilize his/her endowment, gifts, and abilities, to experience a full life, living, loving, cherishing, and contributing. This constitutes the republic of fetal and neonatal physiology. Although limited, in terms of the planetary systems,

galaxies, and expanding universe of science, the field of developmental physiology goes back several centuries. In this synopsis, I would like to consider some aspects of this history as a case study. That is, the manner in which, with sequential and parallel discoveries, persons of more than average ability came together, bringing their different backgrounds, talents, and expertise, to ask critical questions. And by diligence and persistence, often in the face of adversity and not necessarily in agreement, these individuals innovated, developing unique models, and opened new vistas to explore. With these contributions, novel ways of thinking occurred in reasoning and reflection upon a given subject that in many instances contributed to advances in care of the pregnant mother and newborn infant. As acknowledged by others, history is difficult to evaluate, even with the availability of well-documented records. Its exploration has a way of keeping us humble. In interpreting historical events and phenomena, it is important to attempt to know the mind-set and goals of those involved. Thus, in the present survey, insofar as possible I have attempted to enlist and record the opinions of the key investigators in the evolution of this field.

By definition, fetal and neonatal physiology encompasses events from early embryonic development through full development of the fetus, and includes the profound changes at the time of birth, and the first month of life as a newborn infant. Growth and development are a function of both genetic and environmental factors, and these represent a continuum of change that serves to maintain homeostasis of the organism. As an example, the history of research on the fetal circulation, which is valid for the field of fetal physiology in general, has been divided into four eras: the anatomical period from the time of Galen [Claudius Galenus] (131–201 CE) of Pergamum [also Pergamon], those contributions from the time of William Harvey (1578–1657) onward, the period of anatomy and anatomical-based physiological hypotheses, and the era of hypothesis-based experimental research which commenced in the mid to late nineteenth century (for instance, see Barclay et al. 1944).

In addition to increasing basic understanding of fundamental physiologic, biochemical, molecular mechanisms, a critical aspect of advances in fetal and neonatal physiology has been its many contributions to clinical medicine, both obstetrical perinatology and pediatric neonatology. It is by such research that patient care for the gravid mother, fetus, and newborn infant have advanced beyond blind empiricism. Importantly, the field is a model of translational biomedical science at its best. As suggested by the subtitle, beyond its contributions to basic science, fetal and neonatal physiology is exemplary in serving as a bridge "from bench to bedside," to advance clinical care. In an attempt to present some of the advances in translational/clinical science, and to engage a broad audience, from undergraduate to graduate students, medical students to practicing physicians, I have included a number of related contributions in reproductive medicine such as development of the fetal/newborn brain, endocrinology, and pulmonary and cardiovascular biology. With the wide interests of a diverse readership, the volume is organized so that one may select those chapters relevant to ones interests.

In general, major contributions to science, the humanities, the arts, and other areas of knowledge have originated from two processes. The first is conceptual. The polymath Arthur Koestler (1905–1983), in his *The Act of Creation*, described this as

"the bisociation" of two ideas or areas of knowledge, which previously had not been appreciated to be related, igniting the mind of an original thinker. On occasion, such associations may occur in an instant (Koestler 1964). The second is the detailed, laborious, experimental testing of a new, innovative concept or hypothesis in an effort to obtain the evidence that either will support or refute a given idea or question. This stage may take years or decades. In speaking of art and its genesis, Walter Gropius (1883–1969) who forged the utilitarian movement in architecture and fine art known as *Bauhaus* [house of building/building school], observed that art blossoms in rare moments of inspiration by the grace of heaven (Gropius 1970). The same may be said for creative, innovative science.

In physiology, and almost all areas of the biomedical sciences, study of the adult organism has preceded that of the fetus or newborn infant. In fetal and neonatal physiology, the majority of contributions have appropriated major conceptual breakthroughs from general biology, biochemistry, or physiology and applied them to the developing organism. Because the fetus is, in effect, an "astronaut in utero," and the newborn infant, particularly one that is markedly premature, is such a fragile organism, experimental studies to uncover fundamental mechanisms have been neither easy nor straightforward. Thus, the field has been met with challenges from almost every quarter. As the English poet and critic Samuel Taylor Coleridge (1772–1834) observed, "The history of a man for the 9 months preceding his birth, would, probably, be far more interesting, and contain events of far greater moment than all the three-score and 10 years that follow it" (Coleridge 1836, p. 244). And as in his poem "C.L.M." regarding our antenatal experience, John Masefield (1878–1967) wrote:

> In the dark womb where I began
> My mother's life made me a man.
> Through all the months of human birth
> Her beauty fed my common earth.
> I cannot see, nor breathe, nor stir
> But through the death of some of her.
> (Masefield 1927, p. 77)

Fundamentally, scientific research looks to the future, to discover what can be imagined and discovered. And yet there is no escaping its history. Perhaps more than in other pursuits of the intellect, that which is possible in science critically depends upon what has been. Of importance in this regard are not only the facts that have been discovered but also the inspiration to be gained and the appreciation of the achievements of those who have gone before. At the same time science lays bare the paucity of our knowledge. Oliver Wendell Holmes (1809–1894), Professor of Anatomy at the Harvard Medical School, recognized this in his essay "Border Lines of Knowledge in Medical Science." He wrote, "Science is the topography of ignorance: From a few elevated points we triangulate vast spaces, inclosing infinite unknown details …. The best part of knowledge is that which teaches us where knowledge leaves off and ignorance begins" (Holmes 1891, p. 211).

An ineluctable consequence of the growth of science is that in essentially every field of inquiry, as it becomes established the subjects expand, become increasingly

complex, and divide into further subdisciplines. In part, reflecting intellectual insights, this process of fracture is a consequence of discoveries and advancements in technology and instrumentation. In the late nineteenth century, Thomas Henry Huxley (1825–1895) one of the founders of the Physiological Society in the United Kingdom, foresaw the inevitability of reductionism when he observed that it would appear that, "... the scientific, like other revolutions, meant to devour its own children; as if the growth of science tended to overwhelm its votaries; as if the man of science of the future were condemned to diminish into a narrow specialist as time goes on" (Huxley 1864; Bibby 1967, p. 234). In considering special features of biological science, Huxley observed, "... Physiology is *the* experimental science *par excellence* of all sciences; ... that which affords the greatest field for the exercise of those faculties which characterize the experimental philosopher ..." (Huxley 1864; Bibby 1967, pp. 53–54).

In this volume, I have striven to chronicle those discoveries and related matters of most importance and relevance. Although attempting to remain free of bias and give full justice to every vital contribution, I appreciate that as a lone author my perspective is less than perfect. What follows is a singular view of the antecedents of this field of physiology, that of the fetus and newborn infant. In particular, for his role as a catalyst and synthesizer, I detail many aspects of the role of the Oxford pharmacologist-physiologist Geoffrey S. Dawes who worked to develop and mature this intellectual endeavor. As a pioneer who donned the mantle of his predecessors, by his indefatigable labors, Dawes not only personally advanced the science but also did so through the influence of the scholars who worked with him, many of whom went on to distinguished careers at academic centers throughout the world.

Some have viewed the origin of this field in terms of a "big bang" theory, e.g., that it commenced in a blinding flash of academic brilliance at the Nuffield Institute in the early 1950s. As this review documents, that is not quite the case. Albeit, although this subspecialty of physiology matured during this period, its creation and development is a long and complex story. In addition to many only minor breakthroughs, it includes digressions and side roads, blind alleys and dead ends, incorrect ideas, and often some degree of confusion and differing perspectives.

The fetus has been viewed from many perspectives. In 1916, Armenouhie Tashjian Lamson, (1887–1970) the physician director of a free prenatal clinic in Seattle, Washington, published *My Birth: The Autobiography of an Unborn Infant*, a chronicle of her unborn infant's 9-month struggle to develop from a fertilized ovum to an embryo, fetus, and newly born son (Lamson 1916). The narrator, Lamson's fetus, stressed the importance of good food and a salutary environment not only in supporting his growth and development but also that of America as a nation. Lamson used her story of embryonic/fetal development as a commentary on the era in which increasing industrialization, economic influence, immigration, and professionalization were reshaping cultural and intellectual life, social structures, and the political economy of the country. During the course of the twentieth century, the "new fetus" became an entity/persona of considerable cultural influence. In her foreword, regarding this antenatal period Lamson reassured, "knowledge will fill every minute of that person with happiness and a peace that comes only from perfect understanding" (Lamson 1916, p. 7).

Again, the view(s) of the developing fetus has changed dramatically over the course of the past century. In the mid- to late nineteenth century, fetal life was recognized at the occurrence of "quickening" in the fourth or fifth month of pregnancy. By the late twentieth century, ultrasonic images could detect fetal life shortly following conception. In the late nineteenth century, embryologists and obstetricians were beginning to understand the mechanisms of fertilization and development but could neither observe nor interfere in this process. By the late twentieth century, reproductive endocrinologists could manipulate fertility and perinatologists could both diagnose and treat the fetus in utero. In the late nineteenth century, the fetus was neither recognized nor regulated by the law. A century later the fetus was governed by a wide array of tort, property, criminal, and abortion laws. In the late nineteenth century, few knew what a fetus looked like. Today, almost anyone can identify its sonographic image.

Despite the monumental developments of the past century, the meanings ascribed to the fetus are marked by subtle continuities and recurrent questions. Some of these, such as how this creature develops, are embryological. Some, such as what fetal life means and the issue of personhood, are philosophical and theological. Other questions, such as the extent to which fetal life should be protected, are ethical and political. Several scholars have explored various aspects of the transformation of the human fetus into scientific specimens (Morgan 2009), the history of fetal health (Woods 2009), the risk of antenatal complications (Reagan 2010), birth control (Gordon 1976), abortion (Mohr 1978), and the resurgence of so-called "natural" home births (Leavitt 1986).

In such an exercise, a number of general questions require consideration. Beyond the discoveries and contributions of individual scientists, what are the major factors that lead to development of a specialty/subspecialty in science? What is the role of related fields of science in shaping development of a discipline? To what extent does such development depend upon advances in instrumentation and technology? What are the ways by which one can calculate the impact of particular scientists, their academic departments, or institutes? Is it a function of the papers produced, those that are the most highly cited, or the cadre of students, postdoctoral fellows, and others, and their academic careers and productivity? What of the social-cultural issues of the time/era under review? These with others are some of the questions one must consider in exploring the fabric and detailed pattern of this or any other particular subject of biomedical science.

Some specific questions in regard to this field also require consideration. How did the field of fetal and neonatal physiology develop? What were the major factors? Who were the key players? In what way did Geoffrey Dawes uniquely contribute to the advance of this field of research? Who were some of the individuals he mentored? What have been some of the seminal contributions of the field to clinical medicine in care of the pregnant mother and her newborn infant? What factors have influenced the continued advancement of this field? What is the role of the university environment and its culture? *Quo Vadis*, what is ahead for fetal-neonatal physiology, what are its opportunities, and what are our challenges?

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Chapter 2
A Scientific Genealogy: Early Development of Fetal-Neonatal Research

> *Human life proceeds by stages. The life-periods of the human individual are no less real and significant than the geological ages of the earth or the evolutionary stages of life*. Each stage . . . is distinguished by a dominant feature, a leading characteristic, which gives the period its coherence, its unity and its uniqueness.
>
> (Samuel Feldman 1941, p. 53)

2.1 The Beginnings and Some Definitions

From a broad perspective, the human life-span can be considered as two major periods, prenatal life and postnatal life. By definition, the fetus in utero is a developing mammal or other viviparous vertebrate. The word fetus, however, is an adopted Latin word which signified a "bringing forth, breeding, dropping, or hatching" of young (Smith and Hall 2000, p. 296). By metonymy, it came to mean the young or progeny. Thus, strictly speaking, a contemporary equivalent would be parturition and neonate. By common usage, the term has been pushed back in ontogeny, and in humans, medical texts define this as the unborn young from the eighth week of pregnancy following fertilization (or 10 weeks after the onset of the last menstrual period), that time at which the major organs have been formed until the moment of birth. In humans at 8 weeks, it is about 5 cm (2 in.) in length and weighs ~8 g, and the developing head constitutes about one-half the total mass. From weeks 11 to 17 of gestation, the brain, heart, and other organs continue to develop, and subtleties appear in the several structures such as centers of ossification in bones and development of genitalia. At about 16 weeks, a woman who has been pregnant previously will sense fetal movements, "quickening," although this may not occur until about 20 weeks in a nulliparous (having not delivered before) woman. From weeks 18 to 27, development continues with the appearance of many structures such as eyelashes and eyebrows, finger- and toenails, and a fine lanugo hair that covers the body. From weeks 28 to 40, nerve growth with myelinization continues, the amount of body fat increases, the electrocorticogram takes on a cyclic pattern of low voltage high frequency (associated with rapid eye movements

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and other muscular activity and an increased rate of cerebral metabolism), alternating with high voltage low frequency. At this time, the fetus is capable of life independent of the mother's womb, and for the most immature, survival has been enhanced greatly by advances in neonatology. By 37 weeks of gestation, almost all infants can survive independently of neonatal intensive care.

At full term (40 weeks), the *gravid* [weighty] uterus with fetus, placenta, and amniotic fluid weighs 5–7 kg. The fetus/newborn weighs about half of that total, depending upon ethnicity, genome, size of the parents, parity of the mother, and other factors, with males weighing about 100 g more than females. Rather than a passive passenger in the drama of reproduction, the fetus plays a critical and dynamic role in regulating its own growth, development, and, at the appropriate time, its delivery into the world. The so-called maternal-placental-fetal unit constitutes a vibrant, dynamic system of communication with exchange of nutrients from the mother, production of vital hormones by both placenta and fetus (particularly the pituitary gland and adrenal cortex), and other interactions. Only recently is the extreme complexity of these interactions being unraveled. Within the uterus, the fetus experiences a position of protection from the external world, never to be experienced again in life (Reece and Hobbins 2007).

Rather than being just a "small adult," in addition to its dependence for life upon the placenta and its unique cardiac circulatory patterns, the fetus is distinct in many other respects. As may be anticipated, gestation is associated with considerable variation in rates of fetal growth and maturation, these being a function of factors noted above, as well as that of the mother's weight and body mass index, exposure to poor diet and/or toxins (such as tobacco products, alcohol, recreational drugs), altitude of residence, and other factors. Dysregulation of placental structure and/or function, compromised uteroplacental blood flow, and other issues may jeopardize nutrient delivery from mother to fetus. Any of these may result in intrauterine growth restriction (IUGR), the fetus being small for gestational age (SGA). During the third trimester, the developing organism undergoes some of its most vital changes, with completion of the process of growth, development, maturation, and remodeling that continues into the third decade of life. In contrast to many facets of mammalian physiology which were fairly well understood in the mid- to late-nineteenth century, the addressing of these mechanisms of the fetus and newborn did not truly develop until the mid-twentieth century.

Not a physiologist, the physician, rhetorician Sir Thomas Browne (1605–1682) of Norwich, Norfolk, England, whose words opened this essay, recognized the vital importance of fetal development and that moment at which, "... wee arise up and become another creature" (Browne 1964, p. 38), a time during which more changes occur than Browne could have imagined. Beyond isolated studies, what has been described as "the age of adventure" (McCance 1977, p. 134), and might be regarded as the origin of the physiology of the fetus and newborn in its broadest sense, arose in the late 1920s and 1930s in Great Britain. This included studies in biochemistry, endocrinology, neuroscience, and related subject areas.

2.2 Arthur St. George Huggett and Early Studies of Fetal Physiology

As presciently observed by Samuel Robert Means Reynolds (1903–1982), the history of fetal-neonatal physiology is the coming together of "Many Slender Treads…" (Reynolds 1978). In this essay, Reynolds noted that one who must be recognized as playing a vital role in this regard was Arthur St. George Joseph McCarthy Huggett (1897–1968) (Fig. 2.1c) (Reynolds 1978). In the mid-1920s, at St. Thomas' Hospital Medical School, London, working with his chief John Mellanby (later Sir John; 1878–1939) and the senior obstetrician John Fairbairn (1868–1944), "Hugo," as he was known to his friends, became interested in the problem of development of regulation and sensitivity of the respiratory center. In an effort to perform his studies in fetal goats and sheep in as physiologic state as possible, with the fetus attached to the placenta, he immersed the anesthetized mother in saline in a large bathtub that he found discarded on the hospital grounds. Bunsen gas burners, placed beneath the tub, maintained the saline at body temperature. Huggett's initial report presented the first oxyhemoglobin saturation curves of the fetal blood of a mammal (Huggett 1927). From this time, until his death four decades later, Huggett contributed to a number of areas of developmental physiology.

In his initial report, Huggett described studies during the previous century in which investigators used pregnant sheep. He observed, "The sheep… has the necessary requirements [for these studies] but is three to five times the price of a

Fig. 2.1 (**a**) Nathan Zuntz (1847–1920). (**b**) Paul Zweifel (1848–1927). (**c**) Arthur St. George Huggett (1897–1968)

goat" (Huggett 1927, p. 375). Later, Huggett extolled the merits of this approach, so that one may ". . . treat the unborn foetus as an entity and to study it at any foetal age at leisure. It has been of particular service in study of the placental mechanisms, since retraction of the placenta does not occur. It is . . . particularly suitable for foetal investigations, since, by using large animals . . . the foetus is of a convenient size unobtainable in most laboratory animals" (Huggett 1950, p. 102). By use of a Van Slyke manometric apparatus (which only recently had been described; Van Slyke and Neill 1924), and Barcroft saturators (Barcroft 1914), in goats anesthetized with urethane, Huggett first measured the fetal oxygen and carbon dioxide dissociation curves and attempted to determine the mechanism by which these gases exchange across the placenta between maternal and fetal blood. In umbilical venous and arterial blood, Huggett determined the oxygen contents to equal oxyhemoglobin saturations of 45 and 17%, respectively, and the O_2 partial pressures (PaO_2) to equal 41 and 15 Torr, respectively. His fetal oxyhemoglobin saturation curve determined by differential tonometry was, in fact, incorrect, showing an abnormally low blood oxygen affinity (a P_{50} of about 40 rather than 19 Torr). As noted several decades later by Sir Joseph Barcroft (1872–1947) (Fig. 2.2a), although Huggett did not publish dissociation curves for the mother (and, in fact, few oxyhemoglobin saturation curves for any creature were available at that time), Huggett's fetal curves so differed from any curves then known as ". . . to excite the curious" (Barcroft 1946, p. 166). Huggett correctly noted the greater amount of carbon dioxide (CO_2) in fetal blood than that of the mother, and its relatively less effect in decreasing the oxygen affinity of fetal blood (Bohr effect), compared to the adult. His determination of the CO_2 equilibrium curve was quite accurate. Based upon the maternal arterial to fetal arterialized O_2 tension difference of about 45 Torr, and a similar difference in CO_2 partial pressures between the fetal umbilical arteries and the maternal vessels, Huggett concluded that these gases crossed the placenta by diffusion rather than by active secretion, as was believed by some investigators. In addition, by contrasting the O_2 content of carotid arterial blood with that of the umbilical artery, Huggett demonstrated the separation in streams of blood in the fetal central circulation (Huggett 1927). Of critical importance, Huggett's studies stimulated investigation of the interrelations of fetal and maternal blood oxygen affinity and capacity and the placental exchange of respiratory gases by other workers over the next decade and more.

This is not the place to review Huggett's life and accomplishments in detail. That has been recorded by others (Brambell 1970). Upon completion of a medical course somewhat abbreviated by World War I, in 1918, Huggett graduated from St. Thomas' Hospital Medical School. Following a year in military service, with the award of a Beit Memorial Fellowship, from 1922 to 1925, he undertook independent research. With a background in study of the central nervous system and respiratory regulation in the cat, he initiated studies of the fetal respiratory center, placental respiratory exchange, and placental glycogen in the goat (Huggett 1927, 1929, 1930, 1955). Huggett credited the influence of two professors, Fairbairn and Mellanby, for focusing his interest on physiology of pregnancy and the fetus, and in addition to being inspiring teachers, as having "something more" (Brambell 1970).

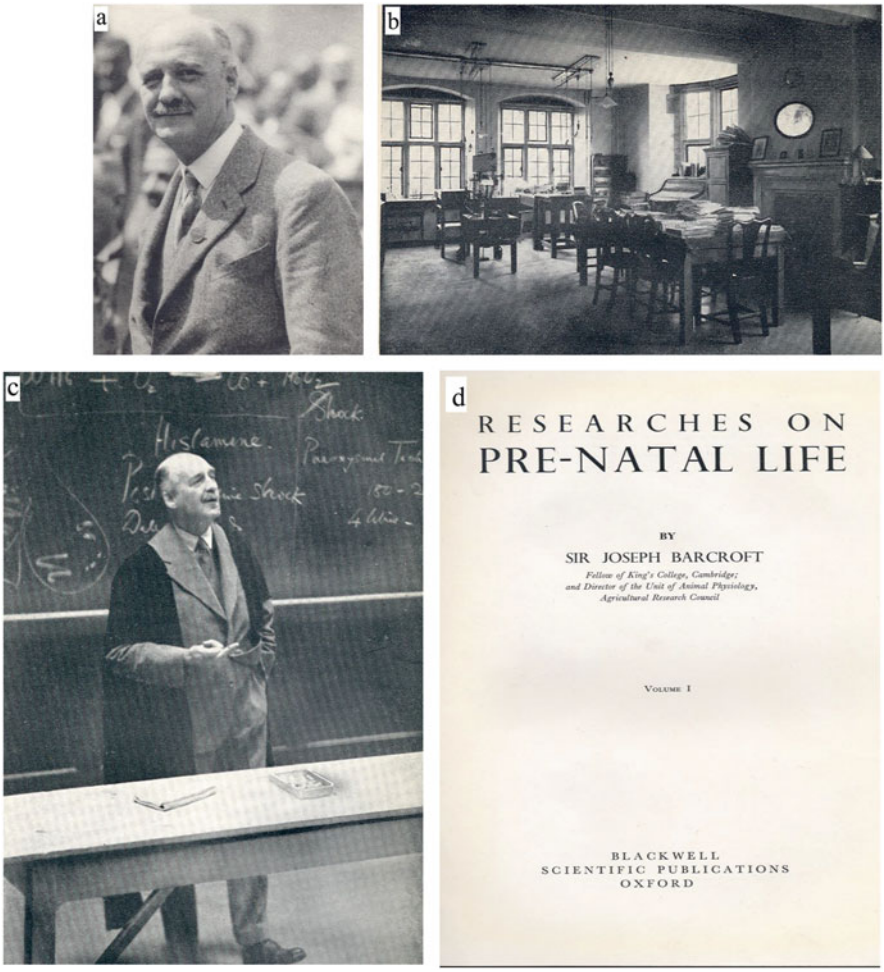


Fig. 2.2 (**a**) Sir Joseph Barcroft (1872–1947). (**b**) Barcroft Laboratory (ca. 1940). (**c**) Sir Joseph Barcroft (1872–1947). (**d**) Title Page (1946)

2.3 Late Nineteenth- and Early Twentieth-Century Contributions by German Physiologists and Others

As has been documented elsewhere, during the latter half of the nineteenth century, German science became preeminent, with its universities serving as world-renown centers for research and creativity (Blackbourn 1998; vom Brocke 1991). To understand the origins of these developments, they must be placed within the context of their time. Friedrich Wilhelm Christian Karl Ferdinand von Humboldt (1767–1835), premier minister of education, governmental functionary and diplomat, in 1810, founded the Humboldt University, Berlin, and worked to initiate a university system that fostered research. During this era, the "Iron Chancellor" Otto

Eduard Leopold von Bismarck (1815–1898), first chancellor (1871–1890) of the unified German Empire, also influenced the support and expansion of the universities and scientific research. In particular, the latter part of the century saw growth of the sciences basic to medicine, with establishment of university departments of bacteriology, pathology, and physiology, within which scientific research was preeminent. In part, this also was the work of Prussian Minister Friedrich Althoff (1839–1908) who in 1872 was instrumental in refounding the University of Strasburg into the Kaiser-Wilhelm University (with the return of Alsace-Lorraine to France following World War I, this was renamed the University of Strasburg) and other institutions of higher learning. By recognizing creative talent and supporting many departments and individual investigators, Althoff expanded the university system. In many respects, development of the research universities in Germany was a consequence of the recognition that science, scholarship, and education with advances in technology contributed to the economic development of the industrial revolution. These were seen as "the fourth factor of production," in concert with the traditional combination of land, capital, and labor. Importantly, the rising demand for highly trained individuals contributed to a related social and economic development (vom Brocke 1991).

This transformation of German intellectual life and culture actually had its origin in the late eighteenth-century Enlightenment, with the rise of literary and philosophical debates and thinking. Rather than a single territorial state in the contemporary sense, Germany consisted of a number of separate territories, with the chief loyalty of its peoples to the church, trade guilds, and feudal lords. With the gradual dissolution of the Holy Roman Empire during the Napoleonic Wars (1806), and creation of the unified German Empire under Bismarck, there developed renewed emphasis in the humanities and science, as well as politics and military endeavors. A belief in *Erziehung und Bildung* [formation or upbringing and education] was central to this culture of progress. Support of the universities acquired its own momentum, with development of academic disciplines and subdisciplines. In particular, following unification, the German state exerted leadership in education, including appointment of teachers and professors and scholarly activity. In part, the goal of this advanced educational system was to produce model citizens and officials to maintain the administrative organization and structure. Thus, education and research was regarded as a "practical need of the state," with high standards and expectations. Both cultural and national optimism supported the concept of scholarship in essentially every field of knowledge. In medicine and the natural sciences, this included preeminence in physics, chemistry, biology, physiology, microbiology, and related disciplines. Professional specialization and scholarly research were given high priority (for discussion, see Blackbourn 1998).

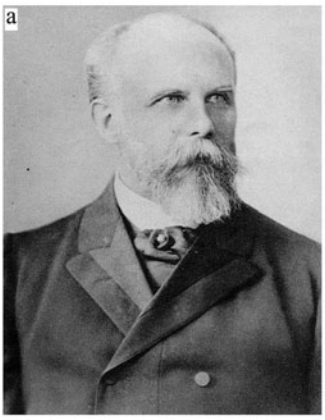
Prior to Huggett by half a century, with his measurement of oxyhemoglobin concentration being greater in the fetal umbilical vein than in the arteries, Paulus [Paul] Zweifel (1848–1927) (Fig. 2.1b) then at the University of Strasburg demonstrated unequivocally that the developing placental mammal consumes oxygen provided from the blood of the mother (Zweifel 1876). At this time, the nature of placental exchange was not understood, however. A common view held that the

fetus was merely an "organ" of the mother, consuming little or no oxygen, and that except for its heart, it grows in an inactive state producing no heat, being warmed by the mother's metabolism. This concept was supported by the prior observation of no apparent difference in the color of blood (e.g., its oxygen levels) between the umbilical arteries and veins (Pflüger 1868). Although two centuries earlier William Harvey had raised the question of how the fetus survives in utero without air (Harvey 1651), there existed no agreement on this matter. Several years later, Paul Bert (1833–1886) first described the oxyhemoglobin saturation [HbO_2] curve (Bert 1878). At Strasburg, because of somewhat limited facilities in the *Frauenklinik* [Woman's clinic], Zweifel worked in the institute founded by Ernst Felix Immanuel Hoppe-Seyler (1825–1895). A leader in physiological chemistry (Hoppe-Seyler 1881), Hoppe-Seyler, had great interest in hemoglobin, being the first to crystallize it and give its name (Hoppe-Seyler 1864). To resolve questions as to the extent to which the fetus consumed oxygen, and if so its source, Zweifel determined to measure the oxyhemoglobin saturation in the umbilical artery and vein of the near-term pregnant rabbit (Zweifel 1876). Based on his clinical experience in handling the pregnant uterus and umbilical cord, in an effort to minimize the effects of exteriorization on the umbilical vessels, with care, he placed the rabbit in a warm saline bath, opening the abdomen and uterus underwater and allowing the placenta to remain in situ attached to the uterine wall. As noted, Zweifel first demonstrated the oxyhemoglobin saturation to be considerably greater in the umbilical vein than the umbilical artery. Upon asphyxiation of the doe, this difference disappeared but reappeared again upon resumption of maternal breathing (Zweifel 1876). As an aside, the technique of immersing a body in a warm saline bath in which to float internal organs had been described several years earlier for the study of the influence of the vagus (10th cranial, parasympathetic) and splanchnic (sympathetic) nerves on gastrointestinal motility (van Houckgeest 1872). There is no evidence that Zweifel was familiar with this report, however. With his study, Zweifel established that, indeed, the fetus consumed oxygen and that the placenta served as a fetal lung (Zweifel 1876). This answered in a definitive manner the question posed by Harvey so many years before and opened the way for studies of fetal metabolism (see below and Barron 1976, 1978).

The following year (1877), Nathan Zuntz (1847–1920) (Fig. 2.1a), in a combined theoretical and experimental approach, also estimated the difference in several variables including O_2 levels in fetal and neonatal blood of the rabbit. When subjected to asphyxia, he observed that the fetus survived longer than the doe. He concluded that per gram of tissue, the fetus required less oxygen than the mother. Again, in part, this idea was based upon his observation that the fetus can derive its heat from the mother. In sheep, Zuntz also demonstrated that in the absence of breathing following delivery, the fetus could be kept alive if the umbilical circulation was intact (Zuntz 1877). A decade later, Isidor Cohnstein (1841–1894) of Heidelberg and Zuntz in Berlin used this technique in rabbits, guinea pigs, dogs, and sheep to explore the question of the distribution of blood volume, hemoglobin, and O_2 concentrations between the placenta and the fetus and the changes such as an increase in blood content in the lungs, following birth in the

newborn. In these studies, they also demonstrated that withdrawing blood from the fetus decreased its blood pressure significantly (Cohnstein 1884; Cohnstein and Zuntz 1884). Because the ages of the fetuses were not given, the preparations were acute in terms of the mothers being anesthetized, the fetus was dead in many instances, and the methods were questionable; some have viewed the results as problematic (Forbes 1955). In a further study, Zuntz measured the decrease in maternal red blood cell count and hemoglobin concentration during the course of human pregnancy (Zuntz 1884). Zuntz went on to become chair of veterinary physiology at Berlin's *Königliche Landwirtschaftliche Hochschule* [Royal Agricultural College] (1881) and a leader in high altitude physiology (Gunga 2009). One to report on the ability of the human fetus to survive anoxia was Eduard Friedrich Wilhelm Pflüger (1829–1910) (Pflüger 1877).

Another who contributed to the physiological chemistry of hemoglobin and blood gases was Thierry Wilhelm Preyer (1841–1897) (Fig. 2.3a), professor of physiology at the University of Jena (Preyer 1871). For studies of respiration in the fetal goat, he used Braam van Houckgeest's and Zweifel's technique of placing an anesthetized near-term goat in a bathtub filled with saline at body temperature.



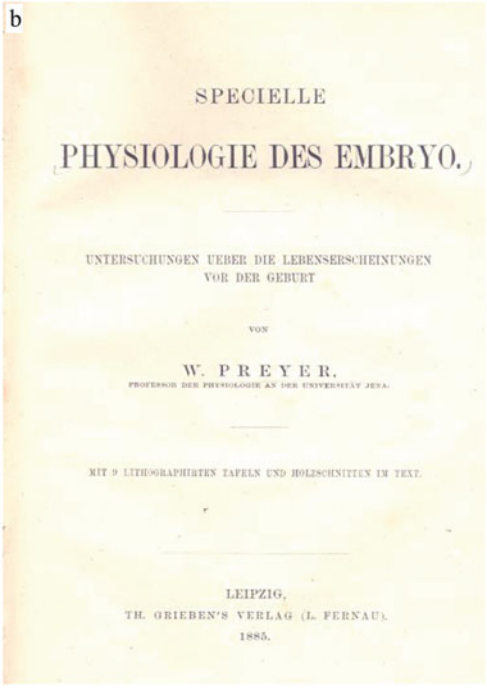


Fig. 2.3 (**a**) Wilhelm Thierry Preyer (1841–1897). (**b**) Title Page (1885). (**c**) *Upper figure*—schema of blood circulation of chick embryo on day 3 of incubation. *Lower figure*—Hen's egg on day 3 of incubation (Preyer 1885). (**d**) Human placenta and uterine wall at 5 months gestation, showing amnion, chorion, villous trees, decidua, spiral arteries, and myometrium (Preyer 1885)

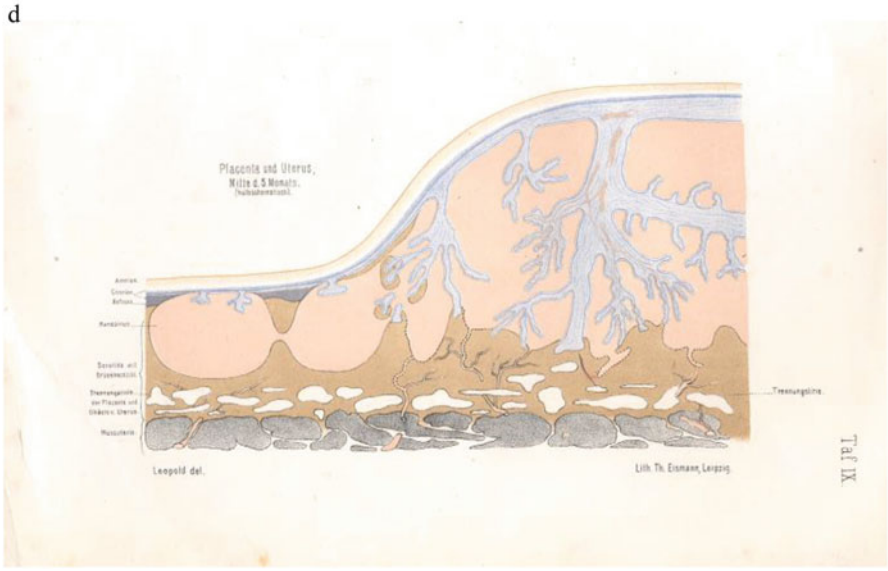


Fig. 2.3 (continued)

Following a hysterotomy incision, the fetus remained submerged and attached to the placenta. A prolific writer, Preyer's *Specielle Physiologie des Embryo; Untersuchungen ueber die Lebenserscheinungen vor der Geburt* [Selective physiology of the embryo; Examination of the life appearance before birth] helped to lay the foundation of this field of research (Preyer 1885) (Fig 2.3b). In many respects, a review of embryonic development and its chemistry, particularly in the chicken egg, this volume consists of nine major sections, most of which have subchapters. These included those on the embryonic heart and circulation of blood, breathing and first movements, nourishment and digestion, heat production, sensation, and growth. A supplement includes three appendices, 552 references to the literature, and nine plates, all but the last of which were drawn by Preyer (1885) (Fig. 2.3c, d). Also considered a founder of scientific child psychology, Preyer wrote *Die Seele des Kindes* [The soul of the child], in which he arranged chronologically his observations on childhood psychological development. In this work, he expressed his belief that the fetus in utero develops with minimal external sensory stimulation (Preyer 1882). Ill health forced Preyer to retire in 1888 at only 47 years of age. Currently, the "Wilhelm Thierry Preyer Award" is presented by the European Society on Developmental Psychology for excellence in research of human development (Geus 1975; Schröder and Young 1995).

The volume *Physiologie des Fötus* by Friedrich Schatz (1841–1920), professor of obstetrics at the University of Rostock, was dedicated chiefly to placental pathology of the twin to twin transfusion syndrome (Schatz 1900). Because of an arterial to venous anastomosis of the placental vasculature between the twins, in the majority of such cases, the recipient fetus is significantly larger and edematous, with a greater blood volume and a fluid-filled bladder. With a relatively high rate of urination into the amniotic cavity, the mother often presents with polyhydramnios and goes into premature labor (Schatz 1900). Other than detailing aspects of comparative size of the twins and some of their bodily organs, their hemoglobin concentrations, hematocrits, and details of the placental vascular anastomosis (see below), the volume addresses little physiology per se. In a 233-page, four-article report, Schatz earlier first reported on measurements of intrauterine pressures within the uterus of a nonpregnant woman. With the use of a liquid-filled balloon, he demonstrated pressure waves from the fundus downward (Schatz 1872a, b, c, d). Other aspects of his life have been reviewed elsewhere (Ludwig 2006).

2.4 Nicholson J. Eastman, Huggett, and Others of the 1930s to 1950s

Throughout the ages, at the time of parturition, a serious problem has been that of asphyxia of the fetus and newborn. Because of his great interest in this issue, and stimulated by the report of Huggett, in a series of studies on fetal blood gas characteristics, Nicholson Joseph Eastman (1895–1973) (Fig. 4.2a), professor of

obstetrics at the Johns Hopkins University in Baltimore, MD, initiated a series of studies on umbilical cord blood gas values at the time of birth. He reported that in these vessels, the O_2 capacity was 21 ± 1 ml dl^{-1} as compared with 15 ± 1 ml dl^{-1} for the mother. (These values equal hemoglobin concentrations of about 16 ± 1 and 11 ± 1 g dl^{-1} for fetus and mother, respectively (Eastman 1930).) Eastman also first compared the O_2 contents of umbilical venous and arterial blood at both normal vaginal delivery and at the time of cesarean section under local anesthesia, values for the latter being 13.3 and 6.3 ml dl^{-1}, respectively, corresponding to [HbO_2] values of 63% and 33%, respectively (Eastman 1930). To determine the extent to which the fetus experiences anaerobic metabolism, and how this may affect the onset of respiration at birth, Eastman measured umbilical lactic acid concentrations at the time of normal and operative delivery. He showed that, compared to the mother, these were within normal limits, ~34 ± 3 mg dl^{-1}, being elevated only in association with asphyxia (Eastman and McLane 1931). Subsequently, Eastman reported the first oxyhemoglobin saturation curves for the human fetus and mother (as well as nonpregnant adult), giving P_{50} values of about 22, 22, and 32 Torr, respectively. In this paper, he also reported the fetal and adult dissociation curves for CO_2 (Eastman et al. 1933).

At this time, Gustav Haselhorst (1893–1955) and Karl Stromberger (1895–1981) of the University of Hamburg-Eppendorf reported much lower values on blood gases of the human fetus, compared to the mother; however, methodological problems probably account for their abnormally low values (Haselhorst and Stromberger 1930, 1931, 1932). Karl-Julius Anselmino (1900–1978) and Friedrich Albin Hoffmann (1843–1924) of Düsseldorf, in papers on *icterus neonatorum*, also reported on the fetal blood oxyhemoglobin curves (Anselmino and Hoffmann 1930, 1931). Because these were determined at a PCO_2 value near zero, however, they cannot be compared with contemporary curves. In 1936, a group from Leningrad reported wide variation in the positions of the human fetal oxyhemoglobin saturation curves at the time of birth, which they ascribed to variations in pH (Leibson et al. 1936). Also about this time, Jacob Roos (1887–1942) and Christiaan Romijn (1910–1988) of the University of Utrecht, the Netherlands, in the cow, confirmed the significantly greater O_2 capacity of blood of the fetus, compared to the adult. They also estimated the O_2 and CO_2 tension gradients across the placenta between the maternal and fetal blood (Roos and Romijn 1937, 1938). During the World War II German occupation of Holland, Roos, who was professor of veterinary physiology, was arrested and sent to the concentration camp at Mauthausen, where he was executed "for trying to escape" (Coppenhagen and van Lieburg 2000). Later, Romijn became professor of veterinary physiology and dean at the College of Veterinary Medicine at Utrecht. An authority in the field of embryonic metabolism and thermoregulation in poultry, he was the recipient of many awards.

Several years following the studies of these early workers, the neonatologist, Clement Smith (Fig. 2.4b), and colleagues of Harvard University presented a detailed study of human oxyhemoglobin saturation curves, giving P_{50} values of about 23 and 28 Torr at pH 7.40 and PCO_2 40 Torr for fetal and maternal blood, respectively. These workers also showed that, following birth, the P_{50} value

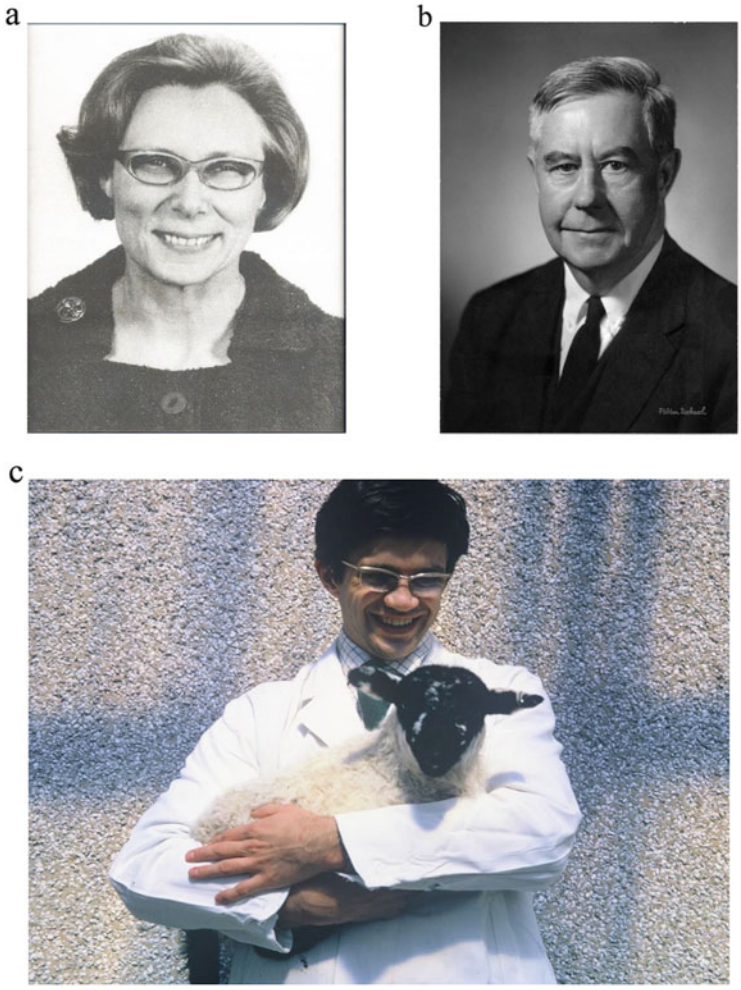


Fig. 2.4 (**a**) Maureen Young. (**b**) Clement Andrew Smith (1901–1988). (**c**) David Mellor holding lamb delivered following 2 months chronic catheterization (1971)

increased to that of the normal adult by 1 month of life (Darling et al. 1941). Later investigators would demonstrate that although in most species studied the oxyhemoglobin dissociation curve of the fetus lies to the left of that of the mother, this is not universally the case, with the half-saturation partial pressure varying from 0 to almost 20 Torr. In addition, the mechanisms responsible for the shift in the fetal dissociation curve have been shown to differ among species (Longo 1987). Importantly, Smith and a colleague attempted to address the issue of the O_2 needs of the newborn infant, including those born prematurely. They noted, "the oxygen economy in the fetus at the moment of birth cannot be taken as representative of the physiologic status which has obtained until that time in fetal life, although there has

been some tendency to accept such an assumption" (Smith and Kaplan 1942, p. 844).

In 1930, Huggett accepted a lectureship in physiology and reader in pharmacology at the University of Leeds, where he remained for 5 years. In addition to continuing the studies of placental glycogen and fetal respiratory reflexes, he had initiated at St. Thomas' (Huggett 1929, 1930), while at Leeds, Huggett collaborated with the chemist Frederick Maurice Rowe (1891–1946) on the anticoagulant properties of azo dyes. Of importance to the field of developmental physiology, in 1932 Huggett visited the laboratory of Joseph Barcroft at Cambridge University, to consult on the measurement of blood volume (Elliott et al. 1934) as well as the use of bisazo dyes. The following year, Huggett acknowledged the "personal aid" of Joseph Barcroft in his determination of fetal lamb blood volume. Regarding this collaboration, Huggett noted, "... I should like to record my thanks to Mr. J Barcroft for much advice, for facilities while at Cambridge, and for the loan of apparatus in London, enabling me to carry out this work" (Elliott et al. 1934, p. 171). It was in conjunction with this visit that Barcroft, at that time a world authority in hemoglobin and the respiratory function of the blood (Barcroft 1914, 1926a, 1934a), became interested in the developing fetus and its oxygenation (Barcroft 1933, 1934b; Roughton 1948). Barcroft also credited Huggett's work on the major differences in O_2 and CO_2 in fetal and maternal blood as stimulating his interest in this field (Barcroft 1946, p. 166).

In 1935, Huggett returned to London, as Chair of Physiology of St. Mary's Hospital Medical School, remaining there until his retirement in 1964. At St. Mary's, he carried a heavy teaching load with only one other full-time faculty member. It was not until the end of World War II (1945) that Huggett was able to resume fetal research in a major way, with the collaboration of D. Pauline Alexander, Hubert G. Britton, Archibald David Mant Greenfield (1917–2005), and D.A. Nixon. A problem that attracted Huggett's interest was that of fetal nutrition and carbohydrate metabolism (Huggett 1941, 1944a, b, 1946) and their relation to placental function (Huggett 1941). He confirmed the relative constancy of placental glycogen under a variety of conditions (Davy and Huggett 1934) and measured the levels of glucose and fructose in blood of the fetus, as well as that of amniotic and allantoic fluids (Huggett et al. 1949, 1951). Huggett also was one of the first investigators to use ^{14}C-labeled carbohydrates (glucose) in the study of transplacental fluxes (Alexander et al. 1955a, b; Huggett and Morrison 1955). In addition to the goat, Huggett investigated a variety of placental and fetal functions in the sheep, rabbit, rat, and mouse (Frazer and Huggett 1970; Huggett and Morrison 1955; Huggett et al. 1949). For instance, by infusing labeled glucose or fructose into the blood of either the pregnant ewe or her lamb, he showed that fructose in fetal blood is derived from glucose. He also demonstrated that while glucose freely crossed the placenta from both mother to fetus and vice versa, fructose crosses only from mother to fetus and is formed in the placenta (Huggett et al. 1951; Nixon et al. 1966). In a number of species, Huggett also contributed to an understanding of the determinants of fetal growth in relation to gestational length (Huggett 1956, 1959; Huggett and Widdas 1951). One of Huggett's coworkers developed a

plethysmograph to use on the umbilical cord (Greenfield 1949), in which the umbilical venous outflow could be obstructed without impeding arterial input (Cooper and Greenfield 1949). By use of this plethysmograph, Huggett and his collaborators made the first measurements of umbilical blood flow in sheep (Cooper et al. 1949) and compared this with that of the guinea pig (Greenfield et al. 1951a, b). To study placental metabolism of carbohydrates, Huggett and coworkers also developed techniques to perfuse the placenta with both recirculation (Huggett et al. 1951) and non-recirculation (Alexander et al. 1955a, b).

During a sabbatical year 1953–1954, Huggett worked with Samuel R.M. Reynolds at the Department of Embryology of the Carnegie Institution of Washington, located in Baltimore, MD. Here, he explored aspects of the transmission of glucose across the placenta in monkeys, as well as in women who were to undergo cesarean section. It was at this time that Reynolds and Huggett developed the method of cannulating the interplacental artery of the rhesus monkey, *Macaca mulatta*. By darkening the operating room and illuminating the uterus with a bright pocket flashlight, they visualized the "intraplacental vessels... as straight or slightly curving vessels with no branching whatsoever" (Huggett 1954a, b; Reynolds 1978; Reynolds et al. 1954). Following these studies, Reynolds went on to make a number of contributions to developmental physiology (Huggett 1955, 1959; Reynolds and Paul 1958).

Working with several other investigators, in 1954 Reynolds organized a week-long special symposium "The Mammalian Fetus: Physiological Aspects of Development," which he chaired. Held at Cold Spring Harbor, on Long Island, NY, over 125 scientists from the USA and Europe participated. These included anatomists, embryologists, physiologists, biochemists, geneticists, and clinicians. The aim of this conference was to evaluate the then current state of knowledge about almost every aspect of the mammalian embryo and fetus, particularly that of the human. Following consideration of some aspects of embryonic development, the group concentrated on what was known and what was not known about a number of aspects of fetal development, metabolism, and physiology (Demerec 1954). The proceedings of this symposium were published as volume XIX of the *Cold Spring Harbor Symposia on Quantitative Biology*, 1954. Uniquely, this symposium was not only the first such meeting devoted to fetal physiology but was attended by almost all of the international investigators in the field. In addition to formal presentations, lively discussions followed. Many have agreed that this conference was a seminal determinant in furthering interest in, and development of, the field of fetal and neonatal physiology. Following this symposium, Huggett summarized much of his work on placental sugar transport in the first of the Josiah Macy Foundation conferences on gestation (Huggett 1955). A decade later, Geoffrey Dawes recalled "The meeting at Cold Spring Harbor in 1954 marked an epoch in the development of studies on the fetus and newborn. That meeting generated an excitement and inspiration that all who attended will remember" (Dawes 1966, p. 74). Still later Dawes stated, "This first post-war international meeting was a turning point for many of us younger physiologists who were fortunate enough to be

a

b

COLD SPRING HARBOR SYMPOSIA
ON QUANTITATIVE BIOLOGY

VOLUME XIX

**The Mammalian Fetus:
Physiological Aspects of Development**

THE BIOLOGICAL LABORATORY
COLD SPRING HARBOR, L. I., NEW YORK
1954

Fig. 2.5 (**a**) Samuel R. M. Reynolds (1903–1982). (**b**) Title Page (1954)

invited, and who there met all those who had already made important contributions to the subject" (Dawes 1980, p. 4) (Fig. 2.5).

Through his studies of fetal homeostasis, oxygenation, glucose metabolism, and other subjects, Huggett's influence continues to stimulate investigative work in these vital areas. Huggett received a number of awards and honors including an honorary PhD (1925) and D.Sc. (1930) conferred by the University of London. He served as Still Memorial lecturer of the British Paediatric Association (1951), De Lee lecturer of the University of Chicago (1953), Claude Bernard lecturer at the *Sorbonne* in Paris (1955), and Purser lecturer at Trinity College, Dublin (1956). He was elected a fellow of the Royal Society (1958), a fellow of the Royal College of Obstetricians and Gynaecologists (1960), and a fellow of the Royal Society of Edinburgh (1965).

Later, Dawes would reminisce that "The modern period of fetal physiology begins with the work of Huggett who during the 1920s [sic] showed that it was possible to undertake acute experimental observations in fetal goats" (Dawes 1980, p. 4). In his biographical memoir of Huggett, Francis William Rogers Brambell (1901–1970) observed:

> There can be no doubt that Barcroft and Huggett, the one wholly and the other partly Irish, working simultaneously but essentially independently and on different aspects of foetal physiology, the one at Cambridge and the other in London, were largely responsible for the revival of interest in this subject. The great advances in this field that have been made during the last 40 years in this country, in the United States of America and in Europe can be traced to the stimulus which their work provided. Huggett's part in this revival has tended to be overshadowed by that of Barcroft, who was far better placed for facilities and

> to attract recruits to his school. Yet Huggett's contribution, with the resources available to him, was great, though the recognition he received during his lifetime was relatively meagre. His paper in 1927 on foetal respiratory exchange in the goat was the starting-point of this revival, though his major personal contribution was the long series of papers on the carbohydrate metabolism of the placenta and foetus. He built up around himself at St. Mary's a notable school whose members are continuing to extend our knowledge of various aspects of foetal life. His place in the history of foetal physiology is honourable and secure.
>
> (Brambell 1970, pp. 359–360)

In a review of several aspects of the field, Robert Alexander McCance (1898–1993), of Cambridge, observed, "Perinatal physiology owes a great deal of debt to a few people; Huggett was one, and his influence on the Physiological Department at St. Mary's Hospital shows it . . .. He introduced Joseph Barcroft to the experimental possibilities of the ruminant and so set the stage for all the work which was subsequently to go on at Cambridge" (McCance 1977, p. 141). Regarding Huggett, David James Mellor of Massey University, Palmerston North, New Zealand recalls:

> Arthur, St George, Joseph, McCarthy Huggett . . . had as many letters after his family name as he did in his given names before it! His qualifications began with FRS, Fellow of the Royal Society, because he had been elected a Fellow on 20 March 1958. He was my first PhD supervisor and I was his last PhD student because he died . . . 22 months after I started my PhD in Edinburgh in September 1966. During those . . . months he shared some of his experiences with me.
>
> He was probably the first person in the UK in the modern era to seriously investigate sheep fetuses. In about 1927 . . . he had immersed the hindquarters of anaesthetized pregnant sheep in warm saline baths and surgically exposed their uteruses and fetuses which he then observed while they floated in the saline. He had long experience of fetal physiology . . . at the St Mary's Hospital Medical School in London. He retired from there in the mid-1960s to become consultant physiologist at the Moredun Research Institute in Edinburgh, Scotland, where our paths crossed in late 1966. Unfortunately, failing health and, sadly, intellectual capacity, limited the academic benefits I could gain from his long experience in the area. However, he eventually became convinced that I had, in fact, received the very extensive, and even for that time, unusual training in experimental surgery of sheep and other mammals that I claimed to have done in Australia during my . . . BSc Honours degree. A feature of the BSc undergraduate major in Physiology at New England University in the 1960s was the duplication of numerous anaesthetised animal models which were used to demonstrate cardiovascular, neurological and other principles established previously by the then giants in the discipline. Also, recovery surgery, including rumen fistulation, exteriorizing carotid arterial loops and adrenalectomy with replacement therapy, for later detailed physiological study, were features in practical class in the third year. Moreover, during my . . . Honours year in 1965 I studied 65 pregnant sheep using epidural anaesthesia, and, as an adjunct, developed an isolated uterus preparation supported by a not very effective heart-lung device which I designed. As a result Huggett approached the UK Home Office on my behalf to have them allocate to me direct copies of all of his experimental Certificates, linked to his Home Office License, which allowed a very wide range of investigative procedures to be conducted on mammalian fetuses. Having accumulated these Certificates since 1927, this action was immensely helpful during my subsequent fetal researches in Edinburgh.
>
> After I had finished my PhD in 1969, I was appointed as Head of the Physiology Department at the Moredun Research Institute to replace Prof Huggett, but not, I hasten to add, on his salary! I then had the astounding good fortune to convince the Director and the

> government department with oversight of the Institute to fund a purpose-built fetal physiology research unit. It was opened in 1971. The unit had two large sheep houses and three smaller rooms containing individual pens for 54 pregnant sheep, a surgery, recovery room and feed preparation room, and, outside, a large covered yard for holding groups of animals. This unit enabled me to run up to 28 chronically catheterised ewes at any one time. The additional pens meant that other ewes could be adapted to the indoor conditions, including the diet, and could be tamed and trained for 6–8 weeks prior to uterine surgery, a practice that was routine in the unit from the outset. The construction and staffing of this unit mark the genesis of the Perinatal Studies Group at Moredun which I led until my departure for New Zealand in 1988. Our remit was to undertake physiological investigations into the causes and prevention of neonatal mortality in lambs.
>
> (Letter from DJM to LDL, 13 July 2009)

Some regard Huggett's most important contribution to science, as that of mentoring Wilfred Faraday Widdas (1916–2008). Following his service as a medical officer in the Royal Army Corps during World War II, Widdas completed his doctorate in physiology under Huggett at St. Mary's Hospital Medical School. With considerable facility in mathematics, his forte was to attempt to explain biologic phenomena in the physical chemical terms of kinetics and in making quantitative predictions. In this regard, Widdas contributed to a number of areas of placental and fetal physiology. For instance, in terms of placental glucose transfer, he calculated that facilitated rather than passive diffusion was required to explain glucose flux rates (Widdas 1952). Following his move to chair the Department of Physiology, Bedford College, University of London, Widdas pursued the studies of carrier-mediated membrane transfer, including that which cannot increase in response to further increase in concentration gradient, now known as "saturation kinetics" (Boyd 2005). In particular, his studies helped to elucidate flux across the erythrocyte membrane under different circumstances (Sen and Widdas 1962a, b). Widdas also emphasized the importance of carrier mechanisms for sugars, amino acids, and other nutrients and the precise regulation required for growth and development of the various fetal tissues, placenta, and amniotic fluid (Widdas 1961). Later, he expanded upon these mechanisms (Widdas 1988). Some have suggested that genes from his nineteenth-century forbearer, the physicist, and chemist Michael Faraday (1791–1867), had a valuable influence on his life (Boyd 2005). In addition to his scientific contributions per se, Widdas served on and chaired the editorial board of *The Journal of Physiology*.

2.5 Joseph Barcroft and a Widening of Interest in Physiology of the Fetus

Prior to the fourth decade of the twentieth century, there was little general interest in the physiology of the fetus or newborn per se. Hitherto, the subject comprised of miscellany of anatomic morphology and comparative anatomy, histology, embryology, and the occasional bit of biochemistry. A seminal figure in respiratory physiology of the first half of the century was Joseph Barcroft (later Sir Joseph)

of Cambridge University. Of Quaker heritage, he grew up in Ulster, Northern Ireland, to which his family had emigrated. In 1888 at age 16, because of his interest in science, Barcroft was sent to Leys School, Cambridge; in 1896 he graduated from King's College. At Cambridge he had been president of the University Natural Science Club, the membership of which during its first 100 years included ten future Nobel Laureates (Pepys 1972). Following graduation, he joined the Physiological Laboratory founded and directed by Michael Foster (later Sir Michael; 1836–1907). During these years and the following several decades, Cambridge was a virtual hotbed of scientific talent. Barcroft's scientific career may be divided into four phases of inquiry: that of oxygen (O_2) consumption in tissues, the definition of the oxyhemoglobin saturation [HbO_2] curve, establishing the limits of human tolerance to high altitude, and pioneering the exploration of various aspects of oxygenation of the fetus in utero. In each of these fields, his contributions opened new fields of investigation. A number of authors have presented various aspects of Barcroft's life and work (Anonymous 1947; Barron 1973; Breathnach 1974; Dale 1949; Dunn 2000; Franklin 1953; Holmes 1970; West 2013; Young 1992). His early work considered oxygen consumption of the salivary gland and the manner in which this increased in response to nerve stimulation, demonstrating that oxygen consumption continued even after the flow of saliva stopped (Barcroft 1900a–c, 1901). These studies lead to the concept of oxygen "debt" in muscle and other tissues, demonstrated for the first time the quantitative study of organ metabolism under various circumstances and during this period, increasingly Barcroft became fascinated by issues relating to tissue oxygen metabolism and respiratory blood gases. In the course of these studies, he developed the differential blood gas manometer, and the tonometer for equilibrating blood with gas mixtures, tools he employed to characterize the properties of hemoglobin exploring its affinity and reversible equilibrium with O_2, thus defining the oxyhemoglobin saturation [HbO_2] curve, and its shift in response to changes in temperature, electrolytes, CO_2, and other factors (Barcroft 1908; Barcroft and Camis 1909; Barcroft and Haldane 1902; Barcroft and Nagahashi 1921; Barcroft and Roberts 1910), and the changes at high altitude (Barcroft 1911; Barcroft et al. 1923) (Barcroft 1899, 1900a, b, 1908; Barcroft and Haldane 1902; Barcroft and Nagahashi 1921; Barcroft and Roberts 1910). In his 1914 *The Respiratory Function of the Blood*, Barcroft summarized his research over the previous decade and a half (Barcroft 1914; second edition, 1926a).

Following World War I, Barcroft extended his studies of high altitude physiology and the mechanisms by which the body acclimatizes to long-term hypoxia (Barcroft et al. 1931). A problem at this time was the extent to which, if any, the pulmonary alveolar epithelium secretes O_2 into the capillary blood, particularly under hypoxic conditions (Barcroft et al. 1920). A related question regarded the extent to which blood oxygen affinity changed with hypoxia, as that at high altitude. In an effort to determine the mechanism of O_2 transfer across the alveolar capillary membrane, Barcroft participated in three expeditions to high altitude: the first in 1910, the second in 1911, and the last in 1921–1922 (Longo 2016). These studies from high altitude were summarized in Barcroft's *Respiratory Function of the*

Blood. Part I, Lessons from High Altitude (Barcroft 1925). With his great interest in hypoxia, he studied blood storage in the spleen and the relation to blood volume (Barcroft 1926b, c; Barcroft et al. 1925). Based on his 1929 Edward Kellogg Dunham (1860–1922) lectures at Harvard University, in 1934 Barcroft published *Features in the Architecture of Physiological Function* (Barcroft 1934a). Of this work, Schack August Steenberg Krogh (1874–1949) stated it to be a volume "... which gives an integration of physiology of such a kind that it ought be read by everyone who is going into experimental work in physiology. It gives the general ideas which cannot be obtained from any other book in existence" (see Franklin 1953, p. 213).

During the later 1920s, Barcroft became interested in blood volume, its stores, and the role of the spleen as a reservoir of stagnant erythrocytes. For the most part, he studied this in dogs in which the spleen had been exteriorized, developing several methods for observing its change in size. Somewhat serendipitously, he observed that the spleen of one of his dogs at rest was contracted to an unusual degree. At autopsy, the animal proved to be pregnant with widely dilated uterine veins (Barcroft and Stevens 1928). This raised in his mind the question of the quantity of blood required by the uterus during the pregnancy. He measured the amount, finding it unusually large, even during the early stages of pregnancy (Barcroft 1932; Barcroft and Rothschild 1932). Thereafter, Louis Barkhouse Flexner (1902–1996), then of the Department of Anatomy at the Johns Hopkins University, spent 2 years as a fellow with Barcroft. With Flexner, Barcroft preformed his initial study in the developmental physiology measuring cardiac output in the fetal goat (Barcroft et al. 1934b).

In reference to his early studies in developmental physiology, David J. Mellor has recalled:

> Prof Huggett said that he had shown Sir Joseph Barcroft how to do his first Caesarian section in sheep. He claimed, and I want to emphasize the words *'he claimed'*, that he anesthetized a pregnant ewe, did a ventro-lateral abdominal incision, and just as he was moving viscera aside in order to draw out the uterus, Barcroft ... elbowed him to one side with words to the effect, "I can take it from here Huggett!", whereupon he promptly incised the rumen!
>
> (Letter from DJM to LDL, 13 July 2009)

In an historical perspective, Barron noted that Barcroft's interest in the course of blood flow through the fetal heart and the timing of the closure of the *ductus arteriosus* arose from his studies on the oxygen environment of the fetal brain and the dramatic changes at the time of birth with arterialization of blood in the newborn lungs (Barcroft 1938; Barron 1979). With his background in studies of blood oxygen capacity and oxyhemoglobin saturation curves, Barcroft compared the O_2 content of umbilical arterial and venous blood with that of the carotid artery in lambs delivered by cesarean section. He concluded that the crossing of the superior and inferior vena caval streams in the heart was essentially complete and that the quantity of blood ejected from the right ventricle via the *ductus arteriosus* equaled that volume in the left ventricle that flowed through the *foramen ovale* (Barcroft 1935a). Barcroft also recorded that during pregnancy, uterine venous

[HbO_2] declined, reaching such low levels that it seemed impossible to maintain a normal state of fetal oxygenation (Barcroft 1935a–d; Barcroft et al. 1940b). The importance of these problems launched him on a full investigation of oxygenation of the fetus.

An example of the manner in which Barcroft could synthesize data, incorporating and expanding upon the work of others, is illustrated by his determination of the fetal and maternal oxyhemoglobin saturation curves. A master in analyzing blood and its oxygen affinity, Barcroft stressed the essential nature of hemoglobin and its physical and chemical environment in determining blood oxygen-binding characteristics. As noted above, Huggett had reported the fetal blood oxygen affinity to be significantly different from that of the adult (although showing it to be less rather than greater, e.g., P_{50} ~40 Torr) (Huggett 1927). With his great interest in this topic, using blood at constant conditions (38°C, PCO_2 ~50 Torr), Barcroft and colleagues reported the correct curves for the newborn goat and its mother (P_{50} = 30 and 37 Torr, respectively) and also compared these at several times from week to week as the fetus matured (Barcroft et al. 1934a; see Windle 1940, pp. 64ff). In a subsequent study, Barcroft and colleagues compared [HbO_2] and O_2 capacity in fetal sheep blood from 63 to 145 days post conception (dpc), reporting on apparent peak in [HbO_2] of ~70% at ~100 days, decreasing from that value until term (145 dpc) (Barcroft et al. 1940a, b). This with other studies (Barcroft 1941, 1942, 1943) contributed to his idea that the "newborn's first breath was the fetus' dying gasp." Thus arose the concept of the fetus being at "Mt. Everest in utero" (Barcroft 1933).

Fortuitously, in the summer of 1934, Barcroft met Donald Henry Barron (1905–1993). At that time, "J.B." or "Jo," as he was known by friends, headed one of the most spacious and well-equipped physiology departments in the UK. Barron, on a fellowship of the National Research Council, USA, had spent 6 months in Berne, Switzerland, and then moved to Cambridge University to work in the laboratory of Edgar Douglas Adrian (First Baron, later Lord Adrian; 1889–1977) and Bryan Harold Adrian Cabot Matthews (later Sir Bryan; 1906–1986) on some aspects of neurophysiology, including spinal cord action potentials (Barron and Matthews 1935). In a chance conversation at afternoon tea, upon learning that Barcroft had purchased 50 ewes for his studies, Barron inquired as whether he proposed to study the functional development of the mammalian nervous system. Admitting that he knew nothing about this topic, Barcroft asked Barron to edify him on the subject. After learning of the little that was known, and questions regarding the controversy as to the applicability of findings in the nervous system of the mole salamander *Ambystomatidae* to mammals, Barcroft invited Barron to join him in studying some aspects of its functional development. For these studies, they would use the sheep as an experimental "model."

In the meantime, to support Barron and their joint research for a year, Barcroft obtained a grant from the Rockefeller Foundation. By use of the technique developed by Huggett, of performing a cesarean section in a warm saline bath to maintain the placental circulation, in November 1934, Barcroft and Barron commenced their

studies on a sheep fetus at 46 days gestation. Fortunately, for the future of the project and the field of fetal physiology, the fetus showed considerable activity, "respiring" spontaneously with rhythmic diaphragmatic movements (Barcroft et al. 1936). In the spring of 1935, shortly before he was made Knight Commander of the British Empire (KBE), Barcroft invited American investigators from the opposing schools of thought regarding neural development to collaborate with him on this line of investigation. Those were William Frederick Windle (1898–1985) from New York University, and a member of George Ellett Coghill's (1872–1941) (see below) department at the Wistar Institute of Anatomy and Biology, Philadelphia, PA, the latter of whom declined the invitation. In the winter of 1935–1936, Barcroft, Barron, and Windle attempted to determine the extent to which the first movements by the fetus represented a response to local reflexes versus mass movements. Although the group could not agree on the interpretation of their findings, they demonstrated that these movements appeared before the central nervous system was fully functional (Barcroft et al. 1936; Barcroft and Barron 1936, 1937, 1939).

Barcroft's studies, demonstrating the importance of the *ductus arteriosus* being patent in the fetus but closing in the newborn, raised the question as to when and by what mechanism this occurs. A serendipitous and fateful event at the March 1937 meeting of the Physiological Society in London significantly influenced the course of fetal physiology. A film made by Barcroft and Barron, "Experimental 'chronic' lesions in the central nervous system of the sheep foetus" (Barcroft and Barron 1937), was shown immediately before the one made by Kenneth James Franklin (1897–1966), a fellow of Oriel College, Oxford, and a colleague, "X-ray cinematographic film of a dogs heart" (Only the Barcroft and Barron abstract was published in the *Proceedings*; Barcroft and Barron 1937). As recorded by Alfred Ernest Barclay (1876–1949) (Fig. 2.6a) and colleagues, "This accidental juxtaposition of the two films suggested to Barron the new line of attack" (Barclay et al. 1944, p. v). Franklin, an authority on the physiology of veins and blood flow in the inferior vena cava (see Franklin and Janker 1936; Franklin and McLachlin 1936a–d), and Barclay, an Oxford radiologist, were using cineradiography to study a number of aspects of the central circulation. Barron has recorded, "The clarity of his pictures was impressive" (Barron 1979, p. 2). Following this meeting on the train returning to Cambridge, Barron suggested to Barcroft the potential value of cineradiology in the study of the long-standing problem of the course of the fetal central circulation and the timing of *ductus* closure. Following some correspondence between Cambridge and Oxford, Barcroft with Barron developed a collaboration with Franklin and Barclay that lasted from 1937 to 1940 (see Barclay et al. 1944, p. vi; Dawes 1994). Barron has recorded some of the vicissitudes of these studies (Barron 1979). Particularly annoying was the fact that, without consultation with either Barcroft or Barron, Barclay and Franklin published a detailed account of these studies, in which they claimed that the *ductus* closed prior to clamping of the umbilical cord or visualization of air in the trachea (Barclay et al. 1938). On later analysis, this erroneous interpretation was shown to have resulted from the obscuring of the *ductus* by pulmonary vessels (Barron 1979). Subsequent cineradiographs of the

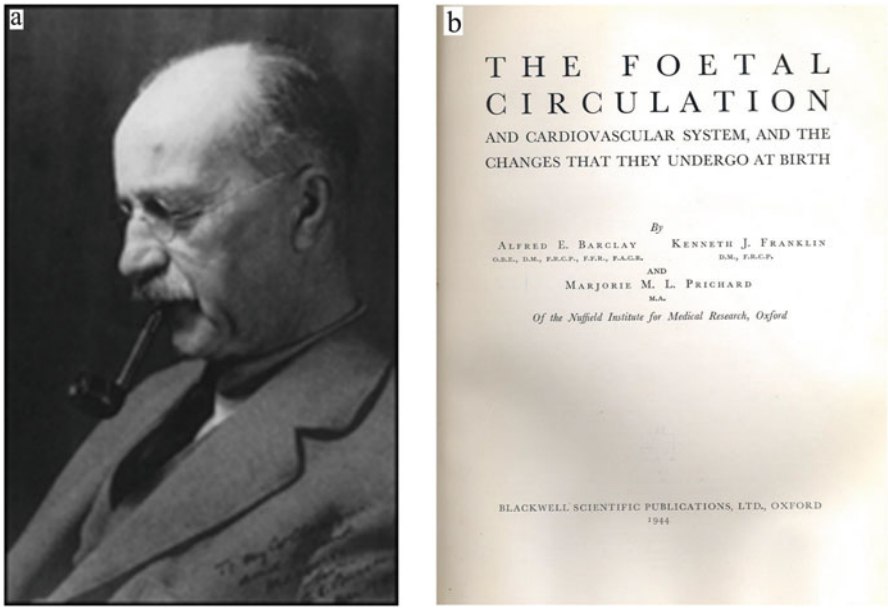


Fig. 2.6 (**a**) Alfred Ernest Barclay (1877–1949). (**b**) Title Page (1944)

pattern of circulation in the fetal heart and great vessels demonstrated closure of the *ductus arteriosus* sometime following birth. In these studies, they also compared the central circulation in the fetus with that of the adult (Barclay et al. 1939). In relation to this fiasco, Barron reminded readers that "The celebrated German physiologist Carl Ludwig [(1816–1895)] is said to have remarked, 'In science, method is everything.' In this study it was!" (Barron 1979, p. 3).

The question of the course of blood flow through the heart and great vessels of the fetus had been of interest since earliest times. Among his voluminous writings, Galen, the Greek physician to the gladiators and others, anatomist and philosopher, and who spent much of his life in Rome, gave the first account of the fetal heart. He described both the *foramen ovale* and *ductus arteriosus*, including details of their fate following birth. In addition, Galen commented upon the relatively small flow of blood through the pulmonary vessels prior to the significant increase following the onset of respiration. Being unaware of the circulation of the blood, his descriptions of the role of the blood and the paths by which it flowed in the great vessels were rather disordered (see Galenus 1914 and below). In his monumental *Exercitatio anatomica de motu cordis…* [Anatomical studies of the motion of the heart] of 1628, following his epochal description of the motions of the heart and circulation of blood in the adult, William Harvey described the essence of blood flow through the heart of the fetus. Because of some of the ambiguous terminology he used for the vessels and other structures, there was room for confused interpretation. Despite Harvey's description being essentially correct, three centuries elapsed before the

anatomical features and valid course of blood flow from the superior and inferior vena cavae (SVC and IVC) through the right atrium, *foramen ovale*, and onto the brain and other organs were established. Even at the commencement of the nineteenth century, three major theories were espoused as to the course of blood flow in the fetal heart following its transit from the superior and inferior vena cavae.

The first of these theories was that of Raphaël-Bienvenu Sabatier (1732–1811), a Parisian surgeon, who described blood entering the right atrium from the inferior vena cava passing directly and without mixing with blood from the superior vena cava, through the *foramen ovale* to the left atrium and ventricle, to be pumped into the aorta, and then onto the brain and upper extremities (Sabatier 1791). This flow of arterialized blood from the placenta to the upper body was believed to account for the relatively more rapid growth of the head and brain, as compared to the hindquarters. The second theory, that of Caspar Friedrich Wolff (1733–1794), placed the orifice of the inferior vena cavae dorsally, between the two atria, with the IVC blood splitting into two streams, each of which entered its respective atria, flowing then into the respective ventricles, and onto the pulmonary artery and aorta, respectively (Wolff 1778). This view, in essence, also was held by Preyer (1885) and one of his students (Ziegenspeck 1905). The third theory was that of Harvey (and essentially that of Galen), e.g., that blood from the SVC and IVC mix in the right atrium before passing through the *foramen ovale* and then to the left atrium, left ventricle, and onto the aorta. In his experiments in deer and other mammals, Harvey described the flow of blood through the *foramen ovale* and it passing into the left atrium and thence into the left ventricle to be pumped into the aorta. It then returned to "The right ventricle, [which] receiving blood from its auricle, propels it through the pulmonary artery and its continuation, . . . the *ductus arteriosus*, to the aorta. . .. In embryos, then, while the lungs are as inert and motionless as though not present, Nature uses for transmitting blood the two ventricles of the heart as if they were one" (Harvey 1928, p. 57).

At the beginning of the twentieth century, Augustus Grote Pohlman (1879–1950), of Indiana University, reviewed the several theories of blood flow through the fetal heart (Pohlman 1907, 1909). Noting the inadequacies of previous studies in the nonliving animal, he conducted a series of experiments in fetal pigs obtained at an abattoir. With a ligature passed around the heart at the atrioventricular groove, by rapid occlusion of ventricular outflow at the end of atrial systole, he first determined that the right and left ventricles contained equal volumes of blood (values not given). In a further study, by inserting capillary tubes into the ventricular lumen and measuring the level to which the blood rose, Pohlman determined that at end-diastole, the pressures in the two ventricles were equal. Then, following injection of either natural or colored cornstarch granules suspended in saline into the SVC or IVC, and counting the particles contained in blood sampled simultaneously from the two ventricles, he concluded, in agreement with Harvey, that blood from the two caval streams mixes thoroughly in the right atrium, prior to ventricular ejection. The first to use such an experimental approach, Pohlman noted that in his studies the fetus was contained in utero removed from the sow some minutes previously and that the thoracic operative interference required for the

experiments may have affected the results (Pohlman 1907, 1909). Later, Pohlman compared the embryonic circulation in reptiles and birds with that of the fetal circulation of several mammalian species. He noted the presence of the *foramen ovale* in those animals with a four-chambered heart and in warm-blooded mammals the requirement following birth, with lung expansion and closure of the *ductus venosus*, to ensure balanced pulmonary and systemic circulations (Pohlman 1924).

Following Huggett's initial study on O_2 and CO_2 in fetal blood, Howard Butters Kellogg (1898–1988) of Northwestern University also examined the course of blood flow from the superior and inferior vena cavae through the heart. As in the experiments of Pohlman, he injected suspensions of either India ink or 10% cornstarch into the umbilical vein or the external jugular vein of ~125 pig fetuses of various ages. Kellogg observed simultaneous blanching or blackening of both ventricular myocardia (Kellogg 1928). He noted that this study confirmed in the fetus that blood from the superior and inferior vena cavae mix. Kellogg stated, "These observations, while not primarily of a quantitative value, constitute definite evidence proving beyond a doubt that the two caval streams do mingle in the right atrium, whereupon both ventricles receive mixed arteriovenous blood. They are especially important, because they involve no subsequent disturbances of the heart" (Kellogg 1928, p. 454). In five dog fetuses, Kellogg further established these findings following umbilical vein injection. In a semiquantitative study in which he counted the starch granules in blood of the ventricles, he calculated that the left ventricle received 16% more blood from the IVC than did the right (Kellogg 1928) (this may have been within experimental error, however). In a subsequent study, he used the Van Slyke micromethod to quantify oxygen and CO_2 content of blood from both ventricles, showing them to be of similar magnitude. Again, this supported the concept of mixing of the two caval streams in the right atrium (Kellogg 1930). Although one may criticize some aspects of Kellogg's methods, his contributions were to deliver the fetuses by cesarean section while maintaining the uterine and umbilical circulations, preventing fetal respiration by placing the amniotic membranes over the fetal head, and in recognizing the value of quantitative blood gas analysis for the study of questions regarding the fetal circulation. These methods would prove of great value to Barcroft and his successors.

In the decade following these studies, Barcroft published a number of contributions on fetal respiration, blood volume, and circulation (Barcroft et al. 1939a, b, 1940a, b). The onset of World War II, however, terminated these productive collaborations. During the war, Barcroft chaired the UK Food Investigation Board and worked to find Britain's Nutrition Society (Franklin 1953). In the early 1940s, Maureen Young (Fig. 2.4), later of St. Thomas Hospital Medical School, worked with Sir Joseph. She has written:

> I assisted "Jo" . . . in a study at Cambridge towards the end of WWII. I had been at the South West London Blood Transfusion Unit for two exciting years when Nora Edkins and Margaret Murray persuaded me to join them in the Department of Physiology at Bedford College, Bedford, as a Demonstrator, to "keep the lamp of learning burning". They had been evacuated to Cambridge where our teaching took place in the theatres and lab when free of their own students. Jo, already in his early 80s, was still working. We all were

> invited to 'assist' at his experiments on foetal sheep, which at this time was delivered into a huge saline bath! At the end of the day, we all were rewarded with a most welcome lamb joint to take home for Sunday lunch.
>
> One day Jo stopped me in the corridor and said that he had asked Dr. Edkins if I might give him a little of my time to help him with a small study. He said, "my fingers and eyes are no longer organs of precision!" The fetuses of pregnant rabbits treated with progesterone became post mature and died 'in utero'. Jo wanted to know if they had outstripped their placental oxygen supply and needed blood samples from the fetuses. He found an animal table on which he could work in his small office and asked Adaire—another delightful 'retired' gentleman in the lab—to teach me how to use the original van Slyke apparatus to measure the blood oxygen content. It was a splendid experience. Taking blood from the carotid artery of the rabbit foetus did not prove a problem, and gave me courage to use the perfused placental preparation later on in my career. The fetuses also provided another small observation, namely that the umbilical cord had a little sphincter only at its junction with the abdomen. Jo found me cutting sections of this one day and said that it should be published in *Nature* (Young 1953), and there it is!
>
> With great charm and marvelous curiosity to the end of his life, Jo joins Widdowson and McCance for creating the stimulus for our interest in and the great progress which has been made in the subject of Perinatal Physiology worldwide during the last eighty years.
>
> (Letter from MY to LDL, May 2012)

In 1944, Barclay, Franklin, and Marjorie Mabel Lucy Prichard collated their radiographic studies in *The foetal circulation and cardiovascular system and the changes that they undergo at birth* (Barclay et al. 1944) (Fig. 2.6b). The authors stated this to be "... the gist of seven years' radiographic, historical, and anatomical research upon the foetal circulation and the cardiovascular system." They observed that although the work was too long and too detailed to appeal to the dilettante, "we believe..., that the more serious student will find himself amply repaid for a few days careful perusal of our account, in view of the great interest in the subject" (Barclay et al. 1944, p. viii). Following a rather extensive historical introduction, in a dozen chapters, the authors presented a wide range of studies. Focused chiefly on the fetal lamb, they also included comparative anatomical studies in a number of mammals including bear, elephant, goat, gorilla, hippopotamus, kangaroo, lion, tiger, and whale. The authors concluded with a consideration of the central circulation in the human fetus and the changes at birth (Barclay et al. 1944). They also presented the caveat that, although these studies were the first of their kind, and thus historic, "... too much reliance should not be placed upon isolated observations, and that schemata which are based solely upon post mortem measurements of vascular channels, or which fail to take into account all the foetal flows, may on occasion be grossly misleading." The authors confessed that this work represented only the beginning, *Fassus me in multis, quae ad foetum spectant, non mihi satisfacere* [In abundance am I bundled, burdened, in regards to observing the fetus, not to my satisfaction] (Barclay et al. 1944, p. 252). A decade later, similar studies with the use of radioangiographic techniques were conducted in the human newborn infant (Lind and Wegelius 1954).

During and following his studies on the fetus, Barcroft summarized his work to date in several lectures and reviews. These included a 1933 address "The conditions of foetal respiration" to the American Association for the Advancement of Science

(Barcroft 1933), the 1935 Croonian Lecture "Foetal respiration" to the Royal Society (Barcroft 1935a), a major review "Fetal circulation and respiration" (Barcroft 1936), the 1941 Cambridge University Linacre Lecture "Respiratory patterns at birth" (Barcroft 1942), and the 1942 Finlayson Memorial Lecture at the Royal Faculty of Physicians and Surgeons, Glasgow (Barcroft 1943). Each of these was notable in the manner which Barcroft posed questions that were clear but provocative for their time. These surveys laid the groundwork for Barcroft's collation of these, and studies with Barron and other colleagues (see Barcroft and Kennedy 1939; Barcroft et al. 1939b), into his last monograph, *Researches in Pre-natal Life, Part I* (Barcroft 1946). In the preface of this work, which he dedicated to Donald H. Barron ". . . to whom the work. . . owes so much," Barcroft stated:

> This work partakes very much of the nature of a will—I hope not my last. In the days of bombs it seemed to me only the due of the many who had given me encouragement and support, not least the Rockefeller Foundation, that I should set down in some connected form such information as I had accumulated concerning pre-natal life; then, if the bomb came my way, the information, for what it was worth, would remain. I say 'in some connected form' because not the least interesting part of the work has been the fitting together of individual items, dealt with in individual papers, into a picture from which a likeness of the organism is commencing to emerge.
>
> A will necessarily deals with the property of the testator at the moment at which that will is made. Some of his projects have come to fruition with assets safely secured, some may look promising, others may be doubtful, yet all must be dealt with. So, in this book, in so far as it is the bequest of such knowledge as I possess, or think I possess, I have put down all that is supported by experiment. There are cases, however, about which the last word has not been said, nor the last experiment completed. These I have indicated for the benefit of such as may wish to undertake future research.
>
> As regards the scope of the book, it purports to deal primarily with researches in which I have had a hand myself, and with observations by others germane to such, but it goes a little further and includes work by colleagues which I have been privileged to see, and even work carried out under the auspices of committees on which I have served. It does not purport to treat on any extensive scale of work with which I have had no personal contact; to take a single instance, the beautiful work carried out at Johns Hopkins University on the initiation of the heart beat—would that I could claim connection with that, but no! I must take up the pulse at the point at which I commenced to observe it.
>
> The general aim, then of this book is to trace the development of function in the mammalian foetus, never losing sight of the fact that one day the call will come and the foetus will be born. Not only has the foetus to develop a fundamental life which will suffice for intra-uterine conditions, but at the same time it has to develop an economy which will withstand the shock of birth, and will suffice, nay more than suffice, for its new environment.
>
> (Barcroft 1946, p. ix)

In 22 chapters, in each of which he clearly stated a specific question to be explored, Barcroft reviewed in depth what was known regarding fetal physiology. Early chapters consider aspects of the maternal and fetal placental vasculature and nutrient exchange, determinants of fetal growth, fetal blood volume, and oxygen consumption. In Chapter V "The relative claims of the foetus and mother to available nutritive material," Barcroft stressed the symbiotic relationship of fetus to mother, at a time the fetus was considered a "parasite." Applying the principle

that nutrient partition among organs was determined by the metabolic rate, he argued that with its relatively high rate of metabolism, the fetus could compete with maternal tissues. The latter two-thirds of *Researches on Pre-natal Life* summarize much of Barcroft's and Barron's work that considered blood pressure and vascular reflexes, fetal blood oxygen capacity and oxyhemoglobin saturation curves, the central circulation with roles of the *ductus venosus* and *ductus arteriosus*, and the onset of respiration at birth. Seven appendices included variations in respiratory activity at birth, measurements of blood sugar, lipids, and the molecular weight of sheep fetal hemoglobin. Also included were recently derived blood gas values obtained by Barron, ". . . from the small arteries and veins going to and leaving a cotyledon, and . . . therefore more fully representative of placental conditions," which suggested a significant decrease near term (Barcroft 1946, p. 285). In several asides in the volume, Barcroft considered the difficulties in acute studies of obtaining reliable samples of fetal blood (umbilical vessels (p. 187) and carotid artery (p. 197) that truly were representative of its physiologic state. He noted that for many studies:

> . . .such results are frankly worthless-some guarantee must be given that the blood in these vessels, so sensitive to any kind of manipulation, is coursing at the normal rate; some guarantee must be given that the foetus is in a normal state; some guarantee must be given, and this is often overlooked, that the circulation in the mother is also normal: and lastly when the worker has satisfied his readers and, what is probably more difficult, himself, that the data are as nearly correct as may be, there remains the questions- to what stage of pregnancy do they refer?
>
> (Barcroft 1946, p. 187)

It would be two decades later that Giacomo Meschia, Donald Barron Henry (1905–1993), and coworkers first reported on the use of chronologically indwelling catheters for measurement in vivo of respiratory gas values in fetal blood that made such measurements of relevance physiologically (Meschia et al. 1965). This methodology places, aided greatly in, the study of the fetus in utero on a firm physiologic basis.

Barcroft had intended to prepare a second volume that would deal with both the nervous system and metabolism. With his demise shortly following this publication, this was not to be, however. It must be recalled that Barcroft was 62 when in 1934 he embarked upon his studies of fetal physiology and upon which he worked for the next decade and a half.

Publication of Barcroft's *Researches in Pre-natal Life*, immediately following the end of World War II, appeared at the beginning of what some call the "golden age" of medical research. Governmental support for biomedical studies increased (see below), as did increasing interest in biology, from cellular and subcellular mechanisms to organ and systems function, and clinical care, including that for the mother and newborn infant. With concomitant technological advances that allowed ever more detailed determinations and studies, Barcroft's volume had considerable impact, both in terms of promoting basic research as well as translational and clinical medicine (Anonymous 1947; Holmes 1970; Young 1992).

One might ask what was it about the Barcroft's research that helped to keep him on the forefront with fresh ideas. Although Barcroft wrote essentially nothing in this

regard, in the preface to his volume on the respiratory function of the blood, he observed:

> At one time, which seems too long ago, most of my leisure was spent in boats. In them I learned what little I know of research, not of technique or of physiology, but of the qualities essential to those who would venture beyond the visible horizon.
>
> The story of the physiological "ventures" will be found in the following pages. Sometimes I have sailed single handed, sometimes I have been one of the crew, sometime I have sent the ship's boat on some expedition without me. Any merit which attaches to my narrative lies in the fact that it is in some sense at first hand. . .I should have like to have called the book, what it frankly is—a log; did not such a title involve an air of flippancy quite out of place in the description of the serious work of a man's life. I have therefore chosen a less exact, though more comprehensive title. . .
>
> After all, the pleasantest memories of a cruise are those of the men whom one has sailed. The debt which I owe to my colleagues, whether older or younger than myself, will be evident enough to any reader of the book. It leaves me well-nigh- bankrupt—a condition well known to most sailors. But I owe another large debt of gratitude to those who, as teachers, showed me the fascination of physiology. . .
>
> (Barcroft 1914, p. vii)

Barcroft repeated this account in his volumes on high altitude (Barcroft 1925, p. vii) and on the respiratory function of the blood, part II hemoglobin (Barcroft 1928, p. v) (Longo 2016).

Following his death, Huggett wrote a tribute to Barcroft in which he cited *Researches on Pre-natal Life* as ". . . a landmark in experimental physiology, a fitting successor to Preyer's volume [of 1885] on the physiology of the embryo in the last century" (Huggett 1947/1948, p. 231). He continued:

> Barcroft's death marks the end of an era in the physiology of the foetus. If he had lived it would have been extended no doubt, but he himself was moving it on to the next phase. This era extends from the early work of Zuntz to the present date, and can be described as the phase of observational physiology. In this phase has been accomplished for physiology the necessary description of the main processes which occur. Previous to this period, physiological knowledge of the embryo and foetus was largely based on anatomical knowledge and inferences from this. After the introduction of the saline-bath technique in the twenties of this century, physiological facts have accumulated rapidly—largely under the influence of Barcroft's drive, personality, and ability to see the essentials of a problem, to dissect it into its component parts, and to inspire workers in the British Isles, in Europe and in America to tackle the several aspects so exposed. Simultaneously there have been three other lines of approach which are now converging in the new phase setting in. These are: the genetic approach; the endocrinological approach arising out of the study of the oestrus cycle and the development of the fertilized egg; and the obstetric approach arising out of the study of the nutrition of the foetus and the physiology of pregnancy, a study which has itself been fortified by the foetal work of the last twenty years. At the end of this phase we therefore have a fair knowledge of the physiological action of the foetus and placenta.
>
> In the new phase we must look forward to a synthesis of all these lines of approach directed to the study of the peculiarly foetal problems of how function is initiated in the embryo and how it can be controlled. In fact, we may as a result be able to visualize the foetus not merely as a passive individual whose growth or development is outside our control, but as one whose care will be within our power as much as is an adult patient—so far as that is the case. The modern applications of biophysics must come into the field of experiment, to explain how the gene, the hormone, the vitamin, and the nutrient react with the mother to produce the newborn.
>
> (Huggett 1947/1948, p. 232)

In his obituary notice of fellows of the Royal Society, the Cambridge biochemist-physiologist Francis John Worsley Roughton (1899–1972) stated of Barcroft:

> It has already been said of Barcroft that he had changed but little in the course of his long life, but retained his youthful freshness of mind and sense of wonder right through till the end of his days. This was due not only to his innate character, but also to his unique habits of work and thought, which endeared him to, and inspired all those who met or worked with him in his daily round. For others who never had that privilege, it is fortunate that he has himself revealed in his permanent writings such vivid examples of the methods and spirit of his research, and such a clear picture of his own personality, scientific and otherwise . . .. He would be apt to choose a field which was not at the moment in the forefront of the battle, . . . however slight his knowledge at the start (and he would be the first to admit it), he would soon be asking shrewd questions and talking in telling fashion about the existing ideas and conceptions of the subject. Then, by some process of intuition, which rarely seemed to fail him, he would succeed in picking out new and salient points of attack which, for one reason or another, had eluded his predecessors. Next he would gather round him one or more younger colleagues and infect them with his own enthusiasm for the new venture. In their company he would buckle to and, if need be, devise methods which were often simple . . ., but would almost always be singularly effective in guiding him quickly to significant results. Thus armed, he and his happy band would forthwith plunge into a series of exhilarating, if sometimes laborious, experiments; these usually brought forth a copious crop of new and often unexpected fruit in a surprisingly short space of time. Then 'he would return to report on what he had seen and done—talking in that simple, exciting and slightly breathless way he had, making all he had discovered seem so self-evident, poking fun at himself and paying generous tribute to his collaborators'. The results, once established to his satisfaction, would then be prepared for final publication with an ease and a gusto which many scientists, who find 'writing up' so irksome, might well envy.
>
> (Roughton 1948, pp. 329–330)

A subsequent symposium devoted to the chemistry and physiology of hemoglobin in Sir Joseph's honor includes tributes to him (Roughton and Kendrew 1949). Later, the Cambridge anatomist, James Dixon Boyd (1907–1968), described Barcroft as:

> . . .concerned always with first principles and taking details in his stride. I shall always remember how he could put one back on the tracks again by pointing out how one's apparently bright thought transgressed one or other of the primary laws.
>
> Personally, nevertheless, I shall always remember him with gratitude, with respect and with real affection for his character and his characteristics. I have known other considerable scientists . . . But I wonder if I shall ever meet one who was so honest, so without guile, so concerned with the truth in the very best sense of this abstraction. And to this one must add friendliness, concern for the welfare of colleagues and friends, and a deep sense of justice. It always rather embarrassed me that he should be so grateful, so honestly and unaffectedly grateful, for the very small services that on several occasions I was able to perform for him. He was kind because that was his nature, not for ulterior motive or design.
>
> And now we shall no longer sense this kindly response, this ready friendliness. And, I can write it honestly and with conviction, something very cherished has gone from my life. I shall remember him with respect, affection and thanks.
>
> (Boyd, in Franklin 1953, pp. 342–343)

Not a great deal has been written of Barcroft's personal life. Probably of Norman origin, the early years of Barcroft family, from the *de Berecrofte* (or "barley-

crafters"; Breathnach 1974) of the thirteenth century to the late eighteenth century, have been traced in the historical survey *Barcroft of Barcroft* (Barcroft 1960). Following the outbreak of World War II, in 1940 Barron returned to the USA and in 1943 joined the Department of Physiology at Yale University, where he continued his studies on the fetal circulation and the placental exchange of respiratory gases (Barron 1946, 1952). In a critical review of the changes in the central circulation from fetus to newborn at the time of birth, Barron placed the entire field into perspective (Barron 1944).

In the mid-1950s, Giacomo Meschia joined Barron in studying placental respiratory gas exchange (Meschia and Barron 1956) and fetal oxygenation at high altitude (Meschia et al. 1961; Metcalfe et al. 1962a, b; Prystowsky et al. 1960). It was in 1965 that Meschia, Barron, and coworkers first reported on the use of chronically indwelling catheters for measurement in vivo of respiratory gas values in fetal blood (Meschia et al. 1965), a technique that was to place study of the fetus in utero on a firm physiologic basis (see below). Importantly, it was with these and related studies, and with the collaboration of several postdoctoral fellows and colleagues, that Barron established the field of fetal physiology in the USA

Acknowledging the inspiration he received from, and debt to, Barcroft, Barron recalled a number of aspects of Barcroft's contributions to life (Barron 1973). In an earlier essay, he had noted:

> I loved Sir Joseph above all men. I loved him for his passionate devotion to the truth; for his charity towards his fellow man in all walks of life; for his devotion to young men and a host of other intangible qualities. To emulate him was and will remain my life's purpose; I can conceive no higher purpose. I have written only of my personal indebtedness to Sir Joseph. My countrymen owe to him the same coin. No one has contributed more generously to the physiological thought of this country than he. The host of students who went through his laboratory, learned his methods and acquired new vistas are spread throughout this country, and they recall with advantage the days and weeks they enjoyed as members of his School. And there are those yet unborn who will catch the spark of his wisdom through the thoughts he put to pen. Few have given so much; fewer there are who had so much to give.
>
> (Barron in Franklin 1953, pp. 339–340)

In his recollections of Barron, David J. Mellor stated:

> I first met Donald Barron through Prof Huggett. He visited Huggett at the Moredun Research Institute for one day in late 1967. I still recall the several hours I was able to spend with them on that day. During the morning I asked Barron dozens of questions about fetal physiology and was delighted by the opportunity to do so and his encouragement. In the afternoon he then outlined details of his work on oestrogen stimulation and progesterone inhibition of fetal respiratory and physical activity. He began this account with the question, "Mellor, have you ever wondered why a colt does not get up and gallop around in the uterus, but within hours of its birth it gallops around in the field?" I had to admit that I hadn't! But I immediately recognized that it was a most intriguing question. At the time I did not appreciate how influential that question would be in directing me much later towards some fruitful paths of physiological thinking, which, now 42 years after he asked me that question, I am still exploring.
>
> (Letter from DJM to LDL, 13 July 2009; also Mellor 2010, p. 94)

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Chapter 3
Oxford and the Development of Physiology, with Notes on the Nuffield Institute for Medical Research

The business and design of the Royal Society is- to improve the knowledge of natural things, and all useful Arts, Manufactures, Mechanick practices, Engynes and Inventions by Experiments-(not meddling with Divinity, Metaphysics, Moralls, Politicks, Grammar, Rhetorick or Logick)…. All to advance the glory of God, the honour of the King…the benefit of his Kingdom, and the general good of mankind.
(Hooke 1663, In C R Weld 1848 p.146)

3.1 William Harvey and the Seventeenth-Century Physiology

As background for appreciating the development of fetal and newborn physiology, undoubtedly some of the earliest studies on the embryo and fetus that can be classified as scientific were done by William Harvey (Fig. 3.1a). He recorded in the early 1630s, shortly following publication of his monumental *Exercitatio anatomica de motu cordis*… (Harvey 1628) investigations on various aspects of generation in deer and other animals that resided at the King's estate. These studies proceeded with the privilege and blessing of King James I (1566–1625; King from 1603 to death) and King Charles I (Charles Stuart; 1600–1649; King from 1625 to his death), for both of whom Harvey served as personal physician. In addition to providing animals from the Royal preserves, Harvey wrote that he had daily opportunity to dissect and study the reproductive and genital organs. Of this patronage, he also credits the King with taking a great interest in his work, for instance, "… my Royal master (whose Physitian I was, and who was himself much delighted in this kind of curiosity, being many times pleased to be an eye-witness, and to assert my new inventions)" (Harvey 1653, p. 397). A Royalist, in anticipation of the Civil War, Harvey later accompanied King Charles to Scotland, and following the Battle of Edgehill (23 October 1642), the first major action of the Civil War, the Royal party settled and set up Court in Oxford. Within several months, Harvey was appointed warden of Merton College. Here, Harvey took advantage of the opportunity to resume studies of the development of hen's egg that he had

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Fig. 3.1 (**a**) William Harvey (1578–1657), (**b**) Sir Henry Wentworth Acland (1815–1900), (**c**) Radcliffe Observatory, (**d**) William Morris, Lord Nuffield (1877–1963)

commenced earlier. He conducted these studies in the rooms of George Bathurst, an Anglican divine of Trinity College, who had a hen to hatch eggs in his chamber which they opened day after day, "That we may the better discover what the... *incubation* hath produced" (1653, p. 80). Relatively uninterrupted by the political upheavals that surrounded him, Harvey pursued his embryological studies. As an aside, it should be noted that during the Civil War, the period of parliamentarian rule during the *Interregnum* and continuing through the Restoration, as a Royalist stronghold, Oxford was fiercely loyal to the Crown.

It was not until a decade later that Harvey's treatise on the generation of animals and embryology, *Exercitationes de generatione animalium*... [Anatomical exercitations concerning the generation of living creatures...], was published. Then, it only was because his friend, the physician and anatomist Sir George Ent (1604–1689), prevailed upon Harvey to allow him to perform "... the meer office

of a midwife: producing into the light this noble Issue of His Brain. . .” committing it to the press. In his “Dedicatory Epistle” Ent wrote,

> A Calmer welcome *this choice Peice* befall,
> Which from *fresh Extract* hath deduced all,
> And for belief, bids it no longer begg
> That *Cafter* once and *Pollux* were an *Egge*
> That both the *Hen* and *Houswife* are so matcht,
> That her Son *Born*, is only her Son *Hatcht*;
> That when her *Teeming* hopes have prosp'rous bin,
> Yet to *Conceive*, is but to *Lay within*.
> *Experiment*, and *Truth* both take thy part:
> If thou canst scape the *Women*! there's the Art.
>
> Live *Modern Wonder*, and be read alone,
> Thy *Brain* hath *Issue*, though thy *Loins* have none.
> Let fraile *Succession* be the Vulgar care;
> Great *Generation's* selfe is now *thy Heire*
>
> (Harvey 1653, pp. xviii–xix)

In this work Harvey rejected the prevailing doctrine of the preformation of the fetus and advanced the fundamental theory, radical for its time, of epigenesis (*per epigenesin*), that all living beings derive from the ovum “by the gradual building up and aggregation of its parts.” Harvey's *Exercitationes de generatione. . .*, the first English edition of which appeared 2 years later (Harvey 1653), thus was a major advance for the study of reproduction. Regarding his theory of epigenesis, Harvey considered this work to be of greater scientific importance than his epic discovery of the circulation of the blood by the pumping action of the heart, as detailed in *De motu cardis. . .* (Harvey 1628). In this work on reproduction, Harvey reported a wealth of observations on many of its aspects in a wide variety of species. As representatives of vivipara, his attention chiefly was devoted to the deer, while the domestic fowl was predominant for ovipara. For Harvey, all life develops from the egg. This principle has been of crucial importance in the history of embryology and is expressed on the frontispiece of this work which depicts the supreme Roman god Jupiter [Jove] opening an egg, inscribed with the fundamental dictum of embryology, *ex ovo omnia* [from the egg everything]. From this egg, in addition to a small human figure, liberated animals and insects fly; these include a bird, stag, fish, lizard, snake, grasshopper, butterfly, and spider. Although the phrase *omne vivum ex ovo* [all life originates from the egg] is often attributed to Harvey, he does not state this explicitly in the text. Harvey speculated that humans and other mammals must reproduce through the joining of an egg and sperm; no other theory was credible. By positing and demonstrating for viviparous animals the same mechanism of reproduction as that observed in oviparous animals, he thus initiated the search for the mammalian ovum. It remained for Carl Ernst von Baer (1792–1876) to make this discovery a century and a half later (Baer 1827; see below). Harvey maintained that *Jovis omnia plena* [All things are full of Deity] so that in the

chicken and all its functions and actions, the *Digitus Dei* [the Finger of God] or the god of nature reveals himself (1653, p. 310). Harvey also wrote a work, *Medical Observations*, a treatise believed to have concerned midwifery. Unfortunately this work was destroyed, with others, at the beginning of the Civil War in August 1642, when Harvey was at Nottingham with King Charles I.

Celebrated for his monumental discovery of the circulation of the blood (Harvey 1628), Harvey was the first to adopt the scientific method for the solution of a biological problem (Singer 1922). In fact, it was Harvey's work on the function of the heart and the circulation in general that led him to consider the circulation of the fetus and its relation to that of the mother. To help grasp the significance of Harvey's epic achievement, one must consider the traditional Galenic concept of the heart, blood vessels, and their contents at this time. Briefly, this doctrine held that the blood was formed in the liver, the dynamic organ that provided vascular pulsations. The heart, in contrast, was believed to be a fibrous sac that dilated or collapsed passively as a consequence of the blood's motion. In the liver, venous blood was mixed with an imaginary essence, the "natural spirits," to become a vegetative fluid, the elixir of nourishment that the veins distributed to the several bodily organs. Within the right side of the heart, venous blood was believed to seep through "pores" in the septum separating the two ventricles, to mix on the left side with air to produce arterial blood. (Also at this time, most workers believed that the heart consisted of only the two ventricular chambers and that the atria were but extensions of the veins joining the heart.) Within the left ventricle, these fluids were imbued with a second essence, "vital spirits" or "pneuma," which entered the lungs with respiration and passed via the pulmonary vein to the left ventricle, before being distributed to various organs. In the brain, "animal spirits" were added to pass through the nerves. Interaction of the "vital" and "animal" spirits was believed to provide movement, such as muscular activity. Rather than circulating, the two varieties of blood were thought to surge as a tide back and forth in the veins and arteries (Frank 1980; Singer 1922; Wright 2012).

In establishing the circulation of the blood as a closed circuit, rather than the ebbing and flowing from the two sides of the heart in two independent systems of arteries and veins, it was Harvey's observation of the relatively slow heartbeats of cold-blooded animals that convinced him that contraction, not relaxation, was the heart's active action. His determination of the heart's output into the aorta demonstrated this to be many times greater than the liver could produce at one time, as was held by established doctrine. In regard to the placental circulation, Harvey stated, "the Extremities of the *Umbilical* vessels, are no way conjoined to the *Uterine* vessels by an *Anastomosis*; nor do extract blood from them. . ." (1653, p. 439). By logic based on his knowledge of the circulation, he held the maternal and fetal circulations to be separate, each following in an opposite direction to the placenta by way of the arteries and returning by the veins.

In his *Exercitationes de generatione*. . ., Harvey raised another question of fundamental importance to developmental biology and life in utero, that of the placenta serving as the lungs for the fetus:

> . . . I shall propose this *Probleme* to the Learned; namely, How the *Embryo* doth subsist after the *seventh moneth* in his *Mothers* womb when yet in case he were borne, he would instantly breath: nay he could not continue one small hour without it? And yet remaining in the *womb*, though he pass the *ninth moneth*, he lives, and is safe without the help of *Respiration* . . . How commeth it to pass, that the *Foetus* being now borne . . . if he have but once attracted the Aire unto his *Lungs*, he cannot afterwards live a minute without it, but dyeth instantly?
>
> (Harvey 1653, pp. 482–483)

He also noted that the "*Embryo* is in no other manner sustained in the *Uterus*, then [sic] the *chicken* in the *Egge*" (1651, p. 240; 1653, p. 440). Based on knowledge that in utero the fetus grows and matures in the absence of air, and at the time of delivery, it dies if the umbilical cord is compressed and it fails to breathe, Harvey postulated that substances absorbed by the umbilical cord stimulated organ development, while the fetus received its main nourishment from the amniotic fluid. Following Giulio Cesare Aranzi [Arantius] (1530–1589) in his *De humano foetu* (Aranzi 1564), Harvey also referred to the placenta as the *hepar uterinum* [uterine liver] and *mamma uterina* [uterine breasts]. Not until a decade later did Marcello Malpighi (1628–1694) of the University of Pisa first describe the capillary bed connecting arteries and veins (Malpighi 1661) and made possible an understanding of the full anatomical basis of regional circulation. Lacking this knowledge, Harvey could not understand completely certain details of the circulatory system in either the adult or the fetus. Perhaps, in part, a secret to Harvey's success was that in his London home, in addition to his "research chamber," he had a "meditating apartment" for contemplation. Several reviews have examined Harvey's contribution in extenso (Bylebyl 1972; Hutchison 1931; Keynes 1966; Meyer 1936; Wright 2012).

"Harvey's question" regarding fetal survival in utero, as it came to be known, aroused the interest of both philosophers and experimentalists. With no clear understanding of respiration or metabolism, however, and without knowledge of the existence of oxygen (the discovery of which did not occur until the following century), Harvey could only speculate on this matter. Many details of the path to discovery of placental respiratory gas exchange, made two centuries later by the young Swiss obstetrician, Paul Zweifel (1876), have been given by Donald H. Barron (1978).

3.2 Other Early Oxford Physiologists

During the seventeenth century, Oxford, the "city of dreaming spires" (Arnold, ~1850), became a center for the advancement of science. A seminal figure in this revolution was the English philosopher, statesman, jurist, and author Francis Bacon, Baron Verulam and First Viscount St. Alban (1561–1626). As opposed to the deductive approach to questions of nature employed by Harvey and others in previous and more contemporary times, in the early-seventeenth-century crucible

of change, Bacon championed an inductive methodology in scientific inquiry, e.g., reasoning from the particular to the general. The "Baconian" method of a systematic approach to what we now call science demanded an evidence-based approach to axioms, general principles, and laws. Serving the Crown in many capacities, including attorney general and lord chancellor, Bacon's thesis was that the most certain path to truth and knowledge of nature was gained by observation. This concept, innovative for its time, was expressed in several works, *The proficience and advancement of learning* (Bacon 1605), *Novum organum* (1620), and *De augmentis scientiarum* (1623) (Spedding et al. 1857–1859). These works helped to transform the way in which knowledge has obtained, recorded, and assessed. In his unfinished *The great instauration*, published after his death (Bacon 1854), Bacon expressed his further vision of a consortium or organization that would acquire knowledge from all over the world and put it to practical use. The essential feature of this scheme was that the growth of knowledge should be a collective activity, with groups of investigators observing the details of natural phenomena. Upon collection of these observations, another group of scholars and philosophers would interpret the results and formulate the laws of nature. Finally, a third group of inventors and entrepreneurs would use these laws to advance human well-being and wealth. Far ahead of his time, Bacon's organizational schema resembles some contemporary institutions of science and technology. Despite his never having performed an experiment, implementation of Bacon's philosophy of the inductive method changed the course of science, and for several generations he served as a symbol for the ideal in science (Hesse 1970; see below).

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As early as the late fifteenth and early sixteenth centuries, the physician-scholar Thomas Linacre (ca. 1460–1524), a graduate of the University of Padua (1496) and founder of the Royal College of Physicians (1518), with the collaboration of several like-minded Renaissance humanists, introduced at Oxford the teaching of Western European medicine (Cawadias 1936). As important as Harvey's discoveries were to establish a point, it was Harvey's rigorous insistence on the experimental method—based on the testing of well-thought-out hypotheses and his own observations—that was even more influential to the history of biomedical science and physiology. Following Harvey in the mid-seventeenth century, a number of physiologists and others worked at the several Oxford colleges, emulating Harvey and contributing to science (Frank 1980; Keynes 1953). These included Nathaniel Highmore (1613–1685), whose *The history of generation...* (1651a) referred to the newly developed microscope to study embryological development in the chick. Highmore also published his textbook *Corporis humani disquisitio anatomica*, which he dedicated to Harvey (Highmore 1651b). Another medical scientist of this period was Walter Charleton (1619–1707), whose *Onomasticon zoicon, plerorumque animalium...* presents the English, Latin, and Greek names for all of the then-known animals (1668). Another of Harvey's acquaintances, and in fact one of his patients during his Oxford stay, was the chemist-physiologist Robert Boyle (1627–1691). Boyle is known primarily for his *Certain Physiological Essays* (1661a), *The Sceptical Chymist* (1661b), and *A Defense of the Doctrine Touching*

the Spring and Weight of the Air (1662, 1682), in which he demonstrated a portion of air to be essential to life and laid the groundwork for the physiology of respiration and the gas laws on the interrelations of pressure and volume. Another of the early Oxford physiologists was Robert Hooke (1635–1703), publishing *Micrographia: Or, Some Physiological Descriptions of Minute Bodies*... (1665), a classic contribution to microscopy. He also described an experiment of preserving animals alive by using a bellows to blow air through their lungs, thereby demonstrating that respiration depends on an adequate supply of fresh air (Hooke 1667).

A number of other seventeenth-century British scientists contributed to the unparalleled pace of scientific discovery. A leader of the Oxford medical scientists of this period was Thomas Willis (1621–1675), a Royalist soldier during the Civil War; following the parliamentary *Interregnum*, he had been rewarded the Sedleian Professorship of Natural Philosophy. He used his position to embark on a bold and innovative project, to map the brain, its blood supply, and its nerves and to determine their function. In the course of this work, Willis published the most complete account of the central nervous system to this time, in which he coined the term "neurology," *Cerebri anatome: cui accessit nervorum*... [the anatomy of the brain with accessory nerves] (1664). Over the next 8 years, Willis took advantage of his experimental studies and clinical observations to write *Affectonum quae dicuntur hystericae et hypochondriacae pathologie spasmodica vindicata*... [Cerebral Pathology] on seizure disorders (1671) and *Two Discourses Concerning the Soul of Brutes*... on neurological and psychological disorders. Another associate was Walter Needham (ca. 1631–1691), who published a seminal work in chemical embryology *Discquisitio anatomica de formatu fetu*, in which he called the placenta the "uterine lung" (Needham 1667). Still another was Willis' junior medical partner, Richard Lower (1631–1691), who among other contributions successfully performed a blood transfusion, *The method observed in transfusing the blood out of one live animal into another* (Lower 1665/1666). He also wrote a major work on the heart describing its "scroll-like" musculature (Lower 1669). A century prior to the discovery of oxygen, by injecting dark venous blood into the pulmonary artery of aerated lungs, in his *Tractatus de corde*, Lower demonstrated that air caused the blood to become bright red in the pulmonary vein. He also presented a creditable account of the fetal circulation (Lower 1669). John Mayow (1643–1679), also of Oxford, in his *Tractatus quinque medico-physici* (1674), first demonstrated that the skeletal muscles produce "animal heat," and stressed the requirement for the fetus to be supplied with "nitro-aerial particles (e.g., oxygen). The Oxford professor of anatomy (and later professor of music), Sir William Petty (1623–1687), a pioneer demographer and biostatician, wrote *Several essays on political arithmetic* (1699). He also was the first to take a census of the population in Ireland. Another notable Oxford scientist of this era, and friend of Lower and Willis, was Christopher Wren (later Sir Christopher; 1632–1723) who, during his tenure as a Savilian Professor of Astronomy, participated in the dissection of brains of a number of mammals, including humans. Wren also attempted to produce narcotic anesthesia by injecting opium, wine, and ale (separately) into veins of a dog by means of a quill and pig's bladder (Little 1975). Wren went on to become one of Britain's foremost architects, who, following the Great Fire of 1666, contributed greatly to the rebuilding of

London, including St. Paul's Cathedral and a number of other sanctuaries and wondrous structures (see Frank 1980).

3.3 Founding of the Royal Society

A critical factor in the evolution of physiology, during the seventeenth century in Britain and several others of the countries of Europe with the virtual explosion in artistic and scientific creativity, men interested in the new science met together for experimentation and discourse. This was a period of unparalleled discovery, and these periodic gatherings led to the organization of scientific societies and academies. In part, this was a consequence of the Renaissance in the arts, humanities, and culture and which for science had begun in Italy a century earlier. With the Galilean (named for Galileo Galilei, 1564–1642) revolution, an unprecedented, almost miraculous leap forward occurred in the art of inquiry and investigation. In Britain, it was a group of "natural philosophers" of such an "invisible college" that beginning about the year 1645 met weekly for the purpose of "improving natural knowledge." In about 1648–1649, under the influence of several of those noted above, the group removed its meetings from London to Oxford. This blossoming of intellectual activity was abetted by the founding of the Oxford Regius Professorship of Medicine (1546) by Henry VIII (1491–1547; King of England from 1509 to death), the establishment of the Bodleian Library (1602), and the creation of a *physic* botanical garden of herbs and other medicinal plants (1651). With the scientific contributions of the worthies, these developments blazed the trail for the foundation in 1660 of the Royal Society (this was founded at Gresham College the leading seat of learning in London, not Oxford) by a dozen "ingenious and curious gentlemen" to form a "College for the Promoting of Physico-Mathematical Experimental Learning" and to publish their discoveries in natural philosophy for all to share. These scholars thus were following the teachings, but not the practice, of the philosopher of the previous century, Francis Bacon, whose writings promoted this almost revolutionary concept. With the Restoration and the ascent of Charles II (1630–1685; King 1660–1685) to the throne, significant advances followed.

Founded initially as "A Society," in 1662 Royal patronage was granted with a charter. Several factors were critical to this success. Following the Restoration, there was a determination to maintain religion and science in separate spheres of influence. "Free thinking" was tolerated more widely, and King Charles II had great interest in natural philosophy. Most important to the Royal Society's profound influence on science per se and to its support by the Crown, the parliament, and the people were the Promethean figures who led out in this enterprise. Formation of the Royal Society, which was to herald a scientific revolution in Great Britain, preceded by only several years the outbreak of the Great Plague of London (1665–1666) (during which bubonic plague was differentiated from typhus) and the Great Fire of London (1666), both of which wreaked widespread devastation. The motto of the Royal Society *Nullius in verba* [accept nothing on authority; take nobody's word for it] was suggested by John Evelyn (1620–1706). That is, if you have an idea,

develop a hypothesis, test it by experiment, and prove it by demonstrating the extent to which it is valid (see Acland 1890, p. 16; Hinshelwood 1960). In fact it was the polymath Evelyn's *Sylva: Or a Discourse of Forest-trees, and the Propagation of Timber in His Majesty's Dominions* (Evelyn 1664) that was the first official publication of the newly founded Royal Society. As Thomas H. Huxley pointed out:

> If these ideas be destined . . . to be more and more established . . . if the spirit be fated . . . to extend itself into all departments of human thought and become co-extensive with the range of knowledge, if as our race . . . discovers . . . that there is but one kind of knowledge, and but one method of acquiring it; then we, . . . may justly feel it is our highest duty to recognize the advisableness of improving natural knowledge, and so aid ourselves and our successors in our course towards the noble goal which lies before mankind.
>
> (Bibby 1967, p. 41)

It was these men of the Royal Society, devoted to the free inquiry and science, who, by their thought and emphasis on publication and peer review, initiated the humane scholarship which helped to create the traditions of modern science and the traditions of Oxford University. A distinguishing feature of the Society was the work of its members in collecting botanical, animal, and mineral specimens from around the world, mapping previously unknown lands, and recording observations from all over the globe. Of critical importance, the core values of the Royal Society have enduring relevance. As other national academies, it has promoted excellence in scientific research. Engaging broadly in the needs of society, it has attempted to stimulate the public understanding of science and demonstrate a commitment to public affairs. The Royal Society also has played a key role in helping political leaders to understand scientific issues and promoting scientific education in general. Publishing papers in its *Philosophical Transactions and Proceedings* from scientists throughout Europe and supporting first-rate investigative work regardless of the country of origin, it helped to establish English as the language of science. Perhaps of most importance, this revolution, from asking "why" to answering "how," flowered into the Enlightenment, the political, cultural, scientific, and educational revolution that gave rise to the modern West (see Bibby 1967; Bronfenbrenner 1938; Bryson and Turney 2010; Dolnick 2011; Gribbin 2005, 2007; Poynter 1970, p. 238; Robb-Smith 1966; Weld 1848). Nonetheless, this is not to say that later during the eighteenth or early-nineteenth century, experimental physiology flourished at Oxford or elsewhere in Britain. In contrast to developments in France and Germany, it did not.

3.4 The Oxford Medical School and Further Developments in Physiology

In considering the development of physiology at Oxford University, one also might consider the "medical school" as the focal point of this study. Some have objected to this term, even referring to it as "A Lost Medical School" (Collier 1904, p. 221), as it only in relative recent times that Oxford has offered a complete education in medicine. As historian Frederick Noël Lawrence Poynter (1908–1979) has noted

regarding medical education in England, ". . . from the sixteenth century onwards, this [topic] has been almost constantly a source of . . . criticism and dissatisfaction". And regarding both Oxford and Cambridge, Poynter concluded, ". . .Medicine was not highly regarded or vigorously pursued as an academic training" (Poynter 1970, pp. 235–236).

In his review of Oxford medical education prior to 1850, Alastair Hamish Tearloch Robb-Smith (1908–2000) quoted Sir James Paget (1814–1899):

> If the reputation of a University is to be measured by the number of medical men it turns out, it must be confessed that Oxford and Cambridge have fallen short of their duty, but if the office of a University be rather to educate men with the capacity for pursuing any profession, and to maintain a high standard of knowledge in its medical graduates, then they have no reason to fear criticism.
>
> (Robb-Smith 1966, p. 52)

During the latter nineteenth and early twentieth centuries, advocates for a medical school at Oxford believed that without such, the University would be starved of talent to conduct biomedical research, thereby being denied the opportunity to become an intellectual center of medical progress. In opposition, others argued that routine clinical instruction and practice would undermine fundamental research and break with tradition, whereby the University devoted itself to advanced research and the humanistic education of the future élite. Those with this view held that the fundamentals of natural science relevant to medicine should be maintained at Oxford, with clinical training to be conducted at the London medical schools with their wealth of the sick and infirm. Because of the fundamental concept of the university as a whole, this issue was divisive and contested bitterly (Webster 1994). Thus, until mid-twentieth century, following their first few years at Oxford, students would transfer to one of the London hospitals to complete their education. Henry Wentworth Acland (later Sir Henry; 1815–1900) (Fig. 3.1b), a major influence in the development of medicine and medical education at Oxford, detailed in a letter to his friend Dr. James Andrew (1831–1897) of London important milestones in the development of basic and clinical medical science at the University and attempted to justify the great need for its providing complete professional education in medicine (Acland 1890).

Poynter has observed that at mid-nineteenth century, the Royal Commission on the University of Oxford had concluded, "Oxford has ceased altogether to be a school of medicine. Those few persons who take medical degrees there, do so with a view to the social considerations which these degrees give or the preferment in the University for which they are needed but study their profession elsewhere" (Poynter 1970, p. 242). In 1845, however, Acland was appointed Dr. Lee's Reader in Anatomy (named for Matthew Lee (1695–1755)), at Christ Church College, and influenced for the good the development of medical education. Acland recorded that this was ". . . before I graduated in medicine" (Acland 1890, p. 93). Two years later (1847) he was elected to the Royal Society. Shortly thereafter, he commenced a campaign to develop a Natural Sciences department, which included the building of a Museum for Natural History, which was to be the center for scientific investigation at the University. ". . . In 1854, a Delegacy was appointed to obtain

plans; however, the progress of the selected structure was opposed at every stage, till the building was completed in 1860" (Acland 1890, p. iii). Although funds for lighting the structure and surrounding area were approved by only two votes of the convocation (oversight committee), that "... for oiling and varnishing all the fine oak window frames of the front being lost, they baked unoiled through the hot summer of 1859 or 1860" (Acland 1890, p. iii). With passage of the Medical Act of 1858 and creation of the General Council of Medical Education and Registration of the UK, Acland represented Oxford University. In addition to its role in defining the "qualified medical practitioner," the Council played a critical role in establishing criteria for, and standards of, medical education. In later years, the Council has been promoting the idea that medical research and teaching be pursued together (Poynter 1958, 1966a, b; Thomson 1958). In addition to the library from the Radcliffe Infirmary, collections collated for the museum included the natural science collection from the Ashmolean Museum, the Anatomical Museum in Christ Church College, and the Geological Museum in the Clarendon Building. Lecture rooms and a hall for scientific meetings also were provided (Acland 1890, p. 19). In 1858, Acland was appointed Regius Professor of Medicine and Litchfield Professor of Clinical Medicine, and the following year, he was appointed to the Thomas Linacre Professorship of Anatomy and Physiology.

In regard to Oxford's role in medical education, Acland asked in the manner of his friend the English writer, critic, and philanthropist John Ruskin (1819–1900) from "Whence do we come? Where are we now? [and] Whither are we going?" (Acland 1890, p. 9). Inspired by the vast anatomical and surgical collection of the Hunterian Museum in London, Acland commenced gathering an "anatomical and physiological" series of anatomical specimens and drawings, some of his dissections being brought from Edinburgh and the Van der Kolk pathological collection from the Netherlands. Also included was a laboratory for comparative pathology and microbiology. To various authorities, including members of Parliament, as models of a university-based medical education and scientific work to be developed at Oxford, Acland held up the universities in Germany, and the newly established Johns Hopkins University and Hospital in Baltimore. Acland also stressed the importance of preventative medicine and public health. Included as one of several appendices, Acland made a special case for the advancement in the study of physiology and pathology at Oxford, writing, "What is derivable for the philosophical student of Biology as a branch of general culture, is a necessity for the student of the healing art, as one of the corner-stones of his profession" (Acland 1890, p. 54). Acland expressed the desire that the University should be made "a place of the most perfect preparation that can be devised" for the study of medicine (Acland 1890, p. 22). He concluded his argument by enumerating the ideal "Character of the Student of Medicine ... such a man any of you may be; but you must begin by learning to stand by the sick-bed, and make it your delight" (Acland 1890, p. 58).

In 1876, the Physiological Society was founded, its initial meeting being held in the home of John Burdon-Sanderson (later Sir John; 1828–1905) of the University College London and an authority on muscle electrophysiology. While chiefly centered by scientists at the London schools, charter members from Oxford

included Edwin Ray Lankester (1847–1929) and Charles John Francis Yule (1848–1905) (Sharpey-Schafer 1927b). Contrary to what one might expect, the Physiological Society was not founded for purely scientific reasons. Rather, in Great Britain, studies on practical physiology were paralleled by the emergence of vocal opposition to the use of animals as experimental subjects. In 1875, a Royal Commission of inquiry into vivisection was established and recommended that all such studies be governed by an Act of Parliament, with investigators licensed by the Home Secretary. Recognizing the need for researchers to have some influence on parliamentary proposals that might unjustifiably interfere with scientific progress, in March 1876, Burdon Sanderson hosted a dinner for a dozen and a half physiologists. They formed a committee, drafted a constitution, and several months later commenced to meet on a regular basis. Initial meetings of this physiological dining club were devoted to sociopolitical considerations. Not until 1880 did scientific presentations and demonstrations become a part of the meetings (Sharpey-Schafer 1927a, b). In an update of activities of the Physiological Society to 1976, William Frederick Bynum then of University College London has emphasized the manner in which the Society has maintained close link to the evolution of physiology in Great Britain (Bynum 1976).

In 1883, Burdon-Sanderson left London to become Oxford's first Waynflete Professor of Physiology. His goal was to develop Oxford as a school of scientific medicine. In part, this had been aided by the Oxford University Commission in 1877 mandating a commitment to experimental physiology with erection of a physiological laboratory. Severe opposition to this proposal by antivivisectionists and others and an ad hominem campaign against Burdon-Sanderson almost aborted this development. In 1895, however, upon Acland's retirement, Burdon-Sanderson was appointed to succeed him as Regius Professor of Medicine. The following year, to strengthen the program in medical education, the new Regius Professor pressed for establishing two new departments, pathology and pharmacology. Within a year, William John Smith Jerome (1839–1929) was appointed lecturer in pharmacology and materia medica. In 1901, the new pathology laboratory opened, which also housed the department of pharmacology. In his Presidential Address at the 1904 Oxford meeting of the British Medical Association, William Collier (1856–1935), physician at the Radcliffe Infirmary, reviewed the growth and development of the medical school. He noted, "... that if the standard of general culture is distinctly raised by all the advantages of university education, ... then as the numbers of those participating in this education is increased the status of the medical profession is certain to be raised ... every encouragement should be given to participate in all the advantages to be gained by a few years experience of university life" (Collier 1904, pp. 223–224).

In 1905, William Osler (later Sir William; 1849–1919), a polymath clinician-historian-scholar who had worked under Burdon-Sanderson two decades earlier, followed as Regius Professor of Medicine. In 1913, the neurophysiologist Charles Scott Sherrington (later Sir Charles; 1857–1952) was appointed Waynflete Professor of Physiology; and in 1920 Archibald Edward Garrod (later Sir Archibald; 1857–1936), a pioneer of biochemical genetics, was appointed Regius Professor of

Medicine, while Benjamin Moore (1967–1922) was appointed Professor of Biochemistry.

A critical development in medical education in England, was the analysis and recommendations of the "Haldane Commission" of 1909, the final report of which was published in 1913 (Royal Commission... 1913). Chaired by Lord Richard Burdon Sanderson Haldane (1856–1928), a barrister, liberal politician, and Lord Chancellor (and brother of Oxford physiologist John Scott Haldane (1860–1936)), the Commission sought to elevate medical training to a proper university education. Influenced greatly by both the German and developing American models, Haldane's views were reinforced by the witness of both Sir William Osler and Abraham Flexner (1866–1959), the latter whom recently had published his *Medical Education in the United States and Canada* (Flexner 1910), as well as *Medical Education in Europe* (Flexner 1912). Osler had been one of the original academicians (Chief of Internal Medicine) at the Johns Hopkins School of Medicine in Baltimore (founded in 1889). In an essay Osler prepared for the *Quarterly Review* regarding the Report of the Haldane Commission, as well as comments regarding Flexner's latest study, he pointed out the major deficiency of the London medical schools.

> It is a remarkable fact ... in the history of medicine in England that a complete medical faculty of a University did not exist until well into the last century. Neither Oxford nor Cambridge has ever had one, nor has London, for the University of the greatest city of the world's greatest empire is a compound educational polyzoon, the units of which, like the polypoides, though highly organized and with admirable vegetative and reproductive organs, are without heart or central nervous system. This higher organization the Commission proposes to supply in the remodeled university; but, in the case of medicine, problems of extraordinary difficulty have to be met.
>
> (Cushing 1925, vol. 2, p. 363)

After discussing the ways in which these challenges could be accomplished, Osler concluded:

> There is a new outlook in Medicine, and a new science is moulding both thought and practice. Vested interests are powerful, old associations and ways are strong, but stronger still, we hope, will be the public and professional opinion in favour of the changes suggested by the Commissioners. London should be the most important medical centre in the world. That it is not this, is due to lack of organization and cohesion. To unite into a great Faculty its scattered forces is one aim of this able and far-reaching report, which will have the active support of all but those whom fear of change not only perplexes but appals.
>
> (Cushing 1925, vol 2, p. 363)

Because of the turmoil of the Great War which erupted soon thereafter, however, this goal was not initiated until much later. In influencing the ultimate change in the philosophy of medical education and the development of physiology in the UK, several factors proved to be important. These included the fact that with the ever-increasing pace of scientific research the curriculum always will contain an element that is obsolete. In particular, fields such as microbiology, pathology, and other basic sciences were rapidly advancing and specialization in medicine was expanding with each organ system being overseen by those who devoted their

lives to understanding the pathophysiology and clinical aspects of specific diseases (Poynter 1970).

Despite the new building for pathology and physiology at the beginning of the century, at the end of the war, Oxford laboratory facilities for the medical sciences for the most part were antiquated and inadequate. The new Department of Pharmacology, under the direction of James Andrew Gunn (1882–1958), commenced in 1912 but was consigned to the attic of the University Museum. During this period and continuing to 1930, the number of Oxford preclinical students rose from 25 to about 50. Credited with providing both impetus and a blueprint for reform of the Oxford medical curriculum were both Sir Walter Morley Fletcher (1873–1933), secretary of the Medical Research Council, and several reports of Abraham Flexner, at this time with the Rockefeller Foundation (Webster 1994).

In 1928, Edward Farquhar Buzzard (later Sir Farquhar; 1871–1945), a Harley Street neurologist and physician to the King, was appointed Oxford's Regius Professor of Medicine. With his vision and personal alliances, Buzzard had a profound influence on the development of physiology and the biomedical sciences. To help further in establishing the Medical School, several years later, the University funded four endowed medical professorships. In 1939, the onset of the World War II presented the unexpected opportunity for Oxford to transition to a full and complete school of medicine, a prospect grasped by Buzzard and his confreres. This development, which previously had engendered so much controversy, was appreciated to be the only responsible reaction to the demands of wartime emergency. Coupled with this was the requirement for many students in the London schools to be dispersed out of the metropolis. Ensuing developments demonstrated the virtues of this scheme. In 1942, a governmental committee on medical education, headed by Sir William Macnamara Goodenough (1899–1951), Chairman of Barclay's Bank, requested the university to outline its long-term commitment concerning the education of medical students. In response, a committee chaired by Buzzard detailed the need for the Oxford clinical school to train the future leaders, teachers, and investigators in medicine. With passage of the National Health Service Act of 1946, the Radcliffe Infirmary, the Churchill Hospital, and several other local hospital facilities became amalgamated under a board of governors into the United Oxford Hospitals. Following planning for further extension, the John Radcliffe Hospital at Headington was opened as a contemporary 1000-bed teaching hospital (Phase I, 1971; Phase II, 1979). The Phase I building included the relocated Nuffield Institute for Medical Research (see below). As noted by one historian, "Thus . . . after more than a century of vacillation, Oxford had come to terms with its role as a complete medical school. It was at last recognized that the presence of large numbers of clinical students . . . need not subvert Oxford's aspirations as a great centre of medical progress" (Webster 1994, p. 343). Several writers have detailed the development of physiology and medical education at Oxford (see Acland 1890; Burdon-Sanderson 1892; Chaplin 1919, 1922; Collier 1904; Franklin 1936; O'Connor 1988; Rolleston 1936; Webster 1994).

3.5 The Nuffield Institute for Medical Research

In view of the seminal role Oxford's Nuffield Institute for Medical Research (NIMR) played in the development of fetal and neonatal physiology and the manner in which its program flourished, it may be of value to consider some of its background. In what would later house the Nuffield Institute, the Radcliffe Observatory opened in 1794 following commencement of its construction in 1772 (Fig. 3.1c). Known as the "Tower of the Winds," after the structure in Athens (~100 BCE) upon which it was based, the structure was funded by the will of John Radcliffe (1650–1714). Known as "the Aesculapius of his age," Radcliffe was physician to King William III (1650–1702; King of England from 1689 to death) and Queen Mary II (1662–1694; Queen of England, Scotland, and Ireland, from 1689 to death) and then to Queen Anne (1665–1714; daughter of King James II (1633–1701); Queen of England, Scotland, and Ireland (1702–1714)) (see Macmichael 1827). Designed by the English architect James Wyatt (1746–1813), the observatory was not erected until 80 years following the donor's death.

The stimulus for situating and building the observatory was the 1769 transit of the planet Venus across the Sun, a phenomenon that occurs in pairs spaced 8 years apart every 105 or 120 years. Tracking the transit from different parts of the world and employing triangulation with a combination of the parallax method (a displacement or difference in apparent position of an object view along two different lines of sight), and Johannes Kepler's (1571–1630) laws of planetary motion, provided to astronomers a more accurate knowledge than previously had been available, of the distance of the earth from the sun, e.g., the "astronomical unit." This then could be used to determine other distances in our solar system, and even to distant stars. Due to a combination of inclement weather and other complications, observations gave few details of the previous transit in 1761. Because the second transit was predicted to be observed optimally in the South Pacific, Lieutenant (later Captain) James Cook (1728–1779) [also later early circumnavigator of the globe] was sent to Tahiti where the phenomenon was observed on 3 June 1769. These measurements, combined with those of other parts of the globe gave the distance to the sun to within 1 percent of what now is known to be correct. In Oxford, the transit was observed by the Astronomer Royal and Savilian Professor Thomas Hornsby (1733–1810). This experience led Hornsby to petition for Radcliffe's funds to erect a proper observatory. Also of note, the transit was followed the next day by a total solar eclipse seen across Europe as well as at Oxford. Accompanying Cook on the *Endeavour's* voyage to the South Pacific was the naturalist Joseph Banks (later Sir Joseph; 1743–1820) who, with the collaboration of Carl von Linné [Linnaeus] (1707–1778) and his binomial classification of plants, animals, and minerals (Linné 1735), helped to enhance British imperial and commercial botanical and agricultural interests. The Radcliffe Observatory was constructed in an East-West alignment, the east wing containing the finest of John Bird's (1709–1776) mural quadrants and the west wing containing teaching instruments. Finally, in 1795 the observatory tower for a large telescope was completed.

In ensuing years, with improved accuracy of the instruments, the eminence of the observatory came from Hornsby's meticulous observations and timing. These allowed production of tables for nautical almanacs that, through accurate determination of longitude, facilitated navigation at sea and thus the ascendency and near dominance of Britain in world trade.

Until 1935, the Radcliffe Observatory was occupied by astronomers who made a number of important discoveries. In 1930, with knowledge that the astronomers would vacate the observatory in the near future, Sir William Richard Morris (later First Viscount, Lord Nuffield) (1877–1963) (Fig. 3.1d), founder of Morris Motors Ltd. in Oxford, purchased this and adjoining land to allow expansion of the Radcliffe Infirmary. Several years later, in 1933, Sir Edward Farquhar Buzzard proposed establishment of the Institute for Medical Research in the observatory. It is said that Buzzard "... from the outset [had] fathered the whole scheme, and whose hope it is that this new department may not only link the science and practice of medicine in Oxford, but may also become a centre of national importance for the prosecution of original research" (Franklin 1936, p. 444).

At this time, Lord Nuffield also was persuaded to endow funds for research. Critically, Sir Hugh William Bell Cairns (1896–1952), who had come to Britain as a Rhodes scholar from Australia to train a neurosurgeon and had worked with Harvey Williams Cushing (1869–1939) in Boston, became a notable and charismatic leader, dedicating himself to creating Oxford as the "Harvard of the British Empire." This he planned to accomplish by establishing a postgraduate school of clinical research, with departments lead by investigator-clinicians of outstanding ability, who would devote themselves to teaching and research. In 1935, Cairns prepared a memorandum on "The desirability of establishing a complete school of clinical medicine in Oxford," which he sent to Buzzard. The following year, in his 1936 Presidential Address "And the Future" to the Oxford meeting of the British Medical Association, Buzzard stressed the importance of university-based research centers for the advancement of medical science and their contributions to raising the standard of health of the people of Britain (Buzzard 1936). Lord Nuffield was present at the address and, with the influence of Buzzard, Cairns, and perhaps others, was convinced to give £2 million (£120 thousand for building, with balance for endowment). In a munificent act, shortly thereafter Lord Nuffield pledged additional funds for the clinical school, which enabled establishment of several Nuffield chairs in clinical subjects (Booth 1989; Minns 1994). Soon, Cairns was appointed the first Nuffield Professor of Surgery, and Leslie John Witts (1898–1982) became the first Nuffield Professor of Medicine. In his 1941 Harveian oration for the Royal College of Physicians in the early years of World War II, Buzzard addressed challenges to medical practitioners, submitting in skeletal form a plan for reorganization of medical education, with increased emphasis on environmental and preventive medicine, social medicine, and the regional organization of medical centers (Buzzard 1942). He emphasized the promise of the Nuffield Institute as a "research center" contributing to the atmosphere essential for education and progress (Booth 1989; Buzzard 1936).

In 1934, James Gunn, the professor of pharmacology, was appointed director of the Nuffield Institute (although astronomers continued to occupy the building for another year). Joining Gunn in 1936 was Alfred Ernest Barclay, a radiologist who had moved from Cambridge following his frustration at receiving little support at that institution (Weatherall 2000). Together with the physiologist Kenneth James Franklin (1897–1966), these investigators focused on cineradiography of the circulation. As has been recorded elsewhere, a remit attached to Lord Nuffield's donation specified that cineradiography should constitute a major focus of the Institute, and later this was specified in the Oxford University Calendar of 1948 (Liggins 1998, p. 113). Establishment of the Nuffield Institute also contributed to development of a postgraduate medical school at Oxford (Webster 1994).

As noted earlier, in 1944 Barclay and colleagues published the gist of their previous 7 years work on cineradiographic and anatomical studies in *The Foetal Circulation… and the changes that they undergo at Birth* (Barclay et al. 1944). In the Preface to this volume, they credited Barcroft and Barron for stimulating their collaborative effort, stating "The foetal studies at the Nuffield Institute were initiated in 1937 when Sir Joseph Barcroft and Dr. D.H. Barron asked if we would co-operate, with our cineradiographic techniques, in the solution of their problem, namely, the determination of the time of functional closure of the ductus arteriosus." As noted earlier, the authors noted that this collaboration arose from Barron seeing, at a March 1937 meeting of the Physiological Society in London, the "X-ray cinematographic film of a dog's heart" by Franklin, immediately following a film "Experimental 'chronic' lesions in the central nervous system of the sheep's foetus" that he had made with Barcroft. In this preface, Barclay and colleagues also observed that in addition to providing "... most of our apparatus," Lord Nuffield "… has shown a personal interest in our results" (Barclay et al. 1944, p. v). The authors emphasized that their chief interest in the Institute was "… to discover more about the *human* subject" (Barclay et al. 1944, p. vi).

In 1943, the Nuffield Foundation was established. Gunn retired in 1946, and Franklin became Acting Director. Soon, however, Franklin left Oxford to chair the Department of Physiology at the University of London. In 1947, Barclay assumed the post as director and appointed Gordon Melville Ardran (1917–1994) as radiologist, a position he retained until 1978, when he assumed the Oxford Chair of Radiology. Later, Ardran served as a consultant to the Atomic Energy Research Establishment at Harwell and contributed to methods of protection from radiation (Golding 1994). With the strength of Oxford's other basic science departments, establishment of the Nuffield Research Institute in proximity to the Radcliffe Infirmary was seen as a critical factor in the establishment of clinical training at the medical school.

In 1948, Geoffrey S. Dawes was appointed Director of the Nuffield Institute, and over a period of several years, the physiology of the developing fetus became the focus of research. In 1970, the Institute moved from the observatory on Woodstock Road to Osler Road, Headington, site of Lord Nuffield's Manor House Estate, where it was connected with a bridge to the new John Radcliffe Hospital,

Departments of Obstetrics and Gynecology and Pediatrics. The observatory then became part of Green College.

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Chapter 4
Geoffrey S. Dawes: A Life in Science

The whole question of imagination in science is often misunderstood by people in other disciplines. Whatever we are allowed to imagine in science must be consistent with everything else we know. Our kind of imagination is quite a difficult game. One has to have the imagination to think of something that has never been seen before, never been heard of before. At the same time, the thoughts are restricted in a straitjacket, so to speak, limited by the conditions that come from our knowledge of the way nature really is. The problem of creating something which is new, but which is consistent with everything which has been seen before, is one of extreme difficulty.

(Richard Phillips Feynman 1918)

4.1 Early Life and Work

In view of his seminal role in the evolution of this field of study, it is perhaps not inappropriate to consider the contributions of Geoffrey Sharman Dawes (Fig. 4.1a). Born in Mackworth, Derbyshire, England, on 21 January 1918, Dawes was the youngest of five children. His father William Dawes (1874–1943), a graduate of Emmanuel College, Cambridge University, was the Vicar of Elvaston with Thulston in Derbyshire. His paternal grandfather, the Reverend Josiah William Dawes (1844–ca. 1914), a farmer and auctioneer, was also Vicar of All Saints Cathedral, Liverpool. His great grandfather was Josiah Belton Dawes (1813–1878). Initially, Geoffrey, called "pugface" by his brothers, attended the preparatory school Shardlow Hall and later the Repton School, also in Derbyshire. In his final year, Dawes was awarded the Senior House Prize for verse, a foretaste of his literary ability and distinction. As a youth, Dawes spent considerable time fishing and hunting in the countryside surrounding the commodious vicarage, Thurlaston Grange, in which he was raised. With a tennis court on the premises, he became first-rate in that sport. Although his parents planned for Geoffrey to follow the family tradition and attend Cambridge, he was awarded a place at New College, University of Oxford, where he gained first class honors in physiology. Following

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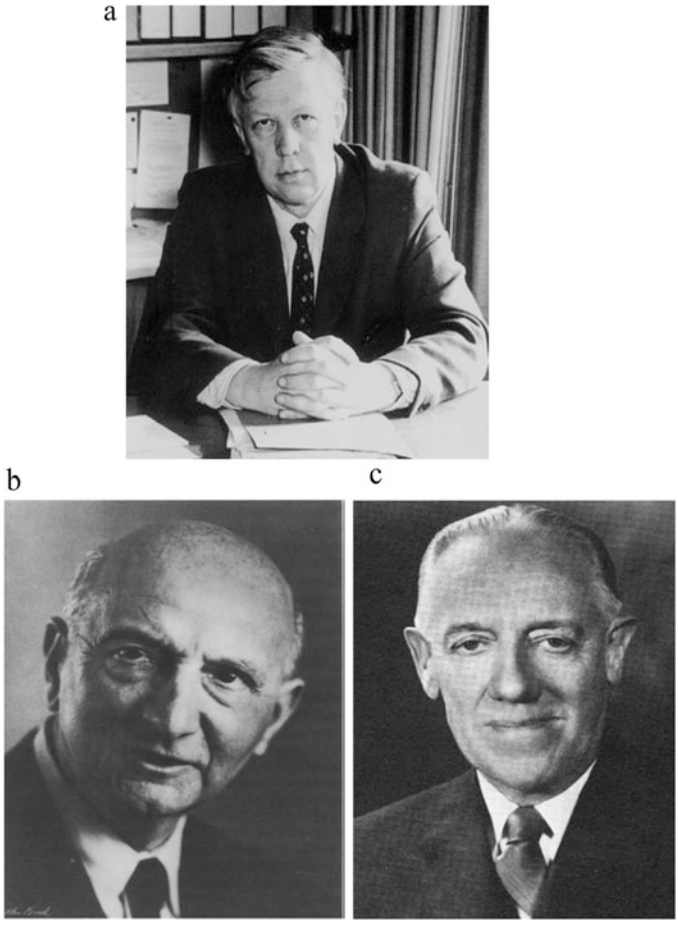

Fig. 4.1 (**a**) Geoffrey Sharman Dawes (1918–1996). (**b**) Otto Krayer (1899–1982). (**c**) Detlev Wulf Bronk (1897–1979)

award of a Bachelor of Science degree (1939), he qualified in medicine (1943). On 15 April 1941, Dawes married Margaret Joan Monk born in 1918 in Singapore, the daughter of Harold Monk (1890–1930), the District Commissioner of Malaya, and Violet Jones (1891–1990). Over the years, they had four children: Caroline Harriet Maunsell, OBE, b. 22 August 1943; Alison Jennifer Williams, b. 3 June 1945; Nicholas William Dawes, DPhil, b. 20 June 1948; and Martin Geoffrey Dawes MD, b. 18 September 1954.

Following obtaining his medical degree, Dawes served as House Physician working with Leslie John Witts (1898–1982), Oxford Professor of Clinical Medicine. In the cataclysm of World War II in Great Britain, every able-bodied man was called into military service. Because of his severe asthma with frequent attacks of *status asthmaticus*, Dawes was exempt from duty, but spent a compulsory year in

clinical practice in St. Giles, working with Robert Emlyn Havard (1901–1985). (Later, Geoffrey's son Martin Geoffrey also worked with Dr. Havard's medical group for several years). In addition to his work as a general practitioner, in medical science "Humphrey" Havard made several contributions to exercise physiology. Also of note, he was the only nonliterary member of the "Inklings," a 1930s and 1940s Oxford writer's group that included the noted authors Clive Staples Lewis (1898–1963) and John Ronald Reuel Tolkien (1892–1973). The "Inklings" provided mutual encouragement, critique, and editorial assistance to one another. At their weekly Thursday evening dinner meetings, often in C.S. Lewis' Magdalen College rooms, the members would read works in progress. Then they often would retire to the St. Giles pub "Eagle and Child" (also called the "Bird and Baby") for continued conversation (Carpenter 1978; Charlton 2009; Glyer 2007; Zaleski and Zaleski 2015). Havard has written of his relations with fellow "Inklings" and his patient J.R.R. Tolkien (Havard 1990).

In 1944, following his posing a rather perceptive question to the Oxford pharmacologist Professor Joshua Harold Burn (1892–1981), Geoffrey was invited to join the latter's research group. Here, he worked on several pharmacologic compounds (atropine and its substitutes, benzamidines, quinidine, the veratrum alkaloids, and quanidines) as they related to the military and the war (Dawes 1945, 1946a, b, c). Following the war (1945), Dawes was elected a Tutorial Fellow in Physiology, the first such scientific Tutor, at Worcester College (Dawes, 1946–1948). Although officially founded in 1714, Worcester College dates from the late thirteenth century when Sir John Giffard (1232–1299) purchased Gloucester Hall and presented it to the Benedictine Order as a "nursery and mansion place" for their novices. Following the Act of Supremacy (1534) with Dissolution of the Monasteries (1537–1540) by Henry VIII (1491–1547, King of England 1509–1547), the monastic buildings were given by the King to the new Anglican See [Latin *sedes*, seat] of the Bishop of Oxford. In the early eighteenth century, the Worcestershire Baronet, Sir Thomas Cookes (1648–1701), left a benefaction for the foundation of a new college. Several notable eighteenth-century architects including Sir George Clarke (ca. 1661–1736) and Nicholas Hawksmoor (ca. 1661–1736) designed the college central buildings, including the chapel and library.

In what was to prove a life-changing experience, Dawes then obtained a Rockefeller Foundation Traveling Fellowship and during the academic year 1946–1947 worked with two authorities in regulation of the cardiovascular system. The first was Otto Krayer (1899–1982) (Fig. 4.1b), Chairman of the Department of Pharmacology at Harvard Medical School and a pioneer in studies of the pharmacology and autonomic regulation of cardiac function. A master mentor, Krayer, inspired investigators in his department to explore important pharmacologic questions and develop new research methodologies (Reiter and Trendelenburg 1982). Dawes was to benefit greatly from this philosophy during his years leading the Nuffield Institute. In Krayer's laboratory, Dawes determined the sites of action of veratrum alkaloids in the lungs and coronary arteries (Dawes 1947, 1951).

For the second half of his fellowship, Dawes moved to the University of Pennsylvania in Philadelphia, where he worked with Detlev Wulf Bronk

(1897–1975) (Fig. 4.1c) in the Johnson Foundation for Medical Physics [named for Eldridge Reeves Johnson (1867–1945) and established in 1929]. "Det" Bronk, a world leader in establishing the field of biophysics, explored several aspects of neurophysiology. With Dawes' background in pharmacology, it appears obvious that Bronk's innovative studies on neural regulation of cardiovascular function and blood pressure probably led Dawes to choose to work in his laboratory. Another factor may have been a seminal 1944 paper Bronk wrote on "The Discovery and Interpretation of Biological Phenomena." In referring to the English philosopher and essayist Francis Bacon, Bronk stressed the importance of the scientist's requirement for freedom of inquiry, the merits of groups of investigators collaborating in fields of inquiry, pursuing clarified directions of research, and the need for cooperation and mutual encouragement in a multidisciplinary program under control of the scholars alone (Bronk 1944). With such freedom and support, Bronk maintained that, "... the ablest minds in the country will ... be recruited for the discovery of natural knowledge" (Bronk 1944, p. 312). As the masterful Director of the Nuffield Institute for almost four decades, it was to these concepts and principles that Dawes promoted. While at the Johnson Foundation, Dawes learned techniques of electrophysiology with the idea of using these to study the mechanism of action of veratrum compounds and their responses and effects on the circulatory and respiratory systems (Dawes 1947; Dawes and Comroe 1954; Dawes and Fastier 1950; Dawes and Feldberg 1949; Dawes and Mott 1950; Dawes et al. 1951a, b). A gifted administrator as well as scientist, during World War II, Bronk had served as a special consultant to the Secretary of War and was Chief of the Division of Aviation Medicine, Committee on Medical Research of the US Office of Scientific Research and Development (OSRD). These experiences and associations led to his appointment in 1945 as foreign secretary of the National Academy of Sciences (NAS) and in 1946 as chair of the National Research Council (NRC). Following Dawes' sabbatical, Bronk assumed the Presidency of the Johns Hopkins University (1949) and the National Academy of Sciences (1950) and received many other honors (Brink 1979). In retrospect, it is tempting to speculate on Bronk's influence on Dawes' thinking in terms of experimental investigation and the nature of the scientific enterprise. Although they published no papers together and in Dawes' writing I can find no definitive evidence, it appears clear that Bronk played more than a minor role in the development of his philosophy of life and career. For instance, Bronk had a great interest in the regulation of the cardiovascular and pulmonary systems and cerebral oxygenation. Regarding the satisfaction of a life as an investigator, he observed "... for we have a rare opportunity to glimpse the essential unity of science. To comprehend this is the final objective of every natural philosopher" (Bronk 1938, pp. 139–142). In his 1953 Presidential Address of the American Association for the Advancement of Science, Bronk stressed the intellectual adventure that constitutes scientific research and inquiry and also the responsibility of scientists to serve in the public arena. He closed with the words from Arthur Dehon Little (1863–1935):

> Ours is the duty and privilege of bringing home to every man the wonders, the significance, and the underlying harmony of the world in which we live to the end that all undertakings may be better ordered, all lives enriched, all spirits fortified.
>
> (Bronk 1954, p. 227)

Upon his return to Oxford in 1947, the Royal Society awarded Dawes a Foulerton Research Fellowship. Throughout his career, Dawes continued to employ electronic technology in his research studies yet would remain a classical organ/systems physiologist-pharmacologist.

The following year, February 1948, the youthful Dawes was invited to become Director of the Nuffield Institute for Medical Research. At this time he was 30 years of age, with eight publications to his credit. Quickly, he dedicated himself to building the Institute. A complicating problem was that Dawes possessed strong reservations about the value of radiology and/or cineradiography as a tool to study physiological mechanisms and resented the stipulation in the Institute's statutes specifying the importance of radiography to the research program. He is said to have clashed frequently with Ardran over this issue (Liggins 1998, p. 114). A year following his appointment as director, Dawes was joined by a physicist-electronic engineer Derek G. Wyatt and the zoologist Joan Culver Mott (1921–1994) (Fig. 4.2b), to collaborate on studies of cardiovascular and respiratory reflexes (Dawes and Mott 1950, 1959a; Dawes et al. 1951a, b, and others; Wyatt 1957, 1961). With David Whitteridge (1912–1994), who left soon thereafter to assume the Chair of Physiology at the University of Edinburgh, he collaborated on single-fiber recording of nerve impulses. Together, with pieces of equipment purchased at war surplus sales, they constructed an oscilloscope, nerve stimulators, and time-basis amplifiers. With Marjorie Prichard, Dawes commenced his studies on the pulmonary circulation (Ardran et al. 1952). Regarding Dawes' appointment, John Guy Widdicombe (1925–2011) (Fig. 4.2c) of University of London, who worked with Dawes in the early 1950s, recalled, "His appointment had been a leap of faith on the part of the Oxford University authorities, fully justified as the years passed. Geoffrey ... [stated] that he had confided to David Whitteridge ... that he had no idea how he would sustain a research programme. Whitteridge had assured him that 'one thing will lead to another' and that is how it worked out" (Letter from JW to LDL, 27 March 2009).

In reflecting upon his contributions to fetal and neonatal physiology, one may consider Dawes' career in four distinct periods: cardiovascular studies of the 1950s through the 1970s, studies on the fetal and newborn asphyxia and the pulmonary vasculature during the 1960s, the exploration of fetal breathing activity beginning in the 1970s, and from the early 1980s until the end of his career investigation of fetal heart rate and its variability.

Fig. 4.2 (**a**) Nicholson J. Eastman (1895–1973). (**b**) Joan C. Mott (1921–1994). (**c**) John G. Widdicombe (1925–2011). (**d**) Dawes Fishing (ca. 1970)

4.2 Dawes and the Fetal Cardiovascular System: The 1950s and 1960s

Following the trauma of World War II, the internal enemy of disease replaced the external axis powers as the greatest threat to the world. As a consequence, commencing with only modest funding, the "golden age" of research in medicine arose phoenix-like from the ashes. Although the war was enormously disruptive to civilization and to life, one side effect of benefit was the advance in instrumentation and technology, which investigators could apply to the furtherance of science. Dawes was one who took great advantage of these developments. It was in 1950 that Samuel Reynolds commenced a year sabbatical at the Nuffield Institute (Reynolds 1978). On the basis of some of his studies at the Carnegie Institution, Reynolds had postulated that umbilical venous return from placenta to fetus was driven by the pulsations of the umbilical arteries within the umbilical cord, rather

than by the *vis a tergo* [driving force]. With use of the angiographic cineradiographic expertise of Ardran and the Nuffield group, he hoped to resolve this question. Of vital importance to his career, Dawes has recorded that stimulated by Reynolds' impending visit, he read both the work by Barclay and colleagues *The Foetal Circulation* (Barclay et al. 1944) and Barcroft's monograph, *Researches on Pre-natal Life* (Barcroft 1946). This experience was an epiphany of sorts, changing his life course. In these two volumes, both Barclay and coworkers and Barcroft outlined a number of functional aspects of the fetal cardiovascular system and its regulation, including that of cardiac output, the pulmonary circulation, the *ductus arteriosus*, the changes at birth, and so forth that were poorly understood or not understood at all. These volumes outlined Dawes' experimental studies for the next two decades. He grasped quickly the "... obvious that there were major gaps in knowledge and the field had a good prospect for development." Of importance, he appreciated:

> ... the possibility that by examining the mammalian organism in the course of development we might be able to identify a period of life at which the organization of the control systems which determine homeostasis is simpler than in the adult, and that we could begin to understand how these mechanisms are assembled to form a coherent whole. It could be that in the fetus one would deal with systems which are free of many of the complexities of independent adult life.
>
> (Dawes 1981, p. 1)

The circulatory system of the fetus is unique in having four major shunts, e.g., the *ductus arteriosus* between the left pulmonary artery and aorta, the *foramen ovale* between the two atria, the *ductus venosus* a channel joining the abdominal portion of the umbilical vein and inferior vena cava, and the placental circulation. During the following years, Dawes and the Oxford group devoted considerable effort to elucidate the roles and dynamics of fetal circulatory physiology (Born et al. 1954, 1956b; Dawes 1961, 1963, 1965a; Dawes et al. 1954, 1955b, c, 1956). While the circulation of the fetus had been described in terms of anatomical systems, Dawes saw that little to nothing was known of either the quantitative aspects or the physiologic regulation of cardiovascular flows or pressures. In addition, he realized the vast ignorance that existed in regard to the pulmonary circulation and the profound changes these systems undergo at birth (Dawes 1981). In particular, he appreciated that there was no rational basis "... of how ventilation in the lungs at birth, caused a redirection of the fetal circulation ... It became evident that this was a more complicated system than it had first appeared ..." (Dawes 1994, p. 2). Several decades later, Dawes would recall, "... the mechanisms that controlled this [the changes in the fetal circulation at the time of birth] continued to provide food for thought and experimentation for years" (Dawes 1994, p. 2). In addition, he understood the importance of the problems of growth and development, the fetal lamb increasing in weight nearly 5000-fold between 40 dpc and term, ~140 dpc (Dawes 1981). In later essays, he stated that in reading Barcroft, he realized that this synopsis "... raised more questions than answers" (Dawes 1984, p. 259), and he reviewed other aspects of the development of his thinking along this line (Dawes 1985).

In a series of studies over the next decade and a half, Dawes demonstrated the manner in which the various elements of the fetal cardiovascular system respond readily to stimuli (reviewed in Dawes 1966). Although the experiments proposed by Reynolds did not support his hypothesis on the role of arterial pulses driving umbilical venous blood flow, the group published their first report on the role of the autonomic nervous system in cardiac reactivity. Sympathetic stimulation or atropine administration increased heart rate, while vagal stimulation, thoracic sympathectomy, or propranolol (alpha-adrenergic receptor blocker) resulted in bradycardia (Dawes et al. 1956). In addition, these workers demonstrated conclusively the remarkable lability of the pulmonary vascular bed and the effect of ventilation of the fetal lung in lowering pulmonary vascular resistance with an abrupt increase in blood flow (Ardran et al. 1952). They also demonstrated that the lowered resistance of the pulmonary vasculature was mediated not only by oxygen, as it also occurred with ventilation with nitrogen, (but not saline) (Ardran et al. 1952; Cassin et al. 1964a, b; Dawes and Mott 1962). In light of these studies, Dawes rejected his original hypothesis that the relatively high pulmonary vascular resistance was a consequence of the "kinking" of small resistance vessels (Dawes and Mott 1962). Although he demonstrated that acetylcholine infusion dilated the pulmonary vascular bed, increasing flow (Dawes and Mott 1962), a more exact mechanistic explanation for these changes had to await the discovery of the vasodilators prostaglandin and nitric oxide. In a later survey of his experiments during this period, Dawes recalled that his studies rarely were carried out in a warm saline bath, as those of Huggett, Barcroft, and coworkers. Rather the anesthetized ewe was placed on her side on an operating table, and the fetus placed on a "smaller adjustable warmed table elevated a few inches and pressed against the maternal abdomen... without tension on the umbilical cord" (Dawes 1994, p. 2). The fact that these studies were conducted under anesthesia, rather than in a chronically catheterized unanesthetized preparation (which was not described until a decade later), would prove a major limitation to their interpretation.

Several questions were germane at this time. Although the Nuffield group had demonstrated the remarkable sensitivity of the fetal pulmonary vascular bed to catecholamines and acetylcholine, the extent to which the variation in pulmonary vascular resistance was related to ventilation per se, as opposed to the role of the autonomic nervous system, remained unclear. In preparations with bilateral thoracic sympathectomy and vagotomy, they demonstrated the lack of importance of the latter nerve (Colebatch et al. 1965). Dawes and collaborators also explored the question whether fetal pulmonary vasodilatation associated with ventilation with the several gas mixtures might arise from pulmonary vascular receptors. They demonstrated this not to be the case, as a single inflation with a low oxygen mixture (3% O_2, 7% CO_2, with no significant change in arterial blood gases) resulted in prolonged vasodilatation, both without and following bilateral thoracic sympathectomy and vagotomy (Colebatch et al. 1965). A further question of relevance was the extent to which increases in arterial PO_2 and/or decrease in PCO_2, as opposed to the liberation of local blood-borne vasodilators, play the dominant role in lowering pulmonary vascular resistance at birth. In a series of studies in premature lambs

combining fetal asphyxia, infusion of oxygenated blood into the pulmonary artery, and in some cases bilateral vagotomy and infusion of hexamethonium (to block sympathetic nerves), the group demonstrated that, indeed, factors in addition to the blood gases played a critical role in this regard (Cassin et al. 1964b). These studies demonstrated conclusively that the basic mechanisms regulating the pulmonary vascular bed had developed long before the time of birth or fetal viability. In addition, these and related studies (Cassin et al. 1964a; Colebatch et al. 1965) contributed to the concept that humoral or other yet to be discovered factors (NO and prostaglandins) play critical roles in this regard.

During the remainder of the 1950s and into the early 1960s, Dawes and his colleagues focused their attention of various aspects of the changes in the circulation at the time of birth with closure of the *ductus arteriosus* and the *foramen ovale* (see Dawes bibliography, Chap. 23). In the normal newborn infant, the *ductus* closes 5–30 min following birth, followed by its gradual obliteration. Immediately following the onset of breathing, the high velocity reversed flow from aorta to pulmonary artery through the partially constricted *ductus arteriosus* creates a vibratory thrill. In these studies on closure of the *ductus* with pulmonary ventilation, these workers demonstrated abrupt constriction with increased oxyhemoglobin saturation, as well as in response to asphyxia with increased PCO_2 (Born et al. 1956a, b). Pulmonary inflation with nitrogen did not constrict the *ductus*. The group also showed that raising fetal [HbO_2] by having the mother breathe 100% O_2 also was followed by *ductus* constriction (Born et al. 1956a, b). These straightforward, well-designed studies demonstrated unequivocally that increased oxygen levels mediated constriction; however, they did not establish the factors causing constriction in the presence of much lower oxygen levels. Dawes proposed that the asphyxial response was mediated by the release of catecholamines, a fact demonstrated by infusion of either epinephrine or norepinephrine (Born et al. 1956a, b). The Nuffield group performed a number of other studies on various aspects of closure of the *ductus arteriosus* (Amoroso et al. 1958; Born et al. 1955; Dawes et al. 1954, 1955a, b). Because the *ductus venosus* constitutes a potential portocaval shunt that bypasses the liver, its closure at the time of birth also is important to newborn well-being. Dawes and colleagues explored aspects of the role of this fetal vascular channel, but abandoned these studies because of the difficulties associated with its investigation, after demonstrating that its closure for up to 5 min had no significant effect on carotid arterial [HbO_2] or arterial blood pressure (Dawes 1968). Other studies concerned regulation of closure of the *foramen ovale* (Dawes et al. 1955c). With his background in pharmacology and cardiovascular reflexes, to a limited extent, he continued to pursue questions related to these (Born et al. 1956a, b; Dawes and Comroe 1954; Dawes and Widdicombe 1953), emphasizing that the fetal heart and blood vessels (other than those of the placenta) are under autonomic control (Dawes 1966).

With his strong interest in, and commitment to, problems of clinical relevance, commencing in 1957, Dawes turned his attention to the oxygenation of fetal tissues, the rates of oxygen consumption, and some aspects of asphyxia on cardiovascular functions (Acheson et al. 1957; Born et al. 1956a, b; Dawes and Mott 1959b). By

determination of oxyhemoglobin saturations in the several vessels, Dawes and colleagues confirmed the early work of Huggett and Barcroft with Barclay and colleagues that descending aortic blood was derived chiefly from the right ventricle and pulmonary trunk via the *ductus arteriosus* (Dawes 1961; Dawes et al. 1954). Within minutes of the onset of ventilation, the direction of flow through the *ductus* reversed, so that the majority of descending aortic flow was from the left ventricle (Dawes 1961; Dawes et al. 1955c). During these years Dawes also collaborated with Kenneth William Cross (1916–1990) of St. Mary's Hospital Medical School, London, on the resuscitation of the newborn infant. Having worked with Huggett, Cross had great interest in anoxia of the newborn (Cross et al. 1953, 1954). In this regard, their studies established that positive pressure ventilation was more satisfactory than the use of either pharmacologic agents or hyperbaric oxygenation (Cross et al. 1959). A distinguished physiologist and student of the newborn, in 1979, Cross was awarded the James Spence Medal of the British Paediatric Association (Wolff 1979).

In the late 1950s, Dawes also became interested in the phenomenon in altricial species (i.e., those helpless at birth requiring food and care for a given period, such as cats, dogs, rats, and humans) of increasing metabolic rate and O_2 consumption in response to exposure to cold. By administrating a curarizating agent, the possibility of shivering was eliminated (Dawes and Mestyan 1963). In their calculation, extra heat production from the liver was eliminated by local measurements with thermocouples (Dawes 1985). On sabbatical at the Nuffield Institute, the Oxford pediatrician Peter Tizard (later Sir Peter; 1916–1993) with Jon Wilfred Scopes (1930–1999) of Queen Elizabeth Hospital, London, showed in rabbits that the epinephrine-mediated increase in O_2 uptake became greatly attenuated within 3 weeks of birth (Scopes and Tizard 1963). Also about this time at the Institute, the pediatric pathologist Michael Dawkins (1931–1965) with David Hull (later Sir David) observed attenuation of adipose tissue deposits around the young rabbit neck and interscapular region over this same newborn period. Upon exposure to cold, this brown adipose tissue (as it was known later) became much warmer than the rest of the body (Dawkins and Hull 1964; see below). This helped to provide a satisfactory explanation of the response to cold in the human infant (Dawes 1985).

A related key issue at this time was the lack of knowledge of the quantitative values of blood flow through the *foramen ovale* or the *ductus arteriosus* and the extent to which outputs of the right and left ventricles were similar or differed. Dawes came to appreciate that measurements of O_2 content and oxyhemoglobin saturation could be used to quantify the relative amounts of blood flow through the major vessels and the individual ventricular outputs. By measuring simultaneously the O_2 content at eight separate sites, he calculated the combined right and left ventricular output flow to the lungs (~10%) and to the placenta (57%), with the balance to the remainder of the fetal organs. He also calculated that a considerable volume of cardiac output transits the *foramen ovale* (40%), *ductus arteriosus* (35%), and aortic isthmus (38%). Although he argued incorrectly that output of the left ventricular greatly exceeded that of the right, he estimated that blood admixture within the heart was of little consequence, because of the large flow

returning from the placenta (Dawes 1961, 1965b). In a highly cited review, Dawes summarized his and others work on the fetal circulation and the changes at birth (Dawes 1961). He later reflected on this series of contributions, "There is a wide variety of distinct physiologic mechanisms, each separate and yet independent, subtle, elusive, and beautiful, which together comprise the circulatory system. . . . If there is one single lesson that has been learned from the last 10 years, it is that the circulation in the fetus and newborn is not a simple, less sophisticated, passive, smaller replica of the adult, but a complex, adaptive mechanism which continues to offer a worthy challenge to the investigator" (Dawes 1966, p. 78).

In recalling the early years at the Nuffield Institute and his involvement in these studies, John G. Widdicombe has written:

> The arrangement of the building at the time was as follows. One passed through the imposing portico to the large entrance hall with its beautiful circular oak table. Here everyone, research workers, technicians, secretaries and visitors came for coffee each morning and tea each afternoon. Democracy was complete. Technicians were valued and made important contributions. In the right wing was first, Geoffrey's room, then Derek Wyatt's (a physicist), then a small room (first occupied by Gwen [Barer] and later by Janet Vaughan, Principal of Somerville College), and then the workshop occupied by Bill Dodson, Arthur Broadway, and an electronics technician. Geoffrey always encouraged his assistants to make their own apparatus, and often there was no choice. In [my] D. Phil. viva Whitteridge, one of the two examiners, asked . . . 'Did you build your own amplifiers?' Thanks to Geoffrey [I] was proud to reply 'Yes'. On the left hand wing was Ardran and the radiology laboratory run by the radiographer Maurice Tuckey. A small room at the back of the hall housed the secretaries. Upstairs on the first floor was the central large laboratory with five [to] six research stations housing, at various times, individuals or pairs: Jesse Thompson (US), John Vane . . . Julius Mestyan (Hungary), Vladimir Kovacik (Slovakia), Kottegoda (Sri Lanka), Egbert Nusser (West Germany) and Karliss [sic] Adamsons. To the right over Geoffrey's room was the sheep room, also used by . . . [me] for respiratory research when outside the lambing season. Smaller rooms to the left housed Prichard and the photographer Eve Sporle. On the third floor was a magnificently furnished but freezing library, from which the telescopes had originally been pushed out to the open balconies on adjoining roofs. Above on the highest roof was a weather station. Outside beyond the Institute was the animal house which contained other small rooms for research staff.
>
> All floors of the Institute (except the entrance hall) had magnificent oak plank flooring. The trouble was that it bounced and was unsuitable of apparatus that had to be vibration free. A solution was found when part of the ground-flooring was lifted; underneath there emerged enormous concrete blocks that Wyatt had installed for the mounting of astronomical equipment, and which provided wonderful stability.
>
> In 1950, during a visit by Sam Reynolds . . . Geoffrey studied the changes in the foetal circulation at birth by cineradiography with a contrast medium, thorotrast. In this and subsequent studies the foetus was delivered and lay beside the ewe, still attached by the umbilical cord to the placenta. They observed the passage of blood through the ductus venosus, the foramen ovale and the ductus arteriosus, channels open in the foetus and newborn but which close soon after birth. This was a defining moment for Geoffrey. He realized that accurate measurements of pressure and flow were not possible with this technique. He largely abandoned radiology for more precise and quantitative techniques. He moved his research from the radiological laboratory to an upstairs room, the "sheep room". The NIMR ceilings were very high, so he had a mezzanine platform built on which was placed Van Slyke gas analysis and other equipment, approached by a ladder. Henceforward there was a yearly programme of research. A farmer/research veterinarian arranged for regular tupping (copulation) of the sheep so that the ewes could be brought in to the

laboratory at known stages of pregnancy. Geoffrey supervised the tupping himself and junior members of his team were not allowed to attend the indelicate procedure.

An hilarious daily event was getting the heavily pregnant and reluctant sheep upstairs to the sheep room. There was a beautiful Regency curved staircase but no lift. We learnt that if the pregnant ewe was pressed to the wall of the stair and surrounded by 4–5 humans she thought she was in a flock and readily followed the animal stream. The ewe was anaesthetized and placed on an operating table with a smaller table alongside, on which the lamb was delivered by Caesarian section and kept warm and moist alongside the ewe and attached to the placenta. This was in the days of post-war deprivation and food rationing; the maternal carcass provided many a Sunday roast joint, its hide a thick floor rug, and the foetus valuable Persian lamb fur for ornament. On one occasion [I] was walking home in the middle of the night after a long experiment, with the spoils on [my] back in a large sack. The police stopped [me]. 'What have you there?' [I] replied honestly but provokingly 'a body!' They laughed and allowed [me] to proceed.

An early important study measured the oxygen content of blood in different parts of the foetal circulation; from these values the flow to different regions could be roughly calculated. However there was a great need for a reliable flow measuring device. John Vane, who arrived in the laboratory to do a D. Phil, and was later awarded a Nobel Prize for his work on prostaglandins, designed a density flowmeter which was an advance on the 'Volkmann-Ludwig type' flowmeter. This was accurate and facilitated much good work. With it the great increase in blood flow and decrease in pulmonary vascular resistance which took place when the newborn lamb was ventilated was now directly measured. An important study in 1954 established the relative blood flow through different parts of the foetal circulation from measurement of oxygen content in the blood at eight different sites; 57% was through the placenta, but only 10% through the lungs. The high placental blood flow meant that the mixing of blood in the right heart was not very important. In 1955 cinematography showed that a cardiac murmur that had been detected in the newborn lamb was associated with reversal of blood flow through the ductus arteriosus. Surgical closure of the ductus caused a fall in arterial O_2 saturation. In the late 1950s two biochemists, Heather Shelley and Michael Dawkins were appointed. With Dr. Shelley it was shown in 1959 that the survival time of the newborn animal (work was now being done on lambs, rats, rabbits and guinea pigs) in hypoxia was dependent on the reserves of carbohydrate in the heart. This was an early finding that was to have important clinical implications.

The Vane flowmeter was clumsy to insert into blood vessels and required a large volume of blood to fill it. Geoffrey had appointed a young physicist (Derek Wyatt) who set about developing a cannulating electromagnetic flowmeter. The technical difficulties involved were great, the chief one being to eliminate all currents except that due to passage of a conducting fluid (blood) at right angles to the imposed magnetic current. The final instrument, many components of which had to be made by hand, was superb and surpassed for very many years any commercially-produced instrument. With this tool, Geoffrey went on to determine the control mechanisms of the foetal pulmonary circulation. Geoffrey and his team demonstrated the factors which controlled blood flow through the foetal and neonatal lungs of the lamb. In 1960 Dawes and Mott showed that the foetal circulation was in a high state of tone; the lung vascular resistance was due to smooth muscle activity and not simply mechanical in origin; it was greatly reduced by the dilator activity of acetylcholine or histamine while, after occlusion, there was reactive hyperaemia. Ventilation of the ewe with 100% O_2 caused vasodilatation, while maternal hypoxia, induced by ventilation with 10% O_2 caused foetal pulmonary vasoconstriction. The demonstration of hypoxic pulmonary vasoconstriction, the reverse of the systemic response to hypoxia which is vasodilatation, was important; this phenomenon is retained in adult life where most think it assists adjustment of local blood flow to local ventilation, although some suppose that it is a vestigial mechanism, useful only in foetal life to reduce flow through the non-functioning lung.

Geoffrey had a great capacity to master new technology. In the early 1950s he taught himself the use of cathode ray tubes while others were still using smoked drums. Later in life he similarly mastered computers and with them was to do important work on foetal heart sounds which had important consequences for the management of pregnancy.

However Geoffrey retained his interest in radiology. He decided to study blood flow through the three-chambered heart of a reptile. At considerable trouble and cost three crocodiles were shipped over from the Nile. Thankfully they were small, about 30–40 cm long, but their teeth looked sharp so plaster tape was put round their snouts. They were kept in a bath tub. On the day of the first, and last, experiment they were anaesthetized, a venous line was inserted, and they were placed on the X-ray machine. Thorotrast was injected and pictures of the heart were taken. However we had not anticipated that all the reptilian scales would be calcified, so that the pictures looked like a heavy snow-storm; it was the end of the research project, and of Geoffrey's interest in radiology.

(Letter from JGW to LDL, 27 March 2009)

Dawes' son, Nicholas William, recalled playing at the "Tower of Winds":

When Pa was Director of Medical Research at the Nuffield Institute, a lot of the experimental work was done using sheep. This early work was done in the Observatory (the "Tower of the Winds"). This building has a gorgeous circular staircase, and the operational work was done in rooms at the top of these stairs. Accordingly, the pregnant sheep had to be 'encouraged' to walk up these stairs, which was a very memorable and extraordinary event for us to watch, from a certain distance of course. . .. When we were young, sometimes one or two of us would go to the lab on weekends with Pa and stay there for a time while he did some work. Pa was very good at blowing glass, and he would make strange objects out it for us to play with. Also, we liked to play with the liquid mercury, which ran about and formed and split into interesting blobs. Quite often some would get away and be lost in the cracks in the floor. When there were sheep there, we would look at them in their pens as well. Sometimes tiny lambs would be brought home to be kept warm by the stove in our kitchen at home overnight. Occasionally we would be allowed to feed them from bottles.

(Letter from NWD to LDL, 18 January 2009)

Nicholas also recollected fond memories of shooting and fishing with his father:

Pa was an excellent shot with the classic English 12 gauge side by side shotgun. He and a group of friends used to go shooting for partridge, hare and pheasant in the winter months at a farm near Oxford where they leased the shooting rights year by year from the farmer. We children were coopted to help as "beaters" in the line walking across the fields, then after a while we would also help carry the game. These shoots occurred most Saturdays, and we would either take a sandwich lunch or, later, meet at a pub before heading off. At the end of the shoot the game would be divided out. We would normally bring some birds home and often a hare, which usually weighed several pounds. This represented a good deal of extra meat for us to have during the week, especially during the 1950s. He would clean the game and taught us how to do so as well.

Pa also fished a great deal. He really enjoyed catching fish for food, considering catching fish one could not eat as being significantly less fun. He taught us all to clean fish, and was very rapid and effective at it, as one would expect from his surgical skills. His carving of beef and other roasts was similarly skillful.

(Letter from NWD to LDL, 18 January 2009)

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Chapter 5
Fetal Asphyxia and the Primate Colony in Puerto Rico

The zoologist is delighted by the differences between animals, whereas the physiologist would like all animals to work in fundamentally the same way.

(Hodgkin 1992, p. 66)

You see things; and you say "Why?" But I dream things that never were; and I say "Why not?"

(Shaw 1921)

5.1 Historical Perspective

From a historical perspective, asphyxia in the newborn infant presents a number of issues. Although defined as "pathological changes caused by lack of oxygen in respired air, resulting in hypoxia and hypercapnia" (Dorland 2007, p. 167), the term is used differently by physiologists, clinicians, and pathologists. In Greek the term means "a stopping of the pulse"; thus, strictly speaking, the word asphyxia is an "infelicity of etymology" (Eastman 1936b, p. 274). As with many definitions, however, once it has been accepted, it may be altered to fit the circumstances. For instance, obstetrical asphyxia is "an imprecise term... frequently based on low Apgar scores alone..." (Cunningham et al. 1989, p. 597). Some prefer the term "neonatal or perinatal depression" (Reece and Hobbins 2007, p. 1234). According to a task force of the World Federation of Neurology Group, it is a "... condition of impaired blood gas exchange leading, if it persists, to progressive hypoxemia and hypercapnia" (Bax and Nelson 1993, p. 1022). For the neonatologist, asphyxia is applied to "infants who require more than 1 min of positive pressure ventilation before the occurrence of sustained respiration." No reference is made to blood gas values. In contrast, hypoxia is the case in which deficient oxygen in the blood (hypoxemia) is associated with the O_2 supply to tissues being reduced below physiologic levels, despite adequate perfusion. Commonly associated with conditions of impaired placental exchange of O_2, asphyxia may result from *abruptio placentae* [premature separation of the placenta from the uterine wall], prolapse of the umbilical cord, maternal shock with hypotension, carbon monoxide intoxication, and other conditions. As noted below, a link between reduced fetal

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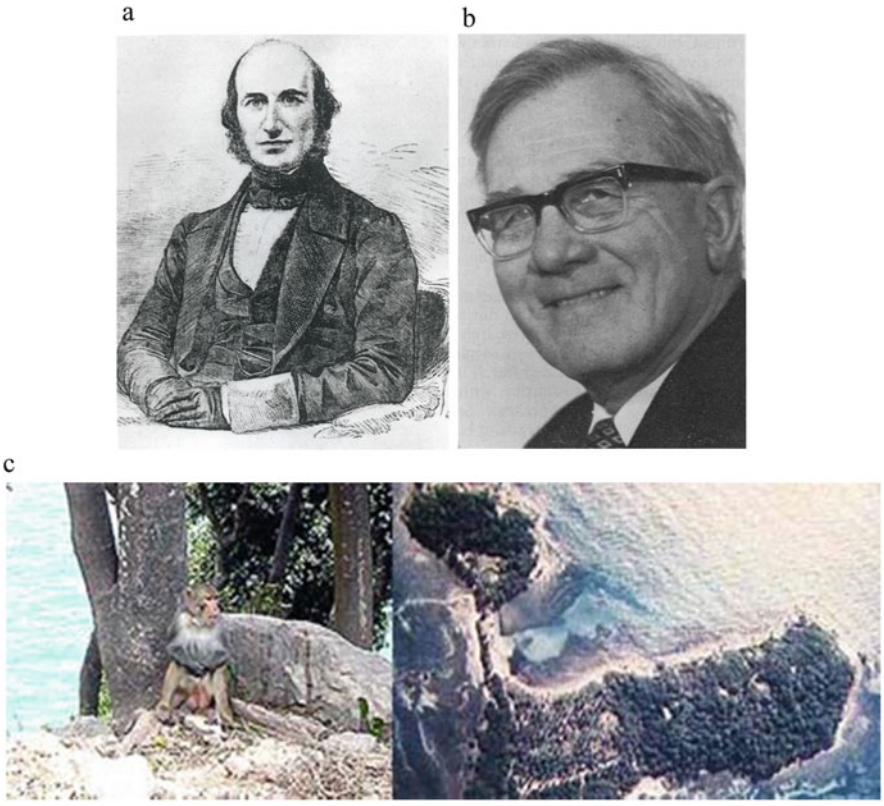

Fig. 5.1 (**a**) William John Little (1810–1894). (**b**) Kenneth W. Cross (1916–1990). (**c**) *Cayo Santiago* rhesus monkey

oxygenation and the development of perinatal complications such as intrauterine growth restriction and subsequent neurodevelopmental handicaps has long been established.

It was William John Little (1810–1894) (Fig. 5.1a), a London orthopedic surgeon, who, in 1861 in a presentation to the Obstetrical Society of London, first proposed the novel thesis in which he defined a causal relationship between abnormal parturition with asphyxia of the newborn to subsequent neurologic damage (Little 1861–1862) and the syndrome that became known as cerebral palsy (Osler 1889). In this "learned bombshell" (Neale 1958, p. 23), Little argued that besides ending in death or recovery, previous medical authors "seem quite unaware asphyxia at birth and abnormal parturition, not unfrequently has. . . a third termination in other diseases." Little's "third termination" included a spectrum of long-term deformities and disabilities. He postulated these were secondary to ". . . interruption of proper placental relation of the foetus to the mother, and non-substitution of pulmonary respiration, 'rather' than from direct mechanical injury" (Little 1861–1862, p. 298), acting on the brains of "too early and

unripe-born foetuses" (p. 314). In an appendix, Little tabulated 47 cases of spastic rigidity of the newborn and 11 related cases (p. 318ff). He noted the varying susceptibility of the developing nervous system to damage at differing ages of gestation. He noted further that many patients exhibited a delay in the appearance of the classical signs, what now would be interpreted as a transitional hypotonic phase. Little, with William Richard Gowers (1845–1915) after him, observed that more extensive motor involvement was correlated with greater intellectual deficiency (Gowers 1888). In association with this syndrome, Little also described erratic learning, short attention span, irritability, destructiveness, aggression, cunning, hebetude, and weakness of every intellectual facility, even complete idiocy.

In the discussion following Little's presentation, one listener recounted a case of a child born with spastic hemiplegia who was shown to have intracerebral bleeding following a "lingering labor" (p. 343). In contrast, many of the audience probably agreed with a discussant who stated "the difficulty . . . in discussing this excellent paper, arose, no doubt, from the entire novelty and originality of the subject" (p. 342). Later Charles West (1816–1898), a founder of the Obstetrical Society of London, observed that he could neither confirm nor deny Little's thesis because, ". . . instances of such termination of abnormal labour had not fallen under his notice" (West, quoted by Neale 1958, p. 24). In closing his presentation to the Obstetrical Society, Little recited the lines from Richard III,

> I that am curtailed of this fair proportion,
> Cheated of feature by dissembling Nature,
> Deform'd, unfinish'd, sent before my time
> Into this breathing world, scarce half made up,
> And that so lamely and unfashionable,
> That dogs bark at me as I halt by them
> (Shakespeare, Richard III, Act I, Scene 1)
> (Little 1861–1862, p. 343)

These few lines from William Shakespeare (1564–1616), Little thought, denoted the clinical and pathological dimensions of cerebral diplegia. Little considered that King Richard's deformity from birth probably represented hemiplegia or other palsy secondary to birth asphyxia. In this conclusion, he drew support from Sir Thomas More's (1478–1535) statement that Richard was born from a breech presentation ["the feet forward" (p. 344)]. Richard III also was said to have been born prematurely, being a difficult delivery and requiring resuscitation (Accardo 1980).

In a previous series of lectures ". . .deformities of the human frame," Little had written ". . . in many instances the spasmodic affection is produced at the moment of birth or within a few hours or days of that event. . .. The subjects were born at the seventh month, or prior to the end of the eighth month of . . . gestation. In two cases the birth occurred at the full period of gestation, but owing to the difficulty and slowness of parturition the individuals were born in a state of asphyxia, resuscitation having been obtained, at the expiration of 2 and 4 h, through the persevering efforts of the acoucheurs" (Little 1843–1844, p. 319). According to a contemporary observer, the eighth and ninth lectures detailed "a peculiar distortion which affects

new-born children which has never been elsewhere described ... the spasmodic tetanus-like rigidity and distortion of the limbs of new-born infants which... [he] has traced to asphyxia neonatorum, and mechanical injury to the foetus immediately before or during parturition" (Anonymous 1854, p. 21). In this early report, only in passing did Little describe spastic diplegia with greater involvement of the lower extremities, among the several types of paralysis (Lecture IX). By the end of the nineteenth century, this was known widely as "Little's Disease" (Longo and Ashwal 1993; Schifrin and Longo 2000).

Probably the earliest study of the effects of hypoxia on the developing mammal was the demonstration in 1670 by the distinguished English chemist Robert Boyle that 1-day-old "Kitlings ... continued 3 times longer in the Exhausted Receiver, than other Animals of that bigness would probably have done" (Boyle 1670, p. 2019). As noted earlier, since the studies of Pflüger (1877), Zuntz (1877), and Zweifel (1876), many others have verified that fetal and newborn animals are more resistant to hypoxemia than are adults (for instance, see Dawes 1968, p. 141ff).

5.2 Eastman and "Mt. Everest in Utero"

As noted earlier, in the early 1930s in conjunction with his studies to understand the genesis of asphyxia of the newborn infant, Nicholson Eastman explored several aspects of maternal-placental-fetal respiratory gas exchange. First, he attempted to define the normal physiologic state and the role of hypoxia, hypercarbia, and/or other factors in the initiation of respiration at birth. As background for these studies, at this time there was little agreement on those factors that initiated newborn respiration at the time of birth. Some attributed this to the sensory stimulation of the process of delivery (for instance, see Preyer 1885, p. 151ff). Eastman argued against this, pointing out that attempts at version and/or the application of forceps to the fetal head failed to initiate breathing. Others held that exposure to air and the change in temperature were major stimuli. Again, Eastman pointed out that respiration began as usual in those infants Ahlfeld had delivered with their mothers immersed in a warm saline bath (Ahlfeld 1888). In a series of studies in which he measured umbilical cord respiratory blood gases at birth, he concluded that for optimal management of *asphyxia neonatorum*, rather than administering CO_2 or performing physical stimulation, "... the chief therapeutic indication ... is for oxygen (or air) ..." (Eastman 1932, p. 50). Importantly, Eastman believed, knowledge of respiratory blood gases could help to understand the genesis of *asphyxia neonatorum* (Eastman 1930, 1932, 1936a, b; Eastman et al. 1933; Eastman and McLane 1931).

In a further study of anesthetic-induced causes of *asphyxia neonatorum*, Eastman reported a rather startling human experiment (Eastman 1936a). To pregnant mothers anesthetized with nitrous oxide ($n = 28$), chloroform ($n = 4$), or ether ($n = 8$) during vaginal delivery or cesarean section, inspired O_2 concentrations from 20% to as low as 5% were given for 5–21 min. In association with light chloroform

or ether anesthesia, fetal arterial and venous [HbO_2] values were reduced only slightly, if at all. When the women inspired a 20% O_2–80% N_2O mixture, umbilical venous and arterial [HbO_2] averaged 41% and 22%, respectively. Assuming pH values of 7.35 and 7.32, these saturations correspond to ~18 and 12 Torr, respectively. In seven subjects who breathed 15% O_2, maternal [HbO_2] averaged 79% ($Pa_{O2} = 43$ Torr), while the umbilical venous and arterial [HbO_2] values were 31% and 15%, respectively, corresponding to P_{O2} values of 16 and 11 Torr, respectively. In three patients who breathed 10% O_2, maternal [HbO2] was 67% ($Pa_{O2} = 33$ Torr), and umbilical venous [HbO_2] and P_{O2} values were 12% and 9 Torr, respectively. Two of these mothers were slightly cyanotic, and the newborn infants showed evidence of asphyxia. In seven subjects respired with 5% O_2 for up to 20 min, maternal [HbO_2] was 59% ($Pa_{O2} = 29$ Torr), and umbilical venous [HbO_2] was 8.6% ($P_{O2} = 7$ Torr). The umbilical arteries of these latter infants were collapsed, presumably due to circulatory failure with inadequate perfusion. All of these newborns were asphyxiated: five were apneic for 5–25 min and, despite resuscitative efforts, two died. Prior to delivery, the hypoxemic fetuses had shown decreased respiratory movements in utero. Eastman concluded by warning physicians of the extreme danger of using <15% inspired O_2 in conjunction with nitrous oxide anesthesia (Eastman 1936a). Although extreme by present-day standards, it must be appreciated that in the mid-1930s when these studies were performed, essentially nothing was known of the relation of maternal oxygenation or anesthesia to the effects on the *fetus* in utero. Numerous subsequent reports have shown a significant drop in fetal arterial O_2 tension in concert with decreased maternal arterial O_2 tension (Longo 1987).

In another 1936 report, Eastman reviewed in extenso various aspects of *asphyxia neonatorum* and others, summarizing in "Chart I" the critical changes in blood gas and pH values (Eastman 1936b, p. 280). On the basis of earlier work that had demonstrated decreased cardiac output as a consequence of asphyxia (Lewis and Mathison 1910), Eastman posited that *asphyxia pallida* of the newborn was a consequence of circulatory failure. He stated, "... that the one urgent need of asphyxiated infants is oxygen. ..." He continued, "that the usual forms of stimulation (including slapping, swinging, bathing, and carbon dioxide inhalation) *produce* depression ... and may even result in irreparable damage to brain cells" (Eastman 1936b, p. 297). As he had concluded previously, "The keynote of the treatment ... is gentleness—gentle removing of mucus from the respiratory passages and the introduction of oxygen at gentle pressures. And in so far as this ideal has been realized throughout, success will follow" (Eastman 1936b, p. 298). Two decades later, in his 1953 Presidential Address to the American Association of Obstetricians, Gynecologists, and Abdominal Surgeons, Eastman reviewed a number of aspects of fetal oxygenation, comparing that state to "Mount Everest in Utero." Emphasizing the problem of cerebral palsy and other neurological sequelae, he pointed out with "... gratification and pride" the record and accomplishments of clinical obstetrics. He concluded, "But obstetrics must look to the future if it is to continue to serve the best interests of our mother and children" (Eastman 1954, p. 711).

To consider anoxia and asphyxia in the newborn infant, Kenneth W. Cross (Fig. 5.1b) of St. Mary's Hospital, London, with Marcel Lelong (1892–1973) of the University of Paris, and Clement A. Smith of the Boston Lying-In Hospital, organized a special symposium in London for 6 days in 1951 (Cross et al. 1953). Held under the aegis of the World Health Organization's Council for International Organizations of Medical Scientists, the group of 17 clinical and basic science leaders reviewed numerous aspects of newborn anoxia/asphyxia in terms of clinical, histological and pathological, biochemical, physiopathological, and therapeutic considerations. An important result of this conference was that it raised the consciousness of neonatologists and others and identified some strategies for investigation of issues such as fetal/newborn anoxic tolerance, the mechanisms of the initiation of respiration at birth, the status of the lung and brain at birth, factors in adequate oxygenation, as well as the prevention and therapy of anoxia/asphyxia (Cross et al. 1953).

5.3 William F. Windle and the Primate Colony at *Cayo Santiago*

Based on the studies of many authors, asphyxia, whether occurring ante-, intra-, or postpartum, has been demonstrated to be one of the most potent factors altering the course of brain development. Based on his series of lectures on "developmental physiology," William F. Windle, then at the University of Pennsylvania, brought together into a small 70 page volume what was known and what was not known regarding *Asphyxia neonatorum* . . .with special reference to its effect upon the brain and subsequent clinical neurological manifestations (Windle 1950). With concern for the relatively high incidence of stillbirths and neonatal deaths (Potter and Adair 1940), Windle asked "How greatly the number of such deaths can be reduced by intelligent application of a knowledge of prenatal respiratory physiology . . ." (Windle 1950, p. 53). In addition to addressing the problem of the number of individuals who suffer permanent damage to the central nervous system (Clifford 1941), he spoke to the ". . . perhaps even more interesting problem . . . [of] possible impairment of mental functions in individuals who were asphyxiated at birth but who escaped clearly defined or persisting symptoms of brain damage" (Windle 1950, p. 53). Windle then reviewed his studies in the guinea pig, chosen because a pregnancy included several fetuses, and the brains were of such size to allow multiple serial section examination (Windle 1950, p. 55). Following asphyxiation for 4.5–23 min, and then resuscitation for one-half hour or more (depending upon asphyxial duration), regardless of the brevity of the insult, essentially all animals displayed ". . . symptoms of a neurological nature after birth" (Windle 1950, p. 56). Windle described both behavioral studies in a maze and the associated neuropathological findings, chiefly in the thalamic nuclei, geniculate bodies, tegmentum of the brain stem, and cerebral cortex (Windle 1950, p. 59; see also Windle 1944;

Windle and Becker 1943; Windle et al. 1944). In addition to aspects of physiology of the fetus and newborn, Windle reviewed other experimental studies of neurological, psychological, and neurohistological changes, particularly those in the guinea pig (Bailey and Windle 1959; Windle and Becker 1943; Windle et al. 1944). After considering the probable relation between asphyxiation in utero or during the neonatal period with cerebral injury and neurological sequelae, Windle concluded considering the analogous issues in humans, stating, "we are prone to blame inferior human mentalities on poor environment or defects in the germ plasm." He then asked, "Can it be that asphyxia at birth is partly responsible?" (Windle 1950, p. 61).

In view of Dawes' many studies and growing reputation in fetal oxygen consumption and related matters, in 1959, Windle invited him to participate in his studies of the effects of fetal asphyxia on the developing brain. An outstanding neuroscientist, at this time Windle served as Chief of Neuroanatomical Sciences at the National Institute of Neurological Diseases and Blindness (NINDB) within the National Institutes of Mental Health at the National Institutes of Health (NIH) in Bethesda, MD. He had led out in forming the Laboratory of Perinatal Physiology of the NINDB within the NIH. As noted earlier, during the winter 1935–1936, Windle had worked with Barcroft and had become intrigued with the antenatal genesis of some neurological conditions (Windle and Barcroft 1938; Windle et al. 1938). To understand better critical aspects of hypoxic-ischemic-induced brain damage in the newborn, Windle with a group of collaborators worked to develop further an existing colony of rhesus monkeys at the *Cayo Santiago* Field Station, a small island just off the southeastern coast of Puerto Rico. This station had been founded in 1938 for a colony of free ranging rhesus monkeys to be used for behavioral, physiological, and genetic research (Windle 1958, 1980). Here, they could have time-dated pregnant animals for their studies (Jacobson and Windle 1960a; De Ramirez De Arellano et al. 1959; Ranck and Windle 1959; Windle 1940, 1945) (Fig. 5.1c). Windle has reviewed some aspects of these studies on the rhesus monkeys, including the major brain regions involved, and comparison with findings in the guinea pig (Windle 1960, 1961, 1963, 1967). He also detailed the establishment of this primate colony, the vicissitudes of its maintenance both logistically and financially, and the work of the Laboratory of Perinatal Physiology (Windle 1980). Other aspects of Windle's contributions have been presented (Clemente 1985).

5.4 The Puerto Rico Studies of Asphyxia

During the years 1959–1962, and also in 1966, Dawes and colleagues, some from the NIMR, worked intermittently with Windle's group (Fig. 5.2a). In light of Dawes' interest in the basic physiologic aspects of clinical problems, he and his colleagues had focused considerable attention to the problem of asphyxia of the fetus and newborn infant and the mechanisms by which the fetus was able to withstand prolonged periods of hypoxia and asphyxia. In an early study in sheep,

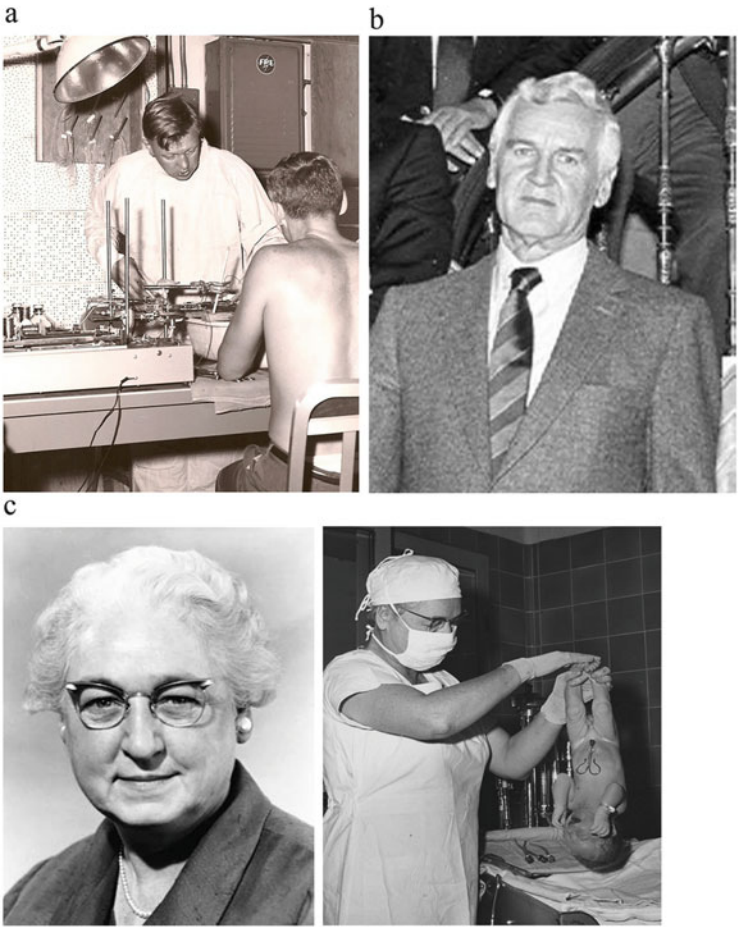

Fig. 5.2 (**a**) Dawes at the primate colony in Puerto Rico (ca. 1960). (**b**) L. Stanley James (1925–1994). (**c**) Virginia Apgar (1909–1974)

Dawes had demonstrated that fetal O_2 consumption ($\dot{V}_{O2}$) per kg body weight remained relatively constant during the last half of gestation, despite the great increase in its body weight. In addition, in both the fetus and newborn lamb, a significant decrease in $\dot{V}_{O2}$ occurred when arterial [HbO_2] was reduced below 35% (Acheson et al. 1957; Cross et al. 1959; see also Dawes 1959). With Kenneth Cross and Joan Mott, Dawes showed that in contrast to adult sheep in which the rate of O_2 consumption was maintained when inspiring 6% O_2 (as contrasted with room air, 20.9% O_2) and cardiac output increased three- to fivefold, in the newborn lamb, $\dot{V}_{O2}$ fell up to 40% with no significant increase in cardiac output. (Note: It was already near the plateau of its cardiac function curve, Gilbert 1980.) On the basis of inferior vena cava blood flow, newborn O_2 consumption appeared to decrease even more severely in the lamb's hindquarters than in the upper body. Cooling the lamb so that

it began to shiver increased $\dot{V}_{O2}$, and this shivering ceased upon breathing 10–20% O_2 (Cross et al. 1959; see also Cross et al. 1953, 1954).

In one of his most highly cited papers, Dawes and colleagues explored the role of glycogen in fetal and newborn cardiac function and the relation of cardiac carbohydrate concentration to survival in control and asphyxiated lambs, guinea pigs, rabbits, and rats (Dawes et al. 1959). Later, Joan Mott reviewed this literature, showing the role of myocardial carbohydrate stores in survival times for several species, at differing ages, ambient temperature, and with other variables (Mott 1961). A critical question regarding fetal asphyxia was its effects on cardiovascular reflexes and pulmonary vascular resistance. In the hypoxemic lamb with [HbO_2] <50%, Dawes and Mott had shown that fetal O_2 consumption fell as blood lactate rose (Dawes and Mott 1959). He and colleagues also demonstrated that perfusion of the pulmonary artery with arterialized blood from the carotid artery reversed vasoconstriction associated with asphyxia, and this was not altered by denervation. Dawes concluded that these responses were secondary to blood-borne hormones, with neural innervation playing little role (Cassin et al. 1964a, b).

In their initial collaboration with Windle, Dawes and his group worked to obtain physiologic data on monkey fetuses from 115 to 158 dpc (term = 168 days) and in the newborns from birth to 12 days of age (Dawes et al. 1960, 1963a). In fetuses in which they had catheterized the femoral artery (and in some cases the brachial or carotid artery), they measured blood pressure and blood O_2, glucose, and lactate concentrations, reporting values similar to those of the sheep. They also showed that oxyhemoglobin saturation in the blood to the upper body (brachial artery) was ~67%, in contrast to that of lower body (femoral artery) of 58% (Dawes et al. 1960). In addition, they determined that the fetal monkey weight doubled during the last one-third of gestation, while that of the placenta did not increase significantly. They also determined that on the basis of body weight, the rate of fetal O_2 consumption was twice that of adult, but essentially the same as adult on the basis of body surface area. In this study they demonstrated the presence of Hering-Brewer respiratory reflexes and that vagal stimulation caused bradycardia. They also showed that following umbilical cord ligation, gasping movements persisted for up to 20 min in the fetus delivered abdominally, but only 7–8 min in those newborns that had been delivered vaginally (Dawes et al. 1960). In related studies in fetal lambs, to establish parameters for fetal survival following asphyxia, Dawes and colleagues studied fetuses at 74–92 dpc (e.g., 0.5–0.7 gestation). In lambs asphyxiated by umbilical cord ligation, infusion of both glucose and sodium bicarbonate resulted in blood pressure, heart rate, and arterial pH falling much more slowly than in those not treated, and survival was prolonged. Infusion of either glucose or alkali alone failed to prevent these changes. These studies supported the idea that maintenance of glycolysis helped to preserve cardiovascular function (Dawes et al. 1963b). At the end of the experiments, they ligated the umbilical cord and recorded the time to last gasp, which decreased from 20+ min at 120 dpc to 10 min or less at term (~160 dpc). These observations confirmed the much greater tolerance of the fetus to anoxia-asphyxia, as compared to more mature animals (Adamsons et al. 1963;

Dawes et al. 1963a), as had been reported in the lamb and other species (Dawes et al. 1963b; Dawes and Mestyan 1963).

Subsequent to their determination of the fetal and newborn responses to asphyxia in both lambs and rhesus monkeys, the group explored methods to alleviate the inevitable adverse sequelae. Following delivery by hysterotomy, near-term fetuses of both species were asphyxiated by placing a rubber bag filled with warm saline over their head and ligating the umbilical cord. In some fetuses, infusion of glucose and alkali was initiated and continued for 1–6 min. "A short while" following the last gasp, the saline-filled rubber bag over the head was removed, the trachea intubated, and resuscitation with oxygen commenced (Dawes et al. 1963a, p. 168). Again, infusion of glucose and sodium carbonate was followed by prolonged maintenance of blood pressure and heart rate, with a greater success of resuscitation. Although those lambs that received alkali-glucose infusion gasped significantly longer than controls and responded better to O_2 insufflation, lambs failed to respond as well as did the monkeys (Dawes et al. 1963a).

In a related study in near-term fetal monkeys delivered by hysterotomy, asphyxiation was produced by umbilical cord occlusion following placement of a saline-filled bag over the head. Alkali and glucose infusion into the umbilical vein was begun 6.5 min after the onset of asphyxia and continued for 4 min. In monkeys so treated, gasping continued longer, and these animals were resuscitated more readily upon ventilation with O_2. Also in treated animals, the blood pressure was maintained better than in untreated controls. These workers also determined that although the solution of sodium carbonate resulted in hepatic hemorrhagic necrosis adjacent to entry of the umbilical vein, infusion of trishydroxymethyl-aminomethane (Tris, with pH adjusted to 8.8) showed no such adverse effect (Adamsons et al. 1963). Thus, maintenance of normal blood pH values was found to prolong breathing movements, facilitate resuscitation, and prevent brain damage.

In another study in *Macaca mulatta*, fetuses and newborns were asphyxiated by being placed in a glass jar into which nitrogen flowed. Windle demonstrated that while the fetuses could be resuscitated successfully as long as 7 min following their last gasp, resuscitation of the newborns begun from 2 to 5 min after last gasp was unsuccessful. Those infant monkeys that survived asphyxia with resuscitation, and which were euthanized 10–13 days later, showed no central nervous system pathology, as compared to the fetuses asphyxiated in utero that displayed symmetrical focal destruction in the inferior colliculi. The authors speculated that this difference in findings may have resulted from greater sensitivity of the more mature newborn heart to the anoxic stress, so that these animals did not survive long enough for the neuropathology to develop (Jacobson and Windle 1960a, b). In subsequent studies, this group attempted to approximate more closely the circumstances of the human infant, in which asphyxiation of the fetus during the latter stages of labor occurs some time prior to the opportunity for treatment. In these instances, resuscitation was more successful, and histological examination of the brains in the animals euthanized 6–26 weeks later disclosed a significant reduction in the incidence and extent of damage in the brain stem and inferior colliculi (Dawes et al. 1964). In experiments on asphyxiated Macaques, the Dawes group coined the term "primary

apnea" to describe failure to breathe soon after delivery, in contrast to "secondary apnea" in prolonged asphyxia with development of acidemia, beyond the last spontaneous gasp (Daniel et al. 1966b). Subsequently, a number of authors have explored physiological and biochemical aspects of fetal asphyxia and its sequelae (for instance, see Jensen 1996; Jensen et al. 1999).

5.5 Virginia Apgar and Evaluation of the Newborn Infant

To develop, to a great extent as possible, a "model" of therapy of the asphyxiated newborn, these workers combined positive pressure ventilation, a standard clinical procedure in neonatal resuscitation, with intravenous glucose and alkali treatment. The primate newborns delivered by cesarean section were asphyxiated for 10–15 min and then ventilated with O_2 while receiving an infusion of glucose-alkali. The duration of time required to establish spontaneous respiration was markedly reduced over those newborns which were ventilated without combined therapy (Adamsons et al. 1964). These studies also demonstrated in the monkey fetus the correlation between the composition of blood samples from the scalp with carotid artery or jugular vein blood (Adamsons et al. 1970). This would help to demonstrate the validity of this technique for use in humans (Saling 1985).

Leonard Stanley James (1925–1994) (Fig. 5.2b), a Columbia University pediatrician and one of the founders of the specialty of neonatology, with Karlis Adamsons Jr., an obstetrician gynecologist and one of the founders of perinatology, continued the studies on asphyxiated primates following their return to Columbia. There, they collaborated with Virginia Apgar (1909–1974) (Fig. 5.2c), anesthesiologist and originator of the five-point scoring system for neonatal well-being; Apgar had chosen the criteria (heart rate, respiratory effort, muscle tone, reflex irritability, and skin color), evaluated at 1 min, in part to obviate the need for intervention during attempts at resuscitation of the newborn, holding that these functions could be determined without compromising the infant's care. Each variable was assigned a score of 0, 1, and 2, with a total score of 10 indicating the most satisfactory condition (Apgar 1953). Some have suggested that APGAR is an acronym for measuring appearance, pulse, grimace, activity, and respiration. As a background of her studies, it is said that one morning in the Columbia Medical Center cafeteria, a medical student asked Apgar how to evaluate properly a newborn infant. "That's easy, you'd do it like this," she responded, writing down the five criteria listed. Then she rushed off to test this idea. After examining these criteria on more than 1000 newborns during 1950 and 1951, she published her findings, and the rest is history (Calmes 1984). Stanley James has recorded a somewhat different scenario of her idea noting that as the former Chair of Columbia's Department of Anesthesiology, wishing to characterize an infant's condition at birth, she completed a course in biostatistics offered by the School of Public Health. She then chose the five signs classically used by anesthesiologists to monitor a patient's condition (James 1976). James noted:

> She wished the score to be simple and easy to apply . . . to enable the physician or nurse to assess the infant's condition rapidly at a glance. She also recognized that time was important and made her observations with a stopwatch which she carried around her neck.
>
> (James 1976, p. 2)

James recalled further:

> As with all of her projects, she approached this with great glee and enthusiasm, and mobilized the interest of the obstetric and anesthesia services. She soon found that the obstetricians all wanted to score their babies 10. Some wished to give 12. From this she concluded that it was important that some person other than the one responsible for the delivery should make the score.
>
> (James 1976, p. 3)

In a "second report," Apgar and colleagues demonstrated that among 15,348 newborns studied, the death rate of those scoring 2 or less was 15%, compared to that for infants with a score of 10, which equaled only 0.13% (Apgar et al. 1958).

Originally, the Apgar score was assigned only at 1 min. However, a twelve-institution "collaborative study of cerebral palsy, mental retardation, and other neurological and sensory disorders of infancy and childhood," which included over 17,000 newborns, presented evidence that repeated scoring at 5 min provided additional prognostic information in predicting neonatal survival and neurologic development (Drage et al. 1964). To a great extent, it was James and colleagues who attempted to correlate the markers identified by Apgar with blood gas and acid-base values (James 1985; James et al. 1958). Of clinical importance, these studies gave credence to including the measurement of pH with that of blood gases and administering alkali, practices which have become standard. James has presented two relatively brief reviews of Apgar's accomplishments and contributions to life (James 1975, 1976). In explaining why she carried basic resuscitation equipment with her at all times, Apgar is purported to have said, "Nobody, but nobody, is going to stop breathing on me" (National Library of Medicine Profiles in Science, http://profiles.nlm.nih.gov/CP/). And it has been said that, "every baby born in a hospital around the world is looked at first through the eyes of Virginia Apgar" (Beck 2009).

In a reflection on the Apgar score, Geoffrey Dawes noted several important contributions, namely, that it required the student of the newborn to pay attention rapidly and critically to each of the five variables enumerated, its emphasis on the importance of accurate timing of postnatal changes in the assessment of health, and it provided an easily understood semiquantitative estimate of the situation (Dawes 1976). In his critique he noted that the score gives the same weight to different variables, for instance, to changes in heart rate as to changes in color. Nonetheless, he admitted that its universal adoption and continued application give ample evidence of its practical utility and being an admirable pragmatic tool. "Advances in technology have not displaced it, time has not impaired it and familiarity has secured its position among the tools of neonatology" (Dawes 1976, p. 4).

As can be appreciated, these collaborative studies of basic scientists and clinicians, which used as experimental "models" both lambs and primates, resulted in a number of contributions to understand more completely fetal asphyxia and acid base balance and suggest the optimal methods of treatment of human newborns

(Adamsons et al. 1963, 1964; Daniel et al. 1966a, b; Dawes et al. 1960, 1963a, c, 1964; James et al. 1958). Based, in part, on the work of Dawes, Windle, and collaborators, the pioneering neonatologist Robert Usher (1929–2006) at the Royal Victoria Hospital and McGill University in Montreal introduced the use of what came to be called the "Usher regime," consisting of intravenous sodium bicarbonate solution with glucose and insulin to correct hyperkalemia and to maintain blood pH within normal limits (Usher 1959). This reduced mortality in preterm infants by about two-thirds (Usher 1961, 1963; and see below).

In a special issue of the *British Medical Bulletin*, Kenneth W. Cross reviewed in extenso *asphyxia neonatorum* in terms of blood gas and pH values and the time course of their changes, respiratory activity including "primary" and "secondary" or "terminal" apnea, the time course of changes in heart rate and blood pressure in association with intermittent positive pressure ventilation and neuronal activity. Of particular value to the neonatologist, Cross detailed several criteria for effective treatment (increase in heart rate, with return of gasping following reestablishment of circulation), pointing out the problems with those that proved not to be effective (Cross 1966).

5.6 In Summary

To a great extent, Windle remains the "unsung hero" of much of the work on asphyxia of the fetus and newborn. It was the study he initiated in the primate, in collaboration with Stan James, Karlis Adamsons, Dawes, and others, that has led to increased understanding of the pathophysiology of asphyxia and its sequelae in terms of damage to the developing brain. In his 1968 monograph *Foetal and Neonatal Physiology*. . ., Dawes included a chapter on "Birth asphyxia. . .," in which he summarized many of the studies by himself and others. Several of the figures (such as Fig. 69 that summarizes changes in fetal arterial blood gases and pH, gasps per min, fetal heart rate, and arterial blood pressure in response to ~12-min asphyxia followed by resuscitation; Dawes 1968, p. 149) have been widely reproduced in a number of volumes (Phibbs 2007).

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Chapter 6
The Pulmonary Vasculature and Dawes' *Foetal and Neonatal Physiology*

> *It is in nature of a hypothesis when once a man has conceived it, that it assimilates everything to itself, as proper nourishment, and from the first moment of you begetting it, it generally grows stronger by everything you see, hear or understand.*
>
> (Lawrence Sterne 1759 pp. 121–122)

6.1 The Pulmonary Vasculature of the Fetus and Newborn

During the 1960s, Dawes with Sidney Cassin (1928–2010), Joan Mott, and other colleagues worked to understand the regulation of pulmonary vascular tone and blood flow in the fetus, with the transition at birth to that of the newborn. As noted earlier, Dawes appreciated that while the circulatory transitions at birth are among the most profound at any time during life, many of the studies reported previously were neither rigorous nor quantitative. Considering their importance, surprisingly little was known concerning the quantitative aspects of these changes or their mechanisms. In a lengthy letter Sid Cassin composed a year before his death, he described his initial interaction with Dr. Dawes and some of the early work of the fetal pulmonary system. Because of the authority of this account, these remarks and insights are given in length:

> I first met Dr. Geoffrey Dawes in 1960 when he visited the Dept. of Physiology at the University of Florida, College of Medicine in Gainesville, FL . . . to give a seminar on the effects of hypoxia on the fetus and newborn. This was of particular interest to me since I had studied the effects of anoxia on the newborn of several species as a doctoral candidate . . . at the University of Texas Medical School in Galveston, Texas. After the seminar we had long discussions on whether the heart or brain of the new born survived an anoxic insult better. Of course we had different opinions and Dr. Dawes invited me to come to Oxford and work in his research laboratory to resolve the problem. I finally made the trip with my wife and four children (all under the age of seven) in 1962. . ..
>
> I was to study the appearance of cerebral oxidative enzymes during fetal maturation using a Warburg apparatus and methodology. Although I managed to generate some data, the approach was not very exciting, and experimental animals were difficult to obtain at appropriate ages. Thus, I requested to work with Drs. Dawes, Mott, and Ross on the fetal pulmonary circulation. The experiments were exciting and the team was outstanding. After

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a meeting of the British Physiological Society where Dr. Dawes and Dr. Leonard Strang had a heated discussion over Leonard's presentation on the fetal pulmonary circulation, Strang was invited to Oxford to see what Geoffrey was up to. As a result Leonard made the trip in from London two or three times a week to participate in the research. I couldn't have asked for a more exciting and imaginative group of scientists with whom to spend a year. . ..

The [sheep] laboratory itself, however, was quite a challenge. It was certainly not equipped [in] the way my own lab back in the States . . . in the old Radcliffe Observatory, . . . Dawes' work area was housed on the second floor. The windows were large and provided good views of the Radcliffe Infirmary, but were not well insulated and in the winter (which was described as the "worst winter in the last 100 years") the cold came right through into the lab. In addition the heating system was not efficient. Most of us ended up wearing heavy winter clothing because of the low temperatures in the lab. Needless to say this made it rather cumbersome to carry out some of the experimental techniques.

(Letter from SC to LDL, 19 September 2009)

Cassin described interactions between Dawes and NIMR Visiting Scientists.

In addition to the worst snow storm and cold weather . . . which we had to combat in order to get into the lab, as well as the indoor temperatures, it is amazing to me now that we accomplished as much as [we] did. The equipment available included an old Warburg and Van Slyke apparatus as well as a very early model Severinghaus blood gas analyzer. Computers were unheard of, and electrical calculators were not available. Ben Ross and I spent many hours working out statistical regression analyses with the then state of the art hand held Curta calculator. There were no elevators so pregnant sheep had to be pushed up the circular stairway from the ground floor to the research lab. On the other hand we all enjoyed the 10 AM and 3 PM tea held every day on the first floor, as well as our connection with the Royal Oak Pub across the Woodstock road from the Nuffield. We all had charge accounts at the pub which did not get paid but once every 3 months. On busy days with our experiments a lunchtime pint of bitters and a half round (small sandwich) were much appreciated. I would point out that Dr. Dawes performed all of the major surgery on the ewe and fetus. His first assistant was Joan Mott and I was allowed to do simple surgical procedures like the tracheotomy on the ewe, and [catheterized] the femoral artery and vein as well as carotid artery and vein . . . on the ewe and the fetus. I also carried out analyses of blood gas contents, pH, pO_2 and pCO_2 on the ewe and fetus. However, all of us seemed to be having continuous discussions about the meaning of experiments, as well as how and what to do next.

Whenever those discussions were at a standstill Geoffrey would invariably bring up some current event or some topic he had recently read about in the *London Times*. None of the rest of us had any knowledge of the topic and Geoffrey would grin and say "everybody knows so and so, how come you do not". At some point we decided to play the same game with [him] . . . Leonard Strang found an obscure topic to read about in one of the London papers and gave us all (except for Dawes) the reference. The next day Leonard asked about the subject and each of us other than Dawes had a comment or two to make about the topic. When we asked Dawes about it, he didn't have a clue and looked very surprised. Well, we didn't let it go but kept pursuing his lack of knowledge of the topic. He apparently, got so upset he had to pull out his inhaler to prevent an asthmatic attack. We then told him about our scheme and he joined us in a bit of laughter. That evening after our experiment was finished we all had a pint of bitters and a few scotch eggs together. However, it did put a stop to Geoffrey's daily irritating approach. He in turn, provided us with a dinner at his house where he served us "jugged hare", something I had never heard of before. This of course was followed by a round of straight scotch malt (also new to me) and interesting discussions.

> Geoffrey's wife Margaret was there and contributed greatly to our discussions. She was unable to get involved to any large extent in the cooking because she was almost blind as a result of retinitis pigmentosa. She did however provide an excellent dessert that evening which was "orange syllabub". However, Geoffrey was an excellent cook as well as a very entertaining and delightful person. . . . At some point in our experimental work, Geoffrey had to leave the Nuffield for an extended period in order to study the effect of asphyxia in the fetal rhesus monkey with L.S. James. It was during this time and almost to the end of sheep season that Joan Mott and I did all of the fetal surgical procedures for our experiments. This experience made it possible for me to carry on fetal pulmonary experiments when I moved back to Gainesville. . ..
>
> (Letter from SC to LDL, 19 September 2009)

After reiterating some of the early work by Huggett, Barcroft, and others, Cassin continued,

> Dawes was not convinced that some of the studies which were reported prior to his interest in the area were as quantitative as they should have been. Thus, in some of his early work, [Dawes] and John Vane . . . developed what they labeled a "Density Flow Meter" (Dawes et al. 1953a). With this device they were able to measure flows from 1 to 500 ml min^{-1}. In early studies by Ardran, Dawes . . . [and colleagues] (Ardran et al. 1952), it was shown that ventilation of fetal lamb lungs produced a fall in pulmonary arterial pressure. In other studies . . . it was shown that there was an increase in pulmonary blood flow when lungs of fetal sheep and a fetal goat were ventilated (Reynolds et al. 1954). However, there were no studies to show what happened to pulmonary vascular resistance after ventilation. Thus, Dawes . . . carried out a series of studies on fetal sheep to measure changes in pulmonary vascular resistance during ventilation (Dawes et al. 1953b). Not only were measurements made of pulmonary arterial pressure and left atrial pressure, but they were able to measure simultaneous flows. . .. Using these techniques they demonstrated decreases in pulmonary vascular resistance [increased pulmonary blood flow with decreased pulmonary arterial pressure] when the lungs were expanded with air, oxygen, [or] nitrogen. However, when the lungs were expanded with saline, pulmonary vascular resistance increased (Dawes et al. 1953b). These studies resulted in further questions concerning why there was a high fetal pulmonary vascular resistance, and the mechanisms that were responsible for the decrease in pulmonary vascular resistance following ventilation.
>
> Prior to birth the fetal lungs are not effective organs of gas exchange . . .. The fetal lungs do, however, consume about 8% of the total fetal oxygen consumption (Dawes et al. 1954) as functions of growth and the process of lung liquid secretion (Adams 1966). Since the pulmonary vascular resistance of the fetal lungs is quite high, most of the right ventricular output goes through the *ductus arteriosus* to the descending aorta. In anesthetized, exteriorized fetal sheep close to term, blood flow through the lungs . . . was about 31 ml kg^{-1} min^{-1} (Dawes et al. 1954) . . . [however], more recent studies on unanesthetized, catheterized fetal sheep using radiolabelled microspheres provided flow rates which were higher (20–40 ml kg^{-1} min^{-1}) (Rudolph and Heymann 1974). Fetal sheep lungs ventilated with air, oxygen or nitrogen showed a marked decrease in pulmonary arterial pressure as well as an increase in flow . . . (Dawes et al. 1953b).
>
> Other experiments on exteriorized fetal sheep demonstrated that using air instead of nitrogen caused a greater decrease in pulmonary vascular resistance (Cook et al. 1963; Dawes and Mott 1962) .. . . Addition of carbon dioxide to a ventilating gas mixture resulted in pulmonary vascular constriction (Cook et al. 1963) . . . In 1962, [we] determined whether inflation of fetal lungs with gas mixtures which cause little or no change in blood gas would cause pulmonary vasodilation .. . . In our initial experiments we used a glass vertical tube and noted that when blood sat in the tube for short times there was a dilator response to blood entering the pulmonary circulation. This was bothersome, since we were not sure of the cause of the dilation. Thus we changed to polyethylene tube and did not see the dilation.

[They also showed the integral role of O_2 in the changes at birth. While ventilation with N_2 resulted in pulmonary vasodilatation, this was much greater with O_2 ventilation] (Cassin et al. 1964a). However, these events clearly stimulated Geoffrey to think about glass and the production of vasodilators. Shortly after I left Oxford in 1963, I wrote to Geoffrey about a former colleague and collaborator . . . at Columbia University [who] was interested in the study of vasodilators. Dr. Dawes invited Anthony Manning Perks [later of the University of British Columbia], to work at the Nuffield in 1964. Subsequently, Dawes, Perks and a new group of visiting scientists were able to demonstrate that kinin formation caused vasodilation in fetal sheep [pulmonary vascular bed] in which flow measurements were made via an extracorporeal loop [with] glass tubes . . . (Campbell et al. 1968). In our experiments, we filled the vertical polyethylene tube with blood, and allowed blood from the tube to flow into the left pulmonary artery while carotid flow was temporarily stopped. This permitted generation of pressure flow curves in which pressure was recorded on the *x* axis and flow on the *y* axis of an XY recorder .. . . The curves were separated into a straight upper portion and a curved small lower portion. Extending the straight upper portion of the conductance curves allowed the estimation of the pressure intercept at zero flow. The conductance are expressed as ml min^{-1} or ml min^{-1} kg^{-1}. A relationship between arterial P_{O2} and P_{CO2} and conductance·kg^{-1} was derived by regression analyses .. . . The results indicated that the more asphyxiated the fetal preparation, the lower the conductance. Also it was established that unventilated fetal sheep had a lower conductance curve than those ventilated with 7% CO_2 in N_2. In fetal sheep at 138 days gestation, we found that ventilation with 7% CO_2 in N_2 had lower conductance curves than those ventilated with air or with N_2 only, but they were still greater than those derived in the fetal state. In additional experiments in this series, we ventilated the fetal right upper lobes of the lungs, while generating conductance curves of the left unventilated lung. Small increases of P_{O2} and decreases in P_{CO2} caused substantial increases in the vascular conductance of the unventilated lung. Similar pressure flow studies we carried out in immature fetal sheep (75–90 days gestation) indicated that pulmonary vascular resistance is elevated in mature fetuses when compared to immature fetuses. [The studies also] demonstrated that acetylcholine (2 μg) as well as isoprenaline (0.2 μg) caused markedly pulmonary vasodilation in the immature fetus (Cassin et al. 1964b).

John "Jack" Thomas Reeves [(1928–2004)] also had the good fortune to work with . . . Dawes . . . in 1967–1968 .. . . The studies demonstrated that in fetal sheep from 0.7 term on exposed to hypoxemia, there was a rise in arterial pressure and femoral vasoconstriction. These effects were eliminated following section of the vagi or aortic nerves, but were unaffected by bilateral section of nerves from the carotid body and sinus (Dawes et al. 1969a). They also demonstrated that sodium cyanide injection into both common carotids simultaneously produced a substantial cardiovascular response. In both cases (aortic and carotid stimulation) there was an increase in arterial pressure (Dawes et al. 1969b). This was thought to increase flow to the placenta and promote transport of oxygen to the fetus. Many of the above described experiments led to further thoughts about the control of pulmonary vascular tone, and the demonstration that other factors such vasoactive substances as well as P_{O2} and P_{CO2} were involved in the regulation of pulmonary vascular resistance.

. . . rhythmical expansion of the lungs with gas mixtures that produced little or no change in arterial blood oxygen or carbon dioxide produced pulmonary vasodilation. As a result experiments we carried out to determine if the changes in pulmonary vascular resistance were dependent on neural supply to the lungs and whether a single brief expansion of the fetal lungs would result in the same decrease in pulmonary resistance as that seen with rhythmical ventilation. It was established . . . that electrical stimulation of the peripheral ends of the vagus on the same side as the pulmonary perfusion resulted in a vasodilation, while sympathetic stimulation resulted in vasoconstriction of near term fetal sheep lungs. This research group also demonstrated that after denervation of unventilated

fetal sheep lungs, acute hypoxia (by occlusion of the umbilical cord) would cause pulmonary vasoconstriction (Colebatch et al. 1965). From these studies it was concluded that regulation of the fetal pulmonary circulation in mature fetal sheep was partly controlled by the nervous system, as well as by a variety of physiological and pharmacological influences, many of which have been studied in detail.

The basic mechanisms by which increased oxygenation or simple expansion of alveoli with gases exert effects on the pulmonary vasculature are still not clearly established. Alterations in PaO_2, or simple dilation of alveoli may activate synthesis and/or release of vasoactive materials such as prostaglandins, thromboxanes, prostacyclin, endothelins and other substances (Cassin 1980, 1990a, b; Tod and Cassin 1997).

(Letter from SC to LDL, 19 September 2009)

At the University of Colorado, "Jack" Reeves, to whom Cassin refers, went on to collaborate with Robert F. Grover, and together they became leaders in cardiovascular physiology, understanding the mechanisms that regulate components of the oxygen transport system from the lungs to the tissues, particularly at high altitude (Moore and Grover 2006; Reeves and Grover 2005).

In considering the contributions of others, Cassin continued:

Attempts have been made to localize the sites in fetal and neonatal lungs for [vascular resistance] responses to ventilation, changes in blood gas tensions and drugs ... The vasoactive substance bradykinin causes a vasoconstriction of pulmonary veins, and vessels proximal to the veins are passively distended in response to an increase in cardiac output (Hyman 1968) .... The Starling resistor model (a collapsible tube through which flow is related to the pressure drop between inflow and surrounding pressure, as long as the surrounding pressure is greater [than] outflow pressure). Raymond Dwight Gilbert applied to experiments on the fetal lung. These studies ... demonstrated ... the average surrounding pressure in the fetal goat lung [to be] about 21 mmHg. Upon ventilation of the lungs with fetal gas (4% O_2 and 6% CO_2 in N_2), this pressure drops to 16 mmHg, whereas when ventilation occurs with air, it ... dropped to about 13 mmHg (Gilbert et al. 1972, 1973). These values are in accord with the shifts in intercepts of the pressure flow curves previously described (Cassin et al. 1964a). The data support the concept of a high critical closing pressure in the fetal lung, with recruitment of vessels accounting for the non linear pressure flow curves and for much of the change in pulmonary flow with initiation of respiration .... Vascular resistance proximal to the Starling Resistor was 4 to 5 times higher ... than the resistance distal to it .... When ventilated with fetal gas [those values fell about one-third] (Gilbert et al. 1972). Still with all these studies the exact site of Starling Resistors in the pulmonary vasculature is unknown. Indirect evidence suggests that they are in both pre and post capillaries .... Injection of bradykinin into the pulmonary artery of the unventilated fetal goat resulted in a significant decrease in [these resistance values] (Gilbert et al. 1973). Experiments involving aerosolization of prostaglandin E_1 in newborn goats suggested that major sites of action and inactivation of prostaglandins are found in the precapillary vessels. If this applies to the unventilated fetus, then perhaps the proximal resistance, which is about 87% of the total resistance across the lungs, is situated on the arterial side of the capillaries. Distal resistance which is about 11% of the total resistance, could be located in the venules. Since PGE_1 presented as an aerosol produces a small decrease in pulmonary vascular resistance, it is probably due to the fact that PGE^1 enters the pulmonary vasculature proximal to the capillaries (Leffler et al. 1977).

(Letter from SC to LDL, 19 September 2009)

Elaborating further on these contributions, Cassin recounted:

Early studies indicated that the fetal arterial carbon dioxide level varied with that of maternal blood and is normally approximately 40 Torr. It was established that changes in pulmonary blood flow following alterations in $PaCO_2$ are large. Unfortunately, in these experiments arterial pH was not maintained at a constant level (Cassin et al. 1964a; Cook et al. 1963). However, Abe Rudolph with Yuan (1966) demonstrated that changes in CO_2 may have been due to changes in pH. In carefully designed experiments in 4 newborn calves they demonstrated that at a pO_2 above 100 Torr, pulmonary vascular resistance increased minimally as pH dropped from 7.39 to 7.18. In contrast when arterial pO_2 was reduced to below 40 Torr, pulmonary vascular resistance increased sharply over the same range of pH values. Gilbert et al. (1972) studied the effects of PaO_2 on calculated proximal pulmonary vascular resistance using the Starling Resistor model and also the relationship of PvO_2 on distal resistance. Rp decreases until a PaO_2 of about 40 Torr and then remains constant. Similarly the distal resistance decreased as the PvO_2 approached 40 Torr and then remained constant (Gilbert et al. 1972).

The fetal pulmonary vasculature is not only reactive to changes in the blood gases but also to many endogenous and exogenous vasoactive substances. Some of these have been described above. Many body tissues synthesize, release, and metabolize prostaglandins. A rather large source of prostaglandin (PG) production is found in the lungs (Pike 1971). [In 1978, Cassin with colleagues demonstrated that indomethacin, a blocker of prostaglandin production, did not alter the postnatal changes in the pulmonary circulation (Leffler et al. 1978). Others also showed that such PG inhibition failed to alter the pulmonary vasodilatation caused by an increase in O_2 (Morin et al. 1988)]. In 1978 Tyler et al. (1978) carried out experiments to evaluate the effects of prostaglandin infusions as well as synthetic analogues of endoperoxide intermediates into the perinatal pulmonary circulation.

Arachidonic acid and dihomo-γ-linolenic acid were used as precursors of mono and bisenoic prostaglandins. Infusion of arachidonic acid into the pulmonary artery of fetal and newborn goats produced an increase in pulmonary vascular resistance and a decrease in systemic arterial pressure. With infusion of dihomo-γ-linolenic acid similar but less marked results were obtained than with the arachidonic acid. Both of these precursors always produced dose dependent increases in pulmonary vascular resistance in fetal and neonatal goats and sheep. These results for arachidonic acid do not agree with responses seen in the adult pulmonary circulation. Thus, while some investigators found that intrapulmonary infusion of arachidonate in dogs (Hyman et al. 1977; Mullane et al. 1979; Wicks et al. 1976), cats (Hyman et al. 1977), and monkeys (Hyman et al. 1977) produced an increase in pulmonary vascular resistance. To complicate matters further, either an increase or decrease could occur in the feline pulmonary circulation following infusion of arachidonate (Hyman et al. 1980). Those investigators noting an increase in pulmonary vascular resistance in adults, suggested that since PGE_2 and PGF_2 increase in pulmonary venous blood, both agents constrict the pulmonary vascular bed. In contrast those investigators noting a decrease in pulmonary vascular resistance following arachidonate have suggested that this is due to an increase in other prostaglandin like materials. PGD_2, PGE_1 and PGE_2 are always pulmonary vasodilators in fetal and neonatal goats and sheep. However, PGF_1- and PGF_2- are always constrictors of the pulmonary circulation in these experimental animals. Thus, the above described effects of arachidonate and dihomo-γ-linolenic acid may be due to a release of the F series prostaglandins.

In terms of leuktrienes, these are produced from the same precursor fatty acids as the prostaglandins, being derived from arachidonic acid via 5^1 lipoxygenase, rather than via cyclooxygenase (Cassin 1990a, b). Saeed and Mitchell (1982) provided suggestive evidence that lipoxygenase activity is present in human fetal lungs at 12–18 weeks of gestation. In addition leukotrienes (LTC4 and LTD4) have been found in lavage fluid of human neonates with persistent pulmonary hypertension (Stenmark et al. 1983). In contrast

fluid from ventilated human neonates without pulmonary hypertension did not have these substances present. In neonatal lambs LTD4 is a powerful constrictor of both pulmonary and systemic circulations (Yokochi et al. 1982).

Gause and coworkers (1988) reported on the effects of LTD4 and the putative [leukotriene] end organ antagonist FPL 57231 on 24 fetal sheep that were delivered by Caesarean section from chloralose anesthetized ewes. At constant pulmonary inflow, bolus injections of LTD4 (0.1–10 μg) resulted in dose dependent increases in pulmonary vascular resistance. FPL 57231 (1.0–10 μg kg^{-1}) blocked the pressor responses to LTD4 and lowered normal pulmonary vascular resistance in a dose dependent fashion. However, FPL 57231 also diminished the pulmonary pressor responses to U46619, a Thromboxane A2 mimic as well as the pressor response to phenylephrine [hydrochloride]. Furthermore the pressor responses to LTD4 were reduced by cyclooxygenase inhibition. In preliminary experiments it was demonstrated that BW 755C (a phenidone derivative which is an inhibitor of both cyclo-oxgenase and 5-lipoxygenase enzymes) did not block the pressor responses to hypoxia. Thus, it appears that FPL 57231 decreases fetal pulmonary vascular resistance by non-specific mechanisms. Also, the action of LTD4 is indirect and by way of the cyclo-oxygenase system. Thus, although exogenous leukotrienes may produce marked pulmonary pressor responses, endogenous leukotrienes are not likely to be responsible for the hypoxic pulmonary pressor responses of fetal lungs or their normally high pulmonary vascular resistance.

Studies concerned with the effect of cyclo-oxygenase inhibition on the pulmonary and systemic circulations were carried out on normoxic and hypoxic premature (0.9 term) and mature (1–14 days of age) goats (Tyler et al. 1975). Cyclo-oxygenase inhibition with either indomethacin or meclofenamate increases the pressor response of the perinatal pulmonary circulation to hypoxic insult. These data were important since indomethacin and melogenamate were used therapeutically to close the ductus arteriosus of preterm infants (Friedman et al. 1976; Heymann et al. 1976) and to prevent premature labor (Wigvist et al. 1975; Zuckerman et al. 1974).

Mechanical stretch or distension of adult lungs may result in their production of prostaglandins of the E series (Edmonds et al. 1969). Hyperventilation of cat lungs results in production and release of PGI_2 (Gryglewski et al. 1978). Thus it appeared to us that the dilator prostaglandins could play a role in the normal decrease of pulmonary vascular resistance during the transition from fetal liquid breathing to air breathing at birth. As a result we investigated the effects of cyclo-oxygenase inhibition with indomethacin on the pulmonary vasodilation that occurs with ventilation at birth. When fetal lungs are ventilated the decrease in pulmonary vascular resistance occurs in two phases: (1) an initial rapid decline that occurs within the first 30 seconds and is not affected by indomethacin, and (2) a slower decline that is diminished by indomethacin and lasts 20–30 min (Cassin 1982). These data support the hypothesis that the dilator substance (PGI_2 and PGE_2) may be involved in the decrease on pulmonary vascular resistance in the transition from fetus to newborn.

(Letter from SC to LDL, 19 September 2009)

Cassin continued, considering the role of endothelium-derived nitric oxide:

One of the most intensively studied mediators in the past 15 years is the endothelium derived relaxing factor, which we now know is nitric oxide (NO) [(Amezcua et al. 1989; Furchgott 1988; Ignarro et al. 1987)]. Synthesis and release of NO can be inhibited by scavengers of NO (hemoglobin and superoxide anion) or inhibitors of guanylate cyclase (methylene blue and LY-83583). Fetal and neonatal isolated pulmonary arteries and veins synthesize NO and this activity increases with postnatal age (Abman et al. 1991; Steinhorn et al. 1993).

(Letter from SC to LDL, 19 September 2009)

It should be noted that inhibition of NO synthesis prevents the postnatal increase in O_2-mediated pulmonary blood flow (Abman et al. 1990). Other studies demonstrated the key role of NO in decreasing pulmonary vascular resistance in response to air (20.9% O_2) ventilation or that with 100% O_2 (Cornfield et al. 1992). Also in the chronically catheterized fetal lamb, O_2-mediated pulmonary vasodilatation was markedly attenuated by blockade of NO synthesis (McQueston et al. 1993; Tiktinsky and Morin 1993). Convincingly, O_2 tension modulates NO production in fetal pulmonary artery endothelial cells (Shaul and Wells 1994). As an aside, NO-induced increases in cyclin guanosine 3′5′-monophosphate-dependent protein kinase phosphorylate, therefore activating the large conductance calcium-sensitive potassium (K_{Ca}) channel resulting in blockade of the L-type Ca^{2+} channel and vasodilatation (Archer et al. 1994; Bolotina et al. 1994). In summary, these studies demonstrate the critical role of NO-mediated in K^{+}_{Ca} channel activation in mediating the pulmonary vasodilatation at birth (Cornfield 2010).

Cassin also considered the clinical implications of these studies.

> Treatment of persistent pulmonary hypertension of the newborn with low dose inhaled nitric oxide has received much attention and appears to be effective treatment of this disease (For instance, see Kinsella et al. 1992, 1993; Roberts et al. 1992). Our group demonstrated that inhaled nitric oxide may also be effective in the treatment of respiratory distress syndrome of prematurity, with improvements of ventilation/perfusion matching and decreased intrapulmonary shunting (Skimming et al. 1995). We also demonstrated that in newborn lambs inhaled nitric oxide can effectively reduce pulmonary hypertension from a variety of causes at doses of 10 to 80 ppm (DeMarco et al. 1996).
>
> (Letter from SC to LDL, 19 September 2009)

Again, this series of studies resulted in several of Dawes' most highly cited papers (Cassin et al. 1964a, b; Colebatch et al. 1965; Dawes 1962c, 1965, 1966a, b; Dawes and Mott 1962). In measurements of blood flow by use of an electromagnetic flowmeter in the perfused near-term fetal lamb lung, in which they injected either acetylcholine (cholinergic agonist) or histamine (vasodilatation and contraction of bronchial smooth muscle), Dawes and Mott established that the high pulmonary vascular resistance was a consequence of inherent vascular tone, rather than by contortion or kinking of the blood vessels. In the unexpanded lung, changing the fetal [HbO_2] by ventilating the ewe with either 100% or 10% O_2 resulted in decreased or increased pulmonary vascular resistance, respectively (Dawes and Mott 1962). Further studies with Cassin of pulmonary arterial pressure versus flow established the dependence of pulmonary vascular resistance on arterial PO_2 and PCO_2 values (flow decreased with low PO_2 and high PCO_2). Lung ventilation with nitrogen resulted in a considerable decrease in pulmonary vascular resistance (Cassin et al. 1964a).

In regard to these studies, Cassin speculated:

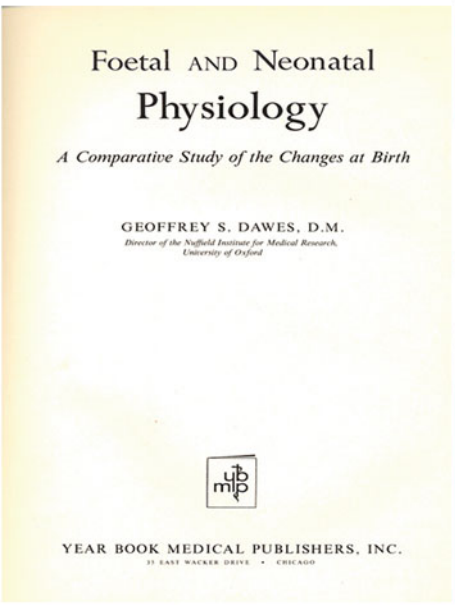
> With the current emphasis on molecular biology and physiological genomics, examining cellular mechanisms of ion channel activity in the developing pulmonary circulation could lead to a better understanding of the regulation of high fetal pulmonary vascular resistance and the events leading to low pulmonary vascular resistance in the postnatal period. Regulation of the pulmonary circulation in the perinatal period involves extensive and complex interactions of anatomic, mechanical, physical, and hormonal factors.

Fig. 6.1 (**a**) Sidney Cassin (1928–2010). (**b**) Geoffrey Dawes; Vicky Arms Pub (Oxford, ca. 1975). (**c**) Title page (1968). (**d**) Geoffrey S. Dawes (ca. 1970)

> Although a number of these factors may contribute to the reduction in pulmonary vascular resistance at birth, a single factor does not seem to be responsible for the high vascular tone seen in the fetus, the maintenance of low vascular tone in the newborn, or the pulmonary vascular response to hypoxia. Basic mechanisms of control of the perinatal circulation continue to be researched actively. Hopefully this work will provide a better understanding of the disorders associated with failure to achieve a dilated pulmonary vasculature at birth as well as rational therapy for this problem.
>
> (Letter from SC to LDL, 19 September 2009)

In 1969, Dawes summarized much of the work of the Nuffield Institute group in regard to the pulmonary circulation of the fetus and newborn (Dawes 1969) (Fig. 6.1).

c



d

Fig. 6.1 (continued)

6.2 Dawes' *Foetal and Neonatal Physiology*

In 1968 Dawes published his magnum opus on fetal and neonatal physiology. Some have wondered regarding the circumstances of this undertaking. As noted earlier, in 1947 Dawes had spent several months at the University of Pennsylvania, at which Julius Hiram Comroe Jr. (1911–1984) chaired the Department of Physiology in the Graduate School of Medicine. As a consequence of their meeting and with mutual interests, in the early 1950s, Dawes and Comroe collaborated on a major review "Chemoreflexes from the heart and lungs." (As an aside, this paper includes essentially nothing on the developmental chemo- or baroreceptor reflexes, Dawes and Comroe 1954.) Following Comroe's move in 1957/1958 to the University of California San Francisco, as first director of the newly established Cardiovascular Research Institute (CVRI), he invited Dawes to spend a sabbatical leave in the Institute to write a book. This Dawes did from April to December 1966. Under the enlightened direction of Comroe, the CVRI included the *crème de la crème* of investigators in cardiac, pulmonary, and vascular biology. Thus, during this sabbatical, Dawes wrote what was to become his classic, *Foetal and Neonatal Physiology* (Dawes 1968). To assist in this endeavor, Comroe provided an editorial staff and medical illustrator. To further his knowledge of contemporary clinical relevance, Dawes attended the weekly UCSF neonatal/perinatal conferences ". . . where he was an imposing presence" (Phibbs 2007, e36). Dawes' resulting volume was

published by Year Book Medical Publishers, the same firm that published Comroe's *Physiology of Respiration* (Comroe 1965).

Later, Dawes recorded that week by week, he wrote the chapters for this monograph, viz., "The Comparative Anatomy of the Placenta," "Oxygen Transfer Across the Placenta," "The Placenta and Foetal Growth," "Maternal Placental and Myometrial Blood Flow," "The Umbilical Circulation," "The Pulmonary Circulation in the Foetus and Newborn," "The Foetal Circulation," "Foetal Blood Gas Tensions and pH," and so forth, 17 chapters in all. An appendix detailed experimental methods such as the management of sheep for experimental studies, methods to improve fetal accessibility, perfusion of the placenta, and the use of electromagnetic flowmeters. Weekly, he would present his latest chapter to Comroe and selected members of his staff for critique. As recounted by Sir Graham "Mont" Liggins (1926–2010), "By the end of the meeting his chapter had been torn to pieces. Lesser men would have been discouraged, but not Dawes, who would return to the next meeting with an edited version and new chapter. The edited chapter satisfied even the sternest critic. The book became the bible of foetal physiologists" (Liggins 1998, p. 118). John Allen Clements, a member of the CVRI faculty, has recalled that during this period,

> He would draft a chapter in his rented apartment in Berkeley, send it to the CVRI, and then meet with Julius [Comroe] and Bill Tooley and me for our critiques. Naturally, it was Julius who had the most useful suggestions. It wasn't all work, though. We also shared dinners at each others' homes, events that allowed Geoffrey's wife Margaret to shine. Although nearly blind from retinitis pigmentosa, she was a brilliantly witty conversationalist. I will never forget her declaiming in that quintessentially English voice and inflection, "Scientists are like dirty little boys, forever peeking under Nature's skirts!"
>
> Back then I found such thoughts hilarious, even exciting, but alas, they're only wistful memories today.
>
> (Letter from JAC to LDL, 1 May 2009)

In the Preface of his work, Dawes observed,

> The Main Theme of this book is the development in the foetus and newborn of the integrated responses which are needed to conserve their energy supply for maintenance of their internal environment, growth and development. For some years it has been apparent that the large and rapidly increasing literature in this field requires a more general treatment than is possible within the scope of a review article. I have attempted to cover the main features of the cardiovascular and respiratory systems, some relevant aspects of energy metabolism, temperature control and growth, and their integration by hormonal and nervous mechanisms. The evidence available is like an unfinished patchwork quilt, derived from many species and incomplete in part. There are other aspects of the physiology of this period of life which have been excluded . . . not because I think they are unimportant, but, because they are peripheral to the main topic.
>
> (Dawes 1968, p. 5)

In his introductory chapter, Dawes stressed the idea that of the many species available for laboratory investigation, each has unique characteristics (such as maternal nutrition, length of gestation, weight at birth, relative organ weights, blood pressure, growth rate, maturity at birth) that present both a challenge and

an opportunity to the investigator attempting to elucidate physiologic principles (Dawes 1968, pp. 13–17).

The work is truly a *tour de force* in synthesizing what was known, as well as defining a number of problems for future study. Dawes is to be credited with quoting widely from the literature and in attempting to unravel controversial issues. For the most part, the studies conducted in fetal sheep and other "models" were performed in acutely anesthetized instrumented animals. Thus, although some of the acute preparation studies remained valid, such as "centralization" of the circulation in response to hypoxia (Campbell et al. 1967a, b) and vascular reflexes (Dawes et al. 1968), others such as those on regulation of specific circulations such as the umbilical (Dawes 1962a, b) required reconsideration. Nonetheless, as noted by one reviewer:

> *Foetal and Neonatal Physiology* is a thoughtful and thought-provoking book by an outstanding investigator in the field. In each chapter the historic background is sketched, experimental work is summarized and evaluated, and, in many instances, clinical experience is integrated with the whole. I am impressed with the author's fair-minded appraisals, not only of others' work, but of his own as well, and with the wealth of alternative interpretations that he finds for observations. The book might have been subtitled "Perspicacity, Perseverance, Pains, and Pitfalls in the Pursuit of Pantology." The pitfalls are given special attention and, with suitable selection and abridgment, another book might emerge: *How Not to Do Research*.
>
> (Chèsley 1968, p. 615)

As the field of fetal and neonatal physiology has matured, Dawes' synthesis has received the encomiums of numerous investigators as a point of reference.

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Chapter 7
Embryology and Early Developmental Physiology

As thou knowest not what is the way of the spirit, nor how the bones do grow in the womb of her that is with child: even so thou knowest not the works of God who maketh all

Ecclesiastes 11:5

I will praise thee; for I am fearfully and wonderfully made: marvelous are thy works . . .

Psalms 139:14

7.1 Origins

One of the wonders of life is the manner in which the single-cell fertilized ovum develops into a sentient human being, with several trillion cells of over 200 individual types each with 10,000 or more proteins and other metabolites interacting in a well-regulated manner. Following ovulation and fertilization, the latter of which normally occurs in the fallopian tube within minutes of ovulation, the zygote (the cell that results from fertilization) divides sequentially to form the morula (a solid ball with 16 cells), then the blastocyst in which a fluid filled cavity forms. At the end of the first week post conception upon reaching the uterus, the blastocyst implants into the endometrium/decidua that lines the uterine cavity. The impetus for implantation is derived from the blastocyst and its metabolic products. The blastocyst invades the uterine decidua and surrounding small blood vessels to obtain essential nutriments to grow. By week three post conception, cell division continues with the inner cell mass giving rise to the embryo. The blastocyst outer cell mass develops into trophoblast cells, the early placenta. Soon the gastrula develops with three layers of cells (germ layers from which body organs and tissues arise). These include the innermost layer, the endoderm, which gives rise to the lungs, digestive organs, and other intra-abdominal viscera; the middle layer, the mesoderm, from which arises the skeleton, muscles, and blood system; and the outer cell layers, or ectoderm, which gives rise to the nervous system, and skin. Recognition of pregnancy by the maternal organism includes a number of processes, including prolongation of the life-span of the ovarian corpus luteum to ensure secretion of progesterone and tolerance by the maternal decidua of the semi-allogenic graft of

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the placenta and fetus. The embryonic period extends until the end of the seventh week of gestation, at which time the major organs have commenced their development. From the eighth week onward, the developing conceptus is referred to as a fetus.

Knowledge of the normal developmental profile of embryo, fetus, and placenta is necessary for understanding many aspects of fetal physiology. By the time of birth, the human newborn will have been transformed from a single cell, 0.1 mm in diameter and weighing $<10^{-9}$ g, to many millions of cells that normally weigh ~3 kg. This miracle of development occurs over a period of 266 days following fertilization (or 280 days, ten lunar months, from the onset of the last menstrual period) (Hamilton and Mossman 1972). The development of the embryo and fetus involves the coordinated regulation of cell division with hyperplasia [increased numbers of cells], hypertrophy [increased cell size], cellular differentiation, and apoptotic cell death, under the orchestrated activity of thousands of genes, growth factors, other proteins, and the temporal influence of many factors that have yet to be discovered. However, details of the many aspects of embryonic/fetal development and its timing have been well described (Benirschke 1993; Hamilton and Mossman 1972; Hamilton et al. 1945; O'Rahilly and Müller 1992). In addition, several reviews of the history of embryology are available (Bodemer 1971, 1973; De Witt 1959; Herrlinger 1951; Horder et al. 1986; Kleiss 1964; Meyer 1939; Needham 1935; Oppenheimer 1967; O'Rahilly 1988). A critical aspect of this developmental regulation are the cellular and molecular signal transduction mechanisms by which determining factors, from the fertilized ovum and embryonic stem cells, result in the generation of different cell types, and, ultimately, their morphology, spatial organization, and function are realized. Not unexpectedly, there is some controversy regarding the degree to which we are approaching an understanding in depth of these mechanisms (Wolpert 1994).

The origins of fetal and neonatal physiology are recognized in early studies of fertilization, embryology, and development. Embryology [Greek for inward folding], the science that describes formation of the embryo, has been of interest for several millennia. Perhaps based, in part, from the study of bird embryos, ancient investigators recognized the correct function of the placenta and umbilical cord. For example, Aristotle (384–322 BCE), the first observational biologist and onetime tutor to Alexander the Great (356–323 BCE), in his great embryological treatises *De generatione animalium* [On the generation of animals] and *De animalibus* [of animals], wrote at length on generation and classified animals on the basis of their embryological characteristics. Included in this first work is the report of his examination of the developing chick on successive days of embryogenesis. Aristotle noted that the umbilical vessels join the placenta and interior uterine wall in a manner similar to the roots of plants, and through them the embryo receives its nourishment (Aristotle 1831–1870). Similarities in early development of birds and mammals were also recognized. Along with the development of practical methods to incubate fertilized chicken eggs, this revelation led to the use of the developing chick as an important vertebrate model organism for embryological research from that time forward (Meyer 1939).

That obstetrical research had its origins in embryology and may have stemmed, in part, from the development of a system for artificial incubation of bird eggs as early as 3000 BCE by the Egyptians and probably also by the Chinese. This original "biotechnology" provided not only an abundant supply of poultry for the table but also a ready source of embryological material for investigation. As Joseph Needham (1900–1995) so clearly pointed out, "... even at the most remote times children were being born, and, though the practitioners of ancient folk-medicine might confine their ideas for the most part to simple obstetrics, they could hardly avoid some slight speculation on the growth and formations of the embryo" (Needham 1934, p. 1). This speculation included the placenta, which was "an easily observed biological phenomenon," and "was regarded as of great importance," since it was thought to "be the [especial] seat of the external soul" (Needham 1934, p. 4).

It is of interest to speculate on embryologic research in ancient times. For example, Rabbinic legend holds that Cleopatra VI (69–30 BCE) "... investigated the process of foetal development by the dissection of slaves at known intervals of time from conception, following the precepts of Hippocrates (460–375 BCE) with regard to hen's eggs" (Needham 1934, p. 47). Although this story may be apocryphal, Cleopatra's Alexandria experiments were often cited by ancient investigators as evidence that sexual differentiation of the male fetus was "complete" about 8 weeks before that of the female fetus. It seems difficult to explain such detailed knowledge of human sexual development unless investigations such as those attributed to Cleopatra had actually been performed. Ancient Indian texts (ca. the sixth century BCE) also demonstrate a relatively detailed knowledge of human embryology, and knowledge in this area as "... likely to have passed in one direction to the other [i.e., between India and the Mediterranean]" (Needham 1934, p. 2).

Beginning in the Renaissance, a number of anatomists gave quite accurate accounts of morphologic aspects of fetal development. For instance, the artist, engineer, and polymath, Leonardo da Vinci (1452–1519) contributed to the embryology of mammals and birds (da Vinci 1913), although his early sixteenth-century drawings did not come to public knowledge until two and a half centuries after his death (O'Malley and de Saunders 1952). Giacomo Berengario da Carpi (ca. 1460–1530), Professor of Surgery at the University of Bologna, advocated the study of fetal development as the tissues are simpler and less well developed than in the adult and in some instances only vestigial in the adult. Andreas Vesalius (1514–1564), the founder of modern anatomy, in the second edition of the *Fabrica*... (Vesalius 1555) differentiated the discoidal placental of man, from the annular placenta in the dog, and the cotyledonous placenta of ruminants. A pupil of Vesalius and discoverer of the pulmonary circulation, Matteo Realdo Colombo (1510–1559) of the Universities of Padua, Pisa, and then Rome in his *De re anatomica*... carefully described the placenta which he first named (Colombo 1559). Gabriele Falloppio (1523–1562) in his *Observationes anatomica*, [anatomical observations] of 1561, noted that the human fetus has a single umbilical vein, in contrast to two in ruminants (Falloppio 1561). Giulio Cesare Aranzi [Arantius] in his *De humano foetu* was the first to maintain the independent and separate

placental maternal and fetal circulations and that organ's ability to purify fetal blood (Aranzi 1564).

Volcher Coiter (1534–1576) in his *Externarum et internarum*. . . [of that that is outside and inside]. . . described development of the chick embryo on 20 successive days (Coïter 1572). Several other investigators of this time detailed aspects of embryological development. For instance, Girolamo Fabrizio (Fabricius de Aquapendente; c. 1533–1619) in his *De formato foetu* (Fabrizio 1604) and *De formatione ovi et pulli* (Fabrizio 1621) and Adrian van der Spieghel (1578–1625) (Spieghel 1626) illustrated their development in a classic manner.

It was Fabricius who first applied the Vesalian method of direct observation to the study of the embryo. With seven full-page plates, he presented the earliest printed figures of the development of the chick. He was the first to establish with any degree of accuracy the role played by the ovary and oviduct in the formation of the hen's egg. He also was the first to describe the germinal disk distinctly. *De formation ovi et pulli* . . . [The formation of the egg and chick] is divided into two parts. The first, in three chapters, deals with the formation of the hen's egg. The first chapter discusses the three bases of animal generation given by Aristotle, the egg, the seed, and the spontaneous generation from decomposing material. In the second chapter, Fabricius describes two functions of the "uterus": the formation of the egg and its subsequent nutrition. The third chapter presents in more detail these functions. The second part of the treatise, also in three chapters, deals with the generation of the chick within the egg and begins with a description of the eggs of various species. The second chapter deals with the three basic functions of the egg: the formation, growth, and nutrition of the chick. He concludes his discussion with the trophic functions of both yolk and albumen. Fabricius then speculates on the various possible causes and conditions on generation, including a discussion of the order in which various parts of the embryo are formed during its development. The last chapter of the treatise returns to teleology to consider the utility of both the egg and the semen of the rooster. In this work, which was published posthumously, Fabricius made several erroneous assumptions, including that for impregnation, the sperm did not enter the ovum but rather stimulated conception from a distance.

At this time, unable to explain how unique organs as the brain, heart, and uterus could have arisen from a single cell, the idea of "preformation" (also called "predelineation" and "predetermination"; Needham 1934, p. 213) held sway. According to this hypothesis, development occurred as a consequence of unfolding and increase in size of organisms that preexisted within germ cells, i.e., that all parts of the embryo and future organism exist completely formed in the germ cell and develop only by increasing in size. The idea was held that sperm contained a miniature human being. An illustration of this view is the 1694 illustration by Nicolas Hartsoeker (1656–1725) in his *Essay de dioptrique*, of a miniature human within a sperm, a so-called *homunculus* [diminutive of man] (Hartsoeker 1694; Fig. 7.1). Some even claimed that the sperm of different species resembled the animal's from which they came. This contrasts with the concept of epigenesis, i.e., that an individual develops by structural elaboration from an unstructured egg rather than by simple enlargement of a preformed entity. This topic of disagreement

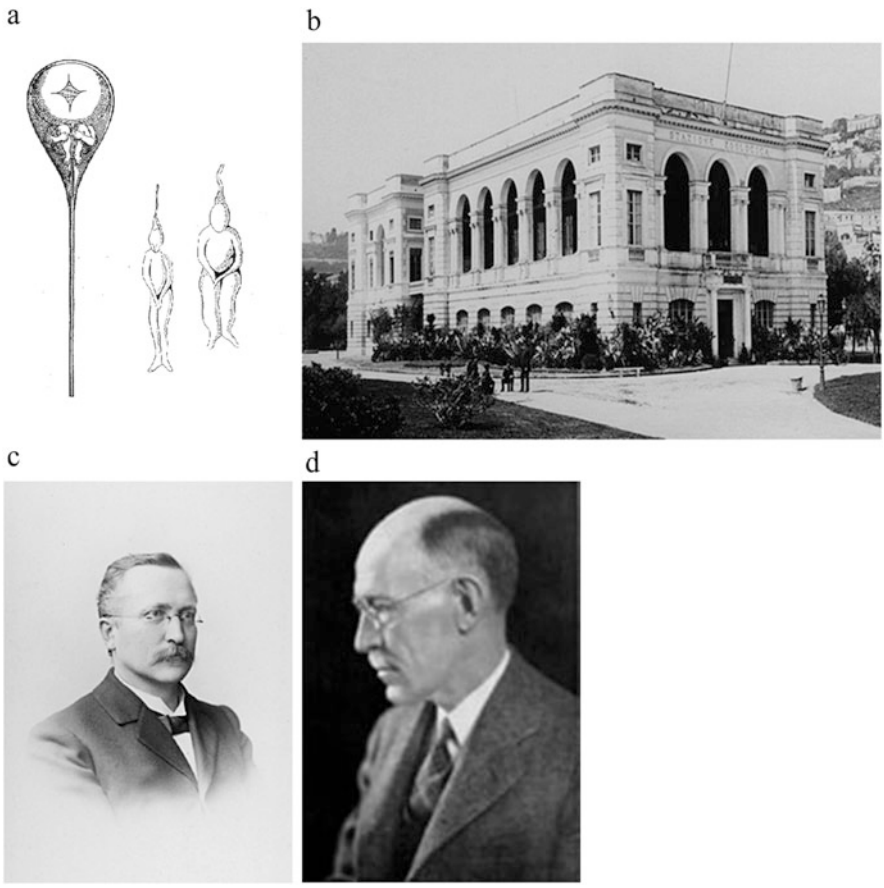

Fig. 7.1 (**a**) Homonculus (Hartsoeker 1694). (**b**) Stazione Zoologica di Napoli (ca. 1880). (**c**) Wilhelm Roux (1850–1924). (**d**) Ross Granville Harrison (1870–1959)

is far beyond the scope of the present essay (for an extended discussion, see Needham 1934).

William Harvey's fundamental treatise on the generation of animals and embryology (Harvey 1651, 1653) is considered the most important book on the subject to appear during the seventeenth century (Norman 1991). He rejected the prevailing doctrine of the preformation of the fetus. Rather, he advanced the theory, radical for its time, of epigenesist (*per epigenesin*), that all living beings derive from the ovum "by the gradual building up and aggregation of its parts." Regarding Harvey's theory of epigenesis, Thomas Henry Huxley (1825–1895) claimed this should give him an even greater claim to the veneration of posterity than his better known discovery of the circulation of the blood (Keynes 1953). Harvey reported a wealth of observations on many aspects of reproduction in a wide variety of species. As representatives of vivipara, he focused his attention chiefly on the deer,

Fig. 7.2 (**a**) Charles Sedgwick Minot (1852–1914). (**b**) Thomas Hunt Morgan (1866–1945). (**c**) Hans Spemann (1869–1941). (**d**) Hermann Joseph Muller (1890–1967)

while that for ovipara was the domestic fowl. For Harvey, all life develops from the egg. This is expressed on the frontispiece which depicts the supreme Roman god Jupiter [Jove] opening a large egg, inscribed with the fundamental dictum of embryology, *ex* (upper half of egg shell), *ovo omnia* (lower half of egg shell), which translates as, "from the egg everything" and from which a number of liberated animals and insects fly (Fig. 7.2). In addition to a small human figure, these include a bird, stag, fish, lizard, snake, grasshopper, butterfly, and spider. Although the phrase *omne vivum ex ovo* [all life originates from the egg] is often attributed to Harvey, he does not state this explicitly in the text (Harvey 1651, 1653).

An opponent of the theory of spontaneous generation, Harvey speculated that humans and other mammals must reproduce through the joining of an egg and sperm. No other theory was credible. By positing and demonstrating for viviparous animals the same mechanism of reproduction as that observed in oviparous animals, he thus initiated the search for the mammalian ovum, which was not discovered until 1827 by Carl Ernst von Baer (1792–1876; von Baer 1827). Harvey maintained that *Jovis omnia plena* [All things are full of *Deity*], so that in the chicken and all its functions and actions the *Digitus Dei* [the Finger of God] or the "God of nature, reveals himself" (Harvey 1653, p. 310).

Nathaniel Highmore of Oxford University collaborated with Harvey in using an early microscope to detail many features of embryonic development (Highmore 1651a, b). Walter Needham (ca. 1631–1691), a Cambridge University medical graduate who studied anatomy at Oxford, reported the first measurements of chemical constituents of the chick embryo, as well as several mammalian species, and gave detailed instructions on dissection techniques (Needham 1667). His volume is divided into seven chapters. In the first, Needham maintained that the uterine arteries must supply nutrients to the uterus and developing fetus. He also refuted the idea that "uterine milk" is identical with lymph. In Chap. 2, Needham considered the anatomy of the placenta, its comparative anatomy among species, and its function. By careful dissection, he demonstrated the *chorion frondosum* [leafy membrane] from *chorion leave* [smooth part of a disappearing chorion membrane] and confirmed that the placenta consists of separate maternal and fetal portions. Importantly, he supported Harvey's doctrine regarding separate maternal and fetal placental circulations. He also upheld Harvey's concept that the umbilical veins convey "nourishing juice" to the fetus for its nutrition. Chapter 3, "the membranes and humours" presents a comparative description of the amniotic and allantoic membranes and their fluid contents. On the basis of his studies, Needham stated, "these liquors … proceed from the blood and seem similar to its serum but they are different from it. For when fire is applied to them in an evaporating basin … they do not coagulate, as the blood-serum always does … In the same way humors differ from themselves before and after digestion, filtration, and the other operations … of nature. All, when distilled, give over a soft and clear water … very like distilled milk" (p. 49ff). Needham also described small solid bodies in the allantoic and amniotic fluid. Chapter 4 is devoted to the vessels of the umbilical cord. In Chap. 5, Needham discusses the *foramen ovale* and the fetal circulation. In Chap. 6, he argues (incorrectly) against the idea of the lungs as an organ of respiration, stating rather that its function is to "comminute the blood and so render it fit for a due circulation." The seventh chapter presents instructions for dissection of the embryo and fetus of various species.

Another original Fellows of the Royal Society, the London physician William Croone (1633–1684), explored generation in the chick. A preformationist and ovist, who believed that a microscopic fetus existed in the egg, Croone claimed to have found rudiments of the chick prior to the egg's incubation (Cole 1944). Another of the preformationists, and with his microscopic studies one who contributed greatly to the development of embryology as a science, was Marcello Malpighi of the

University of Bologna (and several other institutions) who presented the first accurate microscopic description of the chick embryo in which he described the cardiac tube, aortic arches, neural tube, and others (Malpighi 1673a, b). In addition, his seminal contribution *De pulmonibus* [the lungs] (Malpighi 1661), in support of Harvey's thesis on blood circulation, described the capillaries and structure of alveoli in the frog as a closed hydraulic system from arteries to veins. Malpighi also described many aspects of neuroanatomy, sensory receptors, and hematology (Belloni 1974). In his five-volume *arbeit*, Howard Bernhardt Adelmann (1898–1988) provided the first English translation of Malpighi's major works, as well as an authoritative treatise on the development of field of embryology (Adelmann 1966).

It was in the late seventeenth and eighteenth centuries, in the spirit of the new age in science, that embryology became an experimental discipline. During the seventeenth century, many still believed that living organisms, including animals, could arise from spontaneous generation. An alternate theory was the Aristotelian doctrine of the egg being formed in the uterus as a result of activation of the menstrual blood by the male semen, with subsequent differentiation into the embryo and fetus (for a detailed review, see Needham 1934). It was at this time that the idea gained credence that the female testes, as the ovary of birds, were the site of egg formation. Regnier de Graaf (1641–1673), a practicing physician in Delft University of Technology, in his *De mulierum* . . . first thoroughly described the mammalian female gonad and established that this organ produces the ovum (de Graaf 1672). On the basis of a comparative study of the ovaries of mammals and birds, De Graaf concluded that the cell-like protuberances, which had been observed by Vesalius and Falloppio in the ovary of mammals, correspond to the egg of the bird's ovary and that the process of fertilization is similar in every animal. Just as the bird's fertilized egg acquires albumin and a shell, the egg of a mammal becomes fertilized in the fallopian tube, traverses to the uterus, and there develops into the embryo and fetus.

De Graaf assumed incorrectly that the entire follicle was the ovum, an understandable error in this era when the microscope was used only in a limited fashion. Besides describing the follicle, which several authors had noted previously, he described for the first time the corpus luteum. He thus rejected the Aristotelian assertion that the embryo originates solely in the male semen. De Graaf concluded, "Thus, the general function of the female testicles is to generate the ova, to nourish them, and to bring them to maturity, so that they serve the same purpose in the woman as the ovaries of birds. Hence, they should rather be called ovaries than testes . . ., many have considered these bodies useless, but this is incorrect, because they are indispensable for reproduction." De Graaf credited Johannes Van Horne (1621–1670) of Leiden who, with Jan Swammerdam (1637–1680) also of the University of Leiden and Niels Stensen (1638–1686) from Copenhagen, independently developed this hypothesis. The same year, Swammerdam by use of advances in [wax] injection technique (Winsor 1976) reported similar findings (Swammerdam 1672). Several decades later, Antonie van Leeuwenhoek

(1632–1723) of Delft, the Netherlands, described the spermatozoa of humans and other species, among his many other discoveries (van Leeuwenhoek 1693–1718).

As the field of embryological research matured, however, the concept of epigenesis (which originally had been advanced by Aristotle) with gradual and progressive development from the fertilized egg containing the total pattern of the unborn individual into specific organ structures was accepted slowly. Working at the newly established University of Göttingen, Albrecht von Haller (1708–1777) demonstrated in both the chick embryo and human the rate of embryonic and fetal growth, with the rate early in development being much greater than that near term (von Haller 1758). Also at this time, in detailed studies of embryonic differentiation, Caspar Friedrich Wolff (1733–1794) demonstrated several organs developing in "leaf-like" blastodermic layers, thus laying the foundation for the "germ-layer" theory of Heinrich Christian Pander (1794–1865) (Pander 1817) and Carl Ernst von Baer (1827, 1828–1888), to dispose of the concept of "preformation" (Wolff 1759). Also, William Hunter (1718–1783) in his *Gravid uterus* (Hunter 1774), one of the most spectacular anatomical atlases ever published, presented a number of illustrations of the fetus and placenta. Samuel Thomas von Soemmerring (1755–1830) in his *Icones embryonum* (Soemmerring 1799) included splendid engravings of late embryos and early fetuses. In 1778, on a visit to London, Soemmerring met and was inspired by the anatomist-obstetrician Hunter. After seeing the latter's classic work *Anatomia uteri humani gravidi* … (1774), Soemmerring decided to prepare a supplementary volume with illustrations depicting the embryo and fetus during the first half of gestation. In this work based on embryos, most of which were spontaneous abortions, he attempted to convey a vision of true development in addition to that of growth per se (Hopwood 2000). In conjunction with another of his works, Soemmerring noted that his goal was "to select only that which proved to be the most excellent or most perfect specimen among many, in other words, the anatomic norm …" (Choulant 1920, p. 302). Typical of those who prepared such atlases, he sought to depict "types" rather than individuals. In the Preface, Soemmerring reviewed the major works in embryology from that of Fabricius of 1604 and 1612, to his own time. The illustrations were by Christian Köck (1758–1818), whom Soemmerring trained specifically for this atlas and later works.

Many other examples could be cited (Eskes and Longo 1993; Longo and Reynolds 2016). Early in the nineteenth century, von Baer reported his monumental discovery of the mammalian ovum (von Baer 1827), and Johann August Heinrich Nicolai (1796–1882) described development of the skeleton, noting that the femur, which showed no ossification centers in the second month, progressed in length to the newborn period (Nicolai 1829). As noted, his measurements are remarkably close to those determined by ultrasonography in the present day (O'Rahilly 1988).

During the latter part of the nineteenth and early twentieth centuries, biology was transformed from a descriptive accounting of natural history to a science dominated by rigorous, quantitative experimental analysis. In a sense this was initiated by the discovery that individual cells constitute a vital basis for life (Schwann 1838) and by Claude Bernard, who formulated the scientific method of investigation. He also compared glycogen metabolism in the fetal and adult liver

(Bernard 1865). In addition, Hermann Vierordt (1853–1943) of the University of Tubingen, in his anatomical, physiological, and physical data and tables, compiled an encyclopedic compendium on anatomical characteristics, physiologic variables with composition of various organs and tissues, and physical characteristics of the human at different ages (Vierordt 1888). Up to this time, fertilization was poorly understood, and many of the phenomena of reproduction and embryology including chromosomes, mitosis, and other features had not yet been discovered. Jean Louis Prévost (1790–1850) and Jean Baptiste Andre Dumas (1800–1884) in the frog first discovered that fertilization occurs by union of spermatozoa and ovum (Prévost and Dumas 1824), while Rudolph Albert von Kölliker (1817–1905) demonstrated the cellular origin of spermatozoa (Kölliker 1841). Martin Barry (1802–1855) of Edinburgh observed the spermatozoa within an ovum (Barry 1843), and Wilhelm August Oscar Hertwig (1849–1922) demonstrated not only that the spermatozoa enters the ovum but that fertilization occurs by union of the nuclei of the female and male sex cells (Hertwig 1876). Two decades later, Robert Heinrich Johannes Sobotta (1869–1945) detailed remarkably accurate illustrations of the steps involved in the generation of the mouse embryo, commencing with the first divisions of the oocyte and ending with the first mitotic divisions of the embryo (Sobotta 1895). Some of the earliest volumes devoted to embryologic development appeared during this time (e.g., Kölliker 1861; Valentin 1839; see Hintzsche 1973).

In the scientific revolution of the late nineteenth and early twentieth centuries, embryology was at the frontier of biological science. Critical to our understanding of the development of embryology and its physiology is the growth in our knowledge of genetics. These advances proceeded in several stages. Probably the earliest of these was a concept of the German evolutionary biologist Friedrich Leopold August Weismann (1834–1914), a graduate in medicine from Gottingen, who spent most of his professional career at the Albert Ludwig University of Freiburg in Breisgau. Weismann argued that two independent processes or cell types commence with division of the fertilized egg. One type leads to formation of "soma" or cells of the body, the other type constitutes cells of the "germ line," and the egg and sperm are essential for initiation of the next generation. Union of these parent germ cells, he called *amphimixis*, which in his mind led not only to the development of a new being but constituted the principal source of heritable variation in evolution by natural selection (Weismann 1892a). The idea that genetic information cannot pass from "soma" to "germ cell" and on to the next generation became known as the "Weismann barrier" (Weismann 1892c).

In addition, Weismann performed experiments that negated the concept of the French biologist and comparative anatomist Jean-Baptiste Pierre Antoine de Monet, Chevalier de Lamarck (1774–1829) that organisms may inherit traits acquired during their parent's lifetime (Lamarck 1815–1822). For instance, to counter the Lamarckian proposition that a giraffe's neck elongated as it ate from the highest leaves on a tree, and this change would persist in the next generation, Weismann repeatedly chopped off the tails of 1500 rats over twenty generations. Among the over 900 offspring, no rat was born without a tail (Weismann 1892b). In its essence, Weismann's "germ plasm theory" stated that multicellular organisms

consist of germ cells that contain heritable information, while somatic cells contain the data to conduct the functions of their particular cell type. Weismann's work established that heredity is dependent, not on a flow of matter or of energy but of information.

7.2 Stazione Zoologica di Napoli

As noted earlier, in the late nineteenth century, the German universities were preeminent in the support of scholarship in science, medicine, and many other fields. In the scientific culture of this era, the sea was regarded as the origin of the most elemental forms of life and as a valuable source in the endless search for knowledge. Marine organisms became a center of interest for naturalist followers of *Naturphilosophie* [natural philosophy], many of whom searched the oceans for the *Urschleim* [elementary living matter]. The new generation of biologists immersed in this philosophy looked to the sea as a source of knowledge regarding fundamental biological problems. In regard to the development of modern-day embryology, a major factor was the establishment of, and contributions by, investigators at the *Stazione Zoologica di Napoli* (SZN) in 1873–1874 (Fig. 7.1b). Following the Italian *Risorgimento* [resurgence, reorganization] with the unification and creation of the Italian state in the late 1850s–1860s, the spirit of nationalism and return to *Principi fondamentali* [fundamental principles] extended to support the arts and sciences.

At this time, biology was searching for general laws, with attempts to synthesize embryology, phylogeny, and comparative anatomy within the Darwinian paradigm of epistemology and natural selection. Thus, the last several decades of the nineteenth and the first few decades of the twentieth century were the "golden age" of descriptive and comparative embryology. The founder of the *Stazione* in 1872, Anton Dohrn (1840–1909), originally from Pomerania (present day Poland), had trained in Jena with the morphologist Ernst Heinrich Philipp August Haeckel (1834–1919), German Darwinist and author of the proposed biogenic law "Ontogeny recapitulates phylogeny" (Haeckel 1866, 1868). Based on Haeckel's theory of recapitulation, comparative embryology was becoming the cornerstone of morphology. As an aside, Haeckel's diagrams that alleged to compare the embryos of different species and similarities among embryos of higher and lower vertebrate species, including human, became enmeshed in controversy, with questions of visual representation, forgery, scientific fraud, and the interrelations of science and religion (Hopwood 2015). With appreciation for the rich biological diversity of the Bay of Naples, and with his family resources, in 1870 Dohrn commenced building the *Stazione Zoologica*, *Naples Italy* (*SZN*), at his own expense. Presently the center is known as the *Stazione Zoologica of Anton Dohrn*, and its focus is on the biology of marine organisms (Fig. 7.1b).

The importance of the *SZN* extended far beyond its purely scientific contributions. Renowned for its support of the arts and humanistic values, for zoologists and other natural scientists, the "Naples experience" consisted of a mixture of scientific

investigation, the acquisition of state-of-the-art methodologies, and cultural enrichment. To establish this as a unique and world-renowned resource, Dohrn made available the finest scientific instruments, equipment, and core laboratory facilities. To promote its international status, and to help ensure its financial stability, Dohrn inaugurated innovations such as a "table or bench" system in which investigators/institutions rented space for research. Each of the 30 or so individual tables had an accompanying set of cupboards and shelves for chemical reagents, histologic stains, and related materials. In addition, the *SZN* employed a technical staff who not only obtained marine specimens for the investigators but also prepared and supplied such specimens to various universities, museums, and other academic institutions. Additionally, Dohrn included in the *SZN* a large aquarium open to the public and founded several scientific journals. In contrast to most universities, the *SZN* guaranteed independence of scientific pursuits, freedom from teaching, and the opportunity to interact with investigators from all over the world. In its first three decades of existence, more than 2000 investigators from around the globe worked at the *Stazione*. Several writers have presented an analysis of the *SZN*, its origin, its role in biological research and the development of embryology, and its continued role in science, art, and culture to the end of the twentieth century (Fantini 2000; Monroy and Groeben 1985; Morgan 1896; Müller 1996; Whitman 1883).

One of the early major discoveries at the *SZN* was that by Wilhelm August Oscar Hertwig (1849–1922), who in the sea urchin egg established that fertilization requires union of the nuclei of the male and female sex cells (Hertwig 1876). Because the sea urchin sheds large translucent ova which can be maintained in fresh sea water, this led to a number of vital studies in fertilization and early embryonic development. Among the many scientists who worked at the *SZN*, and who were stimulated by Hertwig, was Otto Heinrich Warburg (1883–1970) of Heidelberg and Berlin. Between 1908 and 1914, Warburg used his "Warburg apparatus" (a modification on the Haldane-Barcroft blood gas manometer) to study metabolism in the eggs of the sea urchin. Importantly, he discovered a major increase in oxygen consumption following fertilization and during cell division (Warburg 1908). Warburg also discovered the essential role of an iron containing pigment in "cell respiration," a term he coined, which led him to discover the function of a respiratory enzyme (Warburg and Negelein 1929). Later, this was shown by David Keilin (1887–1963) to be *cytochrome oxidase* (Keilin 1929). Warburg also described anaerobic glycolytic metabolism, as occurs in many cancer cells (Warburg et al. 1924). In recognition of his numerous contributions, Warburg was awarded the 1931 Nobel Prize in Physiology or Medicine.

Among the early American biologists to work at the *SZN* was Charles Otis Whitman (1842–1910), who became interested in the "program of development" and performed classic studies in establishing the analysis of cell lineage. These studies contributed additional evidence to support the concept of "epigenesis" and lay to rest that of "preformation" (Whitman 1878, 1894). Of note, upon returning to the USA, as curator of the Zoological Museum of the University of Chicago, Whitman played a major role in establishing in 1888 the Marine Biological

Laboratory at Woods Hole, MA. Here he became its founding director, continuing for two decades (to 1908) and enlarging his leadership in embryological research (Maienschein 1978; Monroy and Groeben 1985; Whitman 1883). Whitman was a member of the National Academy of Sciences, the Linnean Society of London, and other prestigious societies (Morse 1912).

Another leader who worked at the *Stazione* was Edmund Beecher Wilson (1856–1939), of Columbia University, New York. Credited as being one of the founders of the fields of cell biology and cytogenesis, Wilson stressed the importance of cytological structure and the central role of the nucleus in progressive cell development (Morgan 1942). In his studies of experimental embryology and heredity, he was consumed by the problem of how an entire individual may lie implicit in a single cell, and he explored factors that result in cell segregation into tissues and organs with their functional specialization. In his study of the cell lineage of the marine *polychaete*, *Nereis*, Wilson traced egg cleavage step-by-step through the formation of germ layers in the principal embryonic organs (Wilson 1892). His work also established the idea that the mosaic-like character of ontogeny emerges at early stages at different periods in the several species (Wilson 1894), and with fate maps he followed embryonic cell-by-cell development to establish the normal morphogenetic processes from fertilization onward (Wilson 1904). In his *An atlas of the fertilization and karyokinesis of the ovum*, Wilson was the first to produce clear photographic illustrations of the early history of the fertilized ovum, that of eggs of the sea urchin *Toxopneustes variegates* enlarged about 1000 diameters (Wilson 1895). He obtained recognition for his *The Cell in Development and Inheritance* (Wilson 1896), which, with critical observations and analysis, laid the foundation for the study of the cell and understanding its structure and functions. Another of his monographs which became a classic was *The Cell in Development and Heredity* (Wilson 1925). Wilson made a number of other lasting contributions to both embryology and genetics. For instance, he promoted the idea that the nucleus contains the basis of inheritance, with chromatin as a key element of information and with chromosomes containing the "factors" or "genes." He also discovered the X-Y chromosome system of sex determination (Wilson 1905, 1906, 1914), detailing aspects of gene activation (Lyon 1961), which still are under investigation. Another of the *SZN* investigators who, by their study of chromosomal preparations from sea urchins and other creatures, contributed greatly to this field was Walter Stanborough Sutton (1877–1916), who advanced the idea that "hereditary particles" responsible for Mendelian inheritance were carried by the chromosomes (Sutton 1903).

In 1900, Gregor Johann Mendel's (1822–1884) laws of segregation and of independent assortment or "ratios" in genetic inheritance (Mendel 1886) were rediscovered independently by Carl Franz Joseph Erich Correns (1864–1933) (Correns 1900), Hugo Marie de Vries (1848–1935) (de Vries 1900a, b), and Erich Tschermak von Seysenegg (1871–1962) (Tschermak von Seysenegg 1900). As an aside, but relevant, de Vries first proposed a theory of mutation (de Vries 1901–1903). Mendel, an Austrian monk and head of a monastery in Moravia (former province of central Czechoslovakia), had trained in the physical sciences. In

addition to his work with peas and their physical characteristics, Mendel studied characteristics in several generations of mice, concluding that they occur in fixed ratios. This discovery was critical in helping to illuminate the fundamental discoveries and concepts of Weismann, Wilson, and others on the basis of inheritance and served as a foundation for modern genetics.

In the 1880s, Wilhelm Roux (1850–1924) (Fig. 7.1c), a pioneer experimental embryologist in Breslau (now Wroclaw) and later the University of Halle, by interfering with developing blastocysts from frog eggs, developed the idea of *Entwicklungsmechanik* [developmental mechanics] and raised questions with respect to the developmental forces responsible for determining the planes of symmetry and what after would be called functional adaptation (Roux 1888). Roux also first established principles and techniques of tissue culture. Although some of his ideas of the "mosaic" theory of embryonic development later were refuted (Hamburger 1997), his demonstration that the nucleus of each blastomere is capable of directing a specific independent line of differentiation and that the nucleus contains hereditary particles remains valid (Oppenheimer 1967). Roux raised a number of important questions regarding embryonic development and played a key role in moving embryology from a descriptive science to one of experimentation. Also in this era, the Johns Hopkins embryologist William Keith Brooks (1848–1908) was exploring fundamental questions regarding embryonic development. In a two-part *Science* essay, he explored a number of questions including that of, "Is cell-differentiation inherent or induced?" (Brooks 1902a, b).

> A thoughtful and distinguished naturalist tells us that while the differentiation of the cells which arise from the egg is sometimes inherent in the egg, and sometimes induced by the conditions of development, it is more commonly mixed; but may it not be the mind of the embryologist, and not the natural world, that is mixed? Science does not deal in compromises, but in discoveries. When we say the development of the egg is inherent, must we not also say what are the relations with reference to which it is inherent? When we say it is induced, must we not also say what are the relations with reference to which it is induced? Is there any way to find this out except scientific discovery?
>
> (Brooks 1902b, pp. 490–491)

One of Brooks' graduate students, Ross Granville Harrison (1870–1959) (Fig. 7.1d), went on to a distinguished career at Yale, pursuing developmental questions by definitive experiments. "Harrison's gift was an ability both to frame the questions and to devise ways and means of enticing the embryo to answer them unequivocally" (Oppenheimer 1967, p. 93). Performing much of his early work in Bonn, Germany, then the intellectual mecca for experimental embryology, Harrison advanced techniques for tissue culture. By grafting frog embryos, he created chimeras to explore questions relating to the factors responsible for establishing bilateral symmetry in development (Harrison 1907). Among his many contributions, Harrison also helped to establish the mechanisms by which nerve fibers develop (Harrison 1907, 1910). A recipient of numerous honorary degrees and other awards, he was a member of the National Academy of Sciences and a foreign member of the Royal Society. Following retirement, he chaired the National Research Council (1938–1946), serving as an advisor to the government on many issues (Abercrombie 1961; Nicholas 1960; Oppenheimer 1967).

It was during this period that a Danish plant physiologist and one of the founders of genetics, Wilhelm Ludvig Johannsen (1857–1927) of the University of Copenhagen, coined the term "gene" as a unit of heredity. With the use of quantitative accounting, Johannsen also made a fundamental contribution to biology when he distinguished clearly between the view of the hereditary constitution of an organism and the totality of its genes, the "genotype," and its appearance and function, the "phenotype" (Churchill 1974; Falk 1984). In inbred "pure lines" of peas, beans, and other plants, he also demonstrated a normal distribution of pod sizes, e.g., the existence of two types of variability, that which was heritable (genetic) and nonheritable (now classed as epigenetic) (Johannsen 1905, 1909). His underappreciated conclusion was that identical genotypes do not necessarily produce identical phenotypes. Johannsen has been viewed "...as a bridge over which nineteenth century ideas of heredity ... passed to be incorporated ... into modern genetics ..." (Dunn 1973, p. 115).

Prior to his monumental studies in the fruit fly *Drosophila melanogaster* [Greek for the black-bellied dew lover] at the turn of the century, Wilson's Columbia University colleague, the experimental zoologist and embryologist Thomas Hunt Morgan (1866–1945) (Fig. 7.2b), also had worked at the *SZN*. By studies in his Columbia "Fly Room" laboratory in which *Drosophila* mutations were created, Morgan demonstrated that genes are carried on chromosomes and are the basis of inheritance. This discovery played a critical role in integrating the tenets of Mendelian genetics and chromosomal inheritance into the field of contemporary experimental genetics. The fly's rapid breeding time and its four large chromosomes made it an ideal "model" for examining the manner in which chromosomal events during meiosis and mitosis determine structural features of the adult. A serendipitous finding of a fly with a white eye (rather than the usual red) led Morgan to appreciate the importance of the sex chromosome in many aspects of development (Morgan 1926; Morgan et al. 1915). Despite some controversy regarding the physical nature of the gene and certain aspects of Mendelian inheritance, genetic research extended to other organisms, and the *Entwicklungsmechanik* [mechanistic experimental approach] dominated genetics/embryology. For several decades, Morgan, with his "boys" Colin Blackman Bridges (1889–1938), Hermann Joseph Muller (1890–1967) (Fig. 7.2d), Alfred Henry Sturtevant (1891–1970), and others, worked in the Fly Room to integrate Mendelism with the chromosome theory. Among other projects, this involved the mapping of chromosomes, the discovery of sex linkage, chromosome inversions, nondisjunction, and other phenomena that serve as the basis for understanding transmission genetics (Bridges 1913; Morgan 1910, 1911; Sturtevant 1913).

To a great extent, modern-day genetics is founded upon much of the work and ideas of Wilson, Johannsen, Morgan, and Muller. Each was a member of the US National Academy of Sciences. In 1913, Wilson served as president of the American Association for the Advancement of Science, and in 1933 Morgan was awarded the Nobel Prize in Physiology or Medicine for discoveries concerning the role of the chromosome in heredity (Allen 1976; Morgan 1939, 1941, 1942; Sturtevant 1959).

Among the young stars in the galaxy that surrounded Morgan was Hermann Muller, who later moved to the University of Texas, Austin. With colleagues, Muller studied the rates of natural gene mutation in *Drosophila*. In the course of these investigations, they recorded the first induction of a genetic mutation that of increasing the mutation rate severalfold upon exposure to X-rays (even diagnostic X-rays not being innocuous in this regard; Muller 1927). In 1946, Muller was awarded the Nobel Prize in Physiology or Medicine "for discovery of mutations by means of X-ray irradiation." With his insights into the dangers of the overuse of radiation exposure in diagnostic radiology, and of the nuclear arms race, Muller became a severe critic of the US policy of testing and using nuclear weapons (Muller et al. 1947).

7.3 Embryology Becomes a Science

As in some other fields of the biological sciences, the advancement of embryology was hindered by limitations in microscopic resolution, the lack of serial sections, the less than optimal staining techniques, and the absence of methods of reconstruction. One who did much to correct these deficiencies in the "Vesalius of Embryology" was Wilhelm His Sr. (1831–1904) of the University of Leipzig as well as several other institutions (O'Rahilly and Müller 1992, p. 3). He co-founded and was president of the *Anatomische Gesellschaft* [Anatomical Society]. His' three-volume *Anatomie Menschlicher Embryoen* [Anatomy of Human Embryos] (His, 1880–1885) was an exhaustive, encyclopedic work (vol. 1, Embryos of the First Months; vol. 2). This covered the form and important development until conclusion of the second month of gestation (vol. 3, Along with the history of the organs); His, by scrupulous attention to detail and careful selection, presented for the first time a highly accurate series of illustrations of the developing human embryo in sequence from the second week to the second month of gestation. His approached embryology in an analytic fashion, with numbered stages of development, although later he abandoned this approach for representative "norms." The first and third volumes were accompanied by folio atlases and two series of eight wax models and a set of glass photographs that were sold separately. Many of the drawings were composed by His himself, while the lithography was done by "a very careful artist," C. Pausch. It was said to ". . . mark an epoch of accomplishment in the study of human embryology," and at the same time furnishing ". . . exceptionally numerous suggestions of many problems yet to be solved, with the most promising lines of attack" (Knower 1911, p. 493). Published simultaneously in German and English, the work illustrates the close relationships of investigators on both sides of the Atlantic that trained under or were otherwise influenced by His (Mall 1905).

J. Keibel, who became lecturer at the University of Freiburg, and later at the Universities of Strasburg, Königsberg, and Berlin, contributed numerous morphologic studies in vertebrate embryology. He is particularly noted for his

Normentafeln zur Entwicklungsgeschichte (Normal Plates of the Development of Vertebrates)... (Keibel 1897–1911), an encyclopedic survey of the embryonic anatomy of various vertebrates. He also contributed to an understanding of many specific aspects of development, such as that of the head, and various germ layers (Keibel and Elze 1908). Later, His published *Nomina Anatomica* [anatomical nomenclature], in which he described the subdivisions of the neural tube, *metamerism*, that occurs at different embryonic stages (His 1895; Streeter 1933) and *Die Anatomische Nomenklatur* [Anatomical Nomenclature] (His 1895). It was His' son Wilhelm His Jr. (1863–1934), an internal medicine specialist in Berlin, who discovered the atrioventricular impulse conducting system of the heart, the "bundle of His" (His 1893), and described "heart block" in instances during which these impulses were interrupted.

In addition to His Sr.'s contributions to embryology in general, he advanced knowledge of the neural crest and notochord, the brain, the heart, the lymphatic system, the innervation and nerve plexus in the adventitia of blood vessels, the vascular endothelium, connective tissue, and other tissues/organs (Fick 1904; Oppenheimer 1955, 1967; O'Rahilly 1988). His also planned a multivolume study of the human embryo; however, this was on such a large scale that it was never completed (Mall 1913).

Importantly, His devised the microtome that enabled one to obtain an uninterrupted series of sections, thereby eliminating the vagaries of freehand sectioning, which he used for his embryologic studies (His 1870), as did many others for their work (Bracegirdle 1978; Purkyně 1918–1973). He also introduced the use of paraffin for embedding tissue, formulated the osmium tetroxide fixation procedure, dehydration with alcohol, clearing with oil of lavender, and mounting in Canada balsam (Bodemer 1971; Querner 1972). His, with his colleagues, used a specially devised "embryograph," a prismatic drawing apparatus that allowed him to project the images onto a paper to ensure more accurate and reliable drawings of the microscopic sections. With his assistant F. Steger, they developed the His-Steger wax models of embryonic development that are part of the collections of many museums. Although he was one of the first to seek a causal-mechanical explanation of embryonic development, His relied on morphologic rather than experimental studies. In addition, and importantly to his detailed description of embryonic development, His presented an important classification of different types of tissues in development, tissue histogenesis, the study of embryonic origins (His 1865a, b, 1874). He also promoted the use of photography and the use of lantern slides for classroom lectures. His' series of wax models of the developing tissues also became especially well known. His also advanced anatomic/embryologic instruction by the use of topographical models and introduced the use of standardized embryological charts (Querner 1972). Staining of tissue sections did not, however, become common until the last decades of the nineteenth century (Longo and Reynolds 2016).

Overall, it was the work of His that helped to establish embryology as a modern science. Importantly, he was one of the originators of the "neuron doctrine," that individual nerve cells are the structural and functional unit of the brain. This was

opposed to the "reticulist" view of a complex network, in which all nerves of the brain interconnect to all other cells in a vast reticular network. The neuron theory was established definitively at the end of the nineteenth century by Santiago Ramón y Cajal (1852–1934) (Ramón y Cajal 1892, 1899–1904), by the use of the staining technique developed by Camillo Golgi (1844–1926) (Golgi 1886).

Inspired by his postgraduate training in Leipzig, in the late nineteenth century, Charles Sedgwick Minot (1852–1914) (Fig. 7.2a), lecturer in embryology, and later James Stillman, Professor of Comparative Anatomy of Harvard Medical School, amassed a superb collection of embryological material. Also during this period, he invented the automatic rotary microtome for cutting ultrathin tissue sections for detailed morphological analysis. Cutting the embryos into serial sections, and arranging them in steel cabinets, this collection grew into an unrivaled resource for teaching and research (Councilman 1918). His *Human Embryology*, a work of over 800 pages on which he spent a decade writing, presented a comprehensive summary of issues regarding human development, with the introduction of several novel theories (Minot 1892). Soon thereafter, he introduced the term "cytomorphosis" to describe the structural alterations cells undergo during the course of their development (Councilman 1918; Minot 1908).

Minot made important contributions to the understanding of embryonic development. He amassed a superb collection of embryological material of ultrathin tissue sections. He cut embryos into serial sections and arranged them in steel cabinets, which served as an unrivaled resource for teaching and research (Councilman 1918). His *Human Embryology*, published in 1892, presented a comprehensive summary of human development and introduced several novel theories (Minot 1892). In his *The Problem of Age, Growth, and Death*, he further advanced the descriptions of the structural alterations that cells undergo during the course of their development (Minot 1908). This work included studies of growth and senescence, from the embryonic period through old age, with observations in humans, and diverse species as dogs, cats, rabbits, guinea pigs, chickens, snakes, frogs, salamanders, dogfish, snails, and woodlice.

7.4 Franklin Paine Mall and the Carnegie Institution Department of Embryology

Several decades later, a work by Keibel and Franklin Paine Mall (1862–1917), both students of His, expanded upon His' monumental *Anatomie* of Human Embryology (His, 1880–1885; Keibel and Mall 1910–1912). Mall, a graduate of the University of Michigan, School of Medicine (1883), obtained postdoctoral training in Leipzig under His and Carl Ludwig. Mall recalled that when he first knocked at His' door, he was turned away. After returning a number of times, he was finally accepted (Sabin 1934). With the opening of the Johns Hopkins University School of Medicine in 1893, Mall became the first Professor of Anatomy, where he continued to

make a number of contributions to embryology (Mall et al. 1900–1908). At the Johns Hopkins, Mall helped to make the study of anatomy an independent science of its own and a vital part of medical education. He also founded the *American Journal of Anatomy* (1901). In his 1913 essay "A plea for an institute of human embryology," Mall made the comparison to the benefits of having major facilities for astronomy, anatomy, and other disciplines. He enumerated the justification for a large research institute with a major collection as complete as possible of specimens and a number of investigators in human embryology. Among many other points, he noted:

> The science of anatomy, which in great measure has been a mother of the biological sciences, needs a thorough revision and a new basis, and this is to be obtained through the study of embryology . . . with a very large collection, a competent staff and the very best material equipment the institute would naturally take up problems which bear an anatomy, physical anthropology, comparative embryology, physiology of gestation, pathology and teratology . . . the chief function of an institute of human embryology should be the formation and solution of problems.
>
> (Mall 1913, pp. 1600–1601)

The following year, the Department of Embryology of the Carnegie Institution of Washington was founded in Baltimore, MD, and Mall became its director. In addition to contributing his extensive collection of human embryos, he made numerous contributions to both the science and the teaching of anatomy and embryology. Mall was an important mentor to many of the outstanding anatomists in the USA, and played a critical role in development of the full-time system of educators in medicine (Corner 1974; Sabin 1934).

The establishment of the Department of Embryology in Baltimore by the Carnegie Institute of Washington in 1914–1915 grew out of His Sr.'s idea to promote an intensive study of human embryology and Mall's goal in this regard, as well as his personal collection of 813 embryos (O'Rahilly 1988; Streeter 1933). Following its creation, Mall expanded the collection to include about 2000 specimens, to become the most extensive and most thoroughly studied such grouping of human embryos in the world. George Corner often referred to it as "the Bureau of Standards of human embryology." Corner noted that the original plan and prime interest for the department was to emphasize the embryology of the human species to include hundreds of beautiful examples and the preparation of reconstruction to include morphology, growth, physical anthropology, the study of abnormal specimens, and comparative experimental embryology (Corner 1981). Years later (1950), the collection of insectivore, lemurs, and platyrrhine monkeys established by Professor Hans Bluntschli (1877–1962) of the University of Bern was further added to the Carnegie Collection (O'Rahilly 1988). Mall promoted the publication of many of the Department of Embryology's research studies in the *Contributions to Embryology of the Carnegie Institution of Washington* from 1915 when it was commenced by Mall until 1966 when it ceased publication (O'Rahilly 1988). A member of the National Academy of Sciences, Mall received many awards and honors (Councilman 1922; Sabin 1918, 1934, 1936).

Following Mall's death in 1917, George Linius Streeter (1873–1948) became Director of the Department of Embryology, which he led until 1940. Streeter

stressed that, contrary to the idea of an embryo being an "incompleted" stage of development, it constitutes a living creature and its component parts are not a mere "anlage" [a laying on, primordium] but functioning organs (Corner 1954, p. 263; O'Rahilly 1988). He also contributed to the "larger question" of the morphometrics of growth of the human embryo that included weight, sitting height, head size, foot length, and other measurements so that the stage of embryonic development could be standardized to "Horizons" (Streeter 1920). In his 1927 presidential address to the American Association of Anatomists, Streeter considered archetypes in development. In regard to the brain he observed:

> . . . the brain begins to build its definitive parts before the closure of the neural tube—without going through a preliminary archetypal indifferent three-vesicle stage . . . there seems to be no evidence that the brain wastes any empty gestures toward the past. With no false moves it proceeds directly with the building of an organ appropriate in all its parts for the respective species.
>
> (Streeter 1927a, p. 411)

In conclusion, Streeter admonished:

> We all know very well that advance in knowledge has come about, first of all, through separating the known facts from the surrounding haze of ignorance. These known facts then have been made more significant through classification and coordination, and finally it is through the device of schemata, types and hypotheses that we have been guided in the acquisition of still more facts . . . Facts are desirable possessions, so are theories, but the two should not be confused. Facts should be kept in one pocket and theories in another. One should never forget that diagram, classification, symbolism, and hypothesis are but temporary expedients. They are good servants but poor masters. Like all man-made things they are imperfect, and as new facts come into view they must be revised or discarded . . . In his classical monograph on comparative embryology von Baer places on the back of his title page the Latin slogan: "simplex est sigillum veritatis!"—simplicity is the seal of truth . . . in view of our new knowledge of the remarkable intricacy of nature should we not change it to read: *Complex est sigillum veritatis* [Complexity is the seal of truth].
>
> (Streeter 1927a, pp. 411–412)

With the use of superb photographs from slides, Streeter, with his colleague Chester Henry Heuser (1885–1965), collaborated on a classic detailed study of the early embryology of the domestic pig (Streeter 1927b; Heuser and Streeter 1929). Streeter was a member of the National Academy of Sciences, as well as the recipient of many other awards and honors (Corner 1954, 1976).

Following Streeter's retirement in 1940, George Washington Corner (1889–1981) accepted the call to be the third Director of the Department of Embryology of the Carnegie Institution. Corner was already a distinguished anatomist, histologist, and embryologist and a graduate of the Johns Hopkins University School of Medicine (1913). Following graduation, Corner had worked with Mall, spent 4 years (1915–1919) with Herbert McLean Evans (1882–1971) in the Department of Anatomy at the University of California Berkeley, another period at the Johns Hopkins (1919–1923), and chairman of the Department of Anatomy at the newly founded medical school at the University of Rochester, NY (1923–1940). During these years, Corner established the role of the corpus luteum, progesterone, and many aspects of the regulation of ovulation and the menstrual cycle in the pig sow and rhesus monkey (Allen and Corner 1929; Corner 1929, 1942; Corner and

Allen 1929). Following his 1940 return to Baltimore, where he remained for 15 years, Corner contributed to the embryologic development in primates and humans (Corner 1955). A medical historian of fist rank, Corner authored several important works in this field (Corner 1937, 1944, 1948, 1958, 1963, 1964, 1965). Among those investigators who added to the Carnegie Department of Embryology were George William Bartelmez (1885–1967) (Bartelmez 1933; Bartelmez and Dekaban 1962; Bartelmez and Evans 1926), Bent Boving, Arpad Istran Csapo (1918–1981), Louis Barkhouse-Flexner (1902–1996), Carl Gottfried Hartman (1879–1968), Arthur Tremain Hertig (1904–1990), Chester Henry Heuser (1885–1965), Elizabeth Maplesden Ramsey (1906–1993), Samuel Robert Means Reynolds (1903–1982), and Florence Rena Sabin (1871–1953). Each of these investigators made notable contributions and achievements at this time, which included identification of the earliest "Hertig-Rock human embryos" (Rock and Hertig 1942, 1943). A member of the National Academy of Sciences, Corner received many honors and awards and several important roles in many governmental and other bodies (Gosden 2013; Ramsey 1994). In his final book, *The Seven Ages of a Medical Scientist*, Corner reflected upon his contributions to life:

> I once had a correspondent who held that the universe is no good and should not exist. I disagree with him. I think highly of the universe. The buffets, it gives us, we must take as they come; the benefits we can often help to arrange. I am proud to have been a member of the universe these 90 years past. One accepts, of course, the regulations for enrollment, pays his annual assessment of hard work, and aims to be a useful citizen of the local galaxy. In return, he receives the friendship of other members and the love of those who are near and dear to him.
>
> (Corner 1981, pp. 395–396)

7.5 Other Early Twentieth-Century Developments in Embryology

Another who laid important groundwork in scientific embryology was Richard Assheton (1863–1915) of Trinity College, Cambridge, and lecturer at Guy's Hospital, University of London. Concentrating chiefly on mammals, but also considering embryonic growth in other species, Assheton, in his *Growth in length; embryological essays*, concluded this to be determined by two centers of cellular activity, e.g., that of the "... protoplasmic centre of the fertilized egg ..." and the "... centre ... in the middle of the blastopore ..." (Assheton 1916, p. 24).

Because of limitations in the available technology and methodology of this era, experimental embryology was characterized by descriptions of morphological changes with time and with postulates such as "continuous fields," "gradients," "inducers," and "polarities." A seminal concept that originated at this time was that of a chemical "organizer" responsible for embryological development. This gave rise to a large body of work aimed at discovering the biochemical substances that accounted for morphologic development and progressive growth. One of the pioneers in this line of investigation was Hans Spemann (1869–1941) (Fig. 7.2c)

of Rostock, Germany, and later Director of the University of Freiburg. Spemann's studies using microdissection in newt embryos focused attention on the signaling properties of a group of embryonic cells they termed the "organizer" or "organizer centers" that controlled morphogenesis in development (Spemann and Mangold 1924). In consideration of the formation of the eye of a frog, Spemann mused:

> How is it that the lens begins to grow just at that spot in the epidermis where it is touched by the optic cup and exactly at that moment when the rudiment of the retina invaginates? Do these processes mutually influence each other, either in that the growing lens presses the retina inward or at least causes it to invaginate, or that the retina, while drawing in, starts the growth of the lens? Or do both processes go on independently of each other in self-differentiation of their respective rudiments, and does their exact fitting together depend upon previous and accurate tuning of the parts to a perfect harmony between them?
>
> (Spemann 1938, p. 44)

For much of the past century, the identification of the organizer's neural-inducing signals has been viewed as one of the most important topics in early neural development. For his work in chemical organizers in embryonic induction, Spemann was awarded the Nobel Prize in 1935. Shortly thereafter, a vital contribution was that of Oswald Theodore Avery (1877–1955) and colleagues at the Rockefeller Institute Hospital, who with rigorous experiments in several types of pneumococcus bacteria established that DNA was the "transforming principle" in Mendelian genetic inheritance (Avery et al. 1944; Kay 1970). It was the 1953 discovery of the molecular structure of DNA by James Dewey Watson and Francis Harry Compton Crick (1916–2004) at the MRC Laboratory in Cambridge University (Watson and Crick 1953a), their proposal of the mechanism by which cellular DNA passed on their characteristics, e.g., information with precision (Watson and Crick 1953b), and the manner in which DNA codons in specifying amino acids and their sequence in proteins (Crick et al. 1961), that transformed genetics and biology. In 1962 Watson and Crick shared the Nobel Prize in Physiology or Medicine with Maurice Hugh Frederick Wilkins (1916–2004) of King's College, London.

Thomas Hunt Morgan's pioneering studies in chemical embryology helped to advance the field beyond the phenomenology of descriptive morphology (Morgan 1897, 1907, 1915). He treated development as a system in which reactions could be measured by physical and chemical standards and interpreted in terms of their interrelations. Eloquently, he presented his philosophy of embryology:

> A transparent egg as it develops is one of the most fascinating objects in the world of living beings. The continuous change in form that takes place from hour to hour puzzles us by its very simplicity. The geometric patterns that present themselves at every turn invite mathematical analyses. The constancy and orderliness of the whole series of events, repeating themselves a thousand fold in every batch of eggs, assures us of a causal sequence conspiring to create an object whose parts are adjusted to make a machine of extraordinary complexity.
>
> This pageant makes an irresistible appeal to the emotional and artistic sides of our nature. Hence not without a feeling of jealous regret, the old-fashioned embryologist sees these gems of nature consigned to test tubes for chemical analyses, to centrifuges to disturb their arrangements, to microdissecting instruments to pick them to pieces, and to endless tortures by alterations in the environment to disturb the orderly, normal course of events. For, it is the automatic self-contained perfection of the developmental process that holds

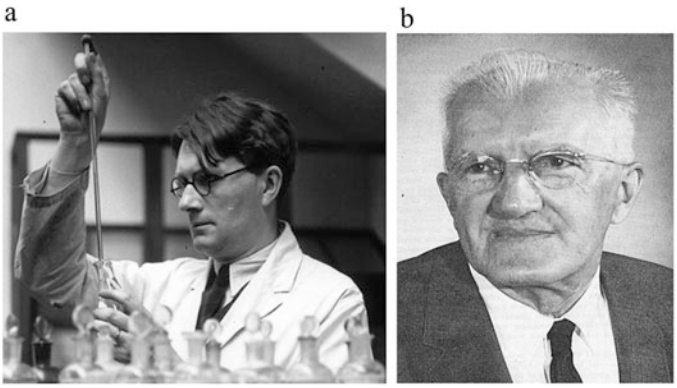

Fig. 7.3 (**a**) Joseph Needham (1900–1995). (**b**) Edward Frederick Adolph (1895–1986)

> our interest. Yet we feel, too, that if the mystery that surrounds the study of embryology is ever to come within our comprehension, we must try not to be sentimental and have recourse to other means than description of the passing show. The recompense, we hope, will be to substitute a more intelligent interest in place of the older emotional response to the order of nature.
>
> (Morgan 1927, p. vii)

Along this line, an important contributor to the biochemical aspects of embryology was Noel Joseph Terence Montgomery Needham (later Sir Joseph, 1900–1995) (Fig. 7.3a), a Cambridge University biochemist whose three-volume *opus* of 1931 with more than 4500 references, *Chemical Embryology*, synthesized essentially everything known about this subject. In this work, inspired, in part, by Preyer's *Specielle Physiologie des Embryo* (Preyer 1885), Needham worked chiefly with the avian egg. Here he attempted to resolve the dispute between "vitalism" and "mechanism" and contribute to "... the increase of knowledge itself," presenting "... the physio-chemical history of embryonic development, from the egg-cell to the losing of the individual into the activity of post-natal life ..." (Needham 1931, p. 38). In this work, Needham proposed a "neo-mechanistic" basis of development with shades of *Weltanschauung* [world view or philosophy] (Needham 1931, 1933; Schröder 1992).

In his *Prolegomena*, Needham acknowledged:

> If the analogy may be permitted, physico-chemical embryology has so far been living an intra-uterine existence. Its facts have been buried in a wide range of scientific journals, and its theories have lain dormant or in potential in reviews of modest scope. Physico-chemical embryology has, indeed, arrived at the stage immediately prior to birth, and all it needs is a skilful obstetrician, for, when once it has reached the light of day and has passed for ever out of the foetal stage, it will be well able to take care of itself.
>
> (Needham 1931, p. 1)

This previously ignored aspect of embryology was fortunate to have as its *accoucheur* one with Needham's background and breadth of knowledge. He promoted the concept of "neo-mechanism as a theory of chemical embryology," which regards "the mechanistic view of the world as a legitimate methodological distortion, capable of application to any phenomenon whatever, and possessing no value

at all as a metaphysical doctrine" (pp. 32–35). In his chapter "The theory of chemical embryology," Needham reviewed the understanding of embryonic development, from the Aristotelian view of the principles of causation: "Efficient, Material, Final, and Formal" Causes (pp. 10–11), which formed the conceptual framework for almost two millennia, to neovitalistic theories of "hormism" or "psychobiology," to "finalism" or "dynamic teleology," to "organicism," to "emergence" (pp. 14–32). Needham emphasized that, in contrast to these rather numinous and mystical concepts of metaphysical materialism, "Biology cannot be [both] philosophical and scientific, emergent and resultant, indeterminate and determinate, teleological and mechanical at one and the same time" (p. 30). He continued that ". . . the most powerful solvent of vitalism . . ." would be contemporary concepts in physics of uncertainty and indeterminacy and the rigor of biochemistry and biophysics (pp. 31–32). Thus, Needham helped to establish embryology beyond a transcendental a priori basis, rejecting the entelechy of vitalism and mechanism, to that of ". . . a branch of exact biology" (Wells 1932, p. 415). Needham concluded, "The physico-chemical embryologist is not committed to any opinion . . .. [but] that the scientific method is one way of describing it and that it is best to apply that method in its full rigour . . ." (Needham 1931, p. 14).

Needham's approach echoed that of the philosopher Robin George Collingwood (1889–1943) that ". . . mathematics, mechanics, and materialism are the three marks of all science . . ." (Needham 1931, p. 36). In his 1932 essay, "Thoughts on the problem of biological organization," Needham observed, "Whatever the nature of these relations may be they form the central enigma of biology, and biology will only be fruitful in the future if this is recognized. . . . The hierarchy of relationships, from the molecular structures of the carbon compounds . . . to the equilibrium between species in ecological wholes . . . will probably be the guiding idea of the future. But one conclusion is clear . . . organization is a problem to be solved . . ." (Needham 1932, p. 92). In an extension of the historical presentation in *Chemical Embryology*, Needham also published his exhaustive survey of embryology from antiquity through the end of the eighteenth century, *A History of Embryology* (Needham 1934). The following year, in his Carmalt Memorial Lecture, Sir Joseph philosophied on the "limiting factors in the advancement of science . . ." as illustrated in the history of embryology (Needham 1935). In this essay, Needham explored those limitations caused by the relation of investigators to their environment, lack of cooperation among researchers, practical and theoretical issues with various methodologies, and achieving a proper balance among speculation, descriptive observation, and experimentation. This latter category reflected an earlier lecture he had given (Needham 1932). In conclusion, Needham cautioned and presented a challenge:

> . . . there can be no doubt that a plethora of observation and experiment is . . . bad for scientific progress. Modern biology is the crowning instance of this fact. What has been well called a "medley of ad hoc hypotheses" is all that we have to show as the theoretical background of a vast and constantly increasing mass of observations and experiments. Embryology in particular has been theoretically threadbare . . . Experimental embryology, Morphological embryology, Physiological embryology, and Chemical embryology form

> today a vast range of factual knowledge, without one single unifying hypothesis, for we cannot dignify the axial gradient doctrines, the field theories, and the speculations on the genetic control of enzymes, with such a position. We cannot doubt that the most urgent need of modern embryology is a series of advances of a purely theoretical, even mathematico-logical, nature. Only by something of this kind can we redress the balance which has fallen over to observation and experiment; only by some such effort can we obtain a theoretical embryology; only by some such effort can we obtain a theoretical embryology suited in magnitude and spaciousness to the wealth of facts which contemporary investigators are accumulating day by day.
>
> (Needham 1935, pp. 17–18)

In later years, Needham became an expositor and scholar of Chinese Science and Civilization (Needham 1954–2004).

Another contemporary scientist who enlarged the horizons of embryology was Paul Alfred Weiss (1898–1989), originally from Vienna and its Biological Research Institute, who emigrated to the USA to work with Ross Granville Harrison at Yale and later worked at both the University of Chicago and the Rockefeller University. In his *Principles of development*. . ., Weiss stressed that the field was analogous to a land in its age of discovery, in:

> . . . a state of flux with rapid and unpredictable advances and fascinating prospects . . . it combines a review of past achievements with a preview of presumable future trends . . .. The growth of our science . . . [is] as the growth of an organism whose food is the ever increasing mass of factual knowledge.
>
> (Weiss 1939, p. iii)

In concert with his detailed review of contemporary embryological concepts, Weiss addressed problematic issues such as development of the nervous system and the integration of behavior (see below).

In further studies of development, one who worked to discover mechanisms by which the structure of an organism develops from germinal material was the zoologist Charles Manning Child (1869–1954) of the University of Chicago. Another "graduate" of the *SZN*, Child in his studies of regeneration established that these metabolic gradients of growth, whether arranged along a polar axis (axial gradient) or spreading outward from a central core, were present not only in the organism as a whole but in the various individual tissues and cells. Further, the gradient was demonstrated in terms of functional components such as oxygen consumption, metabolic rate, enzyme activity, and others. Child's contributions were a frontal attack on the central problem of biology, that is, how the developing organism, structurally composed of various cells, achieves pattern, order, form, unity, and correlation of activities. In an 800+ page 1941 monograph and summary of his lifework, *Patterns and Problems of Development*, Child explored various aspects of the problems of the origin and nature of developmental patterns and their physiologic implications (Child 1941; see Blackstone 2006). The "gradient" theory took its name from his demonstration in *Planaria* that development occurs in graded physiological stages along an axis with relation to adjacent tissues (Hyman 1957). Quite obviously, Child's attempts to solve the mystery of the regulation of cellular and tissue development, one that continues to the present day, were premature before the creation of contemporary cellular and molecular biology. Although beyond the limits of consideration of this review, the concept of

"morphogen gradients" is important in today's developmental biology (e.g., Cooke 1988) as a possible mechanism by which a signal from one cell group in an embryo can act upon, and determine, the development/differentiation/lysis, and thus fate, of surrounding cells.

Other reviews document a broader rooting of experimental embryology during the latter part of the twentieth century (Alexandre 2001; Mulnard 1986). Although strictly speaking, not an embryologist, another who contributed greatly to thought in this domain was the physiologist Edward Frederick Adolph (1895–1986) (Fig. 7.3b) of the University of Rochester. Principally an environmental physiologist, Adolph appreciated the vital importance of understanding the emergence of biologic regulatory mechanisms. In the Preface to his *arbeit*, *Physiological Regulations*, Adolph speculated on what may be a credo for physiologists:

> Physiology seems to me more than a science of individual working parts.. . . Physiological regulations are patterns of processes; the outcome of all those operating characteristics that assure the constancy of a property. . . There is no limit to the patterns of physiological investigation, for every concept adds a pattern of search. Physiology is more than a technology, more than information. It develops new aspects at every turn; as long as it lives it will include the unorthodox.. . . Let not wisdom scoff at strange notions or isolated facts. Let them be explored. For the strange notion is a new vision, and the isolated fact a new clay, possible foundations for tomorrow's science.
>
> (Adolph 1943, pp. v–vi)

In his studies, Adolph sought to identify critical aspects of the ontogeny of the several regulatory systems, in particular those of water balance and renal function, temperature control, oxygen consumption and metabolism, and the blood and circulatory systems. As noted in his analysis of quantitative relationships, the ". . . selected relations among data are manifestations of the processes commonly meant by the term physiological regulations. They provide a quantitative means of visualizing what organisms do to maintain constancy not only of composition, but of energies, forces, structures, and functioning" (Adolph 1943). In his studies, many of which used the developing rat as a model, regulatory systems were categorized into five major types and traced the manner in which their development followed similar lines or differed. Among his conclusions was the manner in which these physiologic systems develop during the antenatal period of fetal maturation, as opposed to suddenly appearing at birth (Adolph 1957).

In his 1968 monograph, *Origins of Physiological Regulations*, Adolph expanded upon the developmental origins of regulatory mechanisms of cardiac and renal function, as well as metabolic and endocrine pathways, with comparisons among species, and with analysis of the general principles of ontogenesis (Adolph 1968a). Subsequently, Adolph explored the basis upon which to compare physiologic developments or "stagemarks" in the fetuses and infants of 16 different species (12 of which he illustrated). Although the ontological sequences of specific functions and landmarks, which appear step-by-step, were quite similar among the species, significant differences were observed (Adolph 1970, 1972). A philosopher of science, Adolph emphasized the role of research in self-education and personal enrichment, in addition to its many contributions to society (Adolph 1968b).

A recipient of many honors, Adolph was awarded the US Presidential Certificate of Merit (1948).

In a continuation of the studies initiated by Spemann, Needham, Weiss, Child, and others, with contemporary methodologies of cellular and molecular biology, investigators such as the 2012 Nobel Laureate Sir John Bertrand Gurdon, formerly of Oxford and currently with the Wellcome Trust/Cancer Research UK (now known as The Gurdon Institute) in Cambridge University, are pursuing the fundamental mechanisms by which cells originate, transmit, and respond to such signals (Gurdon 1962; Gurdon and Bourillot 2001; Gurdon et al. 1999). Considering the mystery of morphogen gradients, Sir John Gurdon has written:

> The question of how a cell can individually recognize and interpret a defined concentration of a factor in the medium remains, to me, one of the most fascinating problems in cell and developmental biology. It seems that a single cell can recognize and interpret as little as a three-fold concentration of a morphogen in its medium. The actual concentration at which morphogens are recognized is in the picomolar range. I believe this to be well beyond the sensitivity of human chemo-reception.
>
> (Letter from Sir JBG to LDL, 11 January 2010)

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Chapter 8
Some Aspects of the Physiology of the Placenta

> *. . . neither is there occasion for returning and refining this blood [of the fetus] in the lungs of the mother, because that office is sufficiently performed in the Placenta until the Foetus is delivered, when its own lungs are put to their proper use.*
>
> (William Smellie 1752, p. 140)

8.1 Late-Nineteenth and Early-Twentieth Centuries

One cannot properly consider the physiology of the fetus without that of the placenta, a fetomaternal organ characteristic of mammalian pregnancy, upon which the fetus is dependent not only for the transfer of oxygen and a variety of nutrients but the elaboration of a number of hormones and growth factors in the "maternal-placental-fetal" complex. Thus, it is perhaps appropriate to review some of the concepts regarding development of that organ and its function, the intervillous space, villous structure, and placental classification. The placenta differs from other organs in being formed by the interaction of both fetal and maternal tissues, shows extreme diversity in its structure among the species, and is of limited lifespan – dare say, a disposable organ. The placenta's unique and specific service as an interface between the mother and fetus, to convey all that is necessary in support of progeny growth while protecting the mother from allograft growth leads to the recognition that this may well be the last organ to differentiate in female mammals. Development of the placenta must be correlated closely with that of the fetus. Near or at term in humans, the placenta weights ~0.5 kg, and throughout pregnancy, by mechanisms poorly understood placental mass, maternal and fetal blood flows, villous surface area for nutrient exchange, and the elaboration of hormones must be matched closely to fetal growth and development. A history of placental biology might be considered from a number of perspectives. These include embryology; variation by species; morphologic form; microscopic cellular organization; maternal and fetal circulations; the interface between maternal and fetal exchange surfaces; respiratory gas and nutrient exchange; metabolic, endocrine, and immunologic functions; and others. Quite obviously, considerations of

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these subjects in detail would require a multivolume treatise far beyond a simple historical discussion. Different aspects of the history of the placenta have been recorded by several authors (Corner 1963; De Witt 1959; Longo and Reynolds 2010; Neale 1970; Ramsey 1977).

Since earliest times, the placenta has been recognized as being of vital importance and at the same time quite mysterious – even somewhat mystical to the origins of the life. For example, in many cultures the placenta has been held as an *alter ego* [my second self], a symbol for the preservation of health and good fortune, and as a talisman in case of danger. In some societies, a sympathetic animism exists between the placenta and the future adult (Ploss et al. 1935). In early Egypt, the placenta was believed to be the seat of the "External Soul." A sculpture on an Egyptian ceremonial slate from Hierakonpolis depicts a Pharaoh, in what appears to be a ceremonial procession, preceded by five attendants, one of whom is bearing a standard interpreted as representing the Royal placenta with umbilical cord – the Pharaoh's "soul" or "secret helper" (Seligman and Murray 1911). The success and prosperity of the kingdom was held to be dependent upon the well-being of the sovereign and the preservation of his soul or "Bundle of Life" (Murray 1930). The Hebrew Scriptures include several references to the placenta, sometimes referred to as the "Bundle of Life" and "External Soul" (for instance, see Deuteronomy 28:57 and I Samuel 25:29; Long 1963; *William Smellie, 1697–1763*). In the folklore of many people of the Pacific Islands, Australasia, and Africa, the placenta is variously regarded as a sibling of the infant, a companion, or soul or otherwise possessing supernatural properties (Longo 1964).

The Greeks recognized the importance of the *placenta* [flat cake] in fetal nutrition and named the outermost embryonic membranes *chorion* [membrane] and the innermost membrane encompassing the fetus *amnion* [bowl]. The Greek philosopher-biologist Aristotle (384–322 BCE) may have been the first to use the term *chorion*, and he also recognized the yolk sac of lower vertebrates (see Aristotle 1831–1870). Because Aristotle based many of his ideas on findings in ruminants and other animals, considerable confusion about many topics was perpetuated. Nonetheless, he did much to establish the science of the study of fetal membranes. In his great embryological treatise *De generatione animalium* [On the generation of animals] (ca. 340 BCE), Aristotle stated that "The [umbilical] vessels join on the uterus like the roots of plants and through them the embryo receives its nourishment" (Aristotle 1831–1870). The Greek physician-anatomist Galen considered embryological development, in his treatises *De formatu foetus in utero* [intrauterine development of the fetus], *De uteri dissectione* [dissection of the uterus], and *De usu partium* [on births] (Galenus 1914). In these works, Galen maintained that the uterine vessels open their mouths and unite with the fetal vessels in the chorionic membranes, thus establishing direct communication between the mother and the fetus. This was concordant with his view that the left ventricle and arteries supplied "vital spirits" or "spiritual blood" to maintain the innate heat of the tissues, whereas the veins supplied the "alimentary blood" to provide pabulum or foodstuffs. His views on this subject were held as dogma until the discovery of the circulation of the blood in the early seventeenth century (Galenus 1914). Galen described four

stages of embryonic development: the seminal state, in which the embryo was a coagulum of semen and menstrual blood; the formation of the *tria principia* [triad of principal organs], the brain, heart, and liver; a third stage in which other structures developed; and finally further growth and maturation of the embryo/fetus (Needham 1934). In the goat, Galen also described fetal movements, including those that followed compression or ligation of the umbilical cord (Duckworth 1962). As noted earlier in Chap. 7, during the Renaissance with its quest for increased understanding of many aspects of life, the anatomist Realdo Colombo coined the term "placenta" and described many of its features, including noting the importance of the umbilical vessels. He wrote:

> At first, Nature spawns the allantois and those multiple veins and arteries that leave through the navel. Then, it makes those vessels fuse to each other, to be sustained. Finally, these vessels divide at the end of their course and form a sort of flat circular cake (that is placenta) . . . Although placed over the allantois, the placenta does not completely surround the fetus and strongly adheres to the uterus. It is therefore not surprising that abundant bleeding occurs during delivery, due to the tearing off of both placental veins and arteries . . . In humans the allantois is a large membrane, which completely encloses the fetus . . . In addition to the two already mentioned membranes, a third one (the amnion) has to be considered. The amnion is an envelope in direct contact with the fetus and contains its sweat and its excrement. The fetus swims in it and is sustained by this fluid, to be less troublesome for the mother.
>
> Don't marvel that no excrements other than urine and sweat are produced. This is due to the cleanness and purity of the blood that feeds the fetus. Thus, it is false what common people believe (i.e. that only menstrual or impure blood reaches the fetus) . . . When the time of delivery comes, these two membranes tear off and come out with the fetus. Obstetricians call "waters" the excremements that flow after the membranes rupture and their coming is considered a sign of imminent birth. Moreover, if the baby is visible when the waters flow, the outcome if considered favourable and easier. This is due to the humidity of excremental liquid that lubricates the mother's genital channel . . . The fetus is nourished through the umbilical cord by its vein. With respect to this . . . it is fed through the umbilical vein with good and perfect blood.
>
> (Colombo 1559; see Pizzi et al. 2012)

From the early sixteenth through the eighteenth centuries, if there was a single dispute that captured the core of placental developmental biology, it was over the degree to which the maternal and fetal placental blood vessels were interconnected.

The mid-nineteenth and early-twentieth centuries were a golden age for research on the placenta and its development. A major point of contention regarded the existence of the intervillous space and its circulation in maternal-fetal exchange. For instance, William Benjamin Carpenter (1813–1885) had described an enclosed space, in which fetal "vascular tufts" were within a cavity, the walls of which were an extension of the membrane lining the maternal veins and sinuses, and that fetal vessels were covered by a layer of cells of maternal origin (Carpenter 1854, pp. 626–627). In contrast, Arthur Farre (1811–1887) wrote, "...the maternal vessels all terminate at once and abruptly upon the inner surface of the decidua. The curling arteries, after passing from the muscular coat of the uterus... through the layer of decidua which forms the roof of the placenta, open directly into the interior of the latter; while the veins commence by equally abrupt openings which

conduct through the decidual layer to the venous sinuses in the uterine walls" (Farre 1859, p. 719). Other investigators wrote of maternal blood filling this space (Bumm 1890; Leopold 1877; Turner 1872, 1876a; Wagner 1851–1859; Waldeyer 1887, 1890). Despite these demonstrations, however, many could not accept the concept of maternal blood flowing through an open cavity in the absence of discrete surrounding walls. Although many workers accepted the presence of maternal blood in this space, a common view was that, rather than being in direct contact, a layer of maternal endothelial membrane separated that blood from the fetal villi (see Boyd and Hamilton 1970; Corner 1963; Pijnenborg and Vercruysse 2008). As may be appreciated, firm evidence of this circulation of maternal blood through the intervillous space awaited both more advanced microscopy and the cineradiographic studies a century later.

It should be noted that even at this time, physiologic functions of the placenta were poorly understood (De Witt 1959). For instance, in a series of lectures at the Royal College of Surgeons, Edinburgh, William Turner (later Sir William; 1832–1916) (Fig. 8.1a) of the University of Edinburgh summarized contemporary knowledge of placental structure, and likened the fetus to a parasite:

> The foetal placenta possesses an absorbing surface; the maternal placenta a secreting surface. The foetus is a parasite, which is nourished by the juices of the mother. . .. As there are, therefore, two sets of secreting structures in the gravid maternal mucosa, the Glands and the Crypts. . . it may be a matter of consideration how far the secreting organs perform similar or different functions in foetal nutrition. . .. The current doctrine that the nutrition of the foetus is provided for by the simple percolation or diffusion of materials through the walls of the vessels from the maternal blood to the foetal blood can no longer be accepted.
>
> (Turner 1876a, p. 114ff)

A *Lancet* reviewer of Turner's treatise wrote, "All Professor Turner's work is so honest and good that it is unnecessary we should do more than direct our reader's attention to the publication of these lectures to induce them to procure a copy for themselves" (Anonymous 1876, p. 541). Despite this somewhat confusing view of placental function, Turner made a number of important contributions to an understanding of placental structure, particularly in regards to its comparative anatomy with striking interspecies differences (Turner 1876a). He also was a founding editor of the *Journal of Anatomy and Physiology* (1866) (Haig 1997; Magee 2003; Turner 1872, 1876b, 1919).

Another issue of disagreement at this time was the nature of the outermost cells of the placental villi in the human. Theodor Langhans (1839–1915) (Fig. 8.1b), when serving as Professor of Pathology at the University of Berne, correctly identified the villous covering of *chorion frondosum* [*chorion villosum*] and *chorion laeve* [*chorion avillosum*] as being of fetal origin and as forming a continuous layer from the early stages of development (Langhans 1870). Several years later, Langhans demonstrated that this membrane comprised two layers, the outermost *Chorionepithel* [chorionic epithelium] of continuous cells, and underlying *Zellschicht* [cell layer] of large, individual, epithelial cells, with cellular boundaries/membranes. We now know these as the *syncytiotrophoblast* [cells

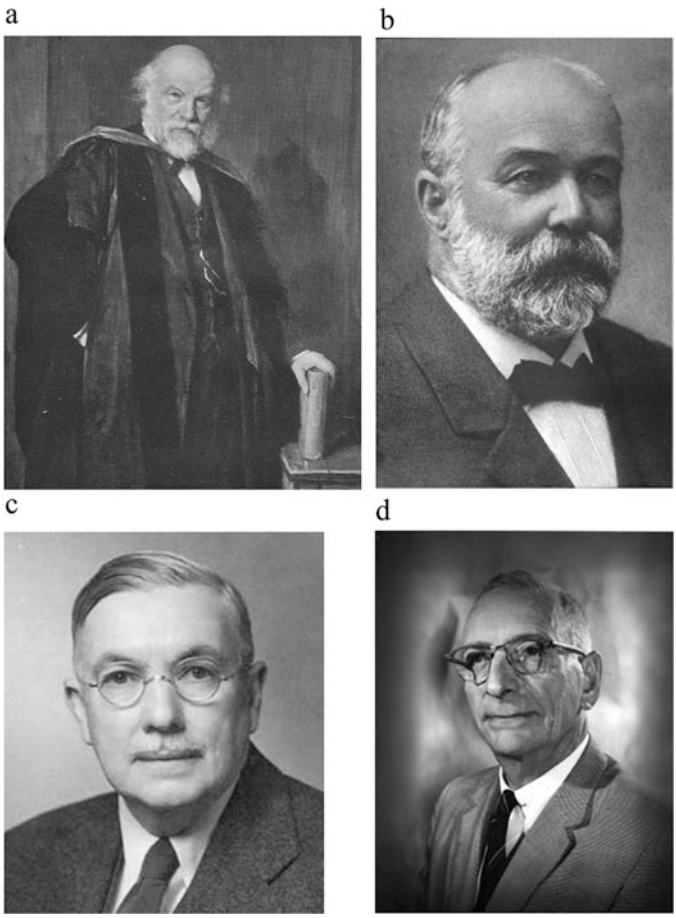

Fig. 8.1 (**a**) Sir William Turner (1832–1916). (**b**) Theodor Langhans (1839–1915). (**c**) George Washington Corner (1889–1981). (**d**) Louis Flexner (1902–1996)

together + trophoblast] and *cytotrophoblast* [cellular + trophoblast], or "Langhans" layer, respectively (Bonnet 1903; Boyd and Hamilton 1966; Langhans 1877, 1882). In regard to the *syncytiotrophoblast* and *cytotrophoblast*, Langhans noted that the epithelium covering the outer surface of the chorion and villi forms a uniform layer of rather homogenous cytoplasm containing nuclei but without a clear division into individual cells. Particularly in younger ova, he observed lines that might represent boundaries of cells of highly variable form, with large, often multiple finely granular protoplasm, and nuclei, the diameter of which were several times that of those in the overlying chorionic epithelium (Langhans 1877).

A related contribution was that of Ambrosius Arnold Willem Hubrecht (1853–1915), Professor of Zoology at the University of Utrecht, who, in contributing to an understanding of the process of implantation, introduced the term

trophoblast [nutrition + germ] to indicate that portion of the blastocyst not contributing to formation of the embryo per se but rather forming the placental villi for the nourishment of the embryo (Hubrecht 1888, 1889). Although working with several species, for these studies Hubrecht selected the placenta of *Erinaceus europaeus* (the hedgehog) (Hubrecht 1889; Pijnenborg and Vercruysse 2013). Hubrecht appreciated the double cellular layer, with the outer layer of nuclei in "nests" not demonstrating mitosis, and the innermost layer of cylindrical cells. According to his original description, trophoblast had limited morphologic significance; however, before long it came to apply to the epithelial derivatives of the outer layer of the blastocyst, the two cell layers described by Langhans. Hubrecht's collection of mammalian embryos and placentas, maintained for many years at the Hubrecht Laboratory in Utrecht (Faasse et al. 1999; Richardson and Narraway 1999), currently is at the *Museum für Naturkunde der Humboldt-Universität*, Berlin (Carter 2008).

Further technical and conceptual contributions to understanding placental morphology were those of Charles Sedgwick Minot. Minot suggested the term *trophoderm* to describe the mature placental cells; however, this never became widely accepted. Nonetheless, he presented a definitive account of the microscopic structure of the human placenta, in one instance describing a section through the uterus and placenta in situ at 7 months gestation, with amnion, chorion, villus trunk, sections of villi in the substance of the placenta, decidua, muscularis, uterine blood-vessel opening into the placenta, and so forth (Minot 1889, 1891). In a later review, Minot noted that the chorion is separated by a dense forest of villi from the decidua, that the termini of some of the villi touch and are imbedded in the decidual tissue, and that the decidua is divided into two strata. He described the section passing through a wide tube, a vein containing blood, that opened into the interior of the placenta (Minot 1903). These contributions did much to help clarify the nature of the maternal-fetal barrier of the placenta. His overview of embryologic-placental development *Human Embryology* (Minot 1892, 1897) was a "classic." A distinguished scientist and member of the National Academy of Sciences, Minot served as President of the American Society of Naturalists (1894–1895), American Association for the Advancement of Science (1901), and the American Association of Anatomists (1904–1905) (Lewis 1914; Morse 1920).

A pioneer in elucidating the intricate histology of the placentas of different mammalian groups, chiefly that in rodents, was Mathias-Marie Duval (1844–1907), Professor of Anatomy and Histology at the *École Supérieure des Beaux-Arts* and the *Faculté de Médecine* in Paris. In particular, Duval is remembered for his research on development of various structures in the placenta detailed in his *Le placenta des rongeurs* [the rodent placenta] (Duval 1892). With the availability of a carefully time-dated series of the mouse concepti, he detailed the successive steps in placental development. For comparison and confirmation, Duval included observations in an undated collection of rat placentas. He identified correctly the several extraembryonic cell layers and first recognized trophoblast invasion. Originally published as a series of journal articles (Duval 1891), the two-volume offprint includes an atlas with 22 engraved plates (Duval 1892). Although contemporary

Table 8.1 Cross species comparison of placental shape and structure

Species	Placental shape	Placental structure
Humans	Discoid	Hemochorial
Primates	Discoid	Hemochorial
Rodents (rats, mice)	Discoid	Hemochorial
Ruminants (sheep, cattle, goats)	Cotyledonary	Epitheliochorial
Pigs	Diffuse	Epitheliochorial
Horses	Diffuse	Epitheliochorial
Carnivores (cats, dogs)	Zonary	Epitheliochorial

advances have superseded many of Duval's interpretations (Adamson et al. 2002; Bridgman 1948; Georgiades et al. 2002; Pijnenborg and Vercruysse 2006; Steven 1975; Vercruysse et al. 2006), his observations and contributions have remained classic.

As is widely appreciated, the gross morphologic and microscopic structural anatomy of the mammalian placenta is quite diverse; thus an additional contribution of this era was a system of its morphologic classification. Table 8.1 lists some of the comparative placental shapes and structures among several species. Even within the specific placental types, there are considerable variations. For instance, the noninvasive cotyledonary singleton placenta of sheep consisting of 60 ± 15 placentomes are classified into four types (A–D) based on their gross morphology (Vatnick et al. 1991). In response to advancing gestation (Robinson et al. 1997) and to stress such as long-term hypoxia at high altitude (Penninga and Longo 1998), the percentage distribution of these placentome types is altered significantly. Although these placentome types have different structures, possible differences in their physiologic function are unknown (Vonnahme et al. 2008).

Thomas H. Huxley, who held that the presence of decidua demonstrated the common descent of a group of mammals, divided mammals into those *non-decidua*; *adeciduate*; *indeciduate*, in which at parturition there was no loss of maternal tissue; and *deciduate*, in which the decidua underwent lysis (Huxley 1864). Soon, however, it came to be appreciated that this view was inexact. Another classification was that of Hans Strahl (1857–1920), of Giessen, who categorized placenta as those *Vollplacenten* [complete placentae], in which at birth the maternal blood space is opened and part of the decidua sloughs off, and *Halbplacenten* [semi placentae], in which the cavity of maternal blood remains intact (Strahl 1902, 1908). In turn, the anatomist-embryologist Arthur Robinson (1862–1948), of Edinburgh and London, suggested the term "apposed placentae" for those instances in which the chorionic membrane is closely applied to the uterine decidua and "conjoined placentae" for those cases in which the layers are fused (Robinson 1904). Another nomenclature suggested was that of *placentae plicate*, with a rather uncomplicated chorionic epithelium, and *placentae cumulatae*, with a complex trophoblastic structure that included lacunae for maternal blood (Assheton 1906). Contributing to an understanding of the changes in relationship of the uterine decidua and attached fetal structures throughout the course of gestation was the

1901 volume *Human placentation*… by John Clarence Webster (1862–1950) (Webster 1901). Including drawings and over 200 microphotographs, it detailed the gestational decidua with invasion of trophoblast cells of placental villi.

Among the various species, a characteristic feature of the placenta is the number of tissue layers that separate the maternal and fetal blood streams. Of physiologic interest is the extent to which these different tissues determine the placenta's function. For this purpose, the classification of placental types most well known to contemporary students of the subject is that of Otto Grosser (1873–1951) (Fig. 8.2), Professor of Anatomy at the University of Prague. In 1907, Grosser published his exhaustive treatise, *Frühentwicklung Eihautbildung und Placentation* [early developmental formation of membranes and placentation] in which he defined the various chorioallantoic placental types on the basis of the number of tissue layers and cell types that, late in gestation, are interposed between the maternal and fetal blood streams, e.g., *epitheliochorial*, *syndesmochorial*,

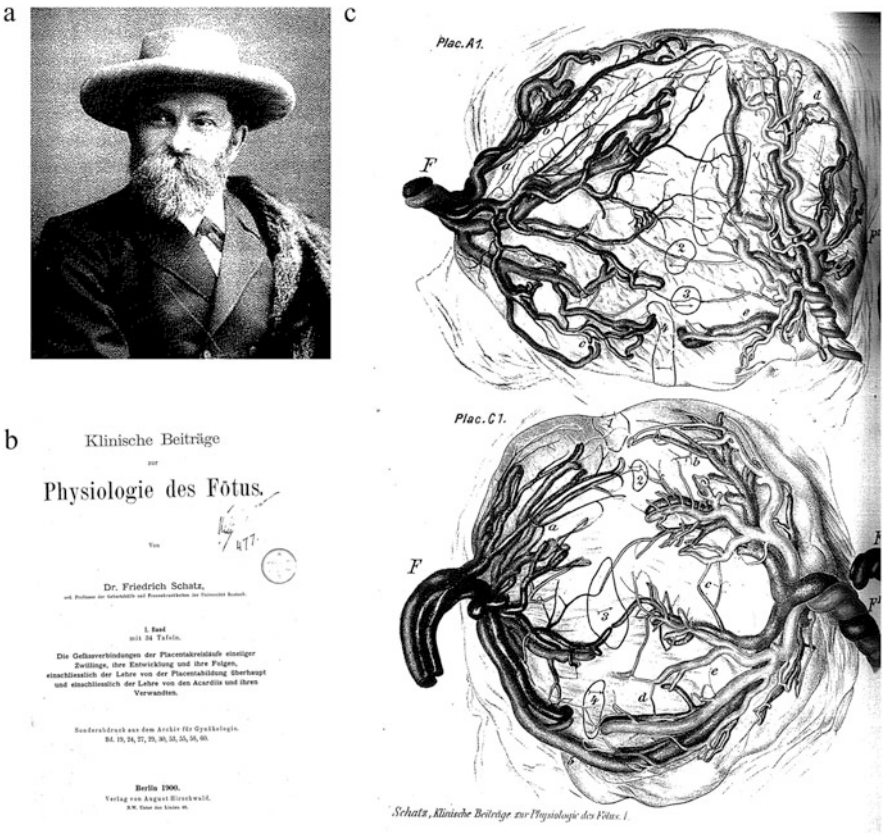

Fig. 8.2 (**a**) Friedrich Schatz (1841–1920). (**b**) Title Page of Physiologie des Fotis. (**c**) Monochorionic "identical" twins

endotheliochorial, *hemochorial*, and *hemoendothelial* (Grosser 1907). Grosser amplified his classification in further publications (Grosser 1908, 1909, 1910, 1927). Table 8.1 details the placental types a la Grosser for several species. Regarding Grosser and his contributions, the embryologist George W. Corner stated (Fig. 8.1c), "... more than any other investigator, [he] has taught us to see in all that [placental] complexity a basically similar pattern in the relation of the maternal to fetal bloodstreams, throughout the mammalian order" (Corner 1963, p. 417). Nonetheless, several considerations have pointed to conceptual problems with Grosser's classification. These include the complexity of the placental barrier in terms of differing mechanisms of exchange, the lack of consideration of the yolk sac, and several aspects of placentation early in development with changes during the course of gestation (Enders 1965a, b; Wislocki 1955). Importantly, contemporary studies using the electron microscope have contributed greatly to understanding placental fine structure and its complexity (Wislocki and Dempsey 1955; Wislocki and Padykula 1961). As Emmanuel Ciprian Amoroso (1901–1982) (Amoroso 1952, 1959a, b; Lawn et al., 1969), Allen Coffin Enders (Carter and Enders 2004; Enders 1965a, b), and others (Björkman 1968) have emphasized, the use of the Grosser classification critically depends upon an understanding of its limitations. For instance, electron microscopic studies have disclosed at least four subdivisions of Grosser's *hemochorial* placental type, e.g., *hemo-trichorial*, *hemo-dichorial*, *labyrinthine hemo-monochorial*, and *villous hemo-monochorial* (Amoroso 1955; Enders 1965a, b).

8.2 Mid-Twentieth Century to the Present: Placental Fine Structure and Function

At mid-century, several issues were paramount in terms of understanding placental morphology and function. An important contributor to an understanding of placental morphology was Harland Winfield Mossman (1898–1991), another member of the Department of Embryology of the Carnegie Institute. In his 1937 monograph on mammalian fetal membranes, Mossman surveyed the placenta and its membranes in several species from both developmental and comparative standpoints (Mossman 1937). Fifty years later, he expanded this treatise into his *Vertebrate fetal membranes* (Mossman 1987). From the standpoint of physiology of the placenta and fetal membranes, this work is important for its presentation of morphologic correlates for biological function. Mossman has described some aspects of these contributions and their impact (Mossman 1991; see also Steven 1975). Further developments concerned elucidation of the fetal component of the placenta suggesting a network configuration of chorionic villi (Stieve 1941). In contrast, the chorionic villi and contained vessels were believed to resemble the limbs and branches of a deciduous tree rooted in the chorion and expanding into the intervillous space (Spanner 1935). An additional issue is that of the relation of

Fig. 8.3 (**a**) James Dixon Boyd (1906–1968). (**b**) William James Hamilton (1903–1975). (**c**) Elizabeth Ramsey (1906–1993). (**d**) Kurt Benirschke

placental to fetal weight. Varying considerably during the course of gestation, at term this normally is about 0.15 ± 0.01. In association with maternal hypoxia or other stress, this ratio usually is increased (see below and Macdonald et al. 2014).

In Great Britain, Amoroso also contributed to knowledge of this subject of early placental development in a number of species (Amoroso 1952, 1955, 1959b), as did Gordon Bourne (Bourne 1962) and James Dixon Boyd (Fig. 8.3a) with William James Hamilton (1903–1975) (Fig. 8.3b) in the human (Boyd and Hamilton 1970). For instance, by examining these tissues in situ attached to the decidua and uterine wall in humans, Boyd and Hamilton traced development of the placenta from the time of implantation to the end of pregnancy. They demonstrated the lack of evidence for Rudolf Spanner's concept of maternal blood flow through the intervillous space; presented evidence for the morphologic changes in the uterine spiral arteries as they course through the decidua, with their invasion of

trophoblastic cells and the development of stromal trophoblastic buds and giant cells; and specialized aspects of chorionic syncytium and the development of interlobular septa. These workers also commenced a statistical analysis of placental and fetal growth (also see Boyd and Boyd 2010). In his Campbell oration to the Ulster Medical Society, Boyd predicted that:

> . . . surely, the placenta will deserve . . . increasing attention, for it is the essential structural basis of the prenatal relationship between mother and child. . . . Derived by differentiation from cells that possess the potentiality of living for seventy, or more, years, its constituents sacrifice themselves after ten lunar months. Built up of disparate cytological elements derived from two heterozygous individuals, it has functions so diverse as to overlap those carried out, in the adult, by lungs, liver, intestinal tract, kidneys, and endocrine glands. . . . For any satisfying explanation of the relation of the unborn child to its mother the darkness of the intra-uterine workmanship must first be made visible and the inscrutability replaced by biological answers to rational questions.
>
> (Boyd 1959b, p. 45)

Other scientists at the Carnegie Institution of Washington who contributed to our current understanding of placental biology include George L. Streeter, George W. Corner, and those who utilized carefully timed pregnancy in the rhesus monkey (*Macaque mulatta*), as well as human tissue, to advance the knowledge of early embryonic development (Corner 1963; Corner et al. 1963; Hartman 1932; Streeter 1920, 1926, 1942, 1945, 1948, 1949; Streeter et al. 1951). At the Harvard Medical School, additional vital contributions to implantation and early embryonic development in humans were made by the anatomist George Bernays Wislocki (1892–1956), the pathologist Arthur T. Hertig, and the obstetrician-gynecologist John Rock (1890–1984) (Hertig 1935, 1945, 1962; Hertig and Rock 1941, 1942, 1943a, b, 1944a, b; Rock and Hertig 1942–1943; Wislocki 1955 1956; Wislocki and Dempsey 1955; Wislocki and Padykula 1961). A number of other individuals have contributed monographs or other writings on placental morphology and its structure (Björkman 1970; Brosens 1965; Gruenwald 1975; Kaufmann and King 1982; Moawad and Lindheimer 1982; Moghissi and Hafez 1974; Strauss et al. 1967; Torpin 1969; Wilkin 1965).

8.3 Some Aspects of the Uteroplacental Circulation and Transplacental Exchange

The miracle of embryonic and fetal development requires the formation of a cardiovascular system in the embryo and fetus and a complex vasculature within both the maternal and fetal side of the placenta (Thornburg and Louey 2013). In regard to the maternal-placental blood flow, several concepts have been suggested (see below). As noted, some of the fetal placental aspects of circulation also had been described (Spanner 1935). The physiological principles that regulate these flows are similar to those of other organs, e.g., driving pressure and vascular resistance. As with other vascular beds, these may, in part, be regulated by a host

of vasoactive chemicals and hormones. (Some aspects of uterine and umbilical placental blood flows are included in the chapter on Maternal Physiology of Normal Pregnancy).

In mammals, the adaptations to pregnancy require sizeable alterations in structure and function of the circulatory system, particularly that of the uterine circulation (Hytten and Leitch 1964; Osol and Mandala 2009; Pijnenborg et al. 2006; Reynolds et al. 2006, 2013). Although maternal red cell mass increases ~30%, that increase of plasma volume is ~50%, resulting in an ~40% increase in blood volume (Longo 1983). In addition to the uterus, remodeling of the human cardiovascular system is accompanied by increases in blood flow to the breast and kidney such that cardiac output increases ~20 to 30% by 30 weeks gestation and then declining somewhat to term (Hytten and Leitch 1964; Longo 1983). The several mammalian species accomplish these changes by different mechanisms, both steroid and protein hormones playing a major role in these changes (Chang and Zhang 2008). Estradiol 17β probably is the most potent steroid in this regulation (Longo 1983; Siiteri and MacDonald 1966), in great part by its stimulation of eNOS with NO production (Rupnow et al. 2001). As would be expected, a number of other hormones and factors play key roles in remodeling of the uterine vasculature (Chang and Zhang 2008; Osol and Mandala 2009; see Chapter on Endocrinology of the Placenta).

As background for an understanding of placental exchange, in his late-eighteenth-century *magnum opus*, the two-volume *Zoonomia* on natural science, bodily functions, and medicine, the British physician, botanist, and poet Erasmus Darwin (1731–1802) devoted a chapter to the placenta (Darwin 1794–1796). Grandfather of both Charles Darwin (1809–1882) and Sir Francis Galton (1822–1911), Darwin appreciated the significance of oxygen, which had been discovered two decades earlier, in respiration of the lungs and fish gills. Observing the change in color from dark to light red as fetal blood passes through the placenta, Darwin postulated that the organ serves as the major organ of oxygenation for the fetus. Erroneously, he believed the fetus' main source of nutrition was the amniotic fluid (Pijnenborg and Vercruysse 2007). It would remain many years before Darwin's enlightened views on placental respiration were more fully valued.

As noted above, the umbilical and placental circulations constitute a shunt in the fetal circulatory system. Derived from the allantois in the early embryo, this is critical for delivery of O_2 and nutrients to the developing organism. As is evident, placental growth, development, and blood flow must keep place with that of the fetus, although the mechanisms by which this synchrony is orchestrated are poorly understood. A rather complex subject in itself, development of this vascular bed has been summarized by others (Anderson and Faber 1984; Burton et al. 2009, 2011; Demir et al. 2006, 2007; Ehrhardt and Bell 1995; Hagen et al. 2005; Hung et al. 2013; Kaufmann and Frank 2004; Kingdom et al. 2000; Reynolds et al. 2005; Vercruysse et al. 2006; Ward et al. 2006). Critical to development of the placenta and its vascular tree is oxygen, hypoxia during its early genesis being associated with increase in a number of growth factors including vascular endothelial growth factor (VEGF), placental growth factor, and others (Ahmed et al. 2000). Rather than being unresponsive, many substances are known to affect placental and umbilical

vessels, and these have been tabulated (Thornburg and Louey 2013). Because of its relevance to complications of pregnancy in the human, a number of "models" of compromised placental blood flow have been described (Gagnon et al. 1994; Giles et al. 1985, 1989; Owens et al. 1986; Robinson et al. 1979; Trudinger et al. 1987). A challenge for the obstetrician-gynecologist-perinatologist, and about which we know little, is to recognize impaired utero- and umbilical perfusion in its early stages. Additionally of importance is the development of therapeutic interventions to correct this pathology.

The major determinant of intrauterine growth is the placental supply of nutrients to the fetus, which occurs primarily by diffusion- and transporter-mediated exchange. These functions depend, in turn, upon the size, morphology, blood supply, and exchange capacity of the placenta, as well as the synthesis and metabolism of nutrients and hormones by the placenta itself. For the physiologist who investigates function at a systems level, the placenta presents distinct problems and is unique in many respects. Essentially every known substance (except perhaps macromolecules) exchanges across the placenta by passive diffusion, facilitated diffusion, active transport, endocytosis, or other mechanisms. In addition, because of its numerous endocrinologic and immunologic functions, for the developing fetus, the placenta serves as a lung, liver, kidney, and so forth. One of the challenges in placental function is that of understanding the mechanisms by which its nutrient exchange is matched to the requirements of fetal growth (Longo 1987). The placenta also presents challenges in terms of the Heisenberg uncertainty principle [named for Werner Heisenberg (1901–1976)], in that the act of quantifying a given function alters the value of the parameter being measured. An example concerns the transfer of specific compounds, ions, carbohydrates, proteins, and so forth from the blood of the mother to that of the fetus (see Dancis 1959; Longo 1972; Meschia 2009; Snoeck 1958; Villee 1960).

A pioneer in these studies was Louis Flexner (Fig. 8.1d) of Johns Hopkins University and the Department of Terrestrial Magnetism, the Carnegie Institution of Washington. With his interest in chemical embryology, stimulated in part by his having spent 9 months working with Barcroft in 1933–1934, with his colleagues, Flexner first used radioisotopes to study placental transfer and, in fact, presented one of the earliest reports of the use of radioisotopes for any biological studies (Flexner and Roberts 1939). In his *Reminisces of early studies of placental function*, Flexner has recounted the manner in which he first used radioisotopes for these studies:

> First of all, I must mention the close relationship that existed at the time between the Department of Anatomy of the School of Medicine, Johns Hopkins University, and the Department of Embryology, Carnegie Institution of Washington. Lewis Hill Weed [1886–1952] was the Director of the Department of Anatomy and additionally was a trustee of the Carnegie Institution. The Department of Embryology, headed by George Linius Streeter [1873–1948], occupied space in a building of the medical school close to the Anatomy Department. My appointment was in Anatomy; my interest at the time was in chemical embryology. I was one of several staff members who felt at home in both departments.

> Each spring, Dr. Streeter arranged a day's program of work in progress in embryology which was attended by Dr. Vannevar Bush [1890–1974], the President of the Carnegie Institution. At the annual meeting in 1937 or 1938, I was invited to talk about work I was doing on the fetal kidney. When the session was over, Dr. Bush came up to me and asked if I'd ever thought of using radioactive isotopes. I remember having answered his question with another, "What's a radioactive isotope?" He quickly made this clear to me, then proceeded to tell me why he had approached me.
>
> The group in nuclear physics at the Carnegie's Department of Terrestrial Magnetism had proposed that the Institution build a cyclotron. Dr. Bush warmly favored the proposal but told me that it was an expensive proposition and that both he and the Trustees of the Institution would find the request easier to meet if the isotopes produced by the cyclotron could be put to biological use. I was left with the invitation to think things over and if I came up with an idea to let him know through Dr. Weed.
>
> (Flexner 1972, pp. 849–850)

Flexner continued:

> The day after my conversation with Dr. Bush, I was in Dr. Weed's office; a few days later, I was in Washington meeting the group in nuclear physics at the Department of Terrestrial Magnetism, in particular, Merle Tuve, Richard Roberts, and Dean Cowie. These physicists could not have been more cordial or more supportive of my interests. At the time, they were dependent upon an electrostatic generator to produce radioisotopes and a string electrometer to measure radioactivity. All limitations considered, they decided that the first effort with my problem should be made with radioactive sodium and, because I lacked an instrument to measure radioactivity, that I should bring pregnant animals from Baltimore to Washington. Shortly afterward, Dr. Roberts and I got down to business, and all went well except for a single episode. I injected precious isotope into an animal which I judged to be at an early stage of pregnancy. When I opened the abdomen and searched for the fetuses, none could be found. And I could not locate a uterus. As has happened often since that time, Dr. Roberts solved the problem. The experimental subject I had chosen was a male.
>
> (Flexner 1972, p. 850)

In part, in an attempt to correlate the Grosser classification of placental layers to function (which proved not to be possible), Flexner and colleagues demonstrated in the rat an increased transfer of sodium and water per gram of placenta as gestation proceeds. They also noted a correlation between the quantity exchanged and the morphologic structure of the placenta (although it is now recognized that factors other than the number of cell layers are probably more important in limiting placental transfer). These workers observed an inverse correlation between the quantities of radioactive sodium exchanged and total fetal mass during the last 4 days of gestation (Flexner and Gellhorn 1942; Flexner and Roberts 1939; Hellman et al. 1948). They concluded: "There is good reason to believe that in the concentrations used here radioactive substances behave like their more common isotopes. This, together with the direct approach which it provides to physiological problems . . . makes the use of radioactive isotopes of unique value in the study of many phases of placental permeability. The method, in addition, is the more valuable because of the ease and exactness of quantitative determinations" (Flexner and Roberts 1939, p. 157). Following the war, Flexner used tritiated water, $^{3}H_2O$, to extend his studies of placental transfer to humans (Flexner 1955; Flexner et al. 1948; Hellman et al. 1948). Introduction of the use of radioisotopes in biological research led to a revolution in the discovery of many details and nuances of

metabolism and other functions, as well as that of the hitherto unsuspected activity and dynamic state of body constituents (for instance, see Davison and Dobbing 1961; Schoenheimer 1946). Jan Job Faber, of Oregon Health Sciences University, has presented a review and critique of Flexner's studies of transplacental clearances (Faber 1999). Flexner also explored the biochemistry of development, neurochemistry, the physiology of the cerebrospinal fluid and meninges, and the basis of memory. Flexner has noted his good fortune to have been "... in just the right place at the right time" (Flexner 1972, p. 850; see also Sprague 1998).

Despite increasing understanding of placental fine structure, the issue of uterine circulation in the intervillous space remained somewhat of an enigma. Following their continued investigation of early embryonic-placental development (Ramsey 1937, 1938), a group of Carnegie Institution investigators, following the lead of Barclay, Franklin, and colleagues several decades earlier, commenced a series of studies in their primates using cineangioradiography to explore this conundrum. Leaders in this endeavor were Elizabeth Maplesden Ramsey (1906–1993) (Fig. 8.3c), Samuel R.M. Reynolds, and their colleagues. Corner has recounted his participation in these studies and in sorting out the mysteries remaining since the time of John Hunter (1728–1793) and his brother William Hunter with Colin Mackenzie (ca. 1715–1775) two centuries earlier, of the "... unexplored maze of the human placenta" (Corner 1963, pp. 417–418). The Carnegie group investigated questions including uterine blood flow regulation and whether, in the human placenta, the pattern of maternal and fetal blood flow was that of a "countercurrent" exchange as had been suggested in studies of the domestic cat (Tafani 1887). This became a topic of interest in comparative placentology, thanks primarily to the histologic studies of Mossman, who demonstrated in the labyrinthine placenta of small rodents and rabbits that maternal and fetal microcirculations appear to run in opposite direction (Mossman 1926).

The physiological relevance of these observations is important as a "countercurrent" exchange pattern is highly efficient, allowing fetal blood exiting the placental capillaries to exchange respiratory gases with maternal arteriolar blood. As a consequence, the oxygen partial pressure of umbilical venous blood would approach that of uterine arterial blood. However, as the human placenta is not labyrinthine, uterine arteries do not carry oxygenated blood all the way to the fetal surface of the organ; rather, that blood is ejected into the intervillous space from the spiral arterial openings in the basal plate. With this arrangement, nonetheless, it would be possible for maternal arterial blood ejected under pressure deep into the fetal surface of the placenta, to be reflected toward the venous openings on the basal plate, creating a stream of blood that runs in opposite direction to that of the fetal blood flowing toward the umbilical vein from the tips of the chorionic villi, which in effect is a countercurrent exchange mechanism (Ramsey 1973).

In the latter nineteenth century, maternal blood was envisaged to enter the intervillous space through orifices high up on pyramidal maternal septa, with drainage returning through venous channels along the placental base, the chorionic villi being bathed in the process (Bumm 1893). A contrasting view postulated maternal arterial entry at the base of the placenta with venous exits only at the

margin, thus effecting an "overflow" type of filling. Entering maternal blood was believed to rise between the septa, which act as dividers, to a subchorial lake from whence it drained into a large circular lake or the marginal sinus. More contemporary methodologies with improved injection materials that permit accurate casts and radioangiographic studies demonstrate an alternate view. In visualizing the intervillous flow pattern in the rhesus monkey, the cineradioangiographic studies of Ramsey and colleagues demonstrated intermittent functioning, with blood being propelled by the *vis a tergo* [force from behind] toward the chorionic villi as arteriolar "spurts" into the intervillous space in a somewhat stochastic process (Brosens et al. 1967, Freese 1966; Martin et al. 1964, 1966; Pijnenborg et al. 1981; Ramsey et al. 1960, 1963, 1967; Sheppard and Bonnar 1974). Ramsey called this intermittent flow, in part a consequence of periodic uterine contraction a "winking and blinking" circulation (Longo and Meschia 2000). In contrast to studies in the rabbit (Faber and Hart 1966) and guinea pig (Moll and Kastendieck 1977), physiological data from the rhesus monkey (Parer and Behrman 1967) and human (Pardi et al. 1992) have failed to provide evidence of countercurrent placental exchange. Another model suggested by histologic studies is the so-called "multi-villous" pattern of intervillous space perfusion (Bartels and Moll 1964). Here, gas exchange has intermediate effectiveness between the countercurrent and concurrent systems, and such a model appears appropriate for the human and other primate placentas.

In concert with these studies, many of which were conducted on the Carnegie monkey colony, a related consideration was that of understanding the more exact anatomic/morphologic relations of maternal and fetal blood flow in the placental exchange area. Among those working on this was Samuel R.M. Reynolds, who had moved to the University of Chicago. Reynolds integrated his and the findings of others of the cotyledonary "structure," with maternal blood from the spiral arteries spurting into the center of an "implanted crown" of anchoring villi, and surrounding third-order umbilical vessels in free villi forming a *tambour* (Bøe 1953; Wilkin 1958). Reynolds also correlated knowledge to that time of the day-by-day and week-by-week morphogenic changes with their functional implications (Reynolds 1966). In an attempt to correlate morphologic features with physiologic functions, Reynolds, working with Roberto Caldeyro-Barcia (1921–1996) and colleagues from the University of Montevideo, Uruguay, and using cinefluoroscopy with the microballoons they had developed to measure uterine myometrial pressures, measured pressures in the intervillous space of the rhesus monkey. They concluded that pressures were highest at the central cavity of the cotyledon, gradually diminishing toward the subchorial lake, supporting the idea of the central entry of uterine blood into the cotyledon intervillous space, and flowing toward the periphery (Reynolds et al. 1968).

Additionally, one must consider the major factors that affect placental O_2 exchange. These include the O_2 partial pressures in maternal and fetal arterial blood, the respective hemoglobin O_2 affinities, maternal and fetal placental blood flows, the anatomical-spatial relations of these blood flows in the exchange area, the placental diffusing capacity, and others (Longo 1987). These are presented in

Table 8.2 Principal factors affecting placental O_2 transfer

Variable	Associated components
Placental diffusing capacity (Dp)	Membrane diffusing capacity (area, thickness, solubility, diffusivity of tissue), capillary blood volume, diffusing capacity of blood (O_2 capacity, Hb reaction rates, concentration of reduced Hb)
Maternal arterial PO_2 (Pma_{O2})	Inspired PO_2 alveolar ventilation, mixed venous PO_2, pulmonary blood flow, pulmonary diffusing capacity
Fetal arterial PO_2 (Pfa_{O2})	Maternal arterial PO_2, maternal-placental Hb flow, placental diffusing capacity, umbilical venous PO_2, fetal O_2 consumption, peripheral blood flow
Maternal Hb-O_2 affinity (Pm_{50})	pH, temperature, P_{CO2}, 2,3-diphosphoglycerate concentration, CO concentration
Fetal Hb-O2 affinity (Pf_{50})	pH, temperature, P_{CO2}, 2,3-diphosphoglycerate concentration, CO concentration
Maternal placental Hb flow rate ($\dot{Q}\,m_{Hb}$)	Arterial pressure, placental resistance to blood flow, venous pressure, blood O_2 capacity
Fetal placental Hb flow rate ($\dot{Q}\,f_{Hb}$)	Umbilical artery blood pressure, umbilical venous blood pressure (or maternal vascular pressure under conditions of sluice flow), placental resistance to blood flow, O_2 capacity of blood
Spatial relation of maternal-to-fetal flow	
Amount of CO_2 exchange ($\dot{V}\,co_2$)	

PO_2 and P_{CO2}, partial pressures of O_2 and CO_2, respectively; Hb, hemoglobin
Adapted from Longo (1987)

Table 8.2. Also of importance is the extent to which the placenta is a substantial barrier limiting exchange between the maternal and fetal circulations. This is of particular relevance to the respiratory gases O_2 and CO_2 and the extent to which this possible barrier increases during the course of gestation. As noted earlier, an original concept was that near term the fetal arterial tensions fell, so that the newborn's first breath was the fetus' "dying gasp." A thoughtful placentologist and early member of the "Barron School," André Eugène Désiré Joseph Hellegers (1926–1979) of Georgetown University, reviewed the development of ". . . opinions about the placenta as a barrier to oxygen." Presenting a Jesuitically reasoned analysis of thesis and antithesis, he examined the idea of the fetus being at "Mt. Everest in utero," with the placental barrier being a "hazard" to the developing conceptus (Hellegers 1969/1970). In addition to a number of other contributions to placental physiology, Hellegers also was a leader in the development of bioethics in America (see below). By measuring Po_2 values in uterine and umbilical veins, several investigators calculated the mean maternal-to-fetal O_2 tension difference to equal 40–50 Torr (Barron 1946; Barron and Alexander 1952; Metcalfe et al. 1967). This suggested that the placenta was a significant barrier to diffusion and was associated with the idea that fetal umbilical Po_2 and oxyhemoglobin saturation values fell dramatically as term approached (Bartels et al. 1962). In near-term

pregnant ewes, the Barron group with Giacomo Meschia also performed the first studies of placental to fetal O_2 exchange at high altitude, on the *alto plano* of Peru. Of interest, at this altitude fetal blood gas values (Metcalfe et al. 1962a), blood volume (Prystowsky et al. 1960), and weights (Metcalfe et al. 1962b) did not differ significantly from those in their near sea level laboratory in New Haven. As noted, in part, these findings also gave rise to the view of the fetus being at "Mount Everest *in utero*" (Eastman 1954), and the "first breath of the newborn" being the "dying gasp of the fetus" (see below). Subsequent studies using carbon monoxide to measure the placental diffusing capacity (a measure of exchange) indicated that the mean maternal-to-fetal Po_2 difference equals only 5–6 Torr (Longo et al. 1967, 1969). This would allow virtual equilibration of maternal and fetal O_2 tensions within the placental microcirculation and suggest that the placenta is not a functional barrier for respiratory gas exchange (Longo 1987; Meschia 2009; Wilkening and Meschia 1992). Table 8.3 presents the normal blood gas and pH values in both maternal and fetal arterial and venous blood (Longo 1987).

A related issue of fundamental interest is that of uneven perfusion of the placenta, somewhat similar to that in the lung. Such nonuniform perfusion can result in unequal oxygenation of fetal blood in portions of the placental microcirculation, so that the blood returning to the fetus via the umbilical vein results from the mixing of blood streams with different oxyhemoglobin saturations. Given the shape of the oxyhemoglobin saturation curve, this mixing would yield an oxygen partial pressure in the collecting vein that is biased toward the lowest Po_2 values in the mixture (Longo and Power 1969; Power and Longo 1969). Ramsey's visual demonstration of nonuniform "winking-blinking" placental perfusion has been substantiated by the use of radioactive microaggregates and of microspheres into the maternal and fetal sheep circulations to show a nonuniform distribution of both maternal and fetal placental blood flows and of the maternal/fetal blood flow ratio (Power and Jenkins 1975; Power et al. 1967, 1972, 1981).

Table 8.3 Normal values of O_2, CO_2, and pH in human maternal and fetal blood

	Maternal uterine		Fetal umbilical	
	Artery	Vein	Vein	Artery
PO_2 (Torr)	95	38	30	22
HbO_2 (% saturation)	98	72	75	50
O_2 content (ml dl^{-1})	16.4	11.8	16.2	10.9
O_2 content (mM)	7.3	5.3	7.2	4.5
Hb (g dl^{-1})	12.0	12.0	16.0	16.0
O_2 capacity (ml dl^{-1})	16.4	16.4	21.9	21.9
O_2 capacity (mM)	7.3	7.3	9.8	9.8
PCO_2 (Torr)	32	40	43	48
CO_2 content (mM)	19.6	21.8	25.2	26.3
HCO^-_3	18.8	20.7	24.0	25.0
pH	7.42	7.35	7.38	7.34

$PO_2$and PCO_2, partial pressures of O_2 and CO_2, respectively; Hb, hemoglobin
Adapted from Longo (1987)

The evolution of ideas about respiratory gas exchange in the placenta has been characterized by a continuous effort to identify the factors of major importance in oxygenation of the fetus. Much of this effort has been in determining the placental diffusing capacity for O_2 (Bacon et al. 1984; Longo et al. 1967, 1969). Without doubt, the critical factors are uterine-placental and umbilical blood flows, maternal and fetal arterial oxygen levels, and placental diffusion characteristics (Longo 1987). Additional considerations of placental physiology, structure, blood flows, maternal-to-fetal exchange, and perfusion techniques have been reviewed by Job Faber and Kent Lu Roy Thornburg of the Oregon Health Sciences University (Faber and Thornburg 1983). In an attempt to understand more completely the physiologic basis and rate limiting factors of respiratory gas exchange in the placenta, a number of authors have developed mathematical models of this process (Bartels and Moll 1964; Bartels et al. 1962; Faber 1977; Faber and Anderson 1990; Faber and Hart 1966; Hill et al. 1972, 1973; Laga et al. 1973; Longo 1987; Longo et al. 1972; Mayhew et al. 1990, 1993; Power and Longo 1969; Power et al. 1972). In a review of these models, the role of placental morphology has been shown to play a major role in the rate limiting factors in respiratory gas exchange (Filoche et al. 1985; Wilson and Ford 2001). These authors also have reviewed several of the challenges that face the experimental investigator of placental function, e.g., species differences in anatomy, physiology, and other aspects, size limitations for given studies, availability of experimental animals, and others, so that "... imperfect comparisons between animals and humans must suffice" (Faber and Thornburg 1983, p. xv). Several symposia also review these and other issues (Wallenburg et al. 1981; Young et al. 1981).

Mid-twentieth century saw a transition from the Carnegie era of embryology to a more contemporary period of other aspects of developmental physiology. A pioneer in exploring the placental maze and gaining an understanding of the development of regulation of growth of trophoblast, particularly in regards to its relation to blood flow and oxygenation, is Graham J. Burton of Cambridge University (Burton et al. 2011; Kato et al. 2017). In response to a query regarding his work, Burton has written:

> Careers often hinge around chance encounters and opportunities. My interest in the placenta was first kindled when I was allocated a research project under the supervision of the comparative placentologist Donald Steven (1975) as part of the third year of my undergraduate degree in Medical Sciences at the University of Cambridge. The project focused on the haemophagous zone of the sheep placenta, where maternal erythrocytes are phagocytosed and broken down by the trophoblast as a mechanism for the transport of iron. This study led to my first publication in 1976 (Burton et al. 1976), but more importantly the exposure to different placental structures and different pathways for transport was to prove critical for the interpretation of human data I acquired over 20 years later.
>
> After qualifying in clinical medicine I returned to Cambridge in 1978 as a junior lecturer in the Department of Anatomy, and have remained at the University ever since. Having been exposed to clinical obstetrics my interests turned towards the human placenta, and in particular the vascular architecture of the fetal villi and how that might be affected by oxygen availability. Many morphological studies at that time were purely descriptive, but the techniques of stereology were being developed and along with Terry Mayhew I applied these to quantify the vasculature and estimate the theoretical diffusing capacity of the

placenta in different pathological conditions (Mayhew and Burton 1997). I was fortunate to collaborate with Olga Reshetnikova who had recently been on a Russian expedition to Kirghizia where they had collected placental samples from different altitudes. Olga brought samples to Cambridge (Reshetnikova et al. 1994), and this sparked an interest in high altitude biology that continues today. My involvement in stereology also led to the most fruitful and enjoyable collaboration of my career, that with Eric Jauniaux.

Eric, a scientific Belgian obstetrician with a PhD in placental pathology, was interested in early placental development, and had been involved in the first study to measure the oxygen concentration within the placenta *in vivo* (Rodesch et al. 1992). Their data indicated a dramatic increase at the end of the first trimester, and Eric wanted to test whether there were any contemporaneous changes in placental structure using stereology. Whilst on a research fellowship in the UK he spent some time in Cambridge analyzing his samples, and then our paths separated for a year or so. The discussions on the oxygen environment lodged in my mind, however, and returned to the fore when I was attempting to develop a model of wound healing in the placenta in order to investigate vertical transmission of the HIV. A major problem was that the syncytiotrophoblast degenerated rapidly *in vitro*, independent of the culture medium and supplements. Oxygen free radicals were a hot topic at that time, and so I decided to look at antioxidant defences and to culture explants under different oxygen concentrations. Amazingly, the defences were low and culture under low oxygen extended the life of the syncytiotrophoblast (Watson et al. 1997, 1998), findings consistent with hypotheses based on Eric's clinical observations.

We then collaborated for many years, Eric measuring the oxygen tension in the first trimester placenta and my group performing molecular analyses of antioxidant defences and oxidative stress in the placental tissues. Our findings supported the earlier work of Hustin and Schaaps (Hustin and Schaaps 1987) and Rodesch (Rodesch et al. 1992), and reinforced the concept that the maternal arterial circulation to the human placenta is not fully established until the end of the first trimester (Burton et al. 1999; Jauniaux et al. 2000, 2001). This concept was hotly contested when first presented, with many questioning how the embryo could possibly survive that long without a maternal circulation. Again, good fortune was on my side, for James Dixon Boyd the eminent embryologist had been Professor of Anatomy in Cambridge, and, despite much pressure over the years, the bulk of his collection of placenta-in-situ slides remains in the department. Aware of other means of fetal nutrition from my days of comparative placentation, I searched serial sections and found examples of the endometrial glands discharching their secretions into the intervillous space (Burton et al. 2002), evidence of histiotrophic nutrition that is the norm for most mammals during the period of organogenesis. Interestingly, Boyd had observed such connections with the glands (Boyd 1959a), but without the benefits of modern imaging had not grasped their full significance. He did surmise, however, in a wonderfully prescient review lectures at his *alma mater* in 1959 that the 'early conceptus is under conditions of distinct anaerobiosis' (Boyd 1959b).

Since then, we have refined our concepts, providing more details of when and how the maternal circulation starts and its influence on remodeling of the primitive placenta into the definitive organ (Jauniaux et al. 2003). We have also shown the reliance of the early placenta on phylogenetically old metabolic pathways that maintain intracellular energy levels despite the low oxygen environment (Cindrova-Davies et al. 2015; Jauniaux et al. 2005). This work continues, and we are currently investigating the endometrial-fetal dialogue that stimulates early placenta development (Burton et al. 2007).

Our finding that low oxygen favours trophoblast development heavily influenced my thinking regarding placenta pathologies, and led me to propose that it is fluctuations in oxygenation, or hypoxia-reoxygenation, that is more damaging than hypoxia alone. This concept was developed further by an obstetrician graduate student, Tai-Ho Hung (Hung et al. 2001, 2002), and later in collaboration with Steve Charnock-Jones and two highly industrious research associates, Hong-wa Yung and Tereza Cindrova-Davies

(Cindrova-Davies et al. 2007). The secturity provided by two Wellcome Trust programme grants enabled us to follow exciting new leads, identifying endoplasmic reticulum stress as a major contributor to placental growth restriction and early-onset pre-eclampsia in the process (Yung et al. 2008, 2014). We have also explored these molecular pathways in high-altitude samples (Colleoni et al. 2013; Yung et al. 2012). Together, these analyses demonstrate a spectrum of changes from physiological homeostatic responses at altitude to frankly pathological in growth restricted placentas. The challenge now is to translate these findings into therapeutic interventions.

It has been a long journey from the sheep haemophagous zone to ribosomal complexes during endoplasmic reticulum stress, with unexpected interesting diversions to be negotiated along the way. Sample availability has been a constant challenge, but otherwise it was made possible by some highly enjoyable collaborations and generous funding, for both of which I am most grateful. The placenta truly is a fascinating organ that demonstrates many unique aspects of biology, and I commend it to any young investigator starting out in their career.

(Letter from GJB to LDL 23 June 2015)

As the primary interface between the mother and fetus, the placenta plays a vital role in nourishing and maintaining optimal fetal growth and development by facilitating the transfer of essential substrates. Thus, yet another area of consideration, and one that could require an entire chapter, is that of the placental transfer of the sugars, amino acids, and other substrates as well as compounds such as iron, copper, and zinc (Battaglia and Meschia 1978; Hill and Young 1973; Yudilevich and Sweiry 1985). Although strictly speaking not entirely from an historical perspective, in an effort to understand the basis and complexity of the processes, some of these will be noted briefly (Table 8.4).

Transplacental glucose and amino acid uptake (as that for other substrates) are dependent upon three steps: uptake from maternal blood by transporters in the microvillous membrane of the syncytiotrophoblast, movement across the cell cytoplasm and their transport across the fetal-facing syncytiotrophoblast basement membrane and cytotrophoblast into the fetal blood. The level of expression of nutrient transporters per unit of surface area is a key factor in this regard. In turn, the activity of the several transporters (Sibley et al. 2005) can be influenced by a wide variety of environmental factors, including hypoxia, under- or overnutrition, placental hormones such as insulin-like growth factors, glucocorticoids, and so forth (Fowden et al. 2008; Jones et al. 2007). For glucose, availability of this primary nutrient for the placenta and fetus depends upon the maternal circulation (Hay 2006; Lager and Powell 2012). Placental glucose uptake and transport occurs down a concentration gradient, where it crosses the placenta by facilitated diffusion (Widdas 1952). This exchange is mediated by a family of sodium-independent glucose transporters (GLUTs) encoded by 14 different genes (Novakovic et al. 2013) that are present in the plasma membrane on both sides of the syncytiotrophoblast (Lager and Powell 2012). The human and sheep placentas have two primary glucose transporters GLUT-1 and GLUT-3 (both of which are insulin independent), with less abundant members GLUT-4 and GLUT-8. In human trophoblast cells GLUT-1 expression is greater on the microvillous than the basal membrane side of the cell, which may suggest that the basal membrane is the rate limiting factor in fetal glucose uptake (Jones et al. 2007; Larqué et al. 2013). In

Table 8.4 Summary of experimental models of placental insufficiency and their impact on the placenta and the fetus

Experimental intervention	Impact on the fetus	Impact on the placenta
Surgical umbilical artery ligation (SUAL)	Isolation and ligation of one hypoxemia umbilical artery close to the IUGR fetal abdomen	Placental infarction causing ↓ umbilical blood flow and ↓ placental substrate transfer
Maternal hyperthermia	Exposing of the pregnant ewe to an environment with an increased IUGR ambient temperature	↓ Uterine artery flow and ↓ placental weight due to ↑ maternal temperature
Placental embolism	Repeated injection of microspheres (15 μm) into the placenta via the hypoxemia Umbilical artery through a catheter hypoglycemia Implanted in the descending aorta or IUGR fetal umbilical vein	Block placental capillaries causing ↓ placental surface area
Uterine carunclectomy	Surgical removal of the majority of hypoxemia the endometrial caruncles from the hypoglycemia uterus of nonpregnant ewes prior to IUGR conception	↓ Placental weight due to ↓ placentomes

sheep, placental GLUT-1 mRNA expression increases throughout gestation, peaking at 120 days in singleton and 140 days in twin pregnancy (Dandrea et al. 2001). In both humans and sheep, GLUT-3 is expressed throughout pregnancy but decreases near term, suggesting a greater role in early- to mid-gestation (Brown et al. 2011; Novakovic et al. 2013). Other evidence suggests that GLUT-3 may be a key transporter in tissues in which glucose is the primary metabolic substrate (Brown et al. 2011).

In general, fetal plasma amino acid concentrations exceed those of the mother reflecting an active transport system of more than 20 different transporters of several systems (Grillo et al. 2008; Lager and Powell 2012; Regnault et al. 2005a, b; Roos et al. 2009). Neutral amino acid transporters, such as those of System A, facilitate the uptake of small, nonessential amino acids (alanine, glycine, serine) concurrently with the uptake of sodium and are in greater concentration in the microvillous plasma membrane. System A consists of three sodium-coupled transport proteins (SNAT-1, SNAT-2, and SNAT-4) encoded by independently regulated genes. Throughout gestation, the activity of System A transporters increases although the individual contribution of the three transporters varies (Lager and Powell 2012). System L amino acid transporters are sodium-independent exchangers of neutral amino acids. Nonessential amino acids are

exchanged for those that are essential with aromatic or branched side chains (leucine, phenylamine) to permit transport against their concentration gradient (Verrey 2003). Cationic amino acid (glutamate, aspartate) transporters (CAT-1, CAT-2, and CAT-4) are present in both the microvascular and basal membranes of the placenta (Ayuk et al. 2000). In turn, several members of anionic amino acid transporters are present in the placenta and appear to increase during the last trimester of gestation (Matthews et al. 1998). These amino acid transporters may be altered significantly in cases of fetal growth restriction (De Vrijer et al. 2004).

Free fatty acids are important precursors of a number of bioactive molecules that are structural components of cells and provide a major source of energy (Lager and Powell 2012). Both esterified fatty acids present as triglycerides, and non-esterified fatty acids are taken up by the placenta, the former increasing during the last third of gestation (Haggarty 2010). Placental fatty acid transfer (particularly of those that are non-esterified) occurs by both simple diffusions (Haggarty 2010). Nonetheless, in late gestation this route may be inadequate for the amounts required, and the syncytiotrophoblast membrane proteins are vital for the uptake of long chain fatty acids (Kazantzis and Stahl 2012). In humans, five fatty acid transport proteins (FATP), FATP-4 and FATP-6, are present on both the microvillous and basal membranes (Campbell et al. 1998). A fatty acid translocase (CD-36) and several fatty acid-binding proteins (FABP, FABP-1, FABP-3, FABP-4, FABP-5) are present in the human placenta (Biron-Shental et al. 2007), and some reside in the murine placenta (Mishima et al. 2011). As may be imagined, each of these molecular species play a vital role in orchestrating optimal maternal-fetal fatty acid exchange and transport, albeit they may play differing roles during the course of gestation.

Of relevance is the role of the placental genes in determining not only the phenotype for the placenta per se (size, morphology including ultrastructure, function, and so forth) but for that of the fetus and its long-term programmed life course as a newborn and adult (see below and Allen et al. 2002; Fowden 2003; Fowden et al. 2006a, b; Heasman et al. 1999; Mellor 1983; Sibley et al. 1997, 2005; Vaughan et al. 2011). These and other contemporary aspects of the biology of the placenta are reviewed in the volume *The Placenta and Human Developmental Programming* (Burton et al. 2011).

During the past decade or so, a considerable body of evidence has demonstrated the vital importance of imprinted genes and epigenetic regulation in normal development and cellular function (Reik and Walter 2001; Reik et al. 1987). In particular, this includes the development and differentiation of various facets of placental growth and metabolism (Ferguson-Smith et al. 2006; Frank et al. 2002; Haig and Graham 1991; Li and Behringer 1998; Moore and Haig 1991; Zechner et al. 2002), as well as that for the fetus (Rahnama et al. 2006; Smith et al. 2006). Apparently unique to mammals, genomic imprinting is the epigenetic-mediated differential modification of the maternal and paternal genetic contributions to the zygote, resulting in the differential expression of parental alleles (e.g., one allele functioning with the other silenced) during development and in the adult (Jablonka and Lamb 2002; Monk 1988; Murrell et al. 2005). For the most part, paternally

imprinted genes enhance, whereas maternally expressed genes suppress, placental growth. The opposite is the case for fetal tissues (Reik and Walter 2001; Tycko and Morison 2002). Imprinting is regulated by epigenetic marks in the genomic imprint control region. These markings are inheritable and result in monoallelic, parent of origin-dependent gene expression. Rather than a single gene, the imprint control regions can regulate the expression of a cluster of genes which can increase in several phylogenetic types. Each imprinted gene network can, in turn, regulate a network of downstream genes engaged in specific cellular functions (Sandhu 2010). A majority of studies on imprinted genes have been conducted in rodents (Hudson et al. 2010; Stadtfeld et al. 2010).

Examples of imprinted genes that affect placental (and fetal) development include the paternally expressed *Igf2* (Baker et al. 1993; Constância et al. 2002), *Mest*, *Peq1*/*Mest* (Lefebvre et al. 1998), *Peg3* (Li et al. 1999), *Phlda2*/*Ip1* (Frank et al. 2002), *Ins1*, *Ins2*, and others, several of which are clustered on mouse chromosome 7. *Phlda*/*2* has been shown to act as a rheostat for placental growth, with fetal IUGR following loss of imprinting and overgrowth after gene deletion (Salas et al. 2004). Those maternally expressed imprinted genes include *Igf 2r*, *H19*, and *Grb10* (Fowden et al. 2006a). Targeted mutation of a paternally expressed imprinted gene has been shown to regulate multiple aspects of placental and fetal development (Curley et al. 2004; Drake and Walker 2004; Reik 2007). The role of imprinted genes that regulate amino acid transport in the murine placenta (*Slc2a* and *Slc38a4*), with genes that regulate growth of the fetus (*Igf2*), also has been described (Constância et al. 2005). The imprinted *Igf-H19* complex plays a critical role in placental nutrient exchange by its modulation of expression and activity of placental-specific amino acid transporters (Fowden et al. 2006a). Of importance, placental imprinted gene products can modulate fetal nutrient supply and growth both by regulating optimal growth and development of all or part of the placenta and by its exchange of nutrients. Examples of genomic imprinting also include X-chromosome inactivation in female mammals (Willard et al. 1993). A complication in the identification of placental imprinted genes is that their expression can vary with sampling site as well as mode of delivery (Janssen et al. 2015). This reinforces the importance of optimizing and unifying collection protocols for studies of the placenta. As may be surmised, a multitude of other genes are involved in development of trophoblast cells and the placenta, the expression of which can change dramatically during the course of gestation (Knerr et al. 2005; Stecca et al. 2002).

Further contributions to understanding placental transport mechanisms, the relation of fetal growth restriction to placental phenotype, the role of imprinted genes in the placenta, and other vital issues have been made by Colin Peter Sibley of the University of Manchester. He has written:

> I became interested in, and started working on, the placenta by serendipity. My PhD work at Queen Elizabeth College at the University of London was on the mechanisms of secretion of steroid hormones by the adrenal cortex. However, the Chair of my department at the time was David Yudilevich (1930–2006) whose interests on nutrient transport mechanisms included those in the placenta. David's laboratory was next to mine and I spent a lot of time rubbing shoulders with his postdocs and PhD students, from whom I started to

understand what a fascinating, and poorly understood organ the placenta was. The Yudilevich laboratory members were particularly experienced in *in vivo* techniques and shared this experience with me whilst I developed a technique for perfusing the rat adrenal cortex *in vivo*. It therefore seemed like fate when, at the end of my PhD work I saw an advert for a postdoctoral position in Tony Firth's laboratory at St. Georges Hospital Medical School for someone to use *in vivo* techniques to investigate the permeability of the capillary endothelium of the guinea-pig placenta. My three years at St. Georges went well, I published my first papers on the placenta, and I was hooked! Both the undoubted importance of the organ and the fact that there was so much important work to be done to understand it enticed me. In this regard the final piece of luck for me was when Robert Boyd (now Sir Robert) who had recently moved to the University of Manchester to become Chair of the Department of Child Health, advertised a Lectureship position for a physiologist with an interest and track record in placental research. The list of suitable candidates must have been very short. I was appointed to this tenured position and with that security was able to build a group with Robert, focusing on mechanisms of placental solute exchange.

In the thirty years or so that I have worked on the placenta, the field has made great progress. This has been aided enormously by the desire and willingness of placentologists to work together and share ideas and data in the best open traditions of science. We all realize that understanding of the organ is hampered by the relative low numbers of researchers in the field e.g. our annual meeting of the International Federation of Placenta Associations attracts at most 300 to 400 attendees. I understand that 10 to 100 times this number of people attend meetings on adult organs, the functions of which the placenta subserves for the fetus; kidney, lung, gut, and others. Why this lack of investigators in the field? I believe there are a number of reasons, large and small, including: a failure until recently to understand that poor, and devastating, pregnancy outcomes such as stillbirth are in many cases caused by placental dysfunction; the terminology—a career studying the 'afterbirth' might not seem so attractive when making choices about research field; of course a lack of money in the field, compared to others, is a major factor and in itself probably has many causes. I believe that key amongst these, however, is the lack of willingness of the pharmaceutical industry because of the risk/cost ratio of such work to devote time and funding to finding treatments for pre-eclampsia and fetal growth restriction, major killers of mothers and babies with their roots in placental dysfunction.

I do believe that placentology has turned a corner in terms of its impact. Thanks to the work of many there is now a solid evidence base of knowledge of placental structure and function. My own group has contributed to this knowledge through (1) demonstrating that there are gestational changes in the activity of nutrient transporters in the placenta in order to meet changing fetal demands (Glazier et al. 1992; Sibley et al. 2004); (2) that there are specific changes in the transfer capacity of the placenta to enable supply to meet fetal demands (Constância et al. 2002, 2005; Godfrey et al. 1998); (3) changes occur in transporter activity in the placenta when a fetus is growth restricted, some of which are causal (e.g. decreased activity of the System A amino acid transporter (Mahendran et al. 1993; Glazier et al. 1997), some of which are adaptive (e.g. calcium transport; Dilworth et al. 2010) and that these are a component of the different placental phenotypes in fetal growth restriction (FGR) (Sibley et al. 2005); finally (4) we have recently contributed to the demonstration that genetic mouse models of pregnancy diseases such as FGR can recapitulate the phenotypes of the human disease and provide preclinical models in which to test potential therapies (Dilworth and Sibley 2013; Stanley et al. 2012).

Through our work, and that of many others, there is now a clear understanding that placental dysfunction is at the root of pregnancy complications and that diagnosing the dysfunctional placenta *in utero*, with a mix of techniques, including measurements of placental hormones in maternal blood and ultrasound and magnetic resonance imaging, will enable a major improvement to obstetric are pathways. It is also encouraging that there now are clinical trials in progress for drugs (sildenafil, melatonin), repurposed from their

> original pharma origins, that focus on placental diseases. Data from this diagnostic and treatment work will inform that of the laboratory scientists and create a virtuous circle in understanding placental function. The next ten years will see a major focus in biomedical science on stratified and personalized medicine. Placentology is well placed to address these challenges for obstetrics. With increasing knowledge of the particular placental phenotype that leads to a particular pregnancy complication there will be an increased understanding of how the placenta normally functions to ensure the perfect pregnancy outcome for most women.
>
> (Letter from CPS to LDL, 8 February 2015)

8.4 Pathology of the Placenta

Although this volume consists of a series of essays on physiology, pathologic changes may lead to profound physiologic dysfunction. The vista of placental pathology and its implications are reviewed in several volumes including *Pathology of the Human Placenta* by Kurt Benirschke (Fig. 8.3d) and Shirley Driscoll, at that time of the Dartmouth and Harvard Medical Schools, respectively (Benirschke and Driscoll 1967; see also Benirschke 2002). Another valuable volume *Pathology of the Placenta* was edited by Harold Fox (1931–2012) of the University of Manchester (Fox 1978). In his *Physiologie des Fötus*, Friedrich Schatz (Fig. 8.2a) presented in detail many aspects of the twin to twin transfusion syndrome with its placental pathology and consequences for the fetal pair (Schatz 1900) (Fig. 8.2c). To a great degree, this 712-page volume consists of a series of papers that he had published previously in the *Archiv für Gynäkologie*. It includes 34 full or double-page plates, many in color, which depict the placental vascular anatomy of monochorionic "identical" twins. In his analysis, Schatz presented an idealized schema of the placenta with central insertion of the umbilical cord and in a meridian-like fashion the alternating arteries and veins passing centrifugally to the placental margin. By performing careful injections of different colored wax Schatz, recognized that most of these cases had an umbilical arteriovenous anastomosis between the placental circulations of the two infants. He demonstrated that in many instances, branches of an artery and vein would delve deep into the placenta, into what he termed a "third circulation" through a "villous district" of the cotyledon. Usually these could not be seen directly from the fetal surface. Schatz previously had reported on such a case of placental anastomosis in twins (Schatz 1875) and later explored this concept more fully (Schatz 1886; see Ludwig 2006).

In such cases the twin's fate depends on the nature of these vascular connections, with the recipient twin being grossly enlarged and edematous with elevated hemoglobin concentration and hematocrit, enlarged organs, and a distended bladder. In turn, the donor twin is small and shriveled with an empty bladder. Some of these cases also demonstrated gross malformations. Because of the recipient twin's elevated urine output, these cases often are associated with maternal polyhydramnios and the onset of premature labor (Benirschke 1958, 1961; Bergstedt 1957; Falkner et al. 1962; Sacks 1959). In a series of reports, the

pathologist Richard L. Naeye of the University of Vermont detailed many consequences for the fetus of the twin to twin transfusion syndrome, including major differences in the relative sizes of various body organs (Naeye 1963, 1964a, b, 1965). For instance, in a study among five twin pairs born from 27 to 30 weeks gestation, the body weight difference between recipient and donor was 294 g. For two pairs born near term, this difference averaged 847 g (Naeye 1963). The recipient twin also demonstrated greater muscle mass about both pulmonary and systemic arteries, as well as significantly larger renal glomeruli (Naeye 1963). In another report of ten such twin pairs from 23 to 30 weeks gestation (which included several pairs from the previous report), not only was there a discordance of about 200 g in body weight, but significant weight differences were seen in the brain, heart, lungs, liver, and other organs. At the cellular level, the number and size of cardiac myocytes were increased significantly in the recipient twin (Naeye 1965). Twin to twin transfusion syndrome has been identified as the most important determinant of growth disparity between identical twins.

8.5 The Human Placental Project

In recognition of the critical importance of the placenta in maternal, fetal, and offspring long-term well-being, in May 2014, leaders at the Eunice Kennedy Shriver NICHD convened a 2-day meeting on the projected 10-year Human Placental Project to brainstorm on several goals. These were to improve current methods and develop new technologies for assessment of placental development, structure, and function, to apply these technologies to real-time evaluation and normal and abnormal placental development and function, to develop and evaluate noninvasive biomarkers for prediction of adverse placental outcomes, to decode the contributions of placental development and function to offspring long-term health and disease, and to develop interventions to decrease the occurrence and severity of placental abnormalities and thus enhance pregnancy and lifelong health (Guttmacher et al. 2014; Guttmacher and Spong 2015; Sadovsky et al. 2014). A second such workshop was held in April 2015 (Guttmacher and Spong 2015) and others are planned. It will be of great interest to follow the developments of this Human Placenta Project. As with the Human Genome Project, one can predict that the use of genomics, other "omics," and imaging technologies will lead to new ideas that will revolutionize maternal and child health.

8.6 Summary and Conclusions

In conclusion, the placenta constitutes the major interface between the mother and fetus. Optimal placental function, maternal and fetal blood flows, its transport of nutrients, and production of hormones ensure adequate substrates for appropriate

growth and development of the fetus. Challenges for the future include the use of advanced microscopy to allow further understanding of the ultrastructure of the human placenta and that of other species. Advances in genomics, epigenomics, and proteomics will see greater understanding of the metabolic, hormonal, immunologic, and other aspects of placentology, and the role that imprinting and these functions play in embryonic and fetal development. Only as endocrinologists, physiologists, biochemists, cellular and molecular biologists, and other scientists work to untangle the "Placental Maze" (Corner 1963), from the nuances of implantation to full development, will we come to a deeper understanding of the mysteries and wonders of the development and function of that wondrous "Bundle of Life" and "External Soul" (Longo and Reynolds 2010).

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Chapter 9
Some Aspects of Endocrinology of the Placenta

Natural science does not simply describe and explain nature; it is part of the interplay between nature and ourselves; it describes nature as exposed to our method of questioning.
(Werner Heisenberg 1959)

9.1 Introduction

The production of hormones that occur in pregnancy and their changes are the most remarkable recorded in mammalian physiology or pathophysiology. These include those of the maternal pituitary gland, adrenal glands, and the thyroid and in particular those of the placenta. Metabolic homeostasis of the pregnant mother and growth of the fetus depend upon combined precise regulation of maternal nutrient storage, its mobilization with placental growth, nutrient transport, and fetal uptake and utilization. Critical to these processes, in addition to placental steroid production, are the placental somatogenic and lactogenic hormones with those of the maternal pituitary gland act in concert to integrate the metabolic demands of pregnancy with those of fetal (and later neonatal) development. Dysregulation of these functions may reflect and/or exacerbate placental dysfunction with long-term effects on the offspring's growth and metabolic function. Aspects of placental endocrinology and maternal and fetal well-being are described in several other chapters.

With blossoming of the new science of endocrinology in the early 1900s (Goldzieher 1939; McCann 1988; Medvei 1982; Rolleston 1936), the placenta quickly was recognized as an endocrine organ with activities similar to those of both neurohypophyseal and ovarian hormones (Phillipp 1930; Zondek and Aschheim 1928). Since that time, the complex role of placental hormones, which act as endocrine, paracrine, and autocrine regulators of maternal and fetal physiologic functions, has been defined for a multitude of steroids and proteins (Benirschke and Driscoll 1967; Siler-Khodr 2011). Near-term pregnant women produce each day 15–20 mg of the most biologically active estrogen, estradiol-17β (E_2), 50–100 mg of its metabolite estriol (E_3), 250–600 mg of progesterone (P_4), 1–2 mg of aldosterone, and 3–8 mg of deoxycorticosterone (DOC) (Simpson

L.D. Longo, *The Rise of Fetal and Neonatal Physiology*, Perspectives in Physiology,
https://doi.org/10.1007/978-1-4939-7483-2_9

and MacDonald 1981). Coincident with these manyfold rise in production of steroid hormones compared to that in nonpregnant women are striking increases in circulating plasma concentrations of renin, angiotensinogen, angiotensin II, and cortisol as well as the daily production of large amounts of human placental lactogens (hPL), later called human chorionic somatomammotropin (hCS), chorionic gonadotrophin (hCG), human chorionic thyrotropin (hCT), chorionic ACTH, and probably chorionic thyroid releasing hormone (TRH), luteinizing hormone releasing hormone (LHRH), and somatostatin. A remarkable phenomenon of pregnancy is the manner in which the mother and fetus adjust to this striking hormonal milieu and its changes. Most of these steroid and polypeptide hormones are produced by the chorionic tissue of the placenta, the largest endocrine organ known. During the past several decades, understanding of the importance of placental hormone synthesis and metabolism and associated nuances of placental and fetal development (Kaufmann and Frank 2004) along with knowledge of the critical dependence upon, and interactions among, the maternal endocrine system, the placenta, and the fetal adrenal glands and other organs have led to the concept of the functional "maternal-placental-fetal unit" or "complex" (Diczfalusy 1964). These hormonal relationships are important not only in terms of fetal growth and development (Evseenko et al. 2007; Kingdom et al. 2000) but in the regulation of maternal blood volume in pregnancy (Longo 1983), the initiation of labor (Beshay et al. 2007; Challis et al. 2001, 2005), and long-term sequelae. Nonetheless, surprisingly little is known about the interrelations of the regulation of placental hormones and fetal growth and development (Fowden 1985, 1989; Fowden et al. 2015).

9.2 Steroid Hormones

Although known to be the source of steroid hormones since the early twentieth century, it was not until mid-century that placental biosynthetic pathways began to be unveiled (Ryan 1959). In addition to the ovary, the placenta in higher mammalian species was recognized to produce progesterone. Circulation progesterone fulfills a number of roles, stimulating maternal food intake, suppressing maternal cell-mediated immunity to prevent rejection of the fetus which expresses paternal antigens, and importantly promoting uterine quiescence during the course of gestation. Progesterone also serves as a precursor for estrogen production. However, the human placenta lacks steroid 17α-hydroxylase activity which is required for production of other estrogen steroids, estradiol 17β in particular. Estradiol plays a wide variety of roles during pregnancy, including the stimulation of growth and differentiation of specific tissues, enhancing receptor-mediated low-density lipoprotein cholesterol for placental steroid production, increasing uteroplacental angiogenesis and blood flow, endometrial prostaglandin synthesis, and preparing the breasts for lactation. By aromatization, the placenta converts 16α-hydroxy dehydroepiandrosterone sulfate made by the fetus into estriol (Casey and MacDonald 1997; Simpson et al. 2002). Estriol is secreted into the maternal circulation and

is the main estrogen metabolite excreted in the mother's urine. The fetus also can initiate the pathways for conversion of cholesterol from low-density lipoprotein to the pregnenolone (a reaction known as cholesterol side-chain cleavage) and on to dehydroepiandrosterone sulfate. Thus, the placenta, in concert with several fetal organs, particularly the adrenal gland, plays a unique role in the regulation of steroid hormone production in pregnancy (Casey and MacDonald 1988, 1997; Ryan 1959, 1980).

A key role in the development of the field of placental steroid metabolism and function was Egon Diczfalusy, originally from the University of Szeged, Hungary, who shortly thereafter moved to Stockholm, Sweden, where he became professor and head of the Reproductive Endocrinology Research Unit at the Karolinska Institute. His investigations centered upon the metabolism of steroids in the fetus and placenta, their interconversion, functional roles, and the concept of the placental-fetal unit in which fetal and placental enzymes work in concert to produce estrogens (Diczfalusy 1964, 1970, 1974; Mathur et al. 1970; Mikhail et al. 1963; van Leusden et al. 1971). Although some may question the ethical aspects of these historic studies in women who were having their pregnancies terminated, one must remember that they were performed under strict guidelines of the Medical Research Council of Sweden as the best approach at that time. In the following years, Dr. Diczfalusy became one of the initiators and the Senior Consultant to the Human Reproduction Program of the World Health Organization. He is the recipient of numerous international awards and honors.

During the years that I edited the "Classic Pages" section of the *American Journal of Obstetrics and Gynecology*, in response to my query regarding the development of his ideas along this line, Diczfalusy responded:

> How did I become a reproductive endocrinologist? As a medical student in Hungary, I had been unable to repeat a microbiology study published by the Nobel Laureate Hans von Euler (1873–1964). Subsequently, in 1946 I moved to Stockholm and, during the years 1946–1947, became von Euler's assistant. As it turned out, many of my scientific activities can be characterized as unfinished symphonies, without the qualities of [Franz] Schubert's (1797–1828) work. In 1947, I had the opportunity to work in the Hormone Laboratory in the Department of Obstetrics and Gynecology of Professor Axel Westman at the Karolinska Hospital in Stockholm. Thus, suddenly I was . . . an endocrinologist. In association with the pharmaceutical company AB Leo of Helsingborg, I worked on the metabolism of estrogen phosphates (Aldman et al. 1951). Following attending a 1950 meeting of the Biochemical Society of Edinburgh, I became friends with, and influenced by, Guy Frederic Marrian (1904–1981), Sir John (Jack) Henry Gaddum (1900–1965), and some of the younger endocrinologists who worked with these giants. Later, at the suggestion of Professor Westman and in partial fulfillment of a thesis for a Swedish academic degree, I worked on chorionic gonadotrophin and estrogens in the human placenta (Diczfalusy 1953).
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> That the placenta is an endocrine organ was first suggested by Joseph Halban (1890–1937) in a paper which is one of the great classics in reproductive endocrinology (Halban 1905). In my thesis I went a step further, saying that "there is some reason to believe that the foetal organism actively participates in the metabolism (possibly also in the production?) of oestrogens". The idea that the placenta and foetus may form a functional unit was certainly in the back of my mind in those early years, judged from the summarizing statement of my paper presented at the Symposium of the German Endocrine Society in Bonn in . . . 1955. The German text says: "In summary it should be emphasized that the

placenta represents a polyvalent hormone producer with an unknown regulatory mechanism. We believe therefore that it is a fruitful hypothesis to study the placental and foetal metabolism of hormones together as a unit" (Diczfalusy 1956). This suggestion was received with much skepticism at the Meeting, one of the major arguments against it being: how could such a small fetus produce such quantities of steroids?

When I started my career, the "heroic age" of reproductive endocrinology was over (Parkes 1966). As an example, the most important estrogens were already isolated (Corner 1965; Parkes 1966; Marrian 1966). What was left to our generation was to isolate and study these compounds in sources other than pregnancy urine, to isolate additional metabolites, and to follow the advice given in the epilogue of Marrian's Sir Henry Dale Lecture and unravel the mechanisms of action of steroid hormones, which became the major focus for attention in the 1960s and 1970s (Marrian 1966). Indeed, the Laurentian Hormone Conference . . . signaled as early as 1962 that this would be a very fruitful field of research (Jensen and Jacobson 1962).

Our modest contributions consisted of isolating "old" estrogens from new sources and a few new ones, such as estriol-3-sulphate-16 (17?)-glucosiduronate (Diczfalusy et al. 1964), 15α-hydroxyestradiol (Lisboa et al. 1967), and 15α-hydroxyestriol (Zucconi et al. 1967) from "classical" sources.

The most significant difference between the "heroic age" of reproductive endocrinology and its "merry post-war period" was, however, that the previous noble hobby, science, became a profession, providing for the first time a living for many people engaged in full-time academic research. Medical Research Councils (MRCs) were created in a relatively large number of countries, and the National Institutes of Health, Bethesda, MD, established an almost revolutionary principle to support research conducted outside the U.S.A. Furthermore, in the early 1960s, the Ford Foundation started a multi-million dollar program to support reproductive biology and endocrinology on a truly world-wide basis. Science became again, at least in part, international, as it was before 1914, when, to quote Sir Henry Dale (1875–1968), "we were able to claim that science belonged to the world, knew no frontiers, was one and indivisible (Feldberg 1970)".

The large number of scientists who decided then to become investigators on a full-time basis did not imagine, of course, in the merry years of the late 1950s and early 1960s, that by the 1970s the so-called public faith in unlimited scientific achievements might rapidly fade away, together with a considerable part of the financial support to science, leaving behind a general feeling of malaise and frustration not only in the so-called "public opinion," but also among many disillusioned scientists. At any rate, during the merry post-war period, especially at the beginning of the 1960s everything was great and dandy. This was the case not only in the U.S.A. with a yearly NIH budget exceeding a billion dollars, but also in my own Unit, supported . . . [by both] the Swedish Medical Research Council and the NIH, and from 1962 by a major contribution from the Ford Foundation.

Interruption of gestation for medical and medical-social indications had been legal in Sweden since 1938, the number of second trimester interruptions was considerable, and the Swedish M.R.C. believed this most valuable scientific material should not be wasted. Hence, in the early 1960s my interest became more and more concentrated on the role of the fetus in the endocrinology of gestation, especially after my collaborator Ove Cassmer (1920–1994) demonstrated that severing the umbilical cord, but leaving the fetus *in situ* (a method widely used at that time in Sweden for the interruption of gestation), resulted in a marked drop in urinary estriol levels, but influenced only slightly pregnanediol excretion (Cassmer 1959). A further impetus for our research were the stimulating studies . . . that pregnant mothers bearing anencephalic monsters excrete greatly reduced amounts of estriol in their urine (Frandsen and Stakemann 1961). However, they attributed this to the absence of estrogen synthesis by the hypoplastic adrenal cortex of the anencephalic monsters, whereas I believed it more probably that the placental—rather than fetal—elaboration of

estrogens is impaired in anencephalics, perhaps as a consequence of the lack of a fetal adrenal precursor of placental estrogen synthesis (Diczfalusy 1962). Last but not least, an important prerequisite for our studies was the development of a suitable technique for the perfusion of pre-viable fetuses … (Westin et al. 1958).

Our first paper directly referring to the feto-placental unit in its title was published … [in 1963] (Mikhail et al. 1963). Nils Wiqvist (1923–2005), who later became the Head of the Department of Obstetrics and Gynecology at the University of Gothenburg, was the principal clinical investigator in this and many subsequent studies; his important contributions to our studies deserve considerable credit. I first presented the full concept of the feto-placental unit at a Federation Meeting in 1964 (Diczfalusy 1964). Unfortunately, I could not attend the meeting, but Claude Alvin Villee (1917–2003) was kind enough to read the paper for me.

Obviously, a concept like this is not born as a child of an exceptionally favorable moment of imagination. Its foundations are laid by hundreds of investigators, providing—as George W. Corner noted in his Dale lecture of 1964—all the necessary "pieces of a jigsaw puzzle" to be put together by someone (Corner 1965). That this was the case also as far as the concept of the feto-placental unit is concerned is obvious from reading the careful review of the field by Fred Mitchell in 1967, and also my own review the following year (Diczfalusy 1969; Mitchell 1967). Who then the "someone" will be depends perhaps on fortunate circumstances ('*sors bona, nihil aliud*', [good fortune, nothing else] as the Hungarian hero of the seventeenth century, Count Miklos Zrinyi (1620–1664) put it), and does not really matter much. During more than 60 years of professional life I have learned that by the end of the day all of us become astronauts, traveling with an incredible speed into oblivion. Hence—as a fact of life—the investigators involved will be rapidly forgotten, just as their disagreements, but the concept if it is a useful one will survive.

At any rate, the suggested concept stated that the fetus synthesizes mainly the "primitive" 3β-hydroxy-Δ^5 forms of steroids, such as pregnenolone or dehydroepiandrosterone, which reach the placenta chiefly as 3-sulphates. Furthermore, the placenta is an incomplete steroidogenetic organ, carrying out little, if any, steroid synthesis *de novo*. However, it has four exceedingly active enzyme systems: various types of sulphatases, 3β-hydroxysteroid dehydrogenases, an aromatizing enzyme system, and some other dehydrogenases. Therefore, the placenta converts 3β-hydroxysteroids (and their sulphates) into the corresponding α,β-unsaturated ketones, for instance pregnenolone into progesterone, dehydroepiandrosterone into androstenedione, and testosterone, and 16α-hydroxytestosterone. The C-19 compounds are then rapidly converted by the placenta into the corresponding principal estrogens, estrone, estradiol, and estriol, in an increasing order of quantitative importance. Finally, steroids reaching the fetus from the placenta are exposed to extensive sulphurylation and/or hydroxylation reactions.

Since, in collaboration with Samuel Solomon's (1925–2008) group in Montreal, it was also shown that the adrenals of fetuses perfused with labeled progesterone convert this steroid into 17-hydroxyprogesterone, corticosterone, and cortisol, it appeared highly likely that the midgestation fetus is capable of providing itself with a variety of essential adrenocortical steroids, using placental progesterone as one of the principal substrates for 11α, 17-, and 21-hydroxylations (Bird et al. 1966). Some of these perfusion studies, which thus confirmed the results of previous *in vitro* studies by several investigators in the 1950s and early 1960s, were presented in detail at the Laurentian Hormone Conference (Solomon et al. 1967).

Thanks to a fruitful collaboration with … [a] group in Paris, we could demonstrate not only the formation of various adrenocortical hormones (including aldosterone) by the pre-viable fetus, but also the significant differences between the fetal and placental metabolism of the various unconjugated and conjugated corticosteroids formed (Pasqualini et al. 1970). Furthermore, a long-term collaboration with Mortimer Levitz and Joseph Dancis (1916–2010) and their co-workers in New York enabled us to investigate in depth the

placental transfer and feto-placental metabolism of the various estrogen conjugates formed (Levitz et al. 1967).

During the period between 1963 and 1972 we could explore systematically the fetal, placental and maternal interrelations in the formation of sterols and steroids during pregnancy. This information was published in some 80 "full dress" papers, but we modified only slightly the principal concept of 1964 (Diczfalusy 1969, 1970, 1974).

In a somewhat simplistic enzymological terminology, the "final" concept states that the placenta is not a true steroid-producing organ, as it cannot form *de-novo* from acetate any squalene, lanosterol or cholesterol. These reactions take place in the fetal and maternal organism. On the other hand, the placenta can convert cholesterol in the blood to pregnenolone and various Δ^5-steroids, e.g. pregnenolone, or dehydroepiandrosterone into biologically active Δ^4-compounds, such as progesterone or androstenedione. The placenta also possesses powerful sulphatases, which hydrolyse a variety of steroid sulphates, for instance pregnenolone sulphate, dehydroepiandrosterone sulphate, or estrone sulphate. It also has a potent aromatizing enzyme system, which converts different androgens into the corresponding estrogens, for instance testosterone into estradiol and 16α-hydroxytestosterone into estriol. Thus, the sequence of placental reactions can be visualized as dehydroepiandrosterone sulphate → dehydroepiandrosterone → androstenedione → testosterone → estrone and estradiol. Hence, the placenta can convert cholesterol into C-21 steroids, 3β-hydroxy-Δ^5-compounds to Δ^4-steroids, and androgens to estrogens.

The placenta also possesses various sulphatases and different 3β-hydroxysteroid dehydrogenases which, again with few exceptions, are absent in fetal tissues. Because certain enzymes, such as the cholesterol side-chain cleaving enzyme and the aromatizing enzyme system, are functioning in both compartments, by integration of these functions, the feto-placental unit can indeed elaborate most, if not all, steroid hormones. On the other hand, the human fetus possesses the cholesterol synthesizing enzyme system, steroid sulphurylating enzymes and various hydroxylating enzymes which, with few exceptions, are not present in the placenta.

As the fetus is capable of carrying out a variety of hydroxylations, it can provide itself with all essential adrenocortical steroids through a series of hydroxylations of placental progesterone. Also because the fetus can also elaborate all the corresponding Δ^5-steroids, it is likely that parts of these are converted by the placenta into their biologically active Δ^4-counterparts.

Furthermore, the fetus can also easily remove the steroid side-chain, forming large quantities of dehydroepiandrosterone sulphate from pregnenolone sulphate, via 17-hydroxypregnenolone sulphate without any hydrolysis of the steroid sulphates. The complexity of the reactions involved can be illustrated by recalling that large quantities of dehydroepiandrosterone sulphate formed by the fetal adrenals are then 16α-hydroxylated by the fetal liver, hydrolyzed and further metabolized into the corresponding androgen, 16α-hydroxyandrostenedione, by the placenta, where it is subsequently rapidly converted into the quantitatively most important steroid of human gestation, estriol. Similarly complex mechanisms exist for the formation of other estrogens (Vokal et al. 1970).

The splendid rise of the "Feto-Placental Empire" came to an abrupt end in 1971, when prostaglandins were introduced in Sweden for the termination of pregnancy. This resulted in such success that within a short time it became unethical to interrupt second trimester gestation by laparotomy. We discontinued our work completely by the middle of 1971, and I wonder whether anyone may ever be ably to resume such studies. In retrospect, one of the weaknesses of our approach was that we did not measure the absolute mass of the various steroids, only the amount of radioactive material.

About the time that prostaglandins were introduced for therapeutic abortion, radioimmunoassays for steroids became available. It is interesting to speculate what might have happened if steroid radioimmunoassays had become available some 10 years before the introduction of prostaglandins. Probably, we would have had today a more complete view

of the feto-placental unit, and in much more quantitative terms. As it happened, in the early 1970s the human fetal-placental unit virtually ceased to exist as a suitable topic for investigation, and I became richer with another unfinished symphony.

In the final analysis, every scientific contribution is in a way an unfinished symphony, the basic motives of which are taken up and developed further by the musicians of a new generation. I sincerely hope that some of the fragments presented here may someday entice other investigators to commence anew similar studies. After all, an in-depth study of the "well established facts" still appears to be an efficient way of obtaining basically new information. As François Villon (1431–ca. 1465) stated in one of his immortal ballads:

Rien ne m'est seur que la chose incertaine:
Obscur, fors cequi est tout évident;
Doubte ne fais, fors en chose certaine;
Science tiens a soudain accident

The English translation, as I have seen it in Colin Blakemore's book, is equally appealing (Blakemore 1977):

I put all my trust in things that I doubt;
The obvious alone is unclear.
Certainty never knows what it's about,
And truth from sheer chance will appear.

(Diczfalusy 2005)

The necessity of maternal cholesterol for the placental synthesis of progesterone (Bloch 1945; Hellig et al. 1970) allowed expansion of the placental-fetal unit to the "maternal-placental-fetal unit." In the fetus, steroids are sulfated rapidly by an adrenal sulfatase, resulting in steroid sulfates that are biologically inactive. This sulfation may serve as a protective mechanism to ensure that fetal tissues are not exposed to overly high levels of active steroids. In the placenta, the sulfate molecule must be cleaved prior to 3β-hydroxysteroid dehydrogenase-isomerase complex to convert DHEA or the hydroxylated DHEAs to androstenedione or hydroxylated androstenediones. These androgens then are aromatized to estrone (E_1); 16α-OH estrone or 15α-OH estrone then are converted to estradiol (E_2), estriol (E_3), or estetrol (E_4) by the placental 17β-hydroxulation enzyme. Maternal DHEA-SO_4 serves as 40% of the precursor for E_2 synthesis, and the latter hormones are formed from fetal precursors, as the maternal liver has limited 5α- or 16α-hydroxylase activity (Madden et al. 1978). Thus, while E_3 and E_4 concentrations can serve as indices of fetal well-being, the majority of estrogens are transferred to the maternal circulation. Although placental steroidogenic ability is functional in early gestation, not until days 35–47 post ovulation can placental progesterone production by itself support the maintenance of pregnancy independent of P_4 production by the ovarian corpus luteum (Csapo et al. 1973).

As noted, during the course of gestation, the circulating concentrations of steroid hormones increase dramatically (Tulchinsky et al. 1972) to perform multiple functions (Csapo et al. 1973; Kallen 2004). Other leaders in the biochemical nuances of placental steroid biochemistry and in unraveling the complexity of its metabolism were the group at the University of Texas Southwestern Medical Center. Directed for many years by Paul Cloeren MacDonald, Jr. (1930–1997), this group included the biochemists John M. Johnston, Pentti "Finn" Siiteri (1926–2012), Evan Rutherford Simpson, and others. In the mid-1970s, Cecil Howard Green (1900–2003), founder

of Texas Instruments, and his wife Ida endowed the Center for Reproductive Biology in their name, which MacDonald headed from 1974 until his death (Casey and MacDonald 1988, 1997; Madden et al. 1978; Siiteri and MacDonald 1963, 1966; Simpson and Burkhart 1980a, b; Simpson and MacDonald 1981; Simpson and Miller 1978; Simpson et al. 1978, 1979, 2002; Winkel et al. 1980).

9.3 Polypeptide Hormones

As noted, pregnancy is associated with a dramatic increase in a combination of maternal pituitary and placental somatotrophic/growth hormones, lactogenic hormones, and chorionic gonadotrophin. In contrast to non-primates, humans possess a growth hormone placental lactogen gene cluster on the long arm of chromosome 17 (q22-24), containing five related genes. Growth hormone-N (GH-N) encodes the pituitary growth hormone, GH-V encodes that hormone in the placenta, and hPL A, B, and L (hPL-A, hPL-B, hPL-L) encode placental lactogens/somatomammotropins (Baumann 2009). GH-V is a potent insulin antagonist that stimulates maternal lipolysis (Barbour et al. 2002) impairing insulin action by increasing levels of p85α, a competitive inhibitor of PI3K and (IRS-1)-associated activity (Barbour et al. 2004), thereby reducing muscle GLUT-4 translocation and glucose uptake (Barbour et al. 2005, 2007). Thus, GH-V promotes the mobilization of maternal fat stores during fasting with glucose and other nutrient sparing for transplacental delivery and fetal growth (Gluckman and Low 2011; Newbern and Freemark 2011).

While the major circulating placental lactogen derives from hPL-A and hPL-B genes, the function of hPL-L remains unclear (Fuglsang and Ovesen 2006). In early human pregnancy, GH-N concentrations decline, to become undetectable by 24 weeks. Conversely, after mid-gestation GH-V rises to peak levels at 34–37 weeks (Baumann 2009; Fuglsang and Ovesen 2006). Thus, in essence, placental growth hormone replaces that of the maternal pituitary. Structurally, hPL is homologous to GH; however, functionally it is closer to prolactin (PRL). Throughout gestation, concentrations of both hPL and PRL rise progressively. Secreted by the trophoblast directly into the maternal and fetal circulations, hPL levels in the mother are about 100-fold greater than in the fetus; levels in the fetus peak near term, while those of the mother peak at 32–35 weeks.

The protein hPL or chorionic somatomammotropin (hCS) was discovered independently by several groups: Yosoji Ito and Kyoko Higashi (1961), John B. Josimovich and John Andrew MacLaren (1962), and John J. Sciarra, Selna Lucille Kaplan (1927–2010), and Melvin Malcolm Grumbach. Josimovich with MacLaren (1919–1970) used an immunodiffusion technique to demonstrate a lactogenic protein in the serum of pregnant women that had partial immunologic identity with pituitary growth hormone. They characterized this hormone as a polypeptide and demonstrated cross-reaction between human retroplacental serum and antibody to human growth hormone (hGH). This finding of cross-reactivity meant that retroplacental serum contained a substance similar to, but

different from, hGH. These authors first used the term "human placental lactogen." In addition to other metabolic effects, hPL can potentiate the action of hGH. MacLaren, a research associate in the Department of Obstetrics and Gynecology at the Boston Hospital for Women, Harvard Medical School, and originally from New Brunswick, Canada, had received his doctorate from Vanderbilt University. Josimovich went on to a distinguished career in academic obstetrics and gynecology at the University of Pittsburgh.

Again, several decades ago, when editing a section of the *American Journal of Obstetrics and Gynecology*, I requested Dr. Josimovich to give an account of his early studies. He replied:

> The presence of one or more proteins in human placental extracts that would stimulate crop sac growth in the pigeon was first documented by Yosoji Ito and Kyoko Higashi (Higashi 1961, 1962; Ito and Higashi 1953, 1961). Somatotropic activity of extracts of the human placenta was also demonstrated earlier by Mineko Fukushima (Fukushima 1961). Our efforts to identify and purify human placental lactogen (hPL; hCS) commenced at the Boston Lying-In Hospital and in the Department of Obstetrics and Gynecology, Harvard Medical School. These endeavors demonstrate the importance of research leadership of the chairman of a clinical department, the importance of early financial support for junior investigators, the provision of time for them to carry out such research, the availability of newer techniques at a critical time in the development of a research project, the close proximity of numerous colleagues in allied fields to help in the development of research techniques, and previous training in research methodology.
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> I had received pre- and post-doctoral training supported by the Josiah Macy, Jr., Foundation at the Harvard Medical School in the laboratory of Ernst Knobil (1926–2000), Department of Physiology where the species specificity of human pituitary growth hormone was discovered. Because of this background, in 1960 Duncan Earl Reid (1905–1973) suggested that I embark on a search for the growth hormone-like factor undoubtedly present during human gestation. Dr. Reid, at that time Chairman of Obstetrics and Gynecology and Chief of the Boston Lying-In Hospital, provided me a laboratory at the behest of Roy Orval Greep (1905–1997) at Harvard and familiarized me with the work of Richard Burt (Burt 1960) and others which suggested that many of the metabolic changes seen in late pregnancy could be accounted for by a circulating growth hormone-like factor. I set up the tibial cartilage growth bioassay in hypophysectomized rats developed by Francis S. Greenspan and colleagues (Greenspan et al. 1949). I had intended to determine whether I could repeat the experiments performed by Alexander N. Contopoulos and Miriam Elizabeth Simpson (1894–1991) (Contopoulos and Simpson 1959) in the rat, and by Carl Axel Gemzell (1910–2007) and associates (Gemzell et al. 1955) in the human subject, detecting increased somatotrophic activity. Because Tetsuo Hayashida and Choh Hao Li (1913–1987) in California had shown the usefulness of the double diffusion technique for establishing immunologic similarity between pituitary growth hormones, I thought that the immunologic approach might be simpler than the cumbersome bioassays. The research project was encouraged in a cooperative and kindly manner by Drs. Maurice Solomon Raben (1915–1977) and Edwin Bennett Astwood (1909–1976) of Tufts University. Dr. Raben supplied me with his human growth hormone preparation for immunization and standards. Dr. MacLaren, an immunologist and bacteriologist at the Boston Lying-In Hospital, suggested that I use the Örjan Ouchterlony (1914–2004) method (Ouchterlony 1949, 1962a, b) double-diffusion technique. It is now apparent that the failure of more than one major growth hormone research group to find hPL was primarily because of their choice of the microscope slide—agar diffusion technique. The so-called "slide" technique, which is excellent for conserving valuable antigens and antibody supplies, unfortunately requires that the relative concentrations of antigen and antibody lie within narrow, critical

limits in order for a precipitin line to be detected between the two. Fortunately, Dr. MacLaren suggested that I employ the large Petri dish and large triangular well molds which he had personally received from Dr. Ouchterlony. It later became apparent that our ability to find a substance in the serum of pregnant patients and placental extracts which cross-reacted immunologically with human pituitary growth hormone was made possible by the lack of need for a narrow ratio of antigen to antibody in this particular double-diffusion system. Using my own isoelectric precipitation techniques for purifying placental extractions and C.H. Li's (1957) method with Amberlite column chromatography for isolating pituitary growth hormone from numerous species, I was able to isolate a protein which appeared to be of 80 percent purity or greater in polyacrylamide gel electrophoresis (Josimovich et al. 1963). This protein showed immunologic behavior similar to that of the substance found in pregnancy serum which cross-reacted with the Raben pituitary growth hormone. Once isolated from placental extracts, it was obvious that both somatotrophic and lactogenic potencies should be tested, because of the findings of the lactogenic potency of human pituitary growth hormone by A. Chadwick and colleagues (Chadwick et al. 1961). To my great disappointment, I had never been able to find any somatotrophic activity in the hPL preparations produced in my own or other laboratories in the Greenspan rat tibial growth assay until recently. Li and Yamashiro (1970) pointed out that the injection of material at an acidic pH would reveal such activity. Later, I confirmed this (Josimovich et al, unpublished data). Somatotrophic bioassays by other groups, claiming the finding of growth hormone-like activity, are known not to be totally specific for growth hormone obtained from the pituitaries in lower species. We found lactogenic activity in the pigeon crop assay (Josimovich and MacLaren 1962) and in the rabbit; and so we called this material human placental lactogen. It is also known as human chorionic somatomammotropin . . ..

Although numerous studies of fetal and maternal serum and placental extracts convinced us that the protein was produced in the placenta itself (Josimovich and Atwood 1964), the work of John J. Sciarra and colleagues (Sciarra et al. 1963) and [Drs.] Grumbach and Kaplan (Grumbach and Kaplan 1965) established *in vitro* that the hormone was produced by the fetal trophoblast. The weak but definite somatotrophic activity of the hormone was finally established by Grumbach and colleagues (Li et al. 1968) in hypopituitary children while the promotion of insulin resistance was demonstrated by Paul Beck and William Hamilton Daughaday (1918–2013) (Beck and Daughaday 1967). Chemical studies begun in greater detail by Henry Friesen (1965) in Dr. Astwood's laboratory were carried out to their ultimate by characterization of the chemical structure and comparison with the pituitary growth hormone molecule in the laboratories of Drs. Louis M. Sherwood (Sherwood 1967) and Li (Li et al. 1971).

The complete biological effects of this hormone still remain to be determined. Although hPL is extremely potent as a lactogenic material when studied in birds and lower mammals, its role in preparing the primate breasts for lactation remains to be demonstrated. The somatotrophic activity of the hormone, present by virtue of very high concentrations of low-activity protein, undoubtedly contributes to the insulin resistance, and increased ability to mobilize free fatty acids during fasting seen in late pregnancy in conjunction with estrogens, insulinase, and perhaps other hormones. Further work is in progress on possible influences of hPL on progesterone metabolism, inasmuch as the hormone's most potent effect in lower animals has been a luteotropic effect seen in rodents (Josimovich 1968; Josimovich et al. 1970). That the ovary is not the site of possible action of hPL in the human subject was shown by our studies carried out in collaboration with Arnold Klopper's (1922–2014) laboratory (Stock et al. 1971). Therefore, it remains to be seen whether the action of the low concentrations of hPL found in the fetus, and the high concentrations found in the placenta, significantly affect progesterone metabolism at those sites during pregnancy.

> Our group at the University of Pittsburgh (Josimovich et al. 1970) and others have questioned the clinical value of measurement of maternal serum levels of hPL in the high-risk pregnancies, as first suggested by Badri N. Saxena and coworkers (Saxena et al. 1968). However, we would all agree (Josimovich et al. 1970) that the low levels found in late pregnancy indicate intrauterine growth retardation, as first strongly suggested by the data of William Nelson Spellacy (1934–2015) and colleagues (Spellacy et al. 1967).
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> A recent series of investigations from Liege has identified and synthesized a placental growth hormone (PGH), slightly different in structure from the pituitary hormone (hGH). PGH is more potent as a growth hormone than hPL (Frankenne et al. 1990). Review of my first two articles on hPL reveals that I had discarded a penultimate placental fraction with higher GH activity than resides in the final precipitate. Thus, hPL, according to work I later published, synergizes with placental GH, but it is more potent as a lactogenic substance.
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> (Josimovich 1974)

Since the early studies of Josimovich and others, a number of aspects of lactogenic/somatomammotropic hormones have come to light (Schraenen et al. 2010). For instance, several lines of evidence suggest that they are important for placental and fetal well-being, the hCS concentrations parallel placental mass throughout gestation. In addition, placenta somatomammotropins play a central role in the maternal pancreatic insulin resistance. These include the striking rise in hCS and prolactin levels during pregnancy which is associated with pancreatic beta cell mass expansion with increased maternal insulin secretion, hCS and prolactin which promote beta cell replication in isolated islets and prolong beta cell survival (Fujinaka et al. 2007), and other studies on hCS overexpression or knockout on islet beta cell function (Fleenor et al. 2000; Vasavada et al. 2000). Unfortunately, the mechanisms by which placental somatomammotropins promote beta cell replication and insulin secretion have not been elucidated fully, but recent studies implicate the induction of cell cyclins, cyclin-dependent kinases, and serotonin, with downregulation of forkhead protein and cell cycle inhibitors (Arumugam et al. 2011; Newbern and Freemark 2011). The effects of lactogens and prolactin on islet DNA synthesis and gene expression are potentiated by glucose (Arumugam et al. 2011), providing a mechanism whereby nutrients and hormones may act in concert to promote beta cell insulin production. The multiple factors that regulate hCS synthesis are poorly defined (Siler-Khodr 2011).

Perhaps the best known hormone of pregnancy is human chorionic gonadotrophin (hCG). Synthesized in the syncytiotrophoblast (Midgley and Pierce 1962), but regulated by releasing factors, gonadotrophin-releasing hormone (GnRH I and II isoforms) produced in the cytotrophoblast (Siler-Khodr and Grayson 2001) is a glycoprotein with α and β subunits translated from separate mRNAs (Hussa 1980). In early pregnancy, hCG stimulates both the formation of the corpus luteum which produces progesterone and placental steroidogenesis, with estrogens inhibiting GnRH production by a negative feedback mechanism (Branchaud et al. 1983).

Other protein hormones of the placenta include the insulin-like growth factors (IGFs)-1 and IGF-2, single-chained polypeptides that in conjunction with their receptors IGF-1R and IGF-2R, and in response to specific signals, promote fetal and neonatal growth. Several lines of evidence suggest that placental GH-V regulates maternal IGF-1 production during pregnancy. These include maternal IGF-1

levels correlate with maternal GH-V, but not GH-N, levels (Caufriez et al. 1993), and GH-V overexpression in transgenic mice increases IGF-1 levels 56% and body weight 84% (Barbour et al. 2002). Although IGF-2 is the most abundant of the fetal IGFs, deletion of either *Igf* gene results in reduced birthweight (Fowden et al. 2008). In addition to promoting fetal growth in concert with nutrient availability, IGF-2 stimulates placenta growth and differentiation (Sferruzzi-Perri et al. 2011). Furthermore, IGFs regulate substrate transport and hormone secretion and thus fetal growth both directly by influencing placental nutrient uptake and transport and indirectly by influencing maternal substrate availability (Sferruzzi-Perri et al. 2011). In the human trophoblast, IGF-1 and IGF-2 stimulate both glucose and amino acid uptake (Roos et al. 2009). In the guinea pig, elevated levels of IGF-1 in maternal plasma increase fetal glucose and amino acid uptake (Robinson et al. 1980), while in the sheep such elevation in early pregnancy is associated with elevated maternal plasma glucose and enhanced fetal growth (Sferruzzi-Perri et al. 2011). Also in the sheep, during late pregnancy, IGF-1 administration increases glucose delivery to the fetus. In cultured human trophoblast cells, IGF-1 stimulates System A amino acid uptake (Roos et al. 2009). In a transgenic mouse model with deletion of the gene transcript for placental-specific *Igf* expression, in association with the upregulation of the glucose transporter (GLUT-3) and the amino acid transporter (SNAT-2), during mid-gestation placental growth is restricted, while that for the fetus is maintained. Nonetheless, fetal growth restriction becomes evident near term (Constância et al. 2002, 2005; Sibley et al. 2004).

Cytokines, including interferons, interleukins, tumor necrosis factor-α, leukemia inhibitory factor, and others, also are produced by the placenta (Chaouat et al. 2002; Hauguel-de Mouzon and Guerre-Millo 2006). A related polypeptide cytokine/hormone, leptin, a 16-kDa product of the obese (*ob*) gene, secreted predominantly by white adipose tissue, also may be secreted by the placenta (Briffa et al. 2015; Lea et al. 2000; Lepercq et al. 2001). Under normal physiologic conditions, plasma leptin concentrations reflect adiposity, with elevated levels present in individuals with a high body mass index (BMI) (Garibotto et al. 1998). The chief physiologic role of leptin is to regulate hunger and satiety (Dhillon and Belsham 2011; Elias et al. 1999) by activating a complex of signaling pathways in the arcuate nucleus of the basomedial hypothalamus (Elias et al. 1999; Funahashi et al. 2003; Mastorakos and Ilias 2003; Valassi et al. 2008). During the course of gestation, in response to the elevated energy demands with accumulation of adipose tissue and gain in weight (Tessier et al. 2013), maternal plasma leptin concentrations rise during the first and second trimesters and peak during the third trimester with a return to prepregnancy levels prior to parturition (Schubring et al. 1998). This occurs in concert with development of leptin resistance (Grattan et al. 2007). This increase in leptin is produced by maternal white fat cells and the placenta (Highman et al. 1998; Lepercq et al. 2001). In contrast to leptin's normal role in increasing satiety, human pregnancy is associated with increased food intake. This prevents maternal nutrient depletion to allow increased transplacental nutrient delivery to the fetus (Sparks et al. 1980). This apparent discrepancy in function is a consequence of hypothalamic pregnancy-induced leptin resistance (Grattan et al. 2007; Tessier et al. 2013;

Trujillo et al. 2011). In summary, leptin's primary role is to regulate hunger and satiety by acting on neuropeptide Y (NPY) and proopiomelanocortin (POMC) neurons, with ultimate decrease in hunger and increase in satiety and increase in energy expenditure.

Along this line, leptin has been proposed to play a role in trophoblast invasion of the uterine endometrium/decidua at the time of implantation and to participate in the regulation of placental and fetal growth and function (Hauguel-de Mouzon et al. 2006; Henson and Castracane 2000). Specifically, leptin appears to promote the development of several fetal organs including the brain (Udagawa et al. 2006), heart (Nath et al. 2008), kidney (Attig et al. 2011), and others. Several studies have reported the positive correlation of newborn birthweight to umbilical cord blood leptin levels (Hassink et al. 1997; Jaquet et al. 1998; Marchini et al. 1998; Shaarawy and el-Mallah 1999). By use of in situ hybridization and immunohistochemistry, leptin mRNA and proteins are co-localized in the syncytiotrophoblast and the villous capillary endothelial cells (Lea et al. 2000), providing evidence that the placenta is a source of both maternal and fetal leptins, with this hormone being transported from the trophoblast to both maternal and fetal interfaces (Hoggard et al. 2001; Masuzaki et al. 1997; Wyrwoll et al. 2005). In addition, fetal adipocytes produce leptin (Atanassova and Popova 2000). Thus, fetal leptin levels appear to be correlated to fetal fat mass in a manner similar to that of the adult. The extent to which the mother contributes the fetal leptin is unknown (Clapp and Kiess 1998; Jaquet et al. 1998), and most of the placental generated leptin may arise from the placenta (Lepercq et al. 2001). Nonetheless, the role of leptin in fetal development has not been studied extensively (Hoggard et al. 2001; Hassink et al. 1997; Henson et al. 1998; Masuzaki et al. 1997; Murphy et al. 2006). To activate intracellular signaling, leptin must bind to the ObRb or megalin receptors (Ahima et al. 1996; Lea et al. 2000), resulting in activation of the Janus-kinase, JAK/STAT, and mitogen-activated kinase (MAPK) pathways (Banks et al. 2000). Its increased production in both gestational diabetes and preeclampsia suggests that leptin plays a role in these pathologies of pregnancy. Leptin may also be involved in fetal programming of some diseases in the adult. Fetal growth-restricted infants and children have significantly reduced circulating leptin concentrations (Kieffer et al. 1997; Koklu et al. 2007; Nezar et al. 2009; Pighetti et al. 2003) and leptin dysregulation during pregnancy is associated with altered organ development and function that could result in metabolic and cardiovascular disease in adulthood (Briffa et al. 2015).

9.4 Summary and Conclusions

In summary, steroid, lactogenic, and somatogenic hormones of the placenta (and maternal pituitary gland) integrate to promote the metabolic adaptations of pregnancy with the demands of fetal development. The steroid hormones also play a major role in this regard. Likewise, the lactogens/somatomammotropins and GH-V

promote fetal growth by the mobilization and optimal distribution of maternal nutrient stores, which in combination with IGF-1 and IGF-2 and other hormones increase maternal tissue growth, uteroplacental blood flow, placental nutrient transport, and fetal growth and development. The maternal-placental-fetal unit constitutes an interrelated and interdependent, multifactorial network of enzymes, signaling pathways, and gene regulation of enormous complexity. Quite obviously, the placenta along with the maternal reproductive tissues and fetus is of vital importance, a virtual miracle in its endocrinologic development and function. In addition to its multiple roles in fetal development and maternal well-being, increasingly it is recognized to play an important role in the long-term optimal growth and function of the fetus into newborn life, infancy, childhood, and adulthood.

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Chapter 10
Maternal Physiology of Pregnancy

. . . science is not a highway to discovery but the rocky and often twisting road to reduction of uncertainty in knowledge.
(Robert E. Becker 2014, p. 2263)

10.1 Introduction

During the course of pregnancy, the mother undergoes major physiologic and metabolic adaptations to accommodate the requirements of the developing fetus and to prepare for subsequent delivery, lactation, and care for her newborn infant. To some, to speak of the history of maternal physiology, in a volume devoted to the physiology of the fetus and newborn infant, may appear to be a contradiction in terms. However, as the fetus constitutes a portion of the maternal-placental-fetal "complex" or "unit" (Diczfalusy 1964), and its growth and development would be impossible without the mother, it suggests a rationale for this consideration. In addition, because optimal fetal developmental cannot occur in the absence of profound changes in the physiologic function of almost each of the maternal organ systems, metabolic, cardiovascular, respiratory, renal, neurohumoral, and others, such consideration is vital. The organs of the female reproductive system are among the most dynamic tissues in the human body. Even in the absence of pregnancy, from puberty to menopause, these undergo repeated cycles of growth and involution. To attain such plasticity, the reproductive tissues must respond to blood-borne signals (hormones, growth factors, and cytokines), as well as physical forces (mechanical and osmotic).

Gestation is associated with changes in metabolism, enzyme activities, and fluid balance of essentially every organ system. These have significant effects and consequences on the pharmacokinetic and pharmacodynamics properties of various medications whether for the pregnant women herself or her developing fetus (Costantine 2014). An additional aspect is that, with many fetal pathologic conditions being related to the state of maternal health and well-being (for instance, fetal growth restriction), the interrelations of the physiologic states of these two organisms are many and of great significance. Additionally, without knowledge of these dramatic maternal physiologic changes, one cannot hope to understand the further

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pathologic changes that, in the presence of maternal disease, can affect the developing organism. In part, this is because previously asymptomatic structural lesions (such as aortic or mitral stenosis and hypertrophic cardiomyopathy) may become symptomatic during the course of gestation. Thus, pregnancy can unmask previously unrecognized abnormalities. Nonetheless, as with any of the topics in this volume, rather than an apercu, their full consideration could necessitate an entire volume in its own right. In addition, although some of the information that follows is not strictly speaking historical, it helps to set the stage for what follows.

10.2 Frank E. Hytten and Early Studies on Maternal Physiology

During the mid-twentieth century, a pioneer in the field of maternal physiologic changes in normal pregnancy was Frank Eyvind Hytten, MD (University of Sydney, Australia) and PhD (University of Aberdeen, Scotland). For many years Hytten headed the Medicine Research Unit at the University of Aberdeen. Later, he held that position at the University of Newcastle upon Tyne. Still later, Hytten conducted his studies at the Clinical Research Centre, Harrow, Middlesex.

In considering maternal physiology Hytten observed:

> In pregnancy the body is subject to physiological turmoil on a scale not otherwise experienced in healthy adult life. No system escapes; there are huge changes in cardiovascular and respiratory function, renal function is modified dramatically, and almost all aspects of metabolism are altered.
>
> The widespread nature and extent of the changes in pregnancy are remarkable; they represent a major physiological stress to the woman, and, because they mimic disease states, they are responsible for much diagnostic confusion.
>
> (Hytten 1995, Introduction)

In addition to about one hundred scientific papers on this theme, with the nutritionist Isabella Leitch (1890–1980), Hytten authored several volumes. The first was *The Physiology of Human Pregnancy* with a foreword by Sir Dugald Baird (1899–1986) (Hytten and Leitch 1964). This work contains 14 chapters on maternal blood volume, cardiovascular dynamics, respiration, renal function, alimentary function, hormones, and so forth. In addition, an appendix considers the Prochownick diet (named after Ludwig Prochownick (1851–1923), who, at the end of the nineteenth century, promoted the concept of weight control in pregnancy to reduce the weight of the soon-to-be-delivered newborn infant of a diet rich in protein with decreased carbohydrates and fluids. In his study of four dozen women, Prochownick did not claim to reduce the size, consistency, or ossification of the fetal skeleton but rather to decrease the amount of fat tissue, resulting in a newborn infant that was thinner with less subcutaneous tissue. For the skin covering the head, this, he believed, would increase mobility of the skull bones and facilitate molding and thus ease delivery in women with a narrow pelvis (Prochownick 1889, 1901). In the Hytten-Leitch volume, two other appendices addressed the sex ratio at

birth and multiple gestation for a total of 463 pages. Roughly the first one-half of this volume concerns the major organ systems noted above. Much of the second half considers weight gain in pregnancy by specific organ components, as well as metabolism and nutrient requirements. The concluding chapter (Chap. 14) reviews the literature on childbearing under adverse social, nutritional, and political conditions in developing countries and some of the effects of war and famine (Hytten and Leitch 1964). A major function of this volume was to clarify what was known and what was not known regarding maternal basic physiologic changes with pregnancy, therefore stimulating research in topics that were relatively unexplored. A particular valuable contribution of this work is the author's critique of the methodologies used to obtain the data, as well as an analysis of the population base under study.

Seven years later these authors published the second edition of *The Physiology of Human Pregnancy*, again with the foreword by Sir Dugald Baird (Hytten and Leitch 1971). This revised edition also included a chapter on dietary requirements during pregnancy and an additional appendix on "mean birthweight" by geographic area. With supplementary additions and revisions, the text with index comprises 599 pages, almost 30% longer than the original edition. The authors brought up to date their critical and extensive review of the literature; and sections such as those on nutrition, body composition, and metabolism reflect their expertise in this field (Hytten and Leitch 1971). A minor criticism of both editions of this work is the inclusion of a host of unpublished data. Nonetheless, each volume constituted a stimulation to further investigation in gaining an understanding the physiology of the pregnant woman.

With the increasing complexity of biomedical science, so that few individuals could encompass more than one field or subspecialty, a decade later, in collaboration with Geoffrey Victor Price Chamberlain (1930–2014) of St. George's Hospital Medical School, London, Hytten edited the volume *Clinical Physiology in Obstetrics* with a foreword by Sir John Dewhurst (1920–2007) (Hytten and Chamberlain 1980), to which leading scientists contributed. This 506-page synthesis consisted of 18 chapters in five parts: Cardiovascular and Respiratory Systems, Nutrition and Metabolism, Urogenital System, Endocrine System, and the Placenta. The chapters in each section stressed principles of basic science applied to the process of reproduction in humans. In addition to writing three of the chapters himself, the remainder were authored by a dozen authorities in their field. A second edition of this work appeared in 1991 with the 18 chapters condensed to 445 pages (Hytten and Chamberlain 1991).

In 1998, another edition appeared, this edited by Geoffrey Chamberlain and Fiona Broughton-Pipkin, the latter of Queen's Medical Centre, Nottingham. In his Foreword to this edition (Chamberlain and Broughton Pipkin 1998), Hytten noted that "Up to the 1950s, pregnancy was generally regarded as a disease by Western obstetricians whose daily experience was of life-threatening complications" (Hytten 1998, p. ix). Regarding *The Physiology of Human Pregnancy*, he observed that following the second edition:

> Thereafter, it became obvious that no individual could reasonably do justice to the subject. Professor Geoffrey Chamberlain, with characteristic optimism, persuaded me that we could edit a third edition, put together by friends who were experts in particular aspects of the field, and *Clinical Physiology in Obstetrics* appeared in 1980. It too enjoyed a further edition in 1991, but by now I had begun to lose the energy for keeping abreast, and I am very happy to see a further edition—what I like to think of as a fifth edition of the original book—in the very capable hands of two old friends . . ..
>
> (Hytten 1998, p. ix)

As with the previous volumes, a major thesis of this work was to stimulate research in areas that were *terra incognita* [unexplored lands]. During the 1980s and until 1990, as an editor of the *British Journal of Obstetrics and Gynaecology*, Hytten also authored a number of editorials on various facets of pregnancy, from the standpoint of both the mother and fetus (Altman and Hytten 1989; Davison and Hytten 1987; Hytten 1979, 1985, 1990). Also in 1973 with Tom Lind, Hytten wrote a thin but valuable volume *Diagnostic Indices in Pregnancy* with a foreword by Angus MacBeth Thomson (1914–1989), director of the MRC Reproduction and Growth Unit at Newcastle upon Tyne (Hytten and Lind 1973). In this compilation the authors tabulate physiologic values during the course of gestation of function of variables for the cardiovascular, hematologic, respiratory, and several other organ systems.

As noted earlier, the coauthor with Hytten in the two editions of *The Physiology of Human Pregnancy* (Hytten and Leitch 1964, 1971) was Isabella Leitch. Hytten recorded that he came to know her in the early 1950s at Aberdeen. He noted further that one evening a week over a period of several years, they compiled the material that would constitute a portion of their collaborative treatises (Hytten 2009). In reference to her expertise in nutrition, Hytten observed:

> Pregnancy . . . [is], after all, a nutritional exercise: the acquisition of chemical building blocks to make a fetus; their carriage to the building site; and the removal of waste products. The range of metabolic modifications [constitute] . . . a very intricate and complex undertaking
>
> (Hytten 2009, p. 114)

Hytten also recorded Leitch's contribution following a false lead in the literature on the relation of the weight of a singleton fetus with that of the mother in a number of species. Leitch collated data from 114 species in which they could obtain the birthweight of an entire litter, to derive from the logarithms of each, a straight-line relationship that extended from a 6 g bat to a 79,000 kg blue whale (Hytten 2009; Leitch et al. 1959).

In regard to Dr. Leitch's role as the founder and editor of *Nutrition Abstracts and Reviews*, Hytten linked the journal's success to her critical mind, her standards of integrity, command of several languages, and scientific relationships with a world-wide invisible college of colleagues and friends. In particular, Hytten stressed her contribution to the scientific review articles in their tracing the history of ideas and overall quality. Hytten extolled her specific paper for a 1958 "International Conference on Scientific Information" organized by the US National Academy of Sciences. He quoted her as writing:

> The technique can be used for any quantitative problem as soon as enough bits of information are available to provide a worth-while array of data. It may or may not require, or be suitable for, complex statistical treatment. There are two main points to be noticed: the difficulties of assembly because of the scatter of publications, and the complexity of the subject matter itself which, by its nature, accounts for a large part of the scatter and, at the same time, makes assembly and analysis of the composite a necessity.
>
> (Hytten 2009, p. 115; Leitch 1959, p. 572)

In his 12-chapter, 263-page final volume *The Clinical Physiology of the Puerperium* (Hytten 1995), Hytten reviewed numerous aspects of this period of about 6 weeks, including the recovery of the pelvic organs and tissues, resumption of ovarian function, changes in body weight and composition, lactation, nutrition, and specifics on resolution of the pregnancy-induced changes in several organ systems.

In response to questions regarding his professional life and work, Frank Hytten responded:

> My own physiological beginnings were with fetal and neonatal physiology. I had devised and built a Drinker type "iron lung" suitable for premature infants (Hytten 1947) in my final year of Medicine, and although in retrospect I don't think it was a great idea, it impressed my professor of obstetrics and he got for me a three year research scholarship to spend two years abroad and a final year back in Sydney, after I had finished my two years of hospital house jobs. My first choice was to spend 6 months in Boston with Clement Smith which I did in 1949–1950 . . ..
>
> For most of the rest of my time abroad my professor thought I should go to Aberdeen, Scotland because somebody told him they had a very good special care nursery there. In fact they didn't, but I found myself in the wonderful department which Dugald Baird was starting to build up after the war. He had a powerful position as University professor, senior obstetrician for the whole region of north-east Scotland, and [was] married to a fellow Glasgow graduate who chaired the local medical committee. From his previous experience with well-off ladies in private practice who did well obstetrically compared to the poorest hospital patients who with equal care did badly, Dugald believed that difference was nutritional. The better-off women were taller, healthier, better educated and particularly . . . better nourished. The first member of the research team to be recruited, from the nearby Rowett Research Unit, was Angus Thomson a medical scientist with considerable experience as having been responsible for the nutrition of the troops in India. He was starting to measure the diets of pregnant patients. When I arrived there was no obvious role for a naïve untrained budding obstetrician, but Dugald set me this problem: obstetric outcome was generally measured by stillbirth or perinatal death rates, but this dead or alive criterion was far too crude—was it not possible to grade all the survivors in terms of vitality? I began by measuring muscle strength and then devised a way of measuring sucking strength. After my obligatory year back in Sydney, I was persuaded to return to Aberdeen, and continued where I left off moving to an interest in breast milk composition, and to a study of aspects of breast feeding. And thus my focus shifted from the baby to the mother. It was logical, with the departmental interest in nutrition, that I should look at changes in maternal body composition. After that it was downhill all the way—blood volume, body water, renal function etc. etc. (Hytten and Leitch 1964, 1971).
>
> Meanwhile, the department had grown enormously. Because we had an entire population in our grasp, all women were delivered by the same team in the same place, and record keeping was of an exceptionally high standard, this was an epidemiologists dream. This remarkable archive is still being mined today. So we had our own statistician (Wladyslaw Billewicz, several sociologists led by Raymond Illsley (1919–2013), an endocrinologist Arnold Klopper (1922–2014), eventually a big team which was incorporated into a Medical Research Council unit: the Obstetric Medicine Research Unit. One of the wonderful and

unusual aspects of the Aberdeen unit was that people like me were encouraged and given every opportunity to pursue clinical research, looking after our research patients as much as we wanted, such as antenatal care, but if operative intervention were needed we were not sufficiently practiced to be expected to provide it and there were plenty of clinicians around who would.

At some point, I can't remember when, Dugald asked me to write a chapter for a big obstetric textbook he was editing on nutrition in pregnancy. With the help of Isabella Leitch, who was the ultimate nutritional guru, a very senior nutritionist (she was the same age as my father) working at the Rowett Institute, editor of the influential *Nutrition Abstracts and Reviews*, and a considerable linguist, we wrote the piece for the book, but were left with a great pile of material on aspects of physiology. So we decided to write what I guess was the first book devoted to the physiology of pregnancy. Isabella retired to live with her daughter in Australia, and I wrote an updated second edition alone. By the time a third edition was being asked for by Blackwells much more information had accumulated, and it seemed sensible to involve specialist authors to write on specific aspects of physiology. I had moved to London by then and Geoffrey Chamberlain seemed anxious to push the idea ("You don't need to worry about it Frank, I'll do all the editing"). Big mistake. Bodger Chamberlain was an old friend, but far too ambitious, and a lousy editor who came spectacularly to grief after he took over editing the *British Journal of Obstetrics and Gynaecology*. (That is another long story). So, anyway, *Clinical Physiology in Obstetrics* was born and has been through three editions with a diminishing input from me. You ask about Jack Dewhurst. I never really knew him, but Chamberlain thought he was the sort of prestigious name to write a foreword.

In 1965, Dugald Baird retired, his successor did not have the ability to take over the MRC unit and we were moved, with Angus Thomson as director, to Newcastle upon Tyne, where they gave us a purpose built outfit beside the Princess Mary Maternity Hospital. We continued with much of what we had been doing, I now [became] particularly interested in placental transfer, and with John Davison developing our work on renal function, Tom Lind on carbohydrate metabolism and so on. Angus himself was particularly interested in fetal and child growth so the unit acquired the new name of MRC Reproduction and Growth Unit. The diagnostic indices booklet arose here; I had been collaborating for years with Ciba Geigy providing data for their wonderful *Documenta Geigy* tables, and it was they who suggested I write this small book, and a good opportunity to involve young Tom Lind in some writing.

In 1975, Angus Thomson was due to retire and as his deputy he would have liked me to take over the unit. I felt I needed a change and also felt the unit should have a proper practicing obstetrician as its next director, since we deeply involved with the care of patients. I made some tentative plans to take a small group to Oxford where I could be embedded in the department of an old Aberdeen buddy, Alexander ("Alec") Cuthbert Turnbull (1925–1990), later at Newcastle upon Tyne. For various reasons that didn't work out, but at that time a new rather exciting prospect was evolving. The secretary of the MRC, Sir Harold Himsworth (1905–1993) had planned that when the next new District General Hospital was built he wanted the MRC to add a clinical research unit with an almost equal number of beds to research into those conditions which come into a general hospital. A splendid idea, and a wonderful facility, with a big biochemical division, an animal division, an isotope setup etc. which was embedded in a new hospital at Northwick Park in Harrow, Northwest London. They had already set up various divisions with heavyweights such as Peter Medewar [Medawar] (1915–1987) in immunology, but were looking for someone to start a division on the obstetric/pediatric side. So I was recruited to set up what became the Division of Perinatal Medicine. I was there for about nine years and we made some progress with studies of placental transfer . . .. In the end it was not a great success, partly because once you get to the top you spend most of your time with administrative chores and staff problems for which I had no training and little aptitude.

> So in 1984, with my wife dying of ovarian cancer, I decided to retire. For many reasons, with which I won't bore you, the Clinical Research Centre disintegrated after a few years and most of the activity was moved to the Hammersmith Hospital. I was relieved to retire and bury myself in my five acre garden, but I kept in touch with my editorship of the *British Journal of Obstetrics and Gynaecology* which needed a major overhaul . . ..
>
> Finally, to end this . . . account . . . I went on to write another book. I had for some time been interested in what happens after pregnancy and had accumulated a lot of information. After all this wretched little fetus, who has spent nine months ruthlessly manipulating his mother's physiology and biochemistry in his own interest suddenly bails out leaving his mother physiologically pregnant. How does she extricate herself from that? It was fun to do, and I thought quite an interesting book but none of my publishers thought so, and I had an acquaintance publisher who willingly took it on. Unfortunately he didn't have many contacts, particularly in the States, and although it was well reviewed once or twice, nobody much noticed it and it sank without much of a trace. *It is The Clinical Physiology of the Puerperium*, published by Farrand Press in 1995 . . ..
>
> (Letter from FEH to LDL, 1 September 2014)

As noted, the Aberdeen Obstetric Medicine Research Unit under the direction of Sir Dugald Baird, regius professor of midwifery (later obstetrics and gynecology), included a wide variety of investigators. Among Sir Dugald's many accomplishments, he was one of the first to stress the sociological aspects of the diseases of women, among them the "fifth freedom," that of the freedom from excessive fertility (Baird 1965). This, of course, was in addition to the four freedoms (of speech, to worship a supreme being (God), from want, and fear) articulated by Franklin Delano Roosevelt in his State of the Union Address to Congress on 6 January 1941. Baird also led in the Royal College of Obstetricians and Gynecologists espousing the right of early abortion for women who choose that alternative. Among Baird's recruits, the sociologist Raymond Illsley (1919–2013) led a group of several sociologists in cross-disciplinary investigation of community medicine and related topics. Later, Illsley was head of the Department of Sociology (1964–1971) (Van Teijlingen and Barbour 1996). Reflecting upon his involvement with the obstetric medicine research unit, Illsley noted:

> I did not think of myself as a medical sociologist, or even as a sociologist, but as an ex-economist and town planner, interested in class and poverty, temporarily located in a medical milieu.
>
> (Illsley 1980, p. 160)

Nicholas Illsley of Hackensack University Medical Center and who worked with both Frank Hytten and his father Raymond for a decade as a graduate student and postdoctoral fellow has written:

> I first met Frank Hytten in Aberdeen, Scotland where he was working in the Obstetric Medicine Research Unit (OMRU). I was still at school at the time but I met Frank through my father, Raymond Illsley, who like Frank had been recruited by Sir Dugald Baird to work in a fairly unique, multidisciplinary research environment. This group included among others Frank, a physiologist and physician, the endocrinologist Arnold Klopper, Angus Thomson an epidemiologist and nutritionist, Bill Billiewicz a statistician and my father, a sociologist. Perhaps this interesting mix was one of the sources of Frank's iconoclastic approach, combined with a respectful but irreverent attitude stemming possibly from his

Australian roots. Much of Frank's most cited work was carried out in Aberdeen in combination with colleagues such as Isabella Leitch.

I experienced Frank's broad range of research interests when I went to work in the Division of Perinatal Medicine at the Medical Research Council's Clinical Research Centre in Harrow, London in 1981. Frank supported research into a wide range of new areas in perinatal research including his own nutritional research, research into energy metabolism (Angus Harkness) polyamine transport and function (David Morgan), folate metabolism (Michael Landon), and the research on which I embarked, placental permeability, transport and metabolic measurements using the new placental perfusion model. This new [approach] was Frank's adaptation of a recently devised model and being Australian, his version was upside-down compared to the others. This occurred against the backdrop of an explosion in placental research, especially in London, which he encouraged. This brought together many now-prominent researchers in the field including Leslie Myatt, Colin Sibley, David Yudilevich, Tony Firth, Lopa Leach, and David Bloxham in the information London Placenta Group that, at its zenith in the 1980s, numbered some fifty researchers.

Frank's talent was asking interesting questions and challenging long-held beliefs. He encouraged new research directions and gave wide latitude to the researchers he supervised, knowing some aspects would flourish and some fail. He was an early "translational" researcher, developing basic science research in obstetrics, a field which largely spurned it, but making that research relevant to the improved care of mothers and children.

(Letter from NI to LDL, 16 January 2015)

10.3 The Reproductive Tract in Pregnancy

During the course of pregnancy, significant changes occur in essentially every element of the reproductive tissues: uterus, cervix, vagina, as well as ovaries, and breasts. The uterus, for instance, undergoes an increase of manyfold from a relatively solid organ weighing ~70 g to a 1.0–1.1 kg structure containing approximately 5 l to accommodate the near-term fetus, placenta, and amniotic fluid. Enlargement of the uterus is accompanied chiefly by myometrial cell hypertrophy (enlargement) stimulated by the rise in estrogen and progesterone, rather than hyperplasia (increase in cell number). The myometrial cells are arranged in three layers: an outer hoodlike layer that arches over the fundus, a middle layer in which the smooth muscle cells are configured in a pattern of eight double curve network that can constrict the perforating vasculature following delivery, and a thin inner layer.

From the first trimester onward, the uterus undergoes irregular contractions that normally are painless and may or may not increase in frequency until shortly before term. First described by John Braxton Hicks (1823–1897) in the latter nineteenth century (Hicks 1871), the discovery of these contractions is an excellent example of careful observation by the clinician. Hicks recorded:

> . . . after many years' constant observation, I have ascertained it to be a fact that the uterus possesses the power and habit of spontaneously contracting and relaxing from a very early period of pregnancy, as early, indeed, as it is possible to recognize the difference of consistence—that is, from about the third month . . . It is seldom that so long an interval occurs as that of twenty minutes; most frequently it occurs every five or ten minutes, sometimes even twice in five minutes . . . The constancy with which these contractions of

> the uterus have always occurred to me leaves no doubt on my mind but that is a natural condition of pregnancy irrespective of external irritation.
>
> (Hicks 1871, pp. 219–221)

With great insight, Hicks considered the physiological effects of these contractions, noting:

> In the first place, it will provide for the frequent movement of the blood in the uterine sinus and decidual processes, for as the sinuses of the uterus are so much larger than the supplying arteries, the current is more slow in them than in the ordinary systemic veins. The contraction of the walls through which the sinuses meander tends to send the current onward, and to act somewhat as a supplementary heart. Besides this, it facilitates the movement of the fluid in the intervillal space of the placenta, or in that which is called the placental sinuses … In the second place, the uterine action adapts the position of the foetus to the form of the uterus.
>
> (Hicks 1871, p. 224)

He noted further:

> For the last six years and upwards I have made use of the intermittent action of the uterus as the principal symptom upon which I have depended in the diagnosis of pregnancy. I am not aware that I have been less successful than others in determining the existence of pregnancy; on the contrary, I have felt myself at an advantage in the possession of an additional sign to make up the deficiency or temporary inapplicability of the others; as, for instance, when external noise prevents the heart sounds from being heard.
>
> (Hicks 1871, p. 225–226)

Hicks detailed that, in part, his success followed his keeping his hand in full contact with the uterus of his patients continuously for 5–20 min and that his observations extended over an 8-year period. In the same year that Hicks published his treatise, Friedrich Schatz (1841–1920) of Leipzig and Rostock, Germany, described a fluid-filled *colpeurynter* (tocodynamometer) attached to a smoked drum to measure intrauterine contractions (Schatz 1872), although he did not mention those documented by Braxton Hicks. It would be almost a century before these contractions/pressure curves were quantified during pre-labor, as well as up to 2–4 h postpartum (Alvarez and Caldeyro Barcia 1948, 1950; Hendricks et al. 1962). The Braxton Hicks contractions have been shown to be associated with an increase in intrauterine pressure from 5 to 25 mm Hg (Alvarez and Caldeyro-Barcia 1950) and late in pregnancy may account for the so-called false labor. They also may coincide with a marked increase in fetal body movements and heart rate variability (Mulder and Visser 1987).

As first described by Alfred Hegar (1830–1914) (Hegar 1895), in conjunction with increased vascularity and edema, as early as 1 month following conception, the cervix begins to undergo softening and develops cyanosis. Although containing a small amount of smooth muscle, the cervix consists largely of collagen and elastin-rich connective tissue which, while helping to retain the products of conception within the uterus throughout the course of gestation, undergoes profound changes near term to effect delivery (Ludmir and Sehdev 2000; Mahendroo 2012). In addition, near term the endocervical mucosal glands hypertrophy to occupy about one-half of that tissue mass; and these mucosal cells secrete a tenacious mucus rich

in immunoglobulins and cytokines that obstructs the endocervical canal (Kutteh and Franklin 2001).

A decade earlier, James Read Chadwick (1844–1905), a Boston gynecologist, presented a thorough account of the bluish discoloration of the vagina as early evidence of pregnancy, Chadwick's sign, based on the examination of about 6000 patients, 281 of whom were pregnant, over a period of 10 years. In early pregnancy, the vulvovaginal mucosa undergoes a change in coloration from pink to rose to bluish purple due to the increased vascularity of the uterus, vagina, and associated structures (Chadwick 1887). Chadwick noted, "in scrutinizing the color of this part in a large number of women I early discovered that, while in the majority the bluish tinge appeared over the whole vaginal entrance, there was a fair proportion in which the violet tint was confined to the anterior wall of the vagina, just below the urinary meatus, whence it shaded it off into the normal pink color laterally. This, when distinctly perceptible, I soon found to be, in my practice, an absolutely sure sign of pregnancy . . .. The recognition of this peculiar localization of the blue tint on the anterior wall as a surge sign of pregnancy I feel is the most important new point in this communication" (Chadwick 1887, p. 407).

In addition to the cessation of ovulation and the suspension of follicular maturation, the ovaries also undergo significant changes. Perhaps the most dramatic of these is the transformation of the follicular site into the *corpus luteum* [yellow body], which for the first 6–7 weeks secretes progesterone to aid in the maintenance of the pregnancy (Csapo et al. 1973). In some cases these can develop into a so-called luteoma [yellow tumor] of pregnancy which, consisting of large acidophilic luteinized cells, can grow to a diameter of 20 cm or thereabouts (Sternberg 1963). Alternatively, the *corpus luteum* may develop into sizable theca lutein cysts which secrete large amounts of human chorionic gonadotropin (hCG) (Bidus et al. 2002).

By the beginning of the third month of pregnancy, the breasts increase in size, small veins become visible beneath the skin, and women may experience local tenderness and tingling. The nipples enlarge and become more deeply pigmented and more erectile. By the early third trimester, by gentle massage, a thick, yellowish fluid—*colostrum* [first milk]—can be expressed from the nipples. At this time the *areolae* [diminutive of space] become more broad [broader] and deeply pigmented. Scattered over the areolae, sebaceous glands—the glands of Montgomery (first described by William Fetherstone Montgomery (1797–1859); Montgomery 1837)—become evident. If the increase in breast size is excessive, striations such as those observed over the abdomen may appear. Perhaps paradoxically, breast size and volume of milk production do not correlate well (Hytten 1995).

10.4 Maternal Metabolic Changes in Pregnancy

In response to the demands of a rapidly growing fetus and placenta, the pregnant woman undergoes numerous physiologic changes in her cardiovascular and other systems and metabolic changes in carbohydrate, protein, and fat metabolism. These accommodate the needs of the developing fetus and prepare for subsequent lactation and care of her newborn infant. In early to mid-gestation, maternal food intake increases to promote uteroplacental development. A significant rise in first-phase insulin secretion, in the setting of normal insulin sensitivity, stimulates lipogenesis and reduces fatty acid oxidation, thereby promoting fat storage. Accordingly, maternal leptin levels begin to rise (Highman et al. 1998; Masuzaki et al. 1997). The accumulation of white fat during pregnancy provides an essential energy source for both the pregnant mother and fetus and serves as an energy reservoir for breast milk synthesis during lactation. With the accumulation of water and electrolytes, the growth of selected organs, and fat deposition, the average weight gain during pregnancy is 12.5 kg (27.5 lb.) (Hytten 1991), and the additional energy demands equal ~300 kcal day^{-1} (Hytten 1968; Hytten and Chamberlain 1991). Normal pregnancy is characterized by a state of mild, fasting, hypoglycemia, postprandial hyperglycemia, and hyperinsulinemia (Phelps et al. 1981). Teleologically, this state of relative insulin resistance may help to ensure a sustained postprandial supply of glucose to the fetus. In late normal pregnancy, insulin action is ~50% lower than that in the nonpregnant state (Butte 2000); however, the mechanism(s) of this insulin resistance is/are not understood. Although estrogen and progesterone may, in part, mediate this insulin resistance, plasma placental lactogen, a protein with growth hormonelike action, can result in lipolysis with liberation of free fatty acid which may contribute further to this insulin resistance (Freinkel 1980).

As noted elsewhere, Hytten and Leitch had a great interest in nutrition in pregnancy, a subject with a long history going back to the earliest records (Mussey 1949). For optimal growth and development of the fetal "parasite" (Paddock 1920, p. 75) or "neoplasm" (Murlin 1917, p. 926), even early twentieth-century writers advocated a diet balanced in the major nutritional categories (Murlin 1917; Paddock 1920; Paton 1903). In fact, some evidence suggested that:

> ... so far from being a sacrifice of the individual [mother] for the good of the species, gestation normally may be looked upon as a means employed by the species for the good of the individual [mother].
>
> (Murlin 1917, p. 928)

In regard to both substance and energy metabolism, the author concluded his review pointing out that beyond a period of "parasitism" during the first trimester, the maternal and fetal organisms enjoy "harmonious symbiosis" (Murlin 1917, p. 946).

As can be imagined, the growing conceptus, with that of the uterus and maternal blood hemoglobin and plasma proteins, places great demands on requirements for protein (~1 kg; Hytten and Leitch 1971). Nitrogen balance, i.e., the state of nitrogen

intake versus excretion, progressively increases during the course of gestation, suggesting a more efficient use of dietary protein (Mojtahedi et al. 2002). Other studies have demonstrated that catabolism of maternal muscle protein is not required to meet the demands of the growing maternal and fetal tissues (Kalhan et al. 2003).

As with other metabolites, the concentrations of plasma lipids, both high- and low-density lipoproteins, and apolipoproteins increase appreciably during pregnancy. The second trimester is associated with fat storage (Hytten and Thomson 1968; Pipe et al. 1979; Thomson et al. 1968), principally in central rather than peripheral sites. A hypothalamic "lipostat" has been postulated for energy storage that might work to protect the mother and fetus during periods of prolonged starvation and physical exercise (Hytten and Thomson 1968).

10.5 Pregnancy-Associated Changes in the Endocrine System

Widely dispersed into multiple individual organs and sub-organs that interact via circulating hormones, the endocrine system is one of the most unique and creative systems imaginable to maintain bodily function and homeostasis. In the pregnant woman, this is coupled tightly with those hormonal systems of the placenta and fetus to optimize successful fetal growth, parturition, and the prolonged survival of the species. In many ways, it is all beyond belief.

Located in the *sella turcica* [Turkish chair] on the inferior aspect of the brain, the pituitary gland or hypophysis [to grow below] is the master director of the glandular orchestra. Divided into anterior (adenohypophysis), small intermediate, and posterior (neurohypophysis) lobes, only in the early twentieth century was it demonstrated that while the anterior lobe was essential to life, the posterior lobe was not (Paulesco 1908) and the relation of that portion of the gland to the reproductive system (Crowe et al. 1910). Other studies during the early days of endocrinology helped to clarify the role of the adenohypophysis (Cushing 1910; Evans and Long 1921; Smith 1927; Smith and Engle 1927). In concert with these reports, clarification on the role of the neurohypophysis became established (Dale 1909; Kamm et al. 1928).

In the early twentieth century, growth hormone (GH) was discovered to be secreted by the adenohypophysis by Herbert McLean Evans (1882–1971) and Joseph Abraham Long (1879–1953) (Evans and Long 1921). Maternal pituitary GH or GH-N appears early in pregnancy and is not detected after 24 weeks. Conversely, that GH secreted by the placenta (GH-V) becomes the principal source of that hormone by mid-gestation (Baumann 2009; Fuglsang and Ovesen 2006; Obuobie et al. 2001). Some have proposed that the fall in GH-N levels during early gestation may increase insulin sensitivity, and in combination with insulin, progesterone, and prolactin, may promote maternal fat storage. Following mid-gestation,

the striking rise in maternal levels of GH-V in concert with TNFα, cortisol, and progesterone, with decreased plasma adiponectin (Catalano et al. 2006; Mastorakos and Ilias 2003), may facilitate the emergence of maternal insulin resistance (Ryan and Enns 1988). Despite fetal circulating GH levels being relatively abundant during the second trimester, this does not appear to be the chief regulator for fetal growth (Lønberg et al. 2003), as the anencephalic fetus without a pituitary gland has essentially normal weight and length (Wollmann 2000). During the latter half of pregnancy, however, the level of GH correlates with placental growth hormone insulin-like growth factor 1 (Chellakooty et al. 2004).

The anterior pituitary hormone prolactin (PRL), discovered and isolated by two groups (Riddle et al. 1933; Stricker and Grueter 1929), increases ~10-fold during the course of gestation. Presumably under the influence of estrogen, thyroid-releasing hormone, and serotonin (Andersen 1982), early in gestation, this with the steroids estrogen and progesterone serves to increase food intake and that of mitotic activity in the mammary gland glandular epithelial cells and presecretory alveolar cells. Later, this is associated with galactopoiesis and the production of casein, lactalbumin, lactose, and lipids in preparation for lactation and breast feeding (Andersen 1982; Kauppila et al. 1987). For reasons not well understood, in the mid- to late second trimester and presumably synthesized by the uterine decidua, amniotic fluid prolactin levels peak at levels ~1000-fold greater than during the nonpregnant state. Some regard this a mechanism to impede transmembrane amniotic water flux, to ensure the maintenance of relatively normal amniotic fluid volume and tonicity.

Associated with fat accumulation and increased PRL synthesis, maternal leptin levels increase (Trujillo et al. 2011) as does the agouti-related peptide (AgRP) (Strader and Buntin 2003). Leptin levels continue to rise after mid-gestation as a consequence of its synthesis by both maternal adipose tissue (Highman et al. 1998) and the placenta (Masuzaki et al. 1997). The third trimester of pregnancy appears to be a state of central leptin resistance that enables the mother to maintain relatively high caloric intake despite fat accretion. As GH-V binds with only low affinity to the prolactin receptor, the hyperphagia and relative leptin resistance of pregnancy are likely to be mediated by the lactogens. Direct effect of the lactogens on maternal insulin production and hypothalamic gene expression and indirect effects on leptin action serve to maintain maternal metabolic homeostasis, at the same time providing nutritional substrates for the fetus and newborn infant (Newbern and Freemark 2011).

As with other ductless glands, the thyroid undergoes moderate enlargement as a consequence of increased vascularity and glandular hyperplasia. This is accompanied at mid-gestation by ~50% increases in the major thyroxin-binding globulin, presumably in response to estrogen, with an accompanying increase in serum thyroxine (T4) (Glinoer et al. 1990). In contrast free serum T4 rises somewhat during the second trimester, as does triiodothyronine (T3) (Burrow et al. 1994). Nevertheless, maternal thyroid function may vary considerably during the course of pregnancy (Glinoer et al. 1990). Pituitary-produced thyroid-releasing hormone (TRH) levels remain relatively stable, but may cross the placenta to stimulate

fetal thyrotropin (Thorpe-Beeston et al. 1991). In view of the fact that the glycoproteins placental human chorionic gonadotropin (hCG) and TSH share similar alpha subunits and somewhat similar beta subunits, hCG possesses intrinsic thyrotropic activity. As noted earlier, maternal basal metabolic rate increases ~25% during pregnancy, of which most of the gain is attributable to that of the fetus and placenta.

Parathyroid hormone secretion is stimulated by decreased [Ca^{2+}] or [Mg^{2+}], whereas increases in these ions have the opposite effect. This hormone also acts to increase intestinal absorption, reabsorption by renal tubules, and bone resorption, thus increasing extracellular fluid [Ca^{2+}] and decreasing levels of phosphate. During the first trimester, parathyroid hormone concentration decreases, but then increases progressively throughout the remainder of pregnancy (Pitkin et al. 1979). Overall, despite the increase in maternal blood volume, glomerular filtration rate, and its transfer to the fetus, plasma [Ca^{2+}] remains quite stable, decreasing only slightly (Pitkin 1985). This has suggested to some that a hypothalamic [Ca^{2+}] "set point" is established during pregnancy that acts to stabilize these various functions (Reitz et al. 1977), the net result being a "physiological hyperparathyroidism."

During the course of gestation, as in other times in life, the plasma calcium concentration [Ca^{2+}] is highly regulated, as is that of magnesium, phosphate, and other compounds vital to mineral metabolism. To a great extent, these ions relate to the activities of parathyroid hormone, calcitonin, and vitamin D. Discovered in the early twentieth century (McCollum et al. 1922), vitamin D now is known to consist of multiple secosteroids (i.e., steroids in which one of the steroid rings is broken) that play differing roles in metabolism. As is typical of steroid hormones, vitamin D is now known to undergo several hydroxylations in multiple organs. In the liver, vitamin D (cholecalciferol) is converted to 25-hydroxy vitamin D (calciferol) which is the circulating reservoir of the inactive substrate for the most active metabolite of vitamin D, 1,25-dihydroxy vitamin D. The majority of 1,25-hydroxy vitamin D in the circulation is produced in the kidney, under the control of parathyroid hormone but not the 25-hydroxy vitamin D substrate. During pregnancy, serum 1,25-dihydroxy vitamin D is markedly elevated; however, the stimulus is yet to be discovered. There is also an extrarenal production of 1,25-dihydroxy vitamin D in several organs, including uterine decidua and placenta. Extrarenal production of 1,25-dihydroxy vitamin D is substrate dependent which contrasts with its renal production. In summary, circulating 1,25-dihydroxy vitamin D is available to act via the vitamin D receptor in reproductive and other organs, whereas circulating 25-hydroxy vitamin D is an important determinant of 1,25-dihydroxy vitamin D produced locally in reproductive and other organs (Weisman et al. 1979; Whitehead et al. 1981). As evidence of the activity of these compounds during the course of gestation, essentially every marker of bone turnover increases during pregnancy, and, somewhat surprisingly, these fail to return to the pre-pregnant basal values even 12 months postpartum. This has caused some authors to conclude that calcium and related compounds needed for fetal growth and postpartum lactation are derived, at least in part, from the maternal skeleton (More et al. 2003).

Roy Macbeth Pitkin, formerly chair of the Department of Obstetrics and Gynecology at both the University of Iowa and the University of California, Los Angeles, in response to queries regarding his contributions to the field of calcium metabolism and other areas of research, responded:

> My interest in calcium metabolism arose from clinical encounters during and following my residency at the University of Iowa. The major clinical problems with which I dealt was pregnancy complicated by type 1 diabetes mellitus. Maternal diabetes is well known to be associated with a number of problems in the fetus and newborn, one of them being hypocalcemia within 48 hours of birth. Clearly, this complication reflected something involving the maternal-fetal relationship during late pregnancy. The pathophysiology of another complication in infants of diabetic mothers, neonatal hypoglycemia, had been quite well worked out by this time, and it seemed that something similar could be involved in the case of calcium. Additionally, the fact that the near-term fetus contains about 30 grams of calcium means that normally pregnancy must involve maternal physiologic adjustment (s) to provide for this need.
>
> I began by reviewing the literature to learn about maternal-fetal-neonatal calcium homeostasis. There wasn't much, and the little I found was incomplete, fragmentary, and often contradictory. It was immediately clear that the situation was rather more complicated than that with glucose. Thus, I was led into an interest in calcium metabolism.
>
> Extracellular fluid calcium exists in three forms. The biologically active form, the divalent ion, represents about 45% of total serum calcium, a similar proportion is bound to protein (principally serum albumin) and the remaining 5 to 10% is complexed, mainly to citrate. Total serum calcium levels (about all one could find in the literature) vary widely, however the variation involves mainly the albumin-bound fraction, whereas the level of ionic calcium is regulated very closely. In fact, in studies of large populations of normal women the range of serum ionic calcium is but 10 percent of the mean, and I cannot think of any other substrate so tightly regulated. The system of regulation is complicated, not least because three hormones (parathormone, calcitonin, and 1,25-dihydroxycholecalciferol) are involved.
>
> My studies were collaborative, involving W. Ann Reynolds and Gerald Williams of the University of Illinois who developed immunoassays for calcitonin and parathormone, respectively. Our investigations extended over a number of years and involved studies of normal pregnancy, the menstrual cycle, the neonate at birth and over the first 24 hours, as well as provocative experiments in adult and fetal monkeys.
>
> As I was completing my military service, I confronted what to do next. I had an interest in academic medicine, an interest encouraged by my clinical mentor, William Charles Keettel (1911–1981) of the University of Iowa. I looked at several positions, ultimately deciding on the University of Illinois in Chicago, where I began as Assistant Professor in July 1965. Illinois at the time was an "old fashioned" medical school with a strict full-time faculty. This meant that salaries were low and there was little in the way of money for professional activities. But it also meant that I had abundant free time. I had a little experience in research, having published five first-author papers during the course of a busy clinical residency, and I was aware, at least in a general way, of the importance of focusing on scholarship if one were to be successful in academia. So I thought, and talked with a wide variety of people, especially in the basic sciences, and I spent a great deal of time in the library. Illinois provided, at least for me, the ideal place to start an academic career.
>
> On one of my wanderings through the basic science building (immediately adjacent to the hospital), I happened to meet Samuel R. M. Reynolds, Chairman of the Department of Anatomy. It was a name known to me, for I had perused his classic book *Physiology of the Uterus with Clinical Correlations* (Reynolds 1939) in the departmental library during my residency at Iowa, but I was unaware that he was now at Illinois. I found him most cordial

and inviting. In fact, he seemed to have nothing better to do other than talk with a young and rather naïve clinician who wanted to learn something about science. So it was that when I found his office door open (as it nearly always was), I would go in and sit and talk with him for extended periods.

The work with amniotic fluid analysis in Rh sensitized pregnancies had just come out and it had demonstrated clearly that the amniotic fluid could accurately reflect the fetal status and, moreover, that amniocentesis was reasonable safe. I was interested in applying amniotic fluid analysis to other clinical states (and had actually just completed work showing its efficacy in estimating fetal maturity). Intelligent use of amniotic fluid analysis, it seemed to me, depended on some understanding of the physiology of that fluid, and I found that very little was known of this matter. Sam patiently took me through dealing with this problem, pointing me to what little literature there was, discussing what type of models and design might be appropriate, and helping me see a systematic approach. He suggested using the rhesus monkey because of its closeness to the human and I recall vividly the day he walked to his filing cabinet and pulled out a paper describing a technique of catheterizing the fetal circulation with the fetus in utero and the amniotic sac intact (Reynolds et al. 1954). It was a technique my colleagues and I would find useful in many studies of various types over the ensuing decades. Perhaps more important than this technical "gift" was the rigorous and analytical approach to scientific questions that I learned from him, always given in the most gentlemanly of ways.

It was Sam Reynolds who first told me of the Society for Gynecologic Investigation (SGI) (now the Society for Reproductive Investigation), identifying it as an organization to which I should aspire. His kindness extended to social matters and my wife and I recall a delightful dinner in the apartment he and Mary Lib occupied in the Lincoln Park area. He retired to Pennsylvania a year or two after they left Illinois to go to Iowa in 1968. I saw him last at the SGI meeting in Philadelphia in 1976.

In regards to future prospects, I'm far enough removed from active investigation that I feel singularly unqualified to comment on what the future might bring with respect to maternal-fetal physiology and medicine. Although I haven't given up journal reading entirely, I am anything but up-to-date in current research. I guess I might make one very general observation. The incredible advances of the last decade or two have been, largely if not entirely, at the molecular level and with this effort has come, correspondingly and inevitable, a decreased emphasis on whole-organism (human or animal) research. We need to take care that we don't, as the old adage goes, "throw the baby out with the bath water".

(Letter from RMP to LDL, 17 October 2014)

During the course of gestation, serum total cholesterol level rises, but most of this is secondary to increased cortisol-binding globulin transcortin, as a consequence of the increased estrogen level. Nonetheless, with doubling of cortisol half-life (Migeon et al. 1957), its metabolic clearance rate is somewhat lower. A paradox exists, however, in that early in pregnancy maternal circulating adrenocorticotropin (ACTH) levels are reduced; however, with lengthening gestation, ACTH and cortisol levels rise, especially near term (Challis et al. 2005). Again, as with other endocrine systems, a resetting of maternal hypothalamic-pituitary-adrenal feedback mechanisms and perhaps tissue refractoriness to cortisol have been proposed (Nolten and Rueckert 1981). In addition, such phenomenon may result, in part, from an antagonist action of progesterone on mineralocorticoids to maintain cellular homeostasis (Keller-Wood and Wood 2001). In fact, in studies in the pregnant ewe, these authors demonstrated that a combined elevation of cortisol and aldosterone is required to maintain the normal expansion of plasma volume during the last trimester of gestation (Jensen et al. 2002).

By 15 weeks' gestation, the *zona glomerulosa* [glomerular zone] of the maternal adrenal secretes significantly increased amounts of aldosterone, which increases further as pregnancy advances (Watanabe et al. 1963). Concurrently, renal renin and hepatic angiotensinogen increase, giving rise to elevated plasma angiotensin II, which by stimulating the zona glomerulosa further increases aldosterone secretion (Lumbers et al. 2013). Such an increase in aldosterone may, in part, negate the natriuretic effect of progesterone and atrial natriuretic peptide. A potent mineralocorticosteroid, deoxycorticosterone increases progressively more than 15-fold during the course of pregnancy (Parker et al. 1980). This increased production arises not from the maternal adrenal, however, but is derived from the kidney via estrogen stimulation. In the blood of the fetus, these levels are even higher, which suggests considerable transplacental transfer into the maternal compartment. In contrast, the dehydroepiandrosterone sulfate concentration decreases during the course of gestation, as a consequence of 16alpha-hydroxylation in the maternal liver with conversion to estrogen by the placenta (see below). Although maternal plasma levels of testosterone and androstenedione are increased in pregnancy, presumably by the ovary, as these are converted to 17beta-estradiol by the placental trophoblast, the fetus sees essentially none of these androgens (Edman et al. 1981). Of vital importance, the placenta, with its secretion and metabolism of a host of steroid hormones, protein, endocrine, paracrine, and autocrine regulators, plays a critical role in maternal physiology (Chap. 9; Siler-Khodr 2011).

In woman during the typical range of reproductive years (age 15–45), the pituitary hormones follicle-stimulating hormone (FSH) and luteinizing hormone (LH) regulate production of estrogens and progesterones by the ovary. The most biologically active of the naturally occurring estrogens, 17beta-estradiol (E2), is secreted by the granulosa cells of the dominant ovarian follicle and luteinized granulosa cells by the corpus luteum. The action of estrogen is complex involving two nuclear receptors, estrogen receptor alpha and estrogen receptor beta (Katzenellenbogen et al. 2001), isoforms of separate genes each with distinct differences in tissue expression. Each of these steroid-receptor complexes acts as transcription factors that become associated with the estrogen response elements of specific genes and, therefore, via the synthesis of specific messenger RNAs (mRNAs) that promote the synthesis of specific proteins. In a similar fashion, progesterone, through its receptors (progesterone receptor type A and B), produces its unique activities related to preparation of uterus for implantation and other physiological adaptations.

The maternal ovary is the principal source of systemic estrogens and progesterone up until the midpoint of the first trimester. Before 8 weeks of gestation, production of these steroids shifts from the corpus luteum in the ovary to the placenta (Tulchinsky and Hobel 1973). The placental trophoblast initially rescues the corpus luteum by producing chorionic gonadotropin (hCG, analogous in biological activity to a long-acting LH) to sustain estradiol and progesterone production, but then takes over as the main source for steroidogenesis that, throughout the course of pregnancy, elevates circulating concentrations over 100-fold greater than that before pregnancy. This luteal placental shift effectively creates a

"hyperestrogenic-hyperprogesteronic" state that, at least for progesterone, is an absolute requirement to sustain pregnancy in women (Csapo et al. 1973).

For estrogens, a distinct placental pathway for synthesis differs from that of the ovary, with dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S) derived from the fetal adrenal gland serving as precursors (Ryan 1959). As noted elsewhere, it was shortly after the mid-twentieth century that the observation was made that women with an anencephalic fetus secreted only a small fraction of the amount of urinary estrogen compared to that of a normal pregnant subject (Frandsen and Stakemann 1961). Because of atrophy or poor development of the adrenal glands with the absence of the fetal zone of such infants, these authors proposed that the fetal adrenal must secrete those compounds that promote placental estrogen synthesis. Subsequent studies demonstrated the truth of this conjecture (Baulieu and Dray 1963; Siiteri and MacDonald 1963). Further details of the studies that affirmed this hypothesis were described shortly thereafter (Siiteri and MacDonald 1966).

Endocrine regulation of energy metabolism undergoes dramatic changes during pregnancy to benefit both the fetus and mother. Although maternal adaptation to pregnancy is to a large extent regulated by placental hormones, such as prolactin, placental lactogen, and steroid hormones (Abrams and Pickett 1999), leptin plays a role in food intake and energy expenditure, among many other functions (Sagawa et al. 2002). This hormone, encoded by the gene *LEP*, is produced predominantly in adipose cells (adipocytes). In nonpregnant individuals, serum leptin concentrations correlate with adipose tissue mass and BMI and induce satiety. Circulating leptin levels with both endocrine and paracrine functions increase during the course of pregnancy (Lepercq et al. 2003; Lepsch et al. 2016) in concert with its production by the placenta (Ashworth et al. 2000; Masuzaki et al. 1997), increasing the mass of adipose tissue in the mother and fetus. The methylation of promoter *LEP* is inversely related to tissue leptin expression (Marchi et al. 2011; Melzner et al. 2002) and is associated with pregnancy complications that can compromise fetal development (Bouchard et al. 2010; Hogg et al. 2013; Lesseur et al. 2013). In summary, leptin expression is regulated by epigenetic mechanisms and exerts a number of metabolic and reproductive functions in both the gravid mother and developing fetus.

As noted below, two of the most dramatic changes in maternal physiology are the marked increases in both blood volume and cardiac output. In light of the massive amount of estrogen produced by the placental trophoblast from fetal adrenal gland precursors and the further effect of maternal renal Na^{2+} absorption, water retention, with increased renin-angiotensin and aldosterone, it is not unreasonable that the maternal increase in plasma and erythrocyte volume and cardiac output is, in part, a consequence of regulation by the maelstrom of maternal-placental-fetal endocrinologic milieu (Longo 1983), and this has been borne out experimentally (Carbillon et al. 2000; Ueda et al. 1986).

10.6 Cardiovascular System: Blood Volume

In view of its central and pivotal role in providing oxygenated and nutrition-laden blood to the tissues of the body, the heart and its vascular system play a key role in the added demands of pregnancy upon the pregnant woman. A pioneer in elucidating the unique hematologic and cardiovascular changes of pregnancy was Jack Arthur Pritchard (1921–2003) of the University of Texas Southwestern, in Dallas. Following his obtaining a degree in pharmacology, his graduation from medical school (1942), completion of a residency in obstetrics and gynecology, and a further 2-year fellowship in hematology under Oscar Davis Ratnoff (1916–2008) all at Case Western Reserve University, he became chair of the newly created department of Southwestern and chief of obstetrics at the Parkland Memorial Hospital. In addition to his contributions to an understanding of the hematological problems of pregnancy per se, Pritchard with his colleagues explored aspects of these in eclampsia and other pathologies (Cunningham and Pritchard 1978; Cunningham et al. 1983, 1986; Maberry et al. 1990, 1992; Pritchard et al. 1984, 1991).

Francis Gary Cunningham of the University of Texas Southwestern, who worked with Pritchard for over a decade, has written:

> Jack Pritchard was the consummate clinician and clinical scientist. He was equally meticulous in his approach to patient care and laboratory and clinical research. During his residency at Case Western he developed an interest in preeclampsia and eclampsia and together with coauthors, published seminal observations on what later became known as the HELLP syndrome. His fellowship with hematologist Oscar Ratnoff spawned a lifetime of interest in hematological complications of pregnancy which led to in-depth studies of intravascular coagulation with eclampsia and placental abruption, as well as studies of iron-deficiency anemia, sickle-cell and other hemoglobinopathies, platelet abnormalities, and a myriad of other hematological disorders complicating pregnancy.
>
> After fellowship, Pritchard took the job as chairman of a newly formed Southwestern Medical School in Dallas. He quickly became respected as a clinician and a firm and fair taskmaster. Shortly after his arrival, he implemented a plan to treat preeclampsia and eclampsia in women at Parkland Hospital using an intravenous/intramuscular magnesium sulfate regimen that became adopted internationally. Pritchard's studies of placental abruption and disseminated intravascular coagulation led him to conduct investigations using radiochromium-labelled erythrocytes to study blood volume, red cell mass, and erythrocyte survival times. These studies—carried out before the proscription of the use of minute quantities of radioisotopes in pregnancy—set the standard for measuring blood loss with normal delivery as well as with obstetrical hemorrhage. It was also during these years that he conducted physiological studies of fetal swallowing, breathing, and urination.
>
> (Letter from FGC to LDL, 17 August 2014)

In response to the question as to what motivated Pritchard to become a physician-scientist in an age in which that was most unusual, particularly among obstetrician-gynecologists, Cunningham replied:

> Interesting question, especially because my own career began early in that "period of enlightenment". This was during the 1970s, and as you know, we were surrounded by self-declared experts who espoused their personal ways of doing things simply proven by "because I said so", or "that's the way we've always done it". I think it was this attitude that caused our specialty to move to become evidence based, especially since many of the

> "pronouncements" were downright dangerous, e.g., giving heparin for "DIC" [disseminated intravascular coagulation], etc. I think that Jack was the antithesis of this thinking, i.e., he demanded meticulously performed clinical research in order to "prove" that a method was safe, effective, and pragmatic.
>
> (Letter from FGC to LDL, 24 August 2014)

In a further inquiry regarding his toughness in critiquing the presentations of others at national meetings, Cunningham responded:

> I think you hit the nail on the head with this characterization of "critical thinker". I can see why you might have viewed him as "hostile"! He really wasn't but he didn't suffer fools and poseurs for sure. As an aside . . . he was not one to lavish praise.
>
> (Letter from FGC to LDL, 24 August 2014)

By 6–8 weeks' gestation, plasma volume begins its 40–50% increases (1400 ± 200 ml) to a maximal value of 5000 ± 200 ml at 32 weeks' gestation, the so-called "plethora of pregnancy" (Bernstein et al. 2001; Chesley 1972; Lund and Donovan 1967; Pritchard 1965a). The initial studies along this line were those of Johannes Lindhard (1870–1947) of Denmark who reported a 50% increase in cardiac output using the dye dilution technique (Lindhard 1915). William Joseph Dieckmann (1897–1957) at the Chicago Lying-in Hospital and a colleague (Dieckmann and Wegner 1934) showed an increase in plasma volume of 17% in primiparous subjects and 32% among those that were multiparous with wide variability among individuals with advancing gestation. The average gestational increases in plasma and whole blood volume were 25% and 23%, respectively (Dieckmann and Wegner 1934). Some have attributed the variability in results to the method used, Congo red, which is known to give less accurate results than other dyes (Hytten and Paintin 1963). Soon, others had confirmed the pattern of this increase in both plasma and red blood cell volume (Adams 1954; Hytten and Paintin 1963; Kjellberg et al. 1950; Lowenstein et al. 1950). As noted, the mechanism of this profound increase, which early in gestation anticipates the demands to be met later in pregnancy, is not totally clear but probably reflects the enormous increase in circulating estradiol and other steroids (Longo 1983). With the increase in plasma volume and decrease in serum albumin concentration, colloid osmotic pressure decreases 10–15% (Clark et al. 1989).

In turn, in response to increased erythropoietin production by the kidneys (Choi and Pai 2001), red cell mass slowly increases 20–30% by 350 ± 100 ml at term to complement the pregnancy-induced plasma hypervolemia, compared to nonpregnant values, an increase that reflects increased RBC production per se rather than a prolonged half-life. Again, although the mechanism of this increase is unclear, a number of maternal erythropoietic factors are involved. These include not only those of the maternal system but also involve placental chorionic somatomammotropin, progesterone, prolactin, and other hormones (Jepson 1968). A complicating factor here is that in addition to the 500 mg of iron required to provide the hemoglobin required for this erythropoiesis and the ~200 mg iron required to compensate for daily losses throughout gestation, ~300 mg iron is transferred from maternal stores to supply the demands of the fetus (Hytten 1985;

Pacheco et al. 2013). In conjunction with this increase in erythrocyte mass, the intra-erythrocyte concentration of 2,3-diphosphoglycerate increases during the course of gestation, which lowers the HbO_2 affinity for O_2 (see below). In view of the fact that the plasma volume increase is disproportionate to that of the RBC increase, this physiologic hemodilution or mislabeled "anemia of pregnancy," which becomes maximal by the mid-third trimester, may help to promote relative protection by decreasing blood viscosity and both promote intravillous perfusion and counter any predisposition to thromboembolic events (Peeters et al. 1987).

10.7 Maternal Cardiac Output

The product of stroke volume (that quantity of blood pumped into the aorta during each cardiac cycle) and heart rate, cardiac output (CO) is a quantitative measure of the functional capacity of the heart. Stroke volume is a function of both preload (the pressure and amount of blood returning to the heart) and afterload (the mean arterial blood pressure) and increases 20–30% during pregnancy (San-Frutos et al. 2011). Early in pregnancy, maternal heart rate increases, plateauing at 15–20 beats min^{-1} in the third trimester (San-Frutos et al. 2011). In an early study with the use of the André Frédéric Cournand (1895–1988) technique of right heart catheterization and the Adolph Fick (1821–1901) principle, the pregnancy-associated increase in pregnancy cardiac output from 4.5 ± 0.4 l min^{-1} in the nonpregnant state to 5.7 ± 0.7 l min^{-1} in the 26th to 29th week equals 27% (Hamilton 1949). With improvement of indicator-dilution techniques, the development of continuous photometric dye analysis of arterial blood, and capillary blood assessment by transillumination of the ear, longitudinal studies became possible and demonstrated the 30–40% maximum increase in cardiac output at 24–30 weeks' gestation (Walters et al. 1966). Also about this time, the importance of postural effects on cardiovascular dynamics became clear, with measurements of the pregnant patient in the left lateral, as opposed to supine, position (Lees et al. 1967; Quilligan and Tyler 1959). Although limited data have been obtained from normal pregnant women by means of cardiac catheterization with dye or thermal dilution (Clark et al. 1989), noninvasive methods such as M-mode echocardiography (Mashini et al. 1987) and laser Doppler ultrasound (Easterling et al. 1987, 1990; Ihlen et al. 1984) have provided good correlation with the invasive methods in those instances of appropriate comparison. A caveat is that thoracic electrical bioimpedance, which is influenced by intrathoracic volume, hemoglobin concentration, and chest configuration (each of which changes with pregnancy), correlates poorly with thermodilution, which underestimates cardiac output in pregnancy (Easterling et al. 1987; Masaki et al. 1989; Milsom et al. 1983).

In conjunction with an increase in ventricular wall muscle mass and an increase in end-diastolic volume (Rubler et al. 1977), an early study disclosed that during the course of pregnancy, maternal cardiac output increases 30–50% (chiefly by the increase in stroke volume), this to provide the expanding uteroplacental vascular

bed, as well as that to the breasts, kidneys, and skin, and to the increasing maternal tissue mass (Adams 1954), and this was substantiated in later work. One-half of this occurs during the first week of gestation (Capeless and Clapp 1991). Near term, in conjunction with a decrease in stroke volume, cardiac output decreases ~10% (McLennan et al. 1987; Rubler et al. 1977; Ueland et al. 1969). As compared to a nulliparous woman, parous women demonstrate a significantly greater median cardiac output and cardiac index [CO per unit time · body surface area^{-1}, usually calculated as 1 min^{-1} m^{-2}] in association with higher median stroke volume, heart rate, left ventricular outflow diameter, and a significant lowering in total vascular resistance (Turan et al. 2008). In normal twin or other multiple gestations, maternal cardiac output increases to an even greater extent from the mid-trimester onward (Kuleva et al. 2011).

As noted, pregnancy-associated increase in cardiac output is a function of both an increase in stroke volume and heart rate. A primary component of the early increase in cardiac output, the increase in stroke volume reflects on early increase in ventricular muscle mass and later increase in end-diastolic volume (Rubler et al. 1977; Thompson et al. 1986). In contrast, from 5 weeks' gestation, maternal heart rate increases to a maximal increment of 15–20 beats min^{-1} by ~32 weeks, where it is maintained. Thus, in the late third trimester, maternal cardiac output is primarily maintained by tachycardia. In addition, a concomitant softening of collagen within the entire vascular tree, associated with smooth muscle hypertrophy, results in increased compliance of capacitive (predominantly elastic wall) and conductive (predominantly vascular smooth muscle) that may become evident by week 6 of pregnancy (Spaanderman et al. 2000).

An additional factor that affects cardiac output is maternal posture. For instance, near-term turning from recumbent to the left lateral to supine position can result in a 25–30% decrease in cardiac output (Ueland et al. 1969). This occurs as a consequence of compression of the inferior vena cava by the gravid uterus, with resultant decreased venous return. A significant number (~8%) of pregnant women experience supine hypotensive syndrome, manifest by decreased arterial BP, bradycardia, and perhaps syncope (Holmes 1960), which has potential consequence to the developing fetus, such as "sluice flow" mechanism, in which abrupt increase in uteroplacental outflow pressure may compromise fetal umbilical placental blood flow and oxygenation (Archer et al. 2011; Butler et al. 1976; Power and Longo 1973). Such effects on the fetus may vary during the course of gestation because uteroplacental blood flow increases several hundredfold to between 500 and 1000 ml min^{-1} (Gant and Worley 1989), a shift from ~2% of cardiac output in the nonpregnant state to ~18% at term. The increase in blood flow to the uterus and placenta constitutes ~25% of cardiac output (Thornburg et al. 2000). Blood flow to the kidneys also increases significantly, ~50% (Chesley and Sloan 1964), as does that to the mammary glands and skin (Frederiksen 2001; Katz and Sokal 1980). Thus, clinical implications of uteroplacental blood for fetal development flow must include consideration of gestational age and related parameters.

10.8 Arterial Blood Pressure

Beginning as early as week 7 or 8 of pregnancy, mean maternal arterial blood pressure decreases (Capeless and Clapp 1989). To a certain extent, this early decrease represents incomplete compensation for the decrease in peripheral vascular resistance with the increase in cardiac output. When measured in sitting or standing positions, systolic BP remains relatively stable throughout the course of pregnancy. Diastolic BP, however, decreases ~10 mmHg by ~28 weeks' gestation, followed by a near-term return to normal values (Wilson et al. 1980). When measured in the left lateral recumbent position, however, systolic and diastolic pressures decrease 5–10 and 10–15 mmHg, respectively, below nonpregnant values. Again, this nadir occurs at 24–32 weeks' gestation, rising toward nonpregnant values near term (Wilson et al. 1980). As would be anticipated, the decrease in diastolic pressure to a greater extent than systolic results in a small, but significant, increase in pulse pressure during the early third trimester. In those pregnant women with body mass index (BMI) >30, e.g., obese, these pressures (systolic, mean, and diastolic) are elevated, and resting heart rate is lowered proportionately (Helmreich et al. 2008).

As noted, systemic vascular resistance (SVR) is calculated as

$$\begin{aligned}\text{SVR} = {} & \text{mean arterial pressure} - \text{central venous pressure}\, 80\,\text{dyne-s}\,\text{cm}^{-5} \\ & \times \text{cardiac output}/\text{min}\end{aligned}$$

decreases from as early as 5–6 weeks gestation, primarily as a consequence of the combined actions of progesterone, vasodilatory prostaglandins, and nitric oxide. In addition, and to a certain extent, this decrease may result from the low-resistance arteriovenous fistula-like uteroplacental circulation (Duvekot et al. 1993; Frederiksen 2001; Gerber et al. 1981; Greiss and Anderson 1970).

The measurement of blood pressure can be the cause of confusion. In part, this may be because the Korotkoff phase IV (the point of muffling which is poorly reproducible) is ~13 mmHg higher than phase V (the point of disappearance). Thus, intra-arterial measurements of diastolic pressures may be 15 mmHg lower than those values determined manually (Koller 1982), whereas they may be significantly higher than those measurements determined by automated techniques (Kirshon et al. 1987). Continuous ambulatory BP monitoring has demonstrated circadian rhythmicity with a nadir in systolic and diastolic pressures in the early morning and a peak in the late afternoon and evening (Hermida et al. 2000).

10.9 Cardiovascular Hemodynamics of Pregnancy

With the expanding size of the gravid uterus, the left ventricular mass mother's heart increases; its axis is oriented to an extent upward and rotated forward with displacement of its left border (Kametas et al. 2001). As noted, the third trimester of pregnancy is characterized by significant increases in cardiac stroke volume and heart rate to increase cardiac output, in concert with significant decreases in both systemic and vascular resistance and serum colloid osmotic pressure. These hemodynamic values were determined by placement of Swan-Ganz catheters and arterial catheters in ten normal primiparous women at 35–38 weeks' gestation and again at 11–13 weeks' postpartum (Table 1 in Clark et al. 1989). As observed, no significant differences were seen in mean arterial blood pressure, central venous pressure of pulmonary capillary wedge pressure. The authors postulate that, despite significant increase in blood volume and stroke volume, pulmonary capillary wedge pressure did not change because of ventricular dilatation and the decrease in pulmonary vascular resistance. The authors noted, however, that the pregnant woman is at greater risk for pulmonary edema because of the significant decreased gradient (28%) between colloid osmotic pressure and pulmonary capillary wedge pressure, as compared to her nonpregnant sister (Clark et al. 1989).

In terms of autonomic cardiovascular regulation, power spectral analysis of heart rate and blood pressure variability between 28 and 38 weeks' gestation demonstrated a significant negative correlation between baroreceptor sensitivity and cardiac output, as well as a positive correlation with total peripheral resistance (Jayawardana 2001). This suggests strongly that during the course of gestation, the baroreceptors respond to changes in cardiac output and peripheral vascular resistance. Also during the course of gestation, the circulation time (and presumably blood flow velocity) demonstrates a slight but progressive decline reaching a nadir of 10 (Manchester and Loube 1946) to 16 s (Adams 1954). During the course of labor at term, with "autotransfusion," each uterine contraction can return 300–500 ml of blood from the uterus to the general circulation (Lee et al. 1989). Cardiac output increases 10–30% in the first stage of labor (e.g., dilatation of the os uteri) primarily due to an increase in stroke volume and increases 40–50% in the second stage (e.g., expulsion of the infant from the uterus and vagina) (Lee et al. 1989; Robson et al. 1987; Ueland et al. 1969). Both maternal systolic and diastolic arterial pressures transiently increase 25–35 mmHg during the course of active labor (Robson et al. 1987). Thus, women with compromised cardiovascular function may experience decompensation, especially during the second stage of labor.

Coincident with the dynamic changes in cardiovascular function is the characterization of pregnancy as a "prothrombotic" or "hypercoagulable" state. A severalfold increase is reported in the concentrations of procoagulant factors (factors VII,

VIII, IX, X, and XII), as well as fibrinogen and activated protein C resistance and plasminogen activator inhibitors 1 and 2 (Brenner 2004; Pacheco et al. 2013). This equates into a significant increase in the risk of thromboembolism (Heit et al. 2005). In women with multiple pregnancy, these hemodynamic changes are even more pronounced, cardiac output being ~20% higher, for instance, secondary to a further increase in stroke volume and heart rate, as well as increased left ventricular mass and ejection fraction (Kametas et al. 2003). In the case of medical complications of pregnancy such as preeclampsia, cardiac output may be decreased in association with increased vascular resistance (Bamfo et al. 2008).

During the course of gestation, compliance of the venous system increases progressively. Presumably, this results from the combined effects of circulating progesterone, nitric oxide, vasodilatory prostaglandins, and other endothelium-derived relaxant factors on the venous vasculature SMCs. This significant increase in compliance leads to a decrease in flow velocity and venostasis (Fawer et al. 1978). Because of this effect on venous pooling, decreased venous return, and decreased cardiac output, pregnant women can show increased sensitivity to blockade of the autonomic nervous system.

10.10 Uteroplacental Blood Flow

Several aspects of the uteroplacental circulation have been noted above, and related considerations are presented in other chapters. Nonetheless, because of the critical role of this circulation with delivery of O_2 and nutrients to fetal development, a brief review will be presented here. For every circulatory bed, the physical principles that regulate its blood flow are the difference in arterial and venous pressures times the conductance, or divided by the vascular resistance. Named after Jean Leonard Marie Poiseuille (1799–1869), Poiseuille's law defines the resistance to flow as a function of the vessel length and radius and blood viscosity (Poiseuille, 1840–1841). In addition, vascular shear and wall stress provide signals that regulate endothelial function and remodeling. In most circulatory beds, resistance is at the level of the arteriole which is innervated. Although much of the uterine vascular bed, including the spiral arteries, has such innervation, the umbilical placental vascular bed is without innervation, both beds being sensitive to vasoactive substances such as angiotensin II, arginine vasopressin, arteriolar natriuretic peptide, and catecholamines, those derived from the endothelium including nitric oxide and prostaglandins, and those released from other cells such as estrogens, progesterone, other steroids, and glucocorticoids.

For mammals, adaptations to the pregnant state include immense changes in the maternal circulatory system (Magness and Ford 2014; Thornburg and Louey 2013; Thornburg et al. 2000). To a great extent, these adaptations occur under the influence of sex steroid hormones (Hall et al. 2001; Longo 1983; Siiteri and MacDonald 1966). In humans these changes can vary with racial or ethnic group (Wilson et al. 2007) and also may be a function of diet (Frias et al. 2011). The

change in uteroplacental blood flow with pregnancy also varies widely among those species in which it has been measured, ranging from 10- to 100-fold (Osol and Mandala 2009; Thornburg and Louey 2013). Evidence indicates that increases in uterine blood flow are directed from the myometrium to the placental-fetal interface and are associated with changes in vascular compliance and reactivity (Cipolla and Osol 1994; Osol and Mandala 2009; Rosenfeld 1989). These compliance changes are associated with a severalfold increase in uterine artery diameter (outward remodeling; Cross et al. 2002; Osol and Mandala 2009) and length (radial and axial remodeling; Cipolla and Osol 1994; Moll 2003; Osol and Mandala 2009). In view of its dominant role in the regulation of uteroplacental flow, the signaling mechanisms by which estrogen (and other steroids) has these effects remain to be understood at a deep level (Klinge et al. 2004). As a corollary, the same is true for the multiple mechanisms involved in uteroplacental vascular remodeling.

In regard to studies on uteroplacental blood flow and its regulation, Charles R. Rosenfeld of the University of Texas Southwestern Medical Center, Dallas, has written:

> In 1971, at the suggestion of Dr. Harry Gordon (1907–1988), Dean of Albert Einstein College of Medicine, I joined the laboratory of Giacomo Meschia and Fred Battaglia at the University of Colorado to try my hand in some aspect of developmental and/or reproductive cardiovascular research. I wanted a basic science experience, and Dr. Gordon assured me that in Denver I would receive the laboratory experience and mentoring I was seeking. Forty years later, I can say without hesitation that he was right! Upon my arrival in Denver, I joined another trainee, Allen P. Killam who was an obstetrician, in his ongoing studies of the mechanisms of estrogen-induced increases in uterine blood flow (UBF) in nonpregnant sheep. I was fortunate that this would be a gold mine, and importantly, the studies were immediately successful (Killam et al. 1973). In order to confirm the novel findings made with Killam, i.e., estradiol-17β (E2) acutely increased UBF >10 to 15-fold vs. the 40% in the literature and that surgical stresses markedly attenuated these responses, I modified the animal model, adding the radiolabeled microsphere method previously used in Denver, permitting us to use this in conjunction with chronically implanted bilateral uterine artery (UA) flow probes to do studies remote from surgery (Rosenfeld et al. 1973). This novel model enabled me to obtain continuous measurements of UBF, heart rate and blood pressure and to select designated time points of interest in order to determine the simultaneous changes occurring in cardiac output (CO), its distribution, and multiple organ/tissue blood flows with the microspheres in animals *remote* from surgery. Using this model, we examined changes in multiple hemodynamic parameters in nonpregnant and pregnant ewes across gestation, both before and after systemic doses of estrogens (Rosenfeld et al. 1974, 1976b). For the first time, we could measure simultaneous changes in CO, heart rate, blood pressure, distribution of cardiac output, total UBF, multiple organ blood flows (Rosenfeld 1977), and importantly, blood flow to the tissues that make up the pregnant and nonpregnant uterus, i.e., myometrium, endometrium and sites of implantation or the placenta (Rosenfeld et al. 1974, 1976b). These novel observations set the stage for understanding the differences in the uterine and peripheral vasculature and the changes occurring in reproductive tissues throughout pregnancy. We next began studies of catecholamines since they are endogenous and contribute to the UBF regulation and thus, fetal-placental oxygen and nutrient delivery. In the first of several reports to come, we observed that the relative uterine vasoconstrictor responses were substantially greater than the systemic *pressor* and peripheral vascular responses to α-adrenergic agonists, demonstrating that the uterine vasculature is exquisitely sensitive to these agents (Rosenfeld et al. 1976a).

As can be seen, this was a highly productive training period, resulting in 6 published manuscripts from two years of intense research.

In 1973, I joined the faculty in Pediatrics (although unknown to some, I am a Pediatrician-Neonatologist) and OB/GYN at the University of Texas Southwestern Medical School in Dallas. This was very opportune since Norman Gant in the Department of OB/GYN was interested in the maternal vascular responses to angiotensin II (ANG II) in pregnancy, but was sorely limited in his studies in women and thus, unable to study the mechanisms involved. In the *Journal of Clinical Investigation* (Rosenfeld and Gant 1981) we first reported that the systemic responses to ANG II in nonpregnant and pregnant ewes were *identical* to those reported by Eduardo Talledo and Leon C. Chesley (Talledo et al. 1968) in women. That is, pregnant women *and* sheep are refractory to infused ANG II; notably, their dose response curves and 20 mmHg pressor responses were *identical*, demonstrating that the model could be used to study the ANG II responses in pregnancy. We (Naden and Rosenfeld 1981) next reported that when compared to α-adrenergic agonists, ANG II had the "opposite" effects on the uterine and peripheral vasculature, i.e., the pressor responses *exceeded* the uterine vasoconstrictor responses over a wide range of physiologic doses, resulting in increases in UBF due to the simultaneous increases in perfusion pressure. Using Doppler flow technology, Erkkola and Pirhonen (1992) later confirmed this in women, providing further validation of the model. Over the next several years we investigated the mechanisms responsible for these differences (Magness and Rosenfeld 1986; Naden and Rosenfeld 1985; Naden et al. 1984, 1985; Rosenfeld and Jackson 1984). We found that local UA nitric oxide (NO) and prostaglandins might contribute (Magness et al. 1985; Yoshimura et al. 1991), but it was the presence of type 2 ANG II receptors (AT2R) in UA smooth muscle that explained this (Cox et al. 1996a; Mackanjee et al. 1991). We also reported that women had *identical* UA AT2R expression (Cox et al. 1996b), accounting for >85% of binding in both species throughout the reproductive cycle, and making the UA unique vs. other vascular beds which predominantly express >95% AT1R, the classical receptor (Cox et al. 1996a). Additionally, Blair E. Cox and I unmasked a modest autoregulatory response in the pregnant ovine uterus and ANG II-mediated α-stimulation during these studies (Cox et al. 2000, 2004).

Because the uterine vascular bed was "always" protected from or refractory to ANG II, we wondered about the fetal vasculature since fetal ANG II synthesis and circulating blood levels were reported to be quite high. In subsequent studies, we reported that the fetus was also refractory to ANG II due to an ANG II metabolic clearance rate ($MCR_{ANG\ II}$) >700 ml min^{-1} kg^{-1} that occurred within the placenta, i.e., >90% uptake in one pass (Rosenfeld et al. 1995). Additionally, AT2R were found to be the major receptor in *all* fetal vascular beds except the umbilical artery where AT1R predominated (Cox and Rosenfeld 1999; Kaiser et al. 1998). Notably, the umbilical artery smooth muscle was also shown to have advanced biochemical development vs. all other fetal vascular beds, making it unique within the fetal compartment (Arens et al. 1998). In later studies, we reported that peripheral vascular AT2R did not transition to the adult AT1R phenotype until a month after birth, thereby protecting the neonate from the marked elevations in ANG II synthesis and blood levels and decreased $MCR_{ANG\ II}$ seen immediately after birth (Cox and Rosenfeld 1999; Velaphi et al. 2002). Despite these advances in maternal-fetal physiology, it remained unclear what accounted for the development of uterine and systemic refractoriness to ANG II seen in normotensive pregnancy. Recently, we suggested that NO *and* activation of large conductance calcium-activated K^+ channels (BK_{Ca}) might contribute (Rosenfeld et al. 2014). Thus our quest to understand ANG II physiology in the mother, fetus and neonate was addressed in large part over time. However, it remains unclear what accounts for this refractoriness to ANG II and in particular, its loss in hypertensive women, e.g., those with gestational hypertension and/or pre-eclampsia. Could it be changes in BK_{Ca} channel subunit expression and/or function or changes in another channel, e.g., K^+ or Ca^{2+}?

Although we made a special effort to understand ANG II physiology and pharmacology during pregnancy, we did not turn away from the estrogen story and the mechanisms involved in uterine vasodilation during pregnancy. This was important, as we believed estrogens contribute to the mechanisms responsible for rise in UBF during pregnancy and the maintenance of vasodilation in the last third of gestation (Magness et al. 1993; Rosenfeld and Worley 1978; Rosenfeld et al. 1977). Interestingly, few investigators presently studying the cardiovascular effects of estrogens in women cite studies of the uterine circulation in order to understand how estrogens work; yet the pathways appear identical. In Colorado, we had shown that new protein synthesis was essential to E2-mediated vasodilation. Over the next several years we demonstrated that chronic E2 exposure upregulated UA nitric oxide synthase (NOS), both eNOS and nNOS, enhancing increases in NO synthesis soon after a bolus dose of E2 (Rosenfeld et al. 1996; Salhab et al. 2000). However, NOS inhibition, which resulted in a decrease in uterine cGMP synthesis, only partially blocked E2-mediated rises in UBF. Thus another pathway was involved. In studies in nonpregnant ewes it appeared that either BK_{Ca} and/or the voltage-gated K^+ channels (K_V) were involved (Rosenfeld et al. 2002). We choose to first study BK_{Ca} in depth and found it was a major contributor through a cGMP-dependent mechanism in nonpregnant and pregnant sheep. This was seen *in vivo* and *in vitro* with electrophysiology (Rosenfeld et al. 2000). In our initial studies of pregnant ewes, unilateral BK_{Ca} inhibition not only decreased E2-mediated increases in UBF, but also decreased baseline UBF 40 to 50% within 1 to 3 min (Rosenfeld et al. 2001). Upon further study, we were able to decrease basal unilateral UBF up to 80% over a range of doses of tetraethylammonium without modifying uterine cGMP synthesis (Rosenfeld et al. 2005). This suggested that the BK_{Ca} channel was responsible for maintaining basal UBF in pregnancy via estrogens and/or changes in channel function. This was not seen in nonpregnant ewes (Rosenfeld et al. 2000). We then reported for the first time that chronically infused E2 did not alter expression of the BK_{Ca} pore forming α-subunits; however, it up-regulated the accessory β1-subunit in the UA but not elsewhere (Nagar et al. 2005). Importantly, the β1-subunit confers estrogen sensitivity to BK_{Ca} independent of cGMP and channel phosphorylation. Thus, BK_{Ca} channels appear to be responsible for maintaining and possibly increasing basal UBF in ovine pregnancy via a non-cGMP-dependent pathway. We believe this occurs by direct channel activation by estrogens through the BK_{Ca} β1-subunit. In support of this, we observed that the β1-subunit is progressively up-regulated in the last third of ovine pregnancy and falls rapidly after birth, paralleling the abrupt fall in UBF (Rosenfeld et al. 2000). Additionally, UA $PKG_{1\alpha}$ also rose progressively in the last third of gestation, suggesting BK_{Ca} phosphorylation might also contribute to the rise in and/or maintenance of UBF. Lubo Zhang and colleagues have confirmed the importance of BK_{Ca} in elegant studies using electrophysiology. With Ronald R. Magness, we reported that during the ovarian cycle the BK_{Ca} β1-subunit is up-regulated in the follicular phase, and thus, it is likely to be responsible for the estrogen-mediated rise in UBF that normally occurs (Khan et al. 2010). We have identified BK_{Ca} in human UA and shown that they contribute to NO-mediated relaxation as well as modification of adrenergic-mediated contractions (Rosenfeld et al. 2008). This further demonstrate the similarities in women and sheep. If BK_{Ca} are major contributors to the regulation of UBF throughout reproductive periods, what does NO do? Recently, we reported in pregnant sheep that although NO "contributes" to basal UBF, it is **NOT** the major player everyone has suggested (Rosenfeld and Roy 2014). Conventional wisdom always need a little hit!!!! Nothing like controversy to keep the fires going.

Since our prior studies of ANG II and α-adrenergic agonists did not directly compare the two vasoconstrictors or detail the similarities in women and sheep (Magness and Rosenfeld 1988; Naden and Rosenfeld 1981; Rosenfeld and West 1977; Rosenfeld et al. 1976a), we used UA rings collected from both species to address this. In two simultaneously published papers, we demonstrated in women *and* sheep that: 1) UA respond identically to the two

vasoconstrictors; 2) ANG II elicits minimal uterine responses compared to adrenergic agonists; 3) UA expression of α_1-adrenergic receptors is unchanged in pregnancy; and 4) this pattern of response occurs throughout the uterine vascular bed in pregnant sheep, including the placental or cotyledonary arteries (Rosenfeld et al. 2012a, b). This and data accumulating over 30 years suggested to us that the obstetrical anesthesiologist may be infusing the wrong pressor agonist to treat women with hypotension following the induction of regional anesthesia. At the Society of Reproductive Investigation in March 2015 we will present a study of >1000 pregnant women undergoing regional anesthesia to support this! We also have preliminary data for presentation at the meeting that the K_V channel, which is more complex than the BK_{Ca}, is involved in modulating basal UBF and UA vasoconstrictor responses and that one of its >70 α-isoforms is up-regulated in pregnancy.

Thus, we have come a long way since the classic studies of Elizabeth Ramsey and Frank Greiss, whom I always considered an inspiration. Problems that remain include determining the pathology that occurs in women with pre-eclampsia and hypertensive diseases in pregnancy that alters vascular sensitivity to a host of vasoconstrictors. The former is a major challenge as there is ***no*** model for this disease which is specific to women. Thus understanding the normal vascular physiology of uterine and peripheral vasculature in pregnancy is essential, and there is much to be learned. Our data in women and sheep suggest that they are quite similar, but they differ from small mammals such as rats and mice; thus, we need to validate the models as we use them.

I hope this helps in your next endeavor, i.e., understanding the new knowledge that has been acquired over the past 50 years related to UBF, reproduction and fetal well-being. It has been a marvelous journey for me with new chapters to come in the future related to cell signaling and function.

(Letter from CRR to LDL, 9 January 2015)

10.11 The Respiratory System in Pregnancy

In association with the continued increase in uterine size with 4–5 cm upward displacement of the diaphragm during the course of gestation, in association with the upward and lateral displacement of the heart, the rib cage circumference enlarges 5–7 cm (Weinberger et al. 1980). This is associated with about a 20% decrease in pulmonary residual volume, expiratory reserve volume, and thus functional residual capacity (Table 1 in Crapo 1996), probably as a consequence of basilar pulmonary alveolar collapse and atelectasis (Hegewald and Crapo 2011). In turn, maternal tidal volume increases 35 ± 5%, with resultant hyperventilation and hypocapnia (Awe et al. 1979). The maternal respiratory rate remains relatively constant during pregnancy; thus the 30–50% increase in minute ventilation noted as early as the first trimester is attributable to the increase in tidal volume (McAuliffe et al. 2002) in association with elevated levels of circulating progesterone and in basal metabolic rate.

In association with maternal hyperventilation, arterial CO_2 drops from a nonpregnant value of ~40 to ~30 Torr at term. This decrease would appear to facilitate fetal to maternal transplacental CO_2 flux and is accompanied by renal

excretion of hydrogen ions and decrease in serum bicarbonate levels, leading to reduced serum bicarbonate concentration (18–21 mEq 1^{-1}) and reduced buffer capacity. This results in a mild respiratory alkalosis (pH = 7.44), as compared to the nonpregnant state (pH = 7.40). This mild respiratory alkalosis also is associated with a shift to the left of the oxyhemoglobin saturation curve with increased hemoglobin affinity for O_2 (the Bohr effect) with resultant reduced O_2 release to tissues including the placenta. This is compensated for, however, by an alkalosis-stimulated increase in 2,3-DPG within maternal erythrocytes, which, in turn, shifts the oxyhemoglobin curve to the right, facilitating transplacental O_2 exchange to the fetus (Longo 1987; Tsai and de Leeuw 1982). Also in association with the increase in minute ventilation is a 30 ± 10% increase in maternal O_2 consumption, while metabolic rate increases 15% as a consequence of the increased metabolic requirements of the fetus, placenta, and maternal organs and tissues (Crapo 1996). In view of this increase in O_2 consumption, with a decrease in functional residual capacity, the pregnant woman with severe asthma or other pulmonary pathologies may be subject to early decompensation.

10.12 The Kidneys and Urinary Tract in Pregnancy

The maternal kidney with its associated structures and function undergoes profound changes during the course of gestation (Jeyabalan and Lain 2007). These include a 30% increase in renal size as a result of increases in vascular and interstitial volume (Bailey and Rolleston 1971; Christensen et al. 1989), and by mid-gestation dilatation of the urinary collecting system (Rasmussen and Nielsen 1988) with caliceal and urethral dilatation is more common on the right than left side (Fried et al. 1983; Hertzberg et al. 1993; Schulman and Herlinger 1975). The prominence of these changes on the right side appears to result from the combined effects of dextrorotation of the gravid uterus, the location of the right ovarian vein that crosses and may compress the ureter, and/or the protective cushion of the sigmoid colon filling the left pelvis. Although some degree of obstruction may play a role in the physiologic pyelectasis of pregnancy, an associated increase in renal arterial resistance has not been documented (Hertzberg et al. 1993). Hormonal relaxation of urethral smooth muscle by progesterone, relaxin, and/or nitric oxide also may play a role in this phenomena; however, there is no consensus on the role of these hormones in this regard (Jeyabalan and Lain 2007; Marchant 1972). This dilatation of the urinary collecting system is associated with a proclivity to ascending infections of the urinary tract.

By the mid-second trimester, renal plasma flow (RPF) increases 70 ± 10% above the nonpregnant value and then decreases to 50% above that value near term (Dunlop 1981). Reflecting maximal venous return and cardiac output, renal plasma flow is significantly greater when the pregnant subject is in the left lateral recumbent position than when supine, sitting, or standing (Davison and Dunlop 1984; Ezimokhai et al. 1981). The pregnant women's glomerular filtration rate

(GFR), as measured by creatinine clearance, often increases as early as 6 weeks' gestation to ~50% nonpregnant value by the end of the first trimester (Davison and Dunlop 1984). Although the exact mechanisms that account for the changes in renal hemodynamics during the course of gestation are unclear, studies in rodents suggest that these are a consequence of vasodilatation of pre- and post-glomerular resistance vessels, without significant changes in the glomerular capillary pressure per se (Baylis 1987), and these are independent of decreasing serum albumin changes in oncotic pressure (Duvekot et al. 1993). Because the increase in renal plasma flow initially is greater than the rise in GFR, the filtration fraction GFR divided by RPF decreases until the third trimester, at which time the decrease in renal plasma flow results in the filtration fraction to the nonpregnant value (Davison and Dunlop 1984). This pattern of altered filtration fraction parallels that of the change in mean arterial pressure and may be a consequence of the changing levels of progesterone and other circulatory hormones (Davison and Dunlop 1980; Dunlop 1981).

Renal filtration capacity, as estimated by the maximal GFR in response to a vasodilatory stimulus, appears intact in pregnancy, as demonstrated by both studies of amino acid administration and protein loading (Ronco et al. 1988). During the course of gestation, as GFR increases, the functional renal reserve (filtration capacity minus resting GFR) decreases. Thus, in pregnant women with early renal disease, renal function can be assessed by the determination of filtration capacity, but not by functional renal reserve (Ronco et al. 1988). This increase in renal blood flow and GFR can increase significantly the elimination rate of those medications cleared by the kidneys, leading to shorter half-lives.

Maternal renal tubular function undergoes significant changes during the pregnant state. In association with the 50% rise in glomerular filtration rate, the filtered sodium load increases 50% from 20,000 to 30,000 mEg day^{-1}. Among those hormones that favor Na^+ excretion are progesterone which competitively inhibits aldosterone (Barron and Lindheimer 1984), vasodilatory prostaglandins (Davison and Dunlop 1984), and atrial natriuretic factor (Bond et al. 1989; Marlettini et al. 1989). Despite these effects, during the course of gestation, about 1000 mg of sodium is retained and distributed among the intravascular and interstitial compartments of the mother, placenta, and fetus (Hytten 1991). Some of the factors that promote Na^+ reabsorption include aldosterone, deoxycorticosterone, and estrogen in association with components of the renin-angiotensin system, and these are augmented by the postural changes of pregnancy. The net reabsorption of sodium is one of the remarkable responses of the kidney to the pregnant state (Monga and Mastrobattista 2014).

Although the pregnancy-associated increase in aldosterone would promote potassium excretion, a net retention of 300–350 mEg of K^+ occurs, perhaps due to elevated progesterone (Lindheimer et al. 1989), and in association with increased proximal tubular reabsorption (Hytten 1991). In addition, as a result of increased calcium clearance, the excretion of calcium is increased (Roelofsen et al. 1988). This is balanced, however, by increased small intestine Ca^{2+} absorption; thus serum plasma unbound calcium concentration [Ca^{2+}] remains stable. Nonetheless, in association with the decrease in plasma albumin (Pitkin et al. 1979), the total

calcium level decreases ~9.5% from 4.75 mEg l^{-1} at the end of the first trimester to 4.30 mEg l^{-1} at term. Both calcium and phosphorous absorptions from the gastrointestinal tract, which may facilitate mineralization of the fetal bones, are promoted by an early pregnancy rise in calcitriol with suppression of parathyroid hormone (Weiss et al. 1998).

10.13 The Gastrointestinal Tract in Pregnancy

Again, with expansion of the gravid uterus, the stomach and intestines are displaced (which can complicate the diagnosis of acute abdominal emergencies). In addition to mechanical displacement, the elevated progesterone levels contribute to delayed gastric emptying and prolonged gastrointestinal transit times (Parry et al. 1970). Not surprisingly, these changes can be associated with symptoms of nausea, vomiting, increased gastric acidity, gastric reflux, bloating, and/or constipation, conditions that may alter the absorption and metabolism of medications (Broussard and Richter 1998; Costantine 2014). With the threat of aspiration of gastric contents, these changes also can complicate the course of labor and delivery.

10.14 Amniotic Fluid and Its Dynamics

In one of the earliest accounts of the amniotic fluid (AF), Walter Needham (ca. 1631–1691), in a founding treatise on embryology, described several aspects of this fluid compartment within the uterus of pregnant mammals, including that it contained small solid bodies (Needham 1667). Over the generations it has been appreciated that AF serves to cushion the fetus, protecting it from trauma and allowing a milieu for optimal growth and development.

In addition to protecting the fetus, the amniotic fluid possesses bacteriostatic properties to lessen the impact of infection, serves as a reservoir to maintain fetal fluid and electrolyte homeostasis, and by allowing fetal movement is essential for development of its limbs. Studies by Pritchard in the mid-twentieth century established the importance of fetal swallowing in the maintenance of AF volume (Pritchard 1965b, 1966). Several decades later, the concept that the fetal lungs secrete liquid that flows into the AF was established (Harding 1994). In a series of studies in humans and sheep, Robert Allen Brace and colleagues of the University of California, San Diego (now at Oregon Health & Science University), have established and quantified the relative roles of the most important pathways of amniotic fluid formation (fetal urine and lung liquid), its egress (fetal swallowing and fetal blood that perfuses the amniotic surface of the placenta), and other fetal pathways, e.g., the "intramembranous" pathway and the "transmembranous" pathway between AF and maternal blood in the uterus (Brace 1995, 1997; Brace and Wolf 1989; Gilbert and Brace 1989, 1993; see also Seeds 1980).

Early in pregnancy, AF is an ultrafiltrate of maternal plasma, but by the second trimester consists largely of fluid that has diffused through the fetal skin, thus reflecting the composition of fetal plasma (Gilbert and Brace 1993; Lind et al. 1972) and/or its extracellular fluid (Lind et al. 1972), and its volume correlates with fetal weight (Lind and Hytten 1970). By mid-gestation, cornification of the fetal skin disallows this flux, and AF then consists chiefly of urine from the fetus. The fetal kidneys commence urine production at the beginning of the second trimester and by late gestation are producing 800–1200 ml day^{-1} (Gilbert and Brace 1993). This urine contains a higher concentration of creatinine, urea, and uric acid than fetal plasma, in addition to desquamated fetal skin, lanugo, and *vernix caseosa* [cheesy varnish]. During the first one-half of pregnancy, the AF volume increases and then declines to a relatively steady state by 33 weeks (Brace and Wolf 1989). Nonetheless, in mid- to late gestation, it demonstrates rapid turnover with high inflows and outflows, so that there is in effect complete turnover daily (Brace 1997; Gesteland et al. 2009). As noted, in both humans and the pregnant ewe, amniotic fluid volume is regulated within a relatively narrow range by a combination of the excretion of fetal urine, lung secretion for input, swallowing, and transplacental fluid flux for uptake (Tomoda et al. 1987). Recent combined studies in vivo and in amnion cells in culture, however, indicate that the regulation of these fluxes is not clear (Cheung et al. 2014).

In response to my query regarding several facets of his work on recent advances in understanding the regulation of amniotic fluid volume, Brace replied:

> It has long been known that amniotic fluid volumes either above or below the normal range are associated with poor perinatal outcome. In spite of this, there is little understanding of the mechanisms that regulate amniotic fluid volume in humans. Further, attempted therapeutic approaches for treating abnormalities in AF volume largely have been either unsuccessful, have shown only short-term effects, or have undesirable effects on mothers and/or fetus. As an alternative to human experimentation, studies have been conducted in several primate and non-primate species. However, these were descriptive in nature and provided little insight into mechanisms involved. An exception has been recent studies exploring the regulation of amniotic fluid volume in chronically catheterized fetal sheep. The first significant breakthrough occurred a quarter century ago with the observations that amniotic water and solutes are rapidly absorbed from the AF directly into the fetal circulation (Gilbert and Brace 1989, 1990; Gilbert et al. 1991). This trans-amnion transport of amniotic water and solutes into fetal blood is termed 'intramembranous absorption'.
>
> A major advance in understanding intramembranous transport mechanisms was that there are multiple components of intramembranous absorption and these are mediated by both passive and active transport mechanisms (Adams et al. 2005; Anderson et al. 2013; Brace et al. 2004, 2014a; Gesteland et al. 2009). Amniotic water is transferred passively into the fetal circulation presumably through aquaporin water channels within the amnion and by bulk, unidirectional vesicular transport outward across the amnion. Solutes are exchanged between AF and fetal blood by bidirectional passive diffusion and are transported from AF into fetal blood by unidirectional, bulk vesicular transport. Further, the passive component of intramembranous solute transport is size selective with smaller molecules transported at a higher rate (Adams et al. 2005) while large molecules such as albumin are transported only unidirectionally out of the amniotic fluid, but not in the opposite direction (Faber and Anderson 2002). Importantly, not only is the vesicular component the primary determinant of amniotic fluid volume but also, during experimental

studies that altered intramembranous absorption rate, only the unidirectional bulk vesicular transport component is altered.

The most recent breakthrough in understanding of the mechanisms that regulate amniotic fluid volume was the observation that replacing fetal urine with physiological saline caused intramembranous absorption rate to decrease and amniotic fluid volume to increase (Anderson et al. 2013). This observation provides the basis for the concept that fetal urine contains a stimulator(s) of intramembranous absorption that plays an important role in regulating AF volume. This observation was followed the finding that amniotic fluid contains an inhibitor(s) of intramembranous absorption that is non-renal and non-pulmonary in origin and is presumably secreted by the fetal membranes (Brace et al. 2014b). Integration of the concept that amniotic fluid contains competing stimulator(s) and inhibitor(s) of intramembranous absorption provided a major advance in understanding of the amniotic fluid volume regulatory mechanisms because, by using computer simulations, experimentally observed changes in amniotic fluid volume over time could be predicted with surprising accuracy (Brace et al. 2014a).

Collectively, these studies are of vital importance because they provide the foundation for the next generation of advances in understanding the mechanisms of amniotic fluid volume regulation. Trans-amnion transport vesicle type, characteristics and dynamics need to be determined. The identity of the stimulator(s) and inhibitor(s) of intramembranous absorption with provide unique insights into AF volume regulation. This will need to be integrated with an understanding of the cellular and molecular pathways that they use to mediate the regulation of AF volume. These new findings can be translated into an understanding of abnormal amniotic fluid volumes in humans by comparing transport vesicle type and dynamics as well as cellular and molecular pathways in ovine and human amnion.

(Letter from RAB to LDL, 2014)

As noted elsewhere, William Harvey and other early workers believed the amniotic fluid was a major source of nutrition to the fetus, but this is not the case. Containing epidermal growth factor, transforming growth factor beta, and other such proteins, ingestion of AF into the fetal gastrointestinal tract and inhalation into the lung may play a role in the growth and differentiation of these tissues. In laboratory animals the drainage of AF produces pulmonary hypoplasia (Adzick et al. 1984; Alcorn et al. 1977). Thus, the formation of intrapulmonary fluid, and its alternating egress and retention, in association with breathing movements would appear essential to the normal development of the fetal lung. Not until the mid-twentieth century was amniocentesis for genetic analysis first performed (1956); however since that time, this procedure has been employed for a number of aspects of fetal metabolism, maturity, and disease diagnoses. In pathologic states, the AF volume may be altered dramatically. For instance, oligohydramnios may occur in cases of fetal renal agenesis or maternal dehydration. In turn, polyhydramnios may occur in cases of fetal esophageal atresia. Of interest, in women at high altitude (1828 m, ~6000 ft), AF volume was significantly increased, as compared to sea level controls (Yancey and Richards 1994); the mechanism of which is unknown. In general, AF volume abnormalities are associated with poor perinatal outcome (Kamath-Rayne et al. 2014; Ross and Beall 2014).

10.15 Uterine Contractions of Labor

The physiologic mechanisms of uterine contractions have been studied *in extension*, the endocrinologic and biochemical changes responsible for uterine contractions being quite complex. Throughout pregnancy the uterine cervix must remain firm and closed, while the uterine corpus remains quiescent as it enlarges by hypertrophy and hyperplasia. As noted, "propregnancy" factors such as progesterone and prostacyclin (e.g., prostaglandin I_2, PGI_2) inhibit myometrial contractility. Labor with parturition involves synchronization of changes in cervical structure, with uterine contractions leading to dilatation and effacement of the cervix. At the appropriate time, "prolabor" factors stimulate coordinated uterine contractions and mediate remodeling of the cervix (Lye 1994). Studies suggest activation of a cassette of contraction-associated proteins, including oxytocin, gap junction proteins, prostanoid receptors, enzymes for prostaglandin synthesis, and cell signaling proteins (Lye 1994).

In preterm labor, inflammation may be integral to the pathologic process, and this may be the case for term labor as well. A vast number of pro-inflammatory genes including those for cytokines may be upregulated. These include that for nuclear factor kappaB (NK-κB) (Allport et al. 2001; Baeuerle and Baltimore 1996), which in turn regulates a number of inflammatory factors including interleukin-6 and interleukin-8 and cyclooxygenase-2 (COX-2). Intriguingly, some have suggested that this response may be triggered by increased surfactant and surfactant protein A from the maturing fetal lung (Condon et al. 2004), as well as by macrophage activation. Corticotropin-releasing hormone (CRH) expressed by both the pituitary gland and placenta also may play a role in this regard, as its concentration in maternal serum rises during the second half of pregnancy to peak during labor (Smith and Nicholson 2007), perhaps acting via a placental "clock" (McLean et al. 1995).

The clinical data from which one must assess the progress of labor include the pattern and strength of uterine contractions, dilatation of the cervix, and descent of the fetal presenting part. To distinguish readily a normal from an abnormal course of labor, however, long has presented one of the most common and challenging problems to the obstetrician. In an effort to develop a relatively simple, reproducible, and objective measure of the progress of labor and its possible variations in conjunction with analgesia and conduction anesthesia, on the basis of the analysis of 500 primiparous women during labor, Emanuel A. Friedman then of the Sloane Hospital for Women of the Columbia-Presbyterian Medical Center in New York, now professor emeritus of obstetrics and gynecology at the Harvard Medical School, and chief emeritus of obstetrics at the Beth Israel Hospital, Boston, detailed some aspects of the evolution of this idea. Friedman devised a graphic analysis of cervical dilatation measured in centimeters as a function of the duration of an idealized labor. Such a plot resulted in the now familiar sigmoid-shaped curve of the first stage of labor. This includes both prodromal labor, or the "latent" phase, and clinically apparent labor, or the active phase, which consists, in turn, of the

phases of acceleration, maximum slope, and deceleration. In evaluation of the progress of labor, comparison with this normal pattern allows the early recognition of dystocia resulting from uterine inertia, fetal malposition, or abnormality of the birth canal. Based on the analysis of many labor curves obtained from a wide spectrum of clinical conditions, and because of certain similarities in etiology, response to therapy, and prognosis, the aberrations of labor can be considered conveniently in three distinct groups: (1) prolonged latent phase alone; (2) the protraction disorders, e.g., protracted dilatation and protracted descent; and (3) the "arrest" disorders, e.g., secondary arrest of dilatation, prolonged deceleration phase, failure of descent, and arrest of descent (Friedman 1978a, b). Thus, given a specific labor pattern and diagnosis, appropriate management can be instituted, thereby optimizing the outcome for both mother and infant. Undoubtedly use of the "Friedman" curve, and the realization that undue prolongation of labor contributes to increased perinatal mortality, has been, with the use of oxytocin augmentation of some types of uterine dysfunction and the more frequent use of cesarean section, an important factor in the decrease of perinatal morbidity and death. The graphic analysis has been modified by other workers, but the resulting so-called "cervimetric analysis," "cervicograph," and "partogram" display the essential features as originally described (Friedman 1954).

In considering the graphic analysis of labor in the parturient, for the "Classic Pages" section of the *American Journal of Obstetrics and Gynecology*, in 1978 Friedman reminisced:

> The fortuitous conjunction of the professional pathways of a number of stimulating, clinical oriented academicians in an almost ideally fertile, thought-provoking environment conspired to give birth to the concept of the graphic analysis of labor progression. In mid-century, the Department of Obstetrics and Gynecology, College of Physicians and Surgeons of Columbia University in New York, was near the zenith of its development under the inspired leadership of Howard C. Taylor, Jr. (1900–1985) Clinical teaching in obstetric care was being supervised by D. Anthony D'Esopo in a manner characterized by conservatism tempered with inquiry and analysis. The heritage of the great Sloane Hospital for Women, only recently incorporated physically into the melting pot of the Columbia-Presbyterian Medical Center, still prevailed. In the presence of those whose promise was already recognized, including such incomparable clinical laboratorians as Albert A. Plentl (1913–1968) and Donald L. Hutchinson (1924–1973) . . . there was an atmosphere most conducive to the germination of novel concepts. And the "soil" was made especially fertile by the intensity of concern and uniformly high quality of nursing skills brought to bear under the strong influence of Lottie Morrison who ran the obstetric nursing service with both insight and compassion. Perhaps the catalyst in this unique milieu was the person of Virginia Apgar, who at this time was in charge of the obstetric anesthesia service. She served to generate an excitement of curiosity in the best academic sense; no dogma was sacred in her critical incisive mind or safe from her probing inquiry. Her legendary energy was boundless and her enthusiasm infectious.
>
> In this setting, young and impressionable residents in training, such as I, were swept up in a whirlwind of both clinical and laboratory investigation. Unlike the situation that was to prevail later in the 1960s when the embarrassment of National Institutes of Health riches often served as the primary (if not sole) impetus and motivation for the pursuit of research activities, the drive at that time had to be generated from within each individual so inclined. While investigational pursuits were expected and encouraged, they were deemed to be extracurricular in nature and something to be accomplished on an *ad hoc* basis,

superimposed on the otherwise fairly full structured clinical educational program, without formal commitments of time, space, or fiscal allocations. These relative impediments notwithstanding, much important work was undertaken, leading to productive academic careers for many.

Much of the thrust among clinical obstetric studies being conducted during this time centered about caudal anesthesia. The potential of this technique fired the imagination of proponents such as Dr. Apgar, who envisioned it as bordering on the elusive ideal being sought for achieving pain relief in labor that was safe and simple, and would not affect the fetus or the course of labor. With regard to this last aspect, only barest beginnings had been made in objective monitoring of uterine contractility. Techniques were being actively developed. Only two years earlier . . . Roberto Caldeyro-Barcia [and colleagues in Montevideo, Uruguay] had startled a disbelieving obstetric community with reports describing graphic recordings of intrauterine hydrostatic pressure, and later the use of intramyometrial microballoons for this purpose (Alvarez and Caldeyro-Barcia 1949). These reports were instrumental in opening the entire field of physiologic experimentation in human pregnancy as we know it today. In the early 1950s, however, there was still much trepidation about invading the sacrosanct uterus either transabdominally or transcervically. Nonetheless, some work was being done in an effort to obtain contractility patterns from patients in labor before and during caudal anesthesia. The prime mover in this regard was Robert L. Hallet . . . with his keen perception, creativity, and intensity of interest (Hallet 1953). His efforts at Sloane were rewarded by the fine recordings he was able to produce, showing a clear-cut, albeit transient, increase in basal tonus as the anesthesia took effect in association with somewhat diminished intensity and frequency of contractions. While this was obviously a meaningful observation, it did not really provide a measure of the overall effect of the anesthesia on the course of labor, although it inferred inhibition.

The only measures of clinical effect available then were data on the duration of labor, first and second stages and total duration, respectively, and the literature was replete with conflicting testimonial series attesting to the shorter, longer, or unaffected average lengths of labor with caudal anesthesia. Repeating this type of "experiment" was considered likely to be an ill-advised exercise in futility. Instead, we searched for some other, more sensitive, objective means of studying labor and its progression. Others had traveled the same tortuous pathway before, and their monuments, like those of Oxymandias [Greek name for Ramesses II, pharaoh of Egypt, circa 1279 to 1212 BCE] lay scattered half-buried in the sands of our literature. Reports of Leroy Adelbert Calkins (1894–1960) and colleagues at the University of Kansas (Calkins 1941, 1954; Calkins et al. 1930) extended over the preceding two decades and presented a growing mass of objective data bearing on variations in the length of the stage of labor. Calkins was convinced that accurate observations of the resistance of the cervix and of the pelvic floor, together with determinations of the effectiveness of the uterine contractions, were necessary to solve the riddle relating to the extreme variations in the length of labor so commonly encountered in clinical practice. He felt that critical observations of the existing forces of labor and the counteracting resistance would enable him to predict the probable duration of labor, serving to reduce the high rate of operative interference. He urged that the consistency of the cervix, as well as the thickness of its wall and the length of its canal, be accurately determined and recorded. Calkins work culminated in a report detailing the factors relevant to prediction of the length of the first stage (Calkins 1941). He showed that it was possible, on the basis of observations of intensity of contractions, degree of cervical effacement, softening of the cervix, and engagement of the fetal presenting part, to predict the approximate length of the normal first stage. Subsequently, a similar approach was developed for predicting second-stage duration by paying particular attention to the number of contractions it took to evolve the descent process. He emphasized the sequence of contractions rather than the elapsed time, until then considered almost exclusively.

This revived a once-popular method, the *Wehenzahlen* (pain count) technique, first developed and popularized by Eugen Frey (Frey 1929). Frey felt that labor would not evolve without a predetermined given number of sufficiently strong contractions, and that this number was more important than the time factor involved. His attempt at graphic presentation consisted of half-hourly notations of the accumulated number, average duration, and character of the contractions, together with other information on cervical effacement and dilatation, and the degree of engagement. Most nulliparous patients were found to require more than 300 contractions after rupture of the membranes, and most multiparous patients more than 200 (Geisendorf 1937). It is apparent that this approach was the forerunner of more recent techniques, which summate number and intensity of contractions in a similar sequential, but considerably more meaningful, manner. Unfortunately, neither the *Wehenzahlen* technique nor that of Calkins provided means for accurate evaluation of the labor in progress, both being directed to overall summations of factors bearing on total duration, leaving much to be desired with regard to the individual labor in progress.

From the historical point of view, palpatory observations of cervical dilatation can be documented back to 1816 when Ebermaier first described the changes in the cervical os which could be palpated by the examining fingers in the vagina (Langreder 1959). W. Kroenig (1894) and D. Ries (1894) simultaneously and apparently independently of each other, first recommended palpation by way of the rectum in 1894. Inconsistency appears to have characterized the observations obtained by this approach because in 1921 W. Liepmann made a strenuous plea for standardization of cervical dilatation nomenclature. He stressed the practical need for expressing dilatation uniformly in centimeters instead of the then popular series of terms: *Fingerkuppe* (fingertip, roughly 1 cm), *Trauring* (wedding ring, approximately 2 cm), *Damenuhr* (lady's watch, around 3 cm), *Herrenuhr* (man's watch, approximately 5 cm), *Kleinhandtellergrösse* (small palm, about 6 cm), and *Handtellergrösse* (palm size, about 8 cm) (Liepmann 1921).

It is to Willi Wolf (1946) that credit must be given for having recognized, in 1930, the necessity for exact and timely registration of the progress of cervical dilatation. It was he who accomplished the first such reported observations in modern times. Wolf portrayed his concept of *Teileröffnungszeiten* (fractional cervical dilatation time) in a graphic manner, illustrating the dynamic aspect of the labor phenomenon. The latter he termed "*Bewegungsvorgang*" (motion process). He represented this diagrammatically in a retrospective fashion with cervical dilatation plotted against the number of hours prior to delivery. It was clear that the curve could not be constructed until delivery had taken place, since this was used as the primary point of reference. Although he asserted that the course of the curve would provide a significant insight into the operation of the biological forces, his sole published material related to rupture of the membranes and its effect on labor (Wolf 1946).

Theo Koller (1948) and Koller and K. Abt (1950) described a graphic representation of the course of labor, that was said to facilitate the practical control of the entire process and permit scientific study. Their *Partogramm* consisted of a coordinate record, with zero time representing the point at which the membranes ruptured. Cervical dilatation was represented on an irregular scale, the divisions of which were unequal. Several steps in cervical dilatation and the deliver process, each representing a phase, were used. These included the diameters of a 1 to 2 franc coin, a 5 franc coin, a small palm, a palm, complete dilatation, delivery of the baby, and delivery of the placenta. Superimposing curves aligned at zero time and the time of rupture of the membranes, Koller demonstrated that dispersion of the patterns before and after this event is different, that is, the curve is less steeply inclined prior to aminotomy and more rapid following it. Although presenting no data at all on patients with intact membranes, he concluded that the effect seen was the result of aminotomy, which influences the duration of the total period of dilatation and the duration of the period of expulsion (Koller and Abt 1950).

Also in mid-century, Karl Zimmer presented studies with the use of a *Wegzeit-Diagramm* (course-time graph) (Zimmer 1951). He also utilized rupture of the membranes as the central theme. His curves were essentially hyperbolic in pattern, their sigmoid characteristics having been ignored. Although a marked change in slope was recognized to occur normally beyond 3 to 4 cm, no transition was accepted, and pure linearity was insisted upon. The influence of dystocia, inertia, or other conditions, as they related to changes in the curve, was not recognized. Variations in the pattern, both quantitative and qualitative, were not described. The device was used only in its academic role, and strictly on a retrospective basis. Moreover, it had the major fault of centering the curve at the time of rupture of membranes. This deficiency, together with the nonlinear, irregular dilatation scale, and the assumption that linear change exited throughout, was reflected in the rough picture of the course of labor it was able to provide. Furthermore, it offered almost no ongoing means for evaluating a labor in progress (Zimmer 1951).

As stated, the prime objective of most of these early investigations was the effect of aminotomy on labor. Despite the intensive effort that had been expended, essentially ignored was the matter of objective study for purposes of defining the complex phenomenon of labor, establishing norms for its course with which comparisons might be made more accurately and more profitably, and determining meaningful limits of normal. In retrospect, these serious shortcomings tended to nullify a good deal of the value of these foregoing studies. Nevertheless, they did play an important role, serving to form the foundation upon which graphic analytic techniques in current use were built.

Insofar as purely clinical factors were concerned, the subject appeared to have been extensively and exhaustively investigated, and the likelihood that any useful additional information would be forthcoming was very remote. Despite this openly expressed pessimism, the need was too pressing to be ignored, and this naïve disbeliever embarked on a pilot study with the objective in mind of trying to determine whether caudal anesthesia, when transiently applied in the course of an otherwise normal labor, could be shown to influence its progress. I chose at the outset merely to assess periodic levels in each of the six characteristic manifestations of clinical labor that were assumed to advance steadily throughout labor, progressive change being the byword of every definition of labor until then—and still to date. These included: intensity, frequency, and duration of contractions, cervical effacement and dilatation, and descent of the fetal presenting part. Each was expressed in strictly clinical terms, and plotted on square-ruled graph paper against time. From the very first case, it was apparent that progressive cervical dilatation was going to be of greatest significance to us as an index of labor progression, and much excitement was generated among those willing to see.

At this late date, it seems inconceivable that such an obviously simplistic and utilitarian technique had not been developed intuitively, and put to wide use many years earlier. Moreover, the resistance it encountered in achieving acceptance and routine adoption in clinical practice cannot now be fully comprehended. Anecdotally, it might be of interest to note here that the first "acid test" of this technique was a brief comparison of two groups of gravid women, one subjected to the ostensible stimulation of aminotomy, the other not. When no differences were found, to my consternation, the method came close to being summarily abandoned as obviously worthless. It was not until much later that a more extensive study (Friedman and Sachtleben 1963) was able to certify these still controversial (but nonetheless documented) findings. Fortunately, we persisted against the adversity of apparent failure.

Our primary motivating force, as related earlier, came from the great interest that was being generated in the effect of conduction anesthesia on labor. We were painfully aware that we had no satisfactory means to assessing progress in labor or of determining the short-term effect on the course of labor of the anesthetic that was being administered. Although good studies could be accomplished for measuring contractile pattern or total duration of labor, these did not give us the information we desired. Hallet's (1953) objective

> observations of uterine contractility, on one hand, were too sensitive and did not reflect true overall progressive change. Data on total duration, on the other hand, were too insensitive to allow detection of any transient effects of the anesthesia. A simple, objective, reproducible, clinically available tool was thought to be necessary for this purpose. It soon became obvious that evaluation of progress in labor, previously synonymous with a nebulous degree of change, must be made available to us in terms of specific rate of change. This was conceived as a possible solution to our dilemma. We proceeded without prior knowledge of the foregoing work in this area. In retrospect, this was perhaps fortunate as it was thereby possible to avoid the ensnarement's of these prior approaches. The rest is history. A method was devised whereby the rate of change in cervical dilatation could be related in a reasonably precise manner to elapsed time. It was during the long night of 10 June 1953, that the too-frequent periodic examinations I made on the first patient studied (B.N., Unit No. 120448) immediately revealed the now familiar characteristic sigmoid curve of normal cervical dilatation. Since that time, extensive study has revealed that the rate of change, within the limits of acceptable error, is specific for each patient and undergoes predictable alterations during the course of normal labor (Friedman 1954, 1955). This technique has introduced a new dimension to us, and has proven to be useful clinical tool (Friedman 1978b). Stemming from this early seed of discovery and invention wrought by necessity, many tangential explorations have been possible, and as a consequence much has been learned about the course of labor (Friedman 1978a).

The Friedman curve, or partogram, lent itself to quantitation, thus demystifying arcana and serving as a teaching device permitting the course of labor to be comprehended at a glance. In addition to introducing terminology that was descriptive and understandable, it defined the components of assessing labor and facilitated mathematical derivation of normative data for each phase. It also led to the recognition that aberrations of different phases have different causes and widely varying prognoses, as Friedman demonstrated in a series of reports.

The half century since Friedman's initial report have seen an inexorable rise in cesarean section rates with a reevaluation of what constitutes normal labor (Cahill and Tuuli 2013; Millen et al. 2014; Spong et al. 2012). As a consequence, new guidelines have been endorsed by the American College of Obstetricians and Gynecologists (ACOG 2014). These guidelines are based heavily on several reports on the pattern of cervical dilatation and fetal descent as functions of time based upon a high-order curve-fitting program for regression analysis (Laughon et al. 2012; Zhang et al. 2002, 2010a, b). Thus, there is a strong likelihood of different mathematical models used to fit these curves, and perhaps a selection bias (Cohen and Friedman 2015a, b). In view of the controversy that has arisen, I asked Emanuel Friedman and Wayne Roy Cohen to place it all into perspective. The following is their response:

> It is impossible not to be awed and inspired by the process of labor and birth. Curiosity about its nature and mechanisms undoubtedly predates recorded history. Written evidence of efforts to understand labor and to codify its management can be found from ancient civilizations (Graham 1951), but the greatest advances in our understanding awaited the last century. The development of our understanding of the events of labor and delivery progressed along two parallel paths during the 20th century: exploration of the complex biochemical and physiologic mechanisms of uterine smooth muscle contraction occurred along with study of the effects of contractions on clinically measurable markers of the labor process. We will focus primarily on the latter approach.

The events of labor take place over time, and the earliest attempts to distinguish normal from abnormal labor involved simply deciding how long a labor should last. This approach led to vague admonitions such as "never let the sun set twice on a laboring woman" and various rather arbitrary time thresholds (Williams 1903). Such guidelines were based on what by today's standards were simplistic concepts of normality; but they made some sense at the time, given the narrow understanding of labor that prevailed then. It was evident to any practitioner that exceptionally long labors had the potential to cause harm. This reality has escaped the attention of many of today's policy makers who work in environments in which intrapartum fetal death and injury are uncommon, and generally ascribed to forces beyond the clinician's control. One need only see results from the developing world (McClure and Goldenberg 2009; Ngoc et al. 2006) or large-sample studies from the developed world (Friedman and Neff 1987) to understand that the labor and delivery process can be quite hazardous, and that many of its unfortunate sequelae are preventable. Normal parturition is generally an innocuous process for the fetus and requires no medical interference; abnormal labor, however, has considerable potential hazards, most of which are foreseeable and avoidable. Therefore, a critical aspect of avoiding risk requires that the obstetrician be able to differentiate normal from aberrant progress in labor.

As noted, it was beginning in the 1930s that Leroy A. Calkins (Calkins 1941, 1954, 1959) and other investigators in Germany and Switzerland (Frey 1929; Koller 1948; Wolf 1946; Zimmer 1951) made the first attempts to quantify the course of labor in a clinically meaningful way. Calkins focused on trying to define the normal length of the stages of labor and to determine how various maternal features (parity, body proportion, pelvic capacity, and so forth) might influence them. He ultimately focused on the mechanical properties of the cervix and the degree of engagement of the fetal head along with the strength of uterine contractions as the primary means to predict the length of the first stage, a foretaste of the Bishop score (named for Edward H. Bishop, 1913–1995), which appeared more than two decades later (Bishop 1964). As Friedman has recounted, to assess the second stage, Calkins relied on counting the number of contractions, rather than elapsed time, borrowing the concept of *wehenzahlen* [pain count] from the German obstetrician Eugen Frey (Frey 1929) . . . This approach had the virtue of being easily graphed, and thereby interpreted visually. Many efforts to predict the course of labor emphasized the time of rupture of membranes as a pivotal and regulating event, according it what we now know to have been undeserved significance . . ..

These several attempts to help us better understand the manner in which labor progresses over time never achieved widespread clinical acceptance. Their various limitations must have become quickly evident. The work of previous investigators did, however, serve an important purpose because they had in common the general notion that cervical dilatation and fetal descent could be described by a time function. That idea kept knocking hopefully but futilely at the door of progress until it was opened by the insight of Friedman beginning in the 1950s. Friedman's seminal work has served to govern our approach to the assessment of labor ever since.

Friedman determined that measurements of changes in cervical dilatation and fetal station over time were the most useful parameters for the assessment of labor progress. The original dilatation and descent curves were based on, and confirmed by, direct clinical observations made by one examiner on women in labor (Friedman 1954). Subsequently, data from multiple practitioners in a single institution were reported (Friedman 1955, 1956, 1967, 1978a). In both instances, the curves were drawn by hand, the descriptions were empiric, and the statistical analysis basic. Friedman approached his initial investigation with no expectation of what the curves would look like. It soon became clear that all normal labors showed similar S-shaped patterns, differing only in duration and slope of their component parts. From the initial observations it became clear that dilatation followed a sigmoid-shaped pattern, similar to a biologic growth curve. Dilatation, in what was termed the latent phase, initially progressed slowly or not at all. Then, at a point that varied among

individuals, dilatation became much more rapid and linear (the active phase), and then appeared to slow as the cervix neared full dilatation (the deceleration phase).

Later, with the aid of a computer algorithm developed with the Office of Biometry of the National Institutes of Health, a more sophisticated method of assessing labor data was used to analyze over 10,000 nulliparas from multiple institutions (Friedman and Kroll 1969; Friedman and Neff 1987). This confirmed the initial findings regarding the nature of the cervical dilatation-time and head descent-time functions. Raw labor data were plotted on a probit (i.e., the normal probability) scale, to convert the sigmoid curves to straight lines. The maximum slope data were converted to logarithms to normalize their right-skewed distribution. The linearity thus achieved made the data amenable to descriptive statistical study for determining distributions and limits of normal, which have stood the tests of time and clinical applicability.

The first publications describing the graphic patterns of dilatation and descent stimulated the interest of many clinicians, and led to the formulation of criteria that elevated the assessment of progress in labor from a rather arbitrary exercise to one guided by scientific objectivity. Researchers the world over confirmed the basic nature of the original curves, and validated their clinical functionality (Bottoms et al. 1987; Cibils and Hendricks 1965; Drouin and Nkounawa 1979; Duignan et al. 1975; Duncan and Costello 1975; Evans et al. 1976; Friedman and Kroll 1969; Hendricks et al. 1970; Incerti et al. 2011; Juntunen and Kirkinen 1994; Kwast et al. 1994; Ledger 1969; Ledger and Witting 1972; Lekprasert 1972; Melmed and Evans 1976; Peisner and Rosen 1986; Philpott and Castle 1972; Sokol et al. 1977; Studd et al. 1975; Van Bogaert 2006). There have been disagreements over the importance of the latent phase or even the existence of the deceleration phase of dilatation, but the core finding that active phase cervical dilatation progresses linearly, with a lower limit of normal approximately 1.0 cm hr^{-1} in nulliparas, has been remarkably consistent among studies.

Because physical examination to determine cervical dilatation and fetal station can be subject to some error and examiner bias, it was important to verify clinical observations with a completely objective approach. To that end, Friedman used a mechanical cervimeter (Friedman 1956; Friedman and Von Micsky 1963). His work, and that of several other investigators using other tools confirmed the sigmoid nature of progress in dilatation (Eijskoot et al. 1977; Kok et al. 1976; Richardson et al. 1978; Sharf et al. 2007; Van Dessel et al. 1994; Zador et al. 1976). Sigmoid-shaped curves of cervical dilatation have even been described in bovine parturition, suggesting a common pattern of labor among mammalian species (Breeveld-Dwarkasing et al. 2003).

The widespread adoption of the graphic approach to the assessment of labor has had four major benefits. It standardized the definition of normal and abnormal labor progress, thus enhancing patient care and communication among health care workers; it provided a reproducible objective standard that greatly enhanced our ability to do meaningful research about labor; it created a prognostic framework for analyzing an individual labor's likelihood of ending in a safe vaginal delivery; and it produced heretofore unrecognized information about the long-term risks of dysfunctional labor and various delivery techniques.

Use of the Friedman system of labor assessment has allowed us to recognize and to quantify, among other things, the effects of parity, analgesia, maternal obesity, prior cesarean, maternal age, and fetal presentation and position on labor (Chazotte et al. 1990; Cohen et al. 1980; Friedman 1967, 1978a; Verdiales et al. 2009). It has permitted analysis of outcomes associated with different types of labor aberrations (e.g., protraction or arrest of active phase dilatation and of second stage descent), quantified the effectiveness of various treatments, and assessed the impact of labor disorders on the need for cesarean delivery (Bugg et al. 2011; Deaver and Cohen 2009; Friedman 1967, 1978a, b; Friedman and Sachtleben 1961; Gross et al. 1987; Hopwood 1982; Steer et al. 1985; Weizsaecker et al. 2007). No other system of labor management can allow these assessments to be made

in a clinically meaningful manner. Moreover, dysfunctional labor patterns may serve as indicators of both short- and long-term risks to offspring, important considerations in making clinical recommendations to patients (Friedman 1973; Friedman and Neff 1987; Friedman et al. 1977, 1984; Towner et al. 1999).

Considerable evidence demonstrates that labor can be hazardous for the fetus and can result in irreversible injury (Amiel-Tison et al. 1988; Friedman 1973; Friedman and Neff 1987; Friedman et al. 1977, 1984; Sorbe and Dahlgren 1983; Towner et al. 1999; Weizsaecker et al. 2007). The disturbing toll exacted by neglected and exceptionally long labors is evident from experiences in the developing world, where labor-related perinatal mortality and morbidity remain common. Such effects are much more rare in the developed world, but they continue to exist. Their study is daunting for many reasons, in addition to being relatively uncommon. A myriad of genetic and environmental factors influence measures such as IQ scores, speech and language development, educational achievement, and general neurobehavioral functioning. The further from birth an individual is, the greater the opportunity for these factors to influence outcome measures, many of which (like IQ or language development) cannot be measured until years after birth. Thus, while it may not be difficult to identify the immediate signs of hypoxic or mechanical birth injury (death, intracranial bleeding, encephalopathy, fractures, peripheral nerve injury, etc.) determining the long-term effects of such findings in survivors is a formidable and difficult challenge. Moreover, some handicaps linked to birth are not diagnosable until considerable time has elapsed after delivery. Most of the attempts to link aspects of intrapartum events to long-term health and achievement suffer from inadequate sample size and the inability to control for variables that confound or interact with each other.

Data from the National Collaborative Perinatal Project (NCPP), sponsored by the United States Public Health Service from 1958–1974, are the most helpful in this regard, although they are not without shortcomings (Niswander and Gordon 1972). The multicenter study gathered comprehensive accurate information on about 18,000 pregnancies for which detailed data regarding labor progress was available. Friedman was one of the primary investigators on the project and, in collaboration with colleagues, used the data to recognize important features of obstetric care that are linked to neonatal outcome. Up to 8-year follow-up (including IQ testing, speech, language and hearing evaluation, and assessment of mental and motor disorders) was available for many offspring. It is the only database available with such detailed information, and has provided a wealth of useful insights into the influence of hundreds of variables on perinatal outcome (Friedman 1973; Friedman and Neff 1987; Friedman et al. 1977, 1984). The data were, however, collected before the widespread use of continuous electronic fetal heart rate monitoring, and precede the advent of modern neonatal care and many intervening advances in obstetric practice. In particular, cesarean delivery rates were very low during the study epoch, making it difficult to study the benefits and risks of cesarean on outcome. These limitations notwithstanding, no comparable study has yet been done. Of importance, the NCPP was one of very few studies that stratified outcome based on the Friedman classification of dysfunctional labor progress. The stunning results indicated that Friedman's classification of labor abnormalities, which was intended simply to provide a clinical framework for interpreting and managing the events of labor, provided a means to assess immediate and enduring perinatal harm.

Among other things, the NCPP demonstrated the unexpectedly high morbidity (much of it not evident at birth) associated with midforceps deliveries, especially those involving rotation or traction from high pelvic stations. The likelihood of complications was amplified when such deliveries were done in the wake of protraction or arrest disorders of labor. In large part as a consequence of these observations, complex midcavity deliveries have become rare. More important, the NCPP allowed insight into the dangers inherent in the dysfunctional labor patterns described by Friedman. Even when various forms of regression modeling were used to control for effects of dozens of potentially confounding factors such as delivery type, birth weight, socioeconomic status, membrane status, and fetal position,

the relative risk of adverse perinatal outcomes was significantly elevated by the presence of prolonged latent phase, or protraction and arrest abnormalities.

It is unfortunate that there have not been more recent studies to collaborate these observations in the context of contemporary obstetric and neonatal care. We do not know with certainty, for example, whether the adverse effects of an arrest of descent are directly related to the duration of the arrest, and when, or indeed whether, timely intervention by cesarean or instrumental vaginal delivery would neutralize the effects of the arrest. We can make inferences in this regard from our understanding of the mechanisms of injury, but these kinds of questions beg to be addressed directly.

Much of our understanding of the association between dysfunctional labor and fetal injury emerged in parallel with many of the advances in fetal physiology outlined in this volume. The overwhelming emphasis of investigation has been on the potential ravages of fetal oxygen deprivation, which has long been viewed as the predominant cause of intrapartum neurologic injury (ACOG 2003; Longo and Packianathan 1997; Schifrin et al. 2014). During labor when the intramyometrial pressure is sufficiently high it can compress the branches of the uterine arteries that traverse the myometrium, thus inhibiting or interrupting blood (and therefore oxygen) flow into the intervillous space. From the clinical perspective, when this occurs during the peak of uterine contractions of normal frequency and intensity, a healthy fetus deals with it with apparent equipoise. If, however, the fetus's ability to tolerate hypoxemia has been reduced by acute or chronic factors (e.g., placental abruption, infection, or growth restriction) normal contractility can reduce oxygenation to hazardous levels. Even the healthy resilient fetus can be harmed by excessive spontaneous or, more commonly, drug-induced hypercontractility. Such events usually cause abnormal fetal heart rate patterns that are presumed to be hypoxic in origin. Of interest is that hypoxemia itself, even if severe, will only cause brain injury if it associated with ischemia (Longo and Packianathan 1997). The latter occurs when asphyxia is sufficiently severe to result in reduced cardiac function and consequent diminution in brain blood flow.

Recently, focus has begun to shift from concerns about hypoxia to ischemia, and the idea that the fetal brain can be injured during labor by ischemia from excessive intracranial pressure even in the absence of severe hypoxia has piqued the interest of investigators (Ghosh et al. 2011; Mann et al. 1972; Schifrin et al. 2014). In fact, some studies indicate that at least half of babies diagnosed with neonatal encephalopathy show no evidence of severe asphyxia at birth (Ruth and Raivio 1988; Yeh et al. 2012), yet imaging studies are consistent with acute ischemic injury (Cowan et al. 2003).

It has long been appreciated that excessive uterine contractile force can cause trauma to the fetus, particularly in long and difficult labors. In fact, the potential dangers of excessive molding on the brain were pointed out by Hendrik van Deventer (1651–1724) and William Smellie (1697–1763) in the early 18th century, and reinforced by William John Little (1810–1894) later in that epoch (Little 1862; Smellie 1752). Scant attention was addressed to this issue subsequently, and, as noted, the emphasis on understanding the effects of hypoxia relegated concerns about labor-related trauma to the periphery of interest. During the second half of the 20th century, although some studies still suggested trauma as an important issue (Amiel-Tison et al. 1988; Sorbe and Dahlgren 1983; Towner et al. 1999), it received little attention. This was, in part, because changes in obstetric practice, including prominently the major decline in the use of complex forceps operations, had so reduced the incidence of major overt cranial trauma such as tentorial tears, subdural bleeding, and severe skill fractures, that the average obstetrician was unlikely to encounter many such injuries, and the notion that subclinical brain trauma could occur and cause long-term damage was not appreciated until the findings of the NCPP described above began to be appreciated.

The mechanism for tolerance of the fetal brain ischemia induced by uterine contractions relates to compression of fetal brain blood vessels by high pressures within the skull. The

ability of a contraction to generate enough pressure to severely impede intracranial flow depends on several factors: the hydrostatic pressure within the vessels, the strength of the vessel wall (in engineering terms, its collapse strength), and the pressure imposed on the vessel from its surrounding tissue (Schifrin et al. 2014). It is clear that pressures exerted on the skull in contact with the cervix or pelvis can exceed 200 mmHg when the intrauterine pressure measured in amniotic fluid is only about 50 mmHg (Pu et al. 2011; Schifrin et al. 2014). These observations provide an explanation for intrapartum brain ischemia and neonatal encephalopathy caused by dysfunctional labor. The threshold for injury from ischemia probably varies considerably among individual fetuses and depends not only on hypoxic and mechanical stresses, but also other environmental factors (infection, other illness, drugs) as well as genetic predisposition.

Recently, the traditional model of how labor progresses has been challenged by a group of investigators who claimed that the data underpinning the longstanding view promulgated by Friedman were not analyzed optimally, and that modern statistical methods (not available to Friedman) demonstrate a different kind of curve for dilatation and descent according to time (Laughon et al. 2012; Zhang et al. 2010a, b). The results of this work, which utilized high-order polynomial curve fitting and interval-censored regression analysis, was quickly adopted by the American College of Obstetricians and Gynecologists and formed the basis for a wholly new set of guidelines for the assessment and management of labor (ACOG 2014). These guidelines, which ignore a half-century of corroborated research, are a testament to the triumph of theory over experience, and have been sharply challenged (Cohen and Friedman 2015a, b).

As noted, the morphology of the Friedman labor curves was determined by direct observation and confirmed by a number of investigators using mechanical and electronic methods to track cervical dilatation directly and accurately. The primacy of direct observation over theoretical conceptualization or indirect analysis of data in hypothesis testing has been central to the scientific method since the Enlightenment. When the results of an analytic approach differ from those derived from observation, it is important to understand why this has occurred.

Why do these more recent labor curves differ from those of previous observers? One explanation was provided when these authors applied their analytical methods to the same data Friedman had analyzed from the NCPP (Laughon et al. 2012). Friedman's analysis revealed a sigmoid-shaped dilatation curve; that of the later studies an exponential curve, essentially the same as they had found from contemporary labors. Clearly, what had changed was not the nature of progress in labor, but how the data were analyzed. This approach was burdened by a set of selection biases and uncontrolled confounders, which cast doubt on the validity of their findings. Women with rapidly progressing labors tend to present themselves for obstetric care and be first examined at more advanced cervical dilatation than those with longer labor. Thus, the intervals at the distal end of the dilatation curve are likely to have been loaded with progressively more rapid labors. This may explain the exponential nature of the dilatation curve derived in this manner. It may also explain why the descent curve, which was unencumbered by that problem because all patients were present and under observation for their entire second stage, looks very much like that originally reported.

In addition, these later labor curves excluded women delivered by cesarean section. Many of these were undoubtedly having slow, dysfunctional labor patterns that led to the need for cesarean delivery. Their exclusion is likely to have falsely increased the average rate of dilatation in residual study cases, contributing to the exponential appearance of the curves. These workers also excluded women whose cervix was more than 6 cm dilated at admission, probably thus ignoring many of the most rapid labors and contributing to the overall appearance of slow average dilatation. Moreover, the use of high-order polynomial curve fitting is controversial, and can produce distortion in the resulting curves (Montgomery et al. 2012).

> To understand the process of labor it is of obvious value to understand uterine contractility. Coordinated strong episodic contractions of the uterus are necessary for normal labor and delivery. It also stands to reason that abnormal labor could be caused by uterine contractions that are in some manner dysfunctional. Our understanding of the biochemical and biophysical complexities of myometrial contractility has progressed steadily over the last century. What we still lack is the necessary translational research to link the physiologic manifestations of uterine contractility with changes in cervical dilatation and descent. Recent efforts to obtain and interpret electrohysterographic patterns hold some promise in this regard (Euliano et al. 2009; Hayes-Gill et al. 2012) as does an initial foray into the molecular basis of dysfunctional labor (Chaemsaithong et al. 2013). For now, however, the clinician is best served by a system of labor assessment proposed more than 60 years ago, a system that has contributed vastly to our understanding of human labor and birth (Cohen and Friedman 2011).
>
> (Letter from WRC & EAF to LDL, 1 September 2015)

Until more definitive data is available from carefully controlled studies, it remains problematic as to the criteria that constitute a "normal" labor curve.

10.16 Summary

For the pregnant woman, profound anatomical and physiological adaptations in essentially every organ system are essential to meet the metabolic requirements of her own body, as well as that of the developing fetus and the demands of childbirth. An understanding of these changes is critical to recognize pathological deviations in the mother and to optimize a successful outcome for both her and her infant. In addition, these physiologic changes, including those of the metabolic rates of given organs, the binding proteins for various compounds, and their clearance rates, also have numerous implications on pharmacokinetic and pharmacodynamic properties of medications taken by the pregnant woman.

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Chapter 11
Maternal Complications of Pregnancy that Affect Fetal Development

If pregnancy were a book, they would cut the last two chapters.

(Nora Ephron 1983, Chap. 4)

In examining disease, we gain wisdom about anatomy and physiology and biology. In examining the person with disease, we gain wisdom about life.

(Oliver Sacks 1985)

The experience of pregnancy and childbirth transforms the lives of mothers and their families. As it may be anticipated, a large number of medical and surgical complications in the pregnant mother may affect development of the fetus and health of the newborn infant. Since the early nineteenth century (Bard 1807), volumes may be devoted to a consideration of these (Creasy et al. 2014; Reece and Hobbins 2007); however, in the light of space limitations, only several will be considered here.

11.1 Premature Onset of Labor and Delivery

As noted in other chapters, preterm or premature birth, e.g., that occurring prior to 37 weeks' gestation, is near or exceeds 10% of pregnancies in the USA (Hamilton et al. 2015; Martin et al. 2015; March of Dimes Foundation 2009), and these infants are more likely than normal to experience the complications associated with low birthweight and prematurity. In turn, these are the leading cause of newborn morbidity and mortality (Bernstein et al. 2000; Goldenberg and Rouse 1998; Iams 2014; Simhan et al. 2014). Strictly speaking, preterm birth (PTB) defines those neonates born prior to term (about 280 days, 40 weeks). It has become appreciated that births between 37 and 39 weeks are associated with greater short- and long-term morbidity than those after 39 weeks (Mackay et al. 2010). Thus, on the basis of recommendations by both the National Institute of Child Health and Human Development (Spong 2013) and a committee opinion of the

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Table 11.1 Recommended classification of obstetrical deliveries

Very preterm: <28 weeks
Preterm: 28–33 weeks
Late preterm: 34–36 weeks
Early term: 37 weeks through 38 weeks 6 days
Full term: 39 weeks through 40 weeks 6 days
Late term: 41 weeks through 41 weeks 6 days
Postterm: 42 weeks and beyond

Data from ACOG (2013), and Spong (2013)

American Congress of Obstetricians and Gynecologists (ACOG 2013), an updated classification separates early term from full term, later term, and post term (Table 11.1). Recent data demonstrate that maternal and neonatal complications differ in the 6-week gestational age that is accepted as term (37–42 weeks) (MacDorman et al. 2006), the frequency of adverse outcomes being "U" shaped with the nadir at 39–40 weeks' gestation (Spong 2013). In addition, preterm delivery is further classified as late preterm (34–36 weeks), early preterm (28–33 weeks), and very preterm (<28 weeks). A scale based on weight that is used more frequently is low birthweight (<2500 g), very low birthweight (<1500 g), and extremely low birthweight (<1000 g). PTB should include all births, including live births, stillbirths, and pregnancy terminations that occur from 16 weeks (112 days) to 38 weeks 6 days (272 days) (Kramer et al. 2012) (Table 11.1).

Low birthweight refers to those infants born too small, and the term small for gestational age (SGA) has been used to categorize those newborns whose weight is below the 10th percentile for gestational age. Other terms used are fetal growth restriction (FGR) and intrauterine growth restriction (IUGR). In contrast, the term large for gestational age (LGA) is used for those newborns whose weight is above the 90th percentile. Appropriate for gestational age (AGA) designates those infants who weigh between the 10th and 90th percentiles.

Few areas of public health have attracted as much attention during recent years as newborn survival. To place this issue in perspective, about 1.3 million infants are born preterm each year accounting for about 10% of births worldwide (Beck et al. 2010), and the rate has not decreased significantly in the past two or three decades (Martin et al. 2005). In 2013, for the first time in history, premature birth replaced infectious disease as the number one killer of children (Lawn and Kinney 2014; Liu et al. 2012). Direct complications from premature birth account for a global total of almost three quarters of a million deaths during the neonatal period, with an additional 125,000 deaths between the ages of 1 month and 5 years (Lawn and Kinney 2014; Lawn et al. 2010, 2014; Liu et al. 2015). In 2001 of slightly over 4 million live births in the USA, 27.5 thousand (0.7%), about half of which were less than 32 weeks' gestation, died within the first year of life. Overall, PTB before <37 weeks was implicated in two-thirds of these deaths (Mathews et al. 2003). From 1940 to 2001 in the USA, infant mortality (again a large fraction of which is associated with premature birth) fell from about 42 and 75 per 1000 live births for Whites and Blacks, respectively, to about 10 and 17, respectively, for the two ethnic

groups. Two thirds of preterm births occur following the spontaneous onset of labor, with the other third being medically indicated because of maternal complications such as preeclampsia or fetal growth restriction or other problem (Goldenberg et al. 2008). Preterm labor has been defined as "... one syndrome, many causes" (Romero et al. 2014, p. 760).

Of the estimated one and a third to half million children worldwide who are born prematurely every year, well over one million survive with functional immaturity of multiple organ systems, as well as serious long-term neurodevelopmental disabilities at great cost to their families and society (Blencowe et al. 2013; Damus 2008; Hack et al. 1995; Lawn et al. 2005, 2014; Petrini et al. 2009; Wallander et al. 2014). Clearly, these statistics emphasize the need for basic biomedical science to work with societal, nongovernmental, and governmental agencies to gain an understanding of the genesis and to lay the foundations for the prevention and amelioration of this pandemic (Bhutta and Darmstadt 2014). Again, to place this issue in perspective, to a great extent the high rate of morbidity of the immature newborn concerns neurologic development, the severity of which is inversely correlated with gestational age and birthweight. The untoward sequelae in these infants include periventricular leukomalacia, intraventricular hemorrhage, and cerebral palsy, as well as chronic lung disease, and other problems. Striking is the severity of these complications (Adams and Barfield 2008; Bhutta et al. 2002; Hack and Fanaroff 1999; Moster et al. 2008; Petrini et al. 2009; Riley et al. 2008; Stoll et al. 2004; Swamy et al. 2008; Vohr et al. 2000; Wood et al. 2000).

Spontaneous preterm labor syndrome or PTB results from a number of contributing factors (Romero et al. 1994, 2006). Among the many etiological considerations for spontaneous premature onset of labor are chorioamnionitis, intrauterine inflammation, premature rupture of amniotic membranes preeclampsia, decidual hemorrhage, placental abruption, fetal distress, uterine overdistension, less than 6-month interpregnancy interval, short cervix, and cervical insufficiency/incompetence, as well as maternal lifestyle factors such as smoking and drug use (Table 11.2; Bossi 1892; Buhimschi and Norman 2014; Meis et al. 1998). Some have categorized these as distinct phenotypes (Manuck et al. 2015).

In the 1960s, infection of the genitourinary tract and vagina were identified as factors in the causation of premature labor and delivery. Two leaders in this field

Table 11.2 Some factors associated with spontaneous preterm labor

Uterine overdistension
Decreased [action] of progesterone
Dysfunction of decidua
Intrauterine inflammation or infection
Pyelonephritis
Vascular dysfunction, hypertension
Premature rupture of amniotic membranes
[Dysfunction] of cervix
Vaginal infection and/or chorioamnionitis
Maternal stress and lifestyle factors (obese, underweight)

were Edward Harold Kass (1917–1990) and William Ellery, Channing professor of medicine at the Boston City Hospital and Harvard Medical School. Kass, a pediatric infectious disease fellow, published a seminal four-part review of the pathogenesis and prognosis of prematurity, which analyzed the demographics of maternal complications and the important role of bacteriuria in its genesis (Abramowicz and Kass 1966). Further reports from the Channing laboratory presented evidence for the role of bacteriuria and pyelonephritis (Kass 1970, 1973, 1982), as well as T-strain mycoplasmas isolated from fetal membranes (Braun et al. 1971; Kass et al. 1986) in this regard. Kass also pioneered in the pathogenesis of toxic shock syndrome. Studies from Robert L. Goldenberg and his group at the University of Alabama at Birmingham also demonstrated the high association of infection, including chorioamnionitis, in the genesis of premature labor (Goldenberg et al. 2000a, b, 2003, 2008).

Another major issue is the further increase in recurrence of PTB in women that had prior preterm delivery (Laughon et al. 2014). A not uncommon factor in the premature onset of labor and delivery is multiparity, which has seen a significant increase in the past decade or so, due in part to the increase in assisted reproductive technologies. The consideration of increased maternal age in these women with PTB cannot be excluded. As discussed elsewhere, monochorionic diamniotic twins may experience twin-to-twin transfusion syndrome with its associated discordant rates of fetal growth. In this syndrome, the two fetuses may have a varying degree of anemia versus polycythemia, congestive heart failure, and hydrops. Along with a wide variation in amniotic fluid volume, intracerebral lesions, possibly caused by ventricular enlargement, are reported. Outcomes of these pregnancies and in utero efforts to separate the blood supply to each fetus are associated with preterm delivery (Malshe et al. 2017). Adding to the complexity of the PTB syndrome is the finding that the risk of PTB is greater higher in women that themselves had been born preterm (Porter et al. 1997). Maternal gestational age at birth was found to be inversely correlated with risk of PTB and was influenced by maternal age and parity. Collectively, a complexity of current, epigenetic, and transgenerational considerations may impact the overall risk for PTB.

During recent years, work has focused on predictive biomarkers, e.g., physiological or pathological biochemical alterations at the organ, tissue, cellular, or subcellular level that is measurable in biological samples and becomes the signature of the onset of disease underlying a pathologic process, or phenotypic outcome predictability. Quantification of biomarkers have proven useful in many fields of medicine, including reproductive medicine through analyses of amniotic fluid, maternal blood, saliva, vaginal secretions, and others. However, the predictive value of various biomarkers for PTB has been inconsistent to any particular risk factor for PTB (Conde-Agudelo et al. 2011; Menon et al. 2011), and their relation to constituting a cause or being an effect remains unclear (Lockwood and Kuczynski 1999, 2001; Romero et al. 2010a, b; Voltolini et al. 2013). Table 11.3 lists some of these biomarkers that have been used for the diagnosis of preterm labor.

A major challenge in the years ahead is to understand the etiology of PTB and the interrelationships of the placenta, fetal membranes, uterus, and cervix. The

Table 11.3 Maternal and fetal complications associated with obesity in pregnancy

Stage	Complications
Antepartum	Gestational diabetes
	Gestational hypertension
	Preeclampsia
	Spontaneous abortion
	Intrauterine fetal demise
	Premature onset of labor
	Birthweight >4500 g
	Congenital abnormalities
Intrapartum	Increased maternal and fetal morbidity and mortality
	Failed induction of labor
	Failed trial of labor after cesarean delivery
	Shoulder dystocia
	Cesarean delivery
	Operative vaginal delivery
	Operative complications during delivery
Postpartum	Increased maternal and fetal morbidity and mortality
	Maternal hemorrhage
	Maternal wound infections and/or endometritis
	Maternal depression
	Maternal suicide
	Offspring growth trajectory altered
	Offspring childhood and adult obesity

extent to which appropriate animal models (transgenic or other) hold promise for understanding the convergence of consideration in a final common pathway for PTB since the ability to obtain human biopsy material at relevant gestational ages is quite limited. At the cellular and molecular level, a number of issues are apparent—the regulation of candidate genes and epigenetic factors for imprinted and clock genes need to be taken into consideration (Leavitt et al. 2006; Muglia and Katz 2010; Norman and Greer 2005; Pennell et al. 2007; Raju et al. 2014). The current theory is that the transition from a quiescent to contractile myometrial state is accompanied by a shift in signaling from anti-inflammatory to pro-inflammatory pathways (Menon et al. 2016; Keelan 2017). Activation of the uterine decidua involves expression of inflammatory cytokines (tumor necrosis factor-α and interleukin-1 and interleukin-6) and chemokines (e.g., interleukin-8). This is accompanied by increased activity of proteases (matrix metalloprotease-8 and metalloprotease-9, connexin 43, oxytocin, and prostaglandin receptors). Dissolution of extracellular matrix components (fibronectin) and apoptosis also have been implicated in this process (Menon and Fortunato 2007; Moore et al. 2006).

Insights about the parturition process have come from recognition that a focus on the uterus as the source that initiates preterm labor disregards important evidence that remodeling of the cervix begins well before the day of birth in women at term

(Danforth et al. 1974) and in PTB (Goldenberg et al. 2008). Studies in other mammals provide insights into the potential mechanism for prepartum cervix remodeling due to the limited availability of biopsy tissues in primates (Yellon 2016). Complex changes in extracellular matrix proteins (including loss of collagen cross-linking, but not total content, and an increase in glycosaminoglycans) are associated with transformation of the cervix from a dense to soft to ripened structure with little resemblance to the heterogeneous morphology found before midpregnancy or in the nonpregnant state. These morphological changes occur while progesterone in circulation is at or near peak concentrations and are associated for increased compliance to dilate and allow for vaginal delivery. In particular, reduced mature cross-linked collagen is associated with decreased mechanical stiffness and strength in the cervix of mice during the softening phase of remodeling well before labor at term (Yoshida et al. 2014). The prepartum transition from a soft to ripe cervix is also characterized by inflammatory processes before term and in several rodent models of preterm birth (Yellon et al. 2009; Yellon et al. 2013; Kirby et al. 2016). Treatments with progestational agents can forestall cervix remodeling and block preterm birth. Understanding how various factors may regulate the presence of resident macrophages and various macrophage phenotypes (Payne et al. 2012) along with activation of genes specifically related to macrophage (Dobyns et al. 2015) may provide insights for molecular pathways that mediate prepartum ripening.

In a broader context, biomarkers for ripening may prove useful to understand the impact of the cervix metabolome (Ghartey et al. 2015) or vaginal microbiome (Bastek et al. 2011; Vinturache et al. 2016) on the mechanism leading to PTB. Although inflammatory processes in the cervix are conceived to be important for remodeling, the findings also raise the possibility that the cervix immunome may be related to an immunological barrier function to protect the contents of the womb from the vaginal biome. Whether a distinct microRNA profiles in blood of women (Elovitz et al. 2014, 2015) or other biomarkers from a "Pap" smear for a noninvasive method to assess cervix remodeling at the molecular level as pregnancy progresses and the potential for targeted therapies to reduce the rate of PTB. These efforts are consistent with the possibility that the mechanism for labor at term reflects activation of a physiological "common pathway" for which one or more components may be advanced with PTB (Romero et al. 2014).

The importance of progesterone to sustain pregnancy is well established (Swaggart et al. 2015). Progesterone also maintains uterine quiescence by repressing specific genes and increasing expression of the microRNA 200 family to block genes associated with contractile pathways in the myometrium and promotes progesterone catabolism (Renthal et al. 2013; Renthal et al. 2015). For parturition, the concept of a loss of this progesterone blockade and its premature withdrawal was pioneered by the Hungarian obstetrician gynecologist Arpad I. Csapo (1918–1980) (Fig. 11.1) of Washington University, St. Louis, MO. In a series of studies, Csapo demonstrated the efficacy of progesterone (initially from the ovaries and later from the placenta) to block myometrial contractility (Csapo 1956). Loss of this effect was proposed to lead to labor and birth. Although the

Fig. 11.1 Arpad I. Csapo

mechanisms were unknown, Csapo proposed the solution to one of the oldest riddles of mankind, the basis for the maintenance of normal pregnancy, and its termination at full term. Despite the attractiveness of this idea, many leaders in the field disputed the interpretation of his findings, and debate raged among academic departments and in the journals. Later, in formulating the basic mechanisms by which the pregnant myometrium is regulated, Csapo proposed his "seesaw" theory of uterine function, which postulated that during pregnancy the fetus is protected by a balance between factors that promote uterine quiescence (progesterone) and those that stimulate its contraction (prostaglandins) (Csapo 1975). He concluded, "Consideration . . . [of the theory] may be useful since it may predict further relationships in a highly complex biological system and point at informative experiments which might otherwise escape attention" (Csapo 1975, p. 581). Again, Csapo's "seesaw" theory occasioned vigorous debate and ad hominem [to the man] attacks. His epochal work thus serves to illustrate the problem of important concepts being promoted "before their time." The world had to await nearly half of a century before cellular and molecular tools such as quantification of receptor isoforms and coactivators (Nold et al. 2013; Tan et al. 2012), including the role of progesterone in altering the activities of prostaglandins (Patel et al. 1999), and the myometrial sensitivity to oxytocin (Grazzini et al. 1998) became available to help support the concept.

In an effort to minimize or eliminate confusion and promote compatibility so that various studies of PTB can be compared and data sets combined for meaningful meta-analysis, a "harmonized template" for such prospective investigation based on optimal data sets has been suggested (Myatt et al. 2012). From a social perspective, developments such as the "Born too Soon" report (Lawn et al. 2013) and initiatives such as the "Every Newborn Action Plan, the Every Woman, Every Child initiative and World Prematurity Day" are raising awareness of the importance of a normal,

uncomplicated gestation and prematurity as global public health issues that demand governmental, political, and private attention (Lawn and Kinney 2014).

A leader in the field of myometrial contractility and the initiation of labor, Sam Mesiano of Case Western Reserve University, Cleveland, Ohio, responded to several of my questions:

> In the early 1980s, I became interested in the problem of preterm birth as a graduate student in the laboratory of Geoffrey Thorburn at Monash University in Melbourne Australia, and later in the late 1980s and early 1990s as a postdoctoral fellow in the laboratory of Robert Jaffe at University of California San Francisco (UCSF). At Monash University my research focused on the role of the fetal pituitary in the control of fetal growth using the pregnant sheep as an experimental model (Mesiano et al. 1987). Although I studied fetal skeletal growth and the fetal growth hormone-somatomedin axis, an important outcome of the work was that the central role of the fetal hypothalamic-pituitary-adrenal axis in the control of ovine parturition was verified using a highly specific method to perform fetal hypophysectomy. The studies highlighted the complex fetal-maternal hormonal communication, especially in the form of the steroid hormones cortisol, progesterone and estradiol in the control of birth timing. As a postdoctoral fellow at UCSF, I also examined the development and function of the human fetal adrenal cortex (Mesiano and Jaffe 1997). The research demonstrated the unique activity of the feto-placental steroidogenic unit in human pregnancy and its remarkable capacity to produce estrogens and progesterone throughout pregnancy. The work motivated me to pursue my present research into the mechanism by which progesterone promotes human pregnancy and how its actions are modulated to trigger parturition. The research is directly aimed at solving the problem of preterm birth.
>
> Preterm birth is a major socioeconomic problem. Each year it affects 15 million pregnancies and is the principal cause of neonatal morbidity and mortality worldwide, accounting for about 1.1 million neonatal deaths (Beck et al. 2010). Despite decades of intensive clinical and basic research there are no effective therapies to predict and prevent preterm birth. A major obstacle to progress has been that the physiology of human parturition, and especially its trigger mechanism, is not mimicked by common laboratory animal models. Consequently, progress made in unraveling the hormonal control of parturition in animals, although seminal in elucidating key hormones in the mother-fetus dialogue that affect the birth process, contributed little to understanding the pathophysiology of human parturition and preterm birth. It should be acknowledged, however, that pioneering studies by Liggins and colleagues in sheep (Liggins 1994; Liggins and Howie 1972; Liggins et al. 1967, 1973) led directly to the use of glucocorticoid therapy for women with threatened preterm birth to promote fetal lung maturation, which has had a major positive impact on improving the survival of preterm neonates.
>
> The discovery of progesterone originates with the discovery of the corpus luteum (CL) in the late seventeenth century. Historical accounts are provided by Roger Short (Short 1977) and Richard Stouffer (2006). Research into the hormonal control of parturition, and specifically the role of progesterone, originated in the late 1600s when anatomists Regnier de Graaf and William and John Hunter observed in animals that pregnancy was associated with the presence of yellow bodies (i.e., the CL) on the ovaries, the number of which corresponded with the number of fetuses, and that in some animals removal of the ovaries during pregnancy induced parturition, suggesting that the ovaries, and especially the CL, were necessary for the maintenance of pregnancy. That the CL is an organ of secretion was proposed 200 years later by Louis-Auguste Prenant (1861–1927) and this concept immediately motivated investigators to isolate and characterize the substance that maintains pregnancy, referred to as a progestin, produced by the CL. This effort was led by George Corner and Willard Allen at Johns Hopkins, who in 1929 prepared an alcohol extract of pig CL and found that it maintained pregnancy in ovariectomized animals (Corner and Allen 1929). Several years later in 1934, it was discovered that the progestin

was a steroid and thus appropriately given the name progesterone (Allen and Wintersteiner 1934). At around the same time estrogenic steroids were discovered and their effects on uterine growth and contractility were being investigated. In his book on the physiology of reproduction (Corner 1947) George Corner made the following prescient statement: "When it was discovered that estrogenic hormones stimulate the involuntary muscle of the uterus, and that progesterone tends to relax it, an attractive theory of the cause of labor at once suggested itself. We need only suppose that when the end of gestation draws near, the production of progesterone goes down, and estrogenic hormone is thereby allowed to build up contractions on the uterine muscle. This hypothesis is however much too simple…" (Corner 1947, p. 206).

This "much too simple" hypothesis remains valid today and dramatically shaped the thinking of future researchers in the parturition field. During the 1950s to 1970s, one investigator, Arpad Csapo, extensively tested the hypothesis in multiple species. His groundbreaking studies on the role of progesterone in pregnancy maintenance led him to propose the "progesterone block hypothesis", which posits that for most of pregnancy progesterone blocks the onset of labor and that parturition is initiated by withdrawal of the progesterone block (Csapo 1956). With the advent of sensitive and specific immunoassays for progesterone and more detailed studies in a variety of species it became clear that in most animals circulating progesterone levels indeed decrease precipitously (i.e., a systemic progesterone withdrawal) prior to the onset of labor. However, it was soon realized that the prepartum fall in maternal blood progesterone does not occur in humans. Instead maternal blood progesterone levels remain elevated throughout pregnancy and during labor and delivery casting some doubt on Csapo's progesterone withdrawal hypothesis, at least with respect to human parturition. To address this inconsistency Csapo proposed an alternate form of progesterone withdrawal in which target cells in the pregnancy uterus become refractory to the progesterone block. He referred to this as "functional progesterone withdrawal" (Csapo 1975).

In the early 1980s (several years after Csapo's death), it was found that administration of the newly discovered progesterone receptor (PR)/glucocorticoid receptor (GR) antagonist RU486 to pregnant women increased uterine contractility and in most cases induced the full parturition cascade at any stage in pregnancy. It was later found that identical effects were caused by PR-specific antagonists. Those clinical findings supported the core tenet of Csapo's functional progesterone withdrawal hypothesis by showing that normal PR signaling is necessary for the maintenance of pregnancy (i.e., it mediates the progesterone block to labor) and that disruption of PR signaling alone is sufficient to induce the full parturition cascade. Thus, it is hypothesized that human parturition is triggered by specific modulation of PR activity in progesterone target cells in the pregnant uterus to cause functional progesterone withdrawal.

Although the modern era of molecular biology has elucidated some key mechanisms of progesterone-PR action, understanding of progesterone-PR signaling in general, specifically in the gestational tissues of human pregnancy, and how that signaling is regulated in the context of pregnancy maintenance and birth timing, remain an ongoing challenge. Present research is focused on the molecular mechanisms underpinning the progesterone block by asking the fundamental question of how progesterone promotes uterine quiescence and how it's blocking action are withdrawn to trigger parturition. Recent studies have shown that the PR isoforms (PR-A and PR-B) mediate distinct genomic (i.e., they function as distinct ligand-activated transcription factors) activity in the pregnant myometrium and that functional progesterone withdrawal may be mediated by changes in PR isoform transcriptional activity (Mesiano et al. 2011; Tan et al. 2012). An important concept that has emerged in recent years is that progesterone promoted uterine quiescence mainly be acting as an anti-inflammatory agent (Hardy et al. 2006; Tan et al. 2012). This hypothesis assumes that the process of parturition is triggered by tissue level inflammation within

myometrium, decidua and cervix and that for most of pregnancy progesterone acting via the PRs inhibits the responsiveness to pro-inflammatory/pro-labor stimuli. Studies from several laboratories in the US (Mendelson, Mesiano), the UK (Johnson, Bennett, Norman) and Australia (Smith) are showing a key functional interaction between tissue level inflammation and PR signaling such that tissue-level inflammation is linked to (and may actually induce) PR-mediated functional progesterone withdrawal. We have proposed that the pregnant uterus is exposed to an inflammatory load derived from multiple inputs that exert pro-labor signals, and that for most of pregnancy progesterone acting mainly via PR-B attenuates responsiveness to pro-inflammatory/pro-labor stimuli. Importantly, this anti-inflammatory effect is not absolute. An inflammatory load threshold may exist above which inflammatory stimuli alter the transcriptional activity of PR-A to induce a trans-repressive activity that inhibits the anti-inflammatory activity of PR-B to cause functional progesterone withdrawal. Recent studies have shown that the capacity for PR-A to trans-repress PR-B is induced by specific post-transcriptional modifications, especially phosphorylation and SUMOylation (Hagan et al. 2012). Importantly, our recent studies are showing that PR-A trans-repression of PR-B in human myometrial cells is augmented by pro-inflammatory stimuli, supporting the concept that tissue-level inflammation induces PR-A-mediated functional progesterone withdrawal. This may be a key trigger mechanism for human parturition that explains the association of preterm birth with inflammatory states such as chorioamnionitis. The research is revealing novel therapeutic targets and strategies to prevent preterm birth by augmenting PR pro-gestational activity and/or preventing inflammation-induced functional PR withdrawal. The advent of the systems biology/omics era is also providing novel and innovative tools to identify the specific signaling pathways, interactive networks and PR PTMs that regulate not only progesterone action in the human pregnant uterus but the general hormonal control of pregnancy and parturition. It is envisioned that with a deeper understanding of the molecular biology of PR action in the cells of the gestational tissues that progesterone-PR activity will be exploited therapeutically to control uterine contractility and prevent preterm birth.

(Letter from SM to LDL, 17 July 2015)

11.2 Chorioamnionitis

Bacterial infection within the amniotic cavity occurs with surprising frequency with diverse untoward and uncertain effects on the developing fetus, neonatal morbidity, and mortality, in addition to maternal morbidity. Histologic evidence of intrauterine infection occurs more commonly than does that of clinically manifest infection, being present in up to 20% of term deliveries and more than 50% of those that are preterm (Dunn et al. 2017; Romero et al. 2014). Of particular concern, chorioamnionitis often is associated with premature rupture of the membranes (PROM) and preterm delivery (Abramowicz and Kass 1966; Goldenberg et al. 2000, 2003, 2008; Gonçalves et al. 2002; Kass 1970, 1973, 1982). Also called "amnionitis" and "amniotic fluid infection," the term "clinical chorioamnionitis" distinguishes the clinical syndrome of fever and uterine tenderness from asymptomatic colonization, subclinical amniotic infection, or histologic inflammation of the placenta and fetal membranes in the absence of maternal symptoms. Chorioamnionitis has been reported to occur in a wide range (0.5–10%) of pregnancies. The presence of microbes within the uterus is thought to result from an ascending invasion from the vagina and cervix.

Even before the onset of uterine contractions and/or rupture of the amniotic membranes, bacteria from the lower genital tract can ascend into the amniotic cavity. Whether the presence of intrauterine microbes reflects impaired mucosa immunity in these tissues is not known; risk factors for this complication of pregnancy include prolonged labor and/or rupture of the membranes, multiple vaginal examinations while in labor, and internal fetal heart rate monitoring. As with other pelvic infections, clinical chorioamnionitis is usually polymicrobial in origin, the most common species being *Bacteroides* (25%), *Gardnerella vaginalis* (24%), group B *Streptococcus* (12%), other anaerobic streptococci (13%), *Escherichia coli* (10%), and aerobic gram-negative rods (10%) (Duff 2014; Power et al. 2017). Adverse pregnancy outcome and preterm labor has also been linked to bacteria involved in periodontal disease (Madianos et al. 2013). The association of periodontal infection with clinically relevant consequences for pregnancy raises the possibility of systemic transplacental dissemination of inflammatory processes and echoes a late nineteenth-century theory of "focal infection" of the oral cavity underlying regional diseases (Miller 1891).

Whether by ascending infection through the cervix or systemically, microbial-induced preterm labor reflects an inflammatory process, with microorganisms and their products being sensed by pattern recognition toll-like receptors which induce the expression of chemokines (IL-8, C-C motif ligand 2), cytokines (IL-1β and TNFα), prostaglandins, proteases, and the inflammation S100A12/receptor for advanced glycation end products that lead to activation of the common pathway of parturition (Agrawal and Hirsch 2012; Buhimschi et al. 2007; Elovitz et al. 2003; Taki et al. 2012). In about one-third of intra-amniotic infection cases, bacteria are identified in the fetal circulation (Carroll et al. 1995) leading to a fetal systemic inflammatory response with multi-organ involvement (Gomez et al. 1998). Some have proposed that near term such inflammation may have survival value, contributing to host defense against infection and acceleration of lung maturation (Jobe 2010). Evidence suggests that measuring the circulating pro-inflammatory mRNA in women with preterm, prelabor rupture of the amniotic membranes may distinguish patients with chorioamnionitis from those without it, thereby providing improved targeted therapy and appropriate timing of delivery (Stock et al. 2015). A diagnosis of clinical chorioamnionitis requires a high index of suspicion in cases of fever, uterine tenderness, and/or foul odor of the amniotic fluid. This, combined with positive laboratory stains and cultures, allows appropriate therapy.

Evidence also suggests that chorioamnionitis accompanied by elevated levels of pro-inflammatory cytokines may predispose the newborn infant (particularly those that are premature) to severe intracranial hemorrhage and sepsis (Soraisham et al. 2009), cerebral palsy (Shatrov et al. 2010; Wu and Colford 2000), cerebral damage (Dammann and Leviton 1997), as well as to dysregulation of brainstem respiratory control mechanisms (Balan et al. 2012; Jafri et al. 2013). Importantly, chorioamnionitis and other perinatal infections can contribute to a variety of neurodevelopmental disorders, many with long-term consequences (Labouesse et al. 2015). Although many infants born to mothers with histologically proven chorioamnionitis are asymptomatic and appear unaffected, preterm infants so

affected are more likely to develop bronchopulmonary dysplasia (BPD). Overall, the significance of intrauterine infection on the neonate appears to depend upon the timing, severity, extent of infection, and associated inflammatory responses, the overall impact of which may have life-span consequences. Perhaps of greater significance is that the role of infections in preterm birth may be underappreciated because subclinical intrauterine or placental inflammation may be asymptomatic or of an origin not detected by standard diagnostics (Horvath et al. 2014).

11.3 Obesity in Pregnancy

In obesity, energy intake and accumulation of body fat exceeds expenditures. This complex, polygenic, multifactorial condition is often resistant to treatment and associated with numerous health problems, as well as reduced life expectancy (World Health Organization 2016). In the USA and other developed countries, individuals are considered obese when their body mass index (BMI) (weight in kilograms divided by height in meters^2) exceeds 30 kg m^{-2}. Being overweight is defined by a BMI of 25–30 kg m^{-2}. Morbid obesity is defined as a BMI $\geq$ 40 kg m^{-2}. Several East Asian countries use more strict criteria. During the past several decades, the prevalence of obesity has increased dramatically in the USA and Great Britain (Heslehurst et al. 2007). Worldwide, "globesity" has reached unprecedented proportions and has become associated with health problems that affect diverse societies in both developed and developing countries (http://www.who.int/nutrition/topics/obesity/en/, Hossain et al. 2007; Balen et al. 2007; Hruschka et al. 2011; Jungheim et al. 2012; Kim et al. 2014; Yu et al. 2006). The enormous challenges presented by this epidemic stems for the simple question, what is wrong with being obese? In fact, serious multiple lifetime risks of chronic conditions, such as hypertension, heart disease, dysglycemia, type 2 diabetes, kidney disease, bone or joint disease, and cancer (Adams et al. 2006), are linked to obesity. The propensity of specific health consequences in pregnant woman as well as for her offspring is implicated to have significant impact in later life.

Alarmingly, the National Health and Nutritional Examination Survey disclosed that in the USA more than one-third of all women are obese and that among pregnant women more than one half are overweight or obese (Flegal et al. 2012). Among ethnic groups, the rates of obesity differ being 50, 45, and 33% for Black, Hispanic, and White women, respectively (Flegal et al. 2012). Pregnancy weight gain guidelines of the Institute of Medicine, and adopted by the American College of Obstetricians and Gynecologists, based on prepregnancy BMI, are independent of ethnicity, age, or parity (ACOG 2013; Rasmussen and Yaktine 2009). The pregnancy complications of which overweight and obese women are at increased risk include gestational diabetes, hypertension, preeclampsia, cesarean section, and weight retention during the puerperium (Baeten et al. 2001; Cedergren 2004; Ng et al. 2014; Sebire et al. 2001; Vesco et al. 2009; Weiss et al. 2004). For the fetus and newborn infant of the overweight pregnant woman, increased risks include

Table 11.4 Maternal and fetal cellular and molecular consequences of obesity during pregnancy

Maternal	Fetal
Endothelial dysfunction with impaired vasoconstriction and vasodilation	Systemic inflammation
Placental pathology and impaired function increased	Nutrient transport altered
Oxidative stress increased	Hyperlipidemia
Circulating leptin increased	Hypertension
Hyperlipidemia	Cardiovascular development altered
Hypertension	

Adapted from Roberts et al. (2015)

prematurity, stillbirth, congenital malformations, macrosomia, and possible injury at birth, with programming of obesity during childhood and adulthood (Dyer and Rosenfeld 2011; Josefson et al. 2013; Kim et al. 2014; Nohr et al. 2005; Oken et al. 2007; Stothard et al. 2009). The fetuses of overweight and obese mothers also are at risk in the presence of intrapartum, operative, and postoperative complications related to anesthesia. Table 11.3 gives some of the antepartum, intrapartum, and postpartum complications of pregnancy associated with obesity. Table 11.4 presents some of the cellular and molecular consequences. In comparison to patients with a BMI of less than 30, those with a BMI of 30–39.9 demonstrated increased risks of gestational diabetes (odds ratio 2.6–4.0), gestational hypertension (odds ratio 2.5–3.2), and preeclampsia (odds ratio 1.6–3.3) (Weiss et al. 2004). In the same study, the rate of cesarean section for women with BMIs of 29.9 or less, 30–34.9, and 35–39.9 were 20.7, 33.8, and 47.4%, respectively. Obesity also lowers the antenatal detection rate for fetal anomalies such as neural tube defects (Dashe et al. 2009; Shaw et al. 1996; Waller et al. 1994).

In addition, the offspring of obese women, or other large for gestational age infants, are at increased risk for programming of childhood, adolescent, and adult obesity (Barker et al. 2002; Elhddad et al. 2014; Hediger et al. 1999; Lawlor et al. 2011; Parlee and MacDougald 2014; Salsberry and Reagan 2007; Sebire et al. 2001), metabolic syndrome (Brenseke et al. 2013), type 2 diabetes (Fraser et al. 2010; Li et al. 2011a), the development of cardiovascular and cerebrovascular disease (Barker 1995; Blackmore et al. 2014; Drake and Reynolds 2010; Reynolds et al. 2013; Roberts et al. 2015), and a number of other diseases (Ojha et al. 2013). The vital role of the fetal environment in early life programming has been demonstrated in a number of studies (Tarantal and Berglund 2014; Tarry-Adkins and Ozanne 2011; Taylor et al. 2014; Turdi et al. 2013; Vickers 2007; Vido et al. 2014; Waters et al. 2012; Watkins et al. 2003 and see below). Studies in nonhuman primates (Ganu et al. 2012) and rodents (Gheorghe et al. 2009; Goyal et al. 2009, 2010, 2011) have demonstrated aspects of the mechanisms of such programming. David Barker and Kent Thornburg (Barker and Thornburg 2013) have reviewed many aspects of the fetal programming of disease in the adult. The issue of effects of maternal obesity on offspring has been referred to as the "Trojan horse" of developmental plasticity (Parlee and MacDougald 2014). Of particular relevance to

understanding the overall effects of maternal obesity on development of offspring is the increasing number of studies that link obesity in the maternal founder (F_0) generation to problems not only the filial generation (F_1) offspring but in the F_2 (perhaps by effects on the ovarian gametes of their parents) and even subsequent generations (see below and Long et al. 2013; Zambrano and Nathanielsz 2013).

A significant contributor to the morbidity and mortality of cardiovascular disease and related entities is elevated cholesterol, including both low- and high-density lipids and triglycerides. These vary during the course of normal gestation from a nadir during the first trimester, followed by a gradual increase, and peaking near term (Wiznitzer et al. 2009). Elevated triglyceride levels, but not those of LDLs, HDLs, or total cholesterol, are associated with increased risk of gestational diabetes and preeclampsia (Wiznitzer et al. 2009). Elevated triglycerides at the beginning of the second trimester were associated with an increased prevalence of pregnancy-induced hypertension, preeclampsia, and macrosomia in the newborn (Vrijkotte et al. 2012). These authors demonstrated that triglyceride levels >140 mg/dl at 3 months' and >200 mg/dl at 6 months' gestation are excessively high and may reflect a high-risk pregnancy. Aore recent meta-analysis further supports the contention that dyslipidemia with increased triglycerides, in particular, may elevate the risk for spontaneous preterm birth (Moayeri et al. 2017).

Of note, the fetal hypothalamic-pituitary-adrenal (HPA) axis is particularly vulnerable to programming with significant alterations in hypothalamic-pituitary function (Bouret 2010, 2012; Xiong and Zhang 2013). The hypothalamic arcuate nucleus, located in the posterior hypothalamic region beneath and around the third ventricle, serves as a negative feedback system that under normal conditions regulates energy intake and output to maintain homeostasis of body weight and composition of tissues. Two orexigenic proteins, neuropeptide Y and agouti gene-related protein, and two anorexigenic peptides, proopiomelanocortin and cocaine- and amphetamine-regulated transcripts, play key roles in this regulation (Hillebrand et al. 2002). Both insulin and leptin feedback on the hypothalamic arcuate nucleus to regulate feeding behavior, and the profiles of their secretion and circulating concentrations are altered significantly in the offspring of obese rat dams (Kirk et al. 2009; Morris and Chen 2009). These F1 offspring have a high prevalence of hypertension, which probably is due to overactivity of the sympathetic nervous system (Samuelsson et al. 2010). A sobering issue is that a maternal high-fat diet alters fetal brainstem circuits prior to the development of obesity (Zsombok 2015).

As reviewed elsewhere, during late gestation glucocorticoids play a critical role in the maturation of a number of fetal organ systems (Busada and Cidlowski 2017; Fowden et al. 1998). Disorders of the HPA axis are quite common in obesity (McMullen et al. 2012); however, interpretation of the various studies along this line is not straightforward (Zambrano and Nathanielsz 2013). Obesity-associated alterations in glucocorticoid receptors and associated regulatory enzymes, such as 11β-hydroxysteroid dehydrogenase 1 and 2, are associated with profound effects in fetal metabolism (Correia-Branco et al. 2015). In addition to being a standard treatment to accelerate the fetal lung for nearly 5 decades (Liggins and Howie

1972), thyroid hormones are vital to normal fetal growth and development (Springer et al. 2017). In nonhuman primates, a maternal high-fat diet decreased circulating levels of T4 (Suter et al. 2012). As cortisol is critical to normal thyroid development (Thomas et al. 1978), in association with obesity, abnormalities in fetal cortisol secretion and circulation would be expected to compromise fetal thyroid function.

Cardiovascular health in adults appears to be critically dependent upon regulation of fetal cardiomyocyte growth in utero. The number of myocytes in the neonate at birth determines the number for life (Thornburg et al. 2011). As a target of fetal programming, the hearts of offspring of obese mothers show hypertrophy (particularly left ventricle), impaired cardiac function, and modified metabolism including lipid oxidation and lipid storage (Cedergren and Källén 2003). At birth, infants born to obese mothers demonstrate a greater atherogenic lipid profile (Merzouk et al. 2000), a precursor to cardiovascular disease. In mice, cardiac geometry and gene expression were altered significantly, perhaps as a consequence of activation of protein kinase B and the extracellular regulated kinase (ERK) and mammalian target of rapamycin pathway (AKT-ERK-mTOR) (Fernandez-Twinn et al. 2012; Geelhoed et al. 2008). In fetuses of obese sheep at 90% gestation, in addition to elevated circulating levels of glucose and insulin, cardiac function was impaired with myocardial fibrosis (Huang et al. 2010), although this became apparent only at high workloads (Wang et al. 2010). Also, phosphorylation of AMP-activated protein kinase, a cardioprotective signaling pathway, was reduced, and the stress-induced p38-MAPK pathway was increased. In addition, phosphorylation of c-Jun N-terminal kinase and insulin receptor substrate-1 were markedly elevated in these hearts (Wang et al. 2010). It has been suggested that elevations in plasma levels of tumor necrosis factor-α (TNFα) (Oral et al. 1997) and leptin may impede angiogenesis (Bohlen et al. 2007). Undoubtedly, changes in these factors underlie the negative ionotropic effects on the heart of offspring. In obesity in general, lipotoxicity is associated with excessive fat storage in the myocardium with myocyte apoptosis and fibrosis, resulting in contractile dysfunction, impaired diastolic filling, and impaired left ventricular chamber expansion (McGavock et al. 2006). In addition, in humans the thickness of the epicardial adipose tissue is not only a marker of cardiometabolic risk, and correlates with the quantity of visceral adipose tissue, but is associated strongly with the risk of cognitive impairment as determined by a battery of psychometric tests (Mazzoccoli et al. 2014). Again, these maternal obesity-induced changes could predispose the offspring to insulin resistance and cardiac dysfunction later in life. Of relevance, the adipocyte now is recognized as an important endocrine organ as the source of several adipokines, including leptin, adiponectin, interleukin-6 (IL-6), tumor necrosis factor-α (TNFα), and free fatty acids (Shuldiner et al. 2001).

In terms of the maternal vasculature, in women at 20 weeks' gestation, the common carotid artery showed a significant correlation of intima-media thickness (a marker of preclinical atherosclerosis) to BMI (Galjaard et al. 2014). Myometrial arteries ($\leq$500 μm diameter) obtained from obese, but otherwise uncomplicated overweight women at the time of cesarean section showed impaired vasoconstrictor

and vasodilator responses. These included decreased contraction in response to the thromboxane mimetic U46619, but not to arginine vasopressin (AVP; Hayward et al. 2014). In this study, although the vasorelaxation in response to bradykinin was not significantly affected in arteries precontracted with the U46619 thromboxane mimetic, it was slightly less in those vessels precontracted with arginine vasopressin. In a similar manner in AVP contracted vessels, the nitric oxide (NO) donor sodium nitroprusside showed impaired relaxation, while those precontracted with U46619 did not (Hayward et al. 2014). These disparate responses raise the question of the extent to which perivascular fat or relevant signal transduction pathways are dysregulated obese pregnant women (Ramsay et al. 2002). As it is well documented, obesity also is associated with increased circulating concentrations of vasoactive, pro-inflammatory, and prothrombotic factors including C-reactive protein, interleukin-1β, and tumor necrosis factor-α (Madan et al. 2009; Ramsay et al. 2002; Ziccardi et al. 2002). These and other factors enhance local peroxidase production resulting in platelet aggregation and vasoconstriction (Table 11.4).

Women with preeclampsia have a similarly altered endocrine profile (Anim-Nyame et al. 2000; Haugen et al. 2006). In obese pregnant women, aberrant vasoactive adipokines may stimulate systemic-, utero-, and/or placental-vascular dysfunction. For reference, in terms of obesity and the vasculature, male obese Wistar rats at 24 weeks of age showed significantly elevated arterial blood pressure as well as levels of both norepinephrine and epinephrine, with strikingly increased renal artery NE synthesis and content (Olea et al. 2014). In high-fat-fed mice, endothelial-specific production of epoxyeicosatrienoic acids (cytochrome P450-derived metabolites of arachidonic acid that contribute to vascular protection by stimulating vasodilatation and inhibiting inflammation), heme oxygenase-1 (a response protein that plays a cytoprotective role against oxidative stress), and a number of other endothelial-specific protective enzymes and metabolites were decreased significantly in association with adipocyte dysfunction (Abraham et al. 2014).

In a similar manner, lipotoxicity is associated with structural and functional changes in the livers of offspring of obese mothers in several species. Hepatic mitochondrial abnormalities in association with obesity include reduced activity of electron transport chain enzyme complex I, II/III, and IV as well as decreased DNA copy number (Alfaradhi et al. 2014; Bruce et al. 2009; Burgueño et al. 2013). In the progression from nonalcoholic fatty liver disease to steatohepatitis, inflammatory pathways which affect, and are affected by, inflammatory pathways have been shown to be altered. Offspring of overnourished mice dams also had livers with greater numbers of Kupffer cells (stellate phagocytic cells in sinusoids) with impaired phagocytic function, reduced numbers of natural killer T cells, enhanced interleukin-12 and interleukin-8 production, and increased reactive oxygen species (Mouralidarane et al. 2013). Such characteristics of excessive hepatic fat storage are often associated with insulin resistance (Savage and Semple 2010), though the contribution of each factor to programming of disease in offspring has yet to be determined. What is known is that maternal dietary overnutrition is associated with

the accumulation of triglycerides and cholesterol in hepatic steatosis (Ashino et al. 2012; Bruce et al. 2009; Drake et al. 2011; Elahi et al. 2009). Nonalcoholic fatty liver disease in the third trimester of pregnancy in fetuses of nonhuman primates from overnourished mothers is associated with inflammation, oxidative stress, triglyceride accumulation, and gluconeogenic gene activation (McCurdy et al. 2009). In conjunction with peroxisome proliferator-activated receptor γ, coactivator 1α deacetylation, and increased hepatocyte nuclear factor 4α expression, these molecules suggest a mechanism by which excess lipids may reprogram hepatic lipid and glucose metabolism. This possibility is further supported by elevated hepatic triglyceride levels persisted until adolescence with a doubling of percent body fat (McCurdy et al. 2009).

In rats, another study in offspring of overfed mothers found enlarged hepatocytes with increased percent liver weight and lipid accumulation (Shankar et al. 2010). In addition, the sterol regulatory element-binding protein 1 (SREBP1) was increased and identified as an important regulator of altered genes, accompanied by a decreased in PPARα-AMPK signaling (Shankar et al. 2010). The F_1 generation of the obese mouse, in addition to excessive hepatic lipid storage with hepatomegaly, demonstrated marked changes in hepatic function and gene regulation (whereas the F_2 generation showed these changes in skeletal muscle; Attig et al. 2013). For reference and to set the stage for obesity in pregnancy, male obese Wistar rats (fed a high-fat diet commencing at 12 weeks of age from overweight dams) at 24 weeks showed multiple changes in hepatic metabolism. In addition to marked increases in body and liver weight, perirenal, and epididymal fat and plasma leptin, cholesterol, and C-reactive protein, these included increased liver peroxidases and nuclear factor κB and cytoplasmic and mitochondrial superoxide dismutase (Olea et al. 2014). Again, these findings strongly support the idea that exposure to the altered milieu of the overweight mother programs systemic changes in insulin and adiponectin levels which, in turn, alter genes involved in carbohydrate lipid metabolism with fatty acid catabolism (Pruis et al. 2014).

In skeletal muscle from both obese and type 2 diabetic patients, cytokine signaling is suppressed (Rieusset et al. 2004). Lipid peroxidation in skeletal muscle also is decreased (Russell et al. 2003). In sheep, maternal obesity was associated with decreased fetal skeletal myogenesis and development (Zhu et al. 2008), including beta catenin signaling proteins (Du et al. 2010; Tong et al. 2009). Fetuses of obese mothers also showed considerable infiltration of skeletal muscle with adipocytes and a marked inflammatory response and other changes of note (Yan et al. 2010). Mitochondria in skeletal muscle of F_1 offspring of obese dams also show significant alterations in number and respiratory enzyme activity (Chanseaume and Morio 2009; Galgani et al. 2008; Park et al. 2003; Selak et al. 2003; Shelley et al. 2009; van den Broek et al. 2010). Similar dysfunction has been demonstrated in the mitochondria of hepatocytes (Peterside et al. 2003; Shelley et al. 2009). As noted, it was the skeletal muscle of F_2 (but not F_1) generation overfed mice that demonstrated excessive lipid storage and significant changes in epigenetic-mediated gene regulation (Attig et al. 2013). Of potential great significance in terms of programming, these epigenetic-mediated modifications

during fetal development may condition mitochondrial function in later life (Theys et al. 2009).

Adipocytes, the cells of fatty tissue, constitute an extremely active endocrine organ with a network of signaling pathways that enable the organism to accommodate to a wide variety of metabolic stresses. These include starvation, general stress, inflammation and infection, and periods of energy excess. Adipocytes are of three major types: white for energy storage in the form of fat, brown and beige which display energy dissipating capacity by the promotion of energy dissipation via triglycerol clearance, glucose disposal, and the generation of heat for thermogenesis, the latter of which is of particular importance for the newborn infant. The functional pleiotropism of adipose cells depends on its ability to synthesize and release a wide variety of hormones, cytokines, chemokines, growth factors, extracellular matrix proteins, and vasoactive factors, collectively termed adipokines (Rodríguez et al. 2015). Obesity is associated with adipose tissue excess and dysfunction that lead to type 2 diabetes, dyslipidemia, nonalcoholic fatty infiltration of the liver, and/or hypertensive vascular disease, among others. These pathologies are associated with adipocyte hypertrophy and/or hyperplasia, inflammation, impaired remodeling of extracellular matrix, fibrosis, and altered adipokine secretion (Rodríguez et al. 2015).

Fatty acids are important precursors in bioactive molecules that provide a major source of energy and are structural components of cells. Fetal and newborn macrosomia appears to be a consequence of increased percent body fat and total fat mass, rather than lean body mass (Sewell et al. 2006). In the rat, male offspring from obese mothers fed a normal amount of chow postweaning demonstrated a significant increase in the size of fat cells and total mass, and elevated circulating levels of triglycerides, insulin, and leptin, that lead to a predisposition to obesity, hypertension, metabolic syndrome, and associated disorders (Zambrano et al. 2010). Maternal obesity is also associated with the deposition of extra adipocytes and fibrosis in the hearts of the F_1 generation (Dong et al. 2013; Huang et al. 2010).

A more recent perspective is to consider obesity as an inflammatory condition and to affect developmental programming of adult disease (Segovia et al. 2014; Westermeier et al. 2014; Xu et al. 2003; Zhu et al. 2010). For instance, increased expression of lipogenic and inflammatory genes in maternal adipose tissue and the placenta of obese women have been demonstrated before any phenotypic changes become apparent. Among the many genes implicated are the macrophage markers CD-68 and CD-14, mannose-6 phosphate (an indicator of the immune network), and the TLR4/NF-κB signaling pathway. In addition to other target genes, a strong genome-wide associated signal lies in the 1 and 2 regions of the gene FTO, a region that contains 89 common variants that are in high linkage disequilibrium in Europeans ($r^2 \geq 0.8$) across approximately 47,000 nucleotides, which makes identification of the likely caused variant challenging (Dina et al. 2007; Frayling et al. 2007). The *FTO* allele associated with obesity represses mitochondrial thermogenesis in adipocity and their precursor cells in a tissue-autonomous manner. Identification of the *FTO* locus, its upstream regulators, and its downstream target genes has provided a mechanistic basis for the association between *FTO* and

obesity (Claussnitzer et al. 2015). These responses were biphasic, being maximal in early and late pregnancy (Resi et al. 2012). As noted, adipocyte dysfunction in high-fat-fed mice was associated with marked alteration in vascular endothelial function (Abraham et al. 2014). Taken together, these changes with lipotoxicity could result in pathologic increases in cytokines, chemokines, and associated metabolites to compromise function of the heart, vasculature, and other organs.

Mitochondrial function also has been shown to be impaired in the human placenta in association with maternal obesity, a finding with considerable implications for programming of the developing fetus. Cultured primary trophoblast from obese subjects who underwent cesarean delivery showed significant reduction in mitochondrial respiration including O_2 consumption ATP levels and citrate synthase activity and in the ratio of mitochondrial to nuclear DNA. In addition, the expression of several respiratory chain complexes was reduced dramatically, and reactive O_2 species were increased (Mele et al. 2014). In addition to other findings, mice fed a high-fat diet prior to and during pregnancy showed evidence of hypoxia and inflammation in the placenta (Li et al. 2013). In these challenges to developmental processes, the roles of reactive oxygen and reactive nitrogen species in glucose homeostasis and mitochondrial function cannot be overlooked (Taylor et al. 2005). Highly reactive free radicals that contain an unpaired electron react with other free radicals to cause oxidative or nitrative damage to nucleic acids, proteins, and lipids (Iossa et al. 2003; Soucy et al. 2006; Valko et al. 2007). Markers of oxidative/nitrative stress include superoxide dismutase, catalase, glutathione peroxidase, and nitrotyrosine. Each of these markers is increased significantly in the tissues of obese female rats maintained on a high-fat diet for 99 days following weaning (Zambrano and Nathanielsz 2013). Mitochondria are exquisitely sensitive to developmental programming, and dysfunction has been noted in the embryos of obese mouse mothers (Shelley et al. 2009). The mitochondria of pancreatic β-cells also are particularly vulnerable to ROS/RNS, predisposing these animals to hyperglycemia and diabetes (Simmons 2007; Simmons et al. 2005). In mitochondria, microRNAs are intimately involved in fatty acid oxidation; and this altered expression with dyslipidemia can produce dysfunction in oxidative metabolism and is associated with endoplasmic reticulum oxidative stress (Christian and Su 2014). Profound alterations in placental trophoblast oxidative metabolism as well as steroid metabolism (Correia-Branco et al. 2015) may thus underlie the susceptibility in pregnancies of obese mothers to fetal demise in late gestation and to developmental programming that promotes disease in adulthood.

Focus on the placenta as a critical regulator for effects of maternal obesity on the fetus seems warranted because two sources of fatty acids cross the placenta from the maternal circulation to provide major sources of energy to the fetus. Uptake and transport of both esterified fatty acids that are present as triglycerides and the nonesterified fatty acids follow binding to several cellular membrane fatty acid transport proteins in placental trophoblasts (Lager and Powell 2012; Kazantzis and Stahl 2012). Fatty acid transport protein 1 is present in trophoblast cells; however, the cellular localization of the others remains unknown (Campbell et al. 1998). The human placenta also contains at least four isoforms of fatty acid-binding proteins

(Biron-Shental et al. 2007). The placental trophoblastic cells of obese pregnant women have been shown to have increased levels of cellular inhibitors of apoptosis 1 and 2, which, independent of their role in apoptosis, in both pregnancy and obesity play a key role in inflammation and in tumor necrosis factor-α-induced expression of pro-inflammatory cytokines (Lappas 2014). Along this same line, female Sprague-Dawley rats fed a high-fat (HF) diet commencing 16 weeks prior to mating (to normal control fed males), until either E15 DPC (Hayes et al. 2014), at the completion of early (endovascular) trophoblast invasion, or E18, when both interstitial trophoblast invasion and spiral artery remodeling are completed (Caluwaerts et al. 2005; Geusens et al. 2008; Vercruysse et al. 2006). In the placentas and decidua of high-fat fed dams at E15, early trophoblast invasion increased about twofold, with a concomitant increase in tissue remodeling and increase in metalloproteinase 9 expression (Hayes et al. 2014). Furthermore, increased levels of smooth muscle actin surrounding the decidual spiral arteries were observed, suggesting impaired spiral artery remodeling (Hayes et al. 2014). Moreover, maternal obesity, combined with gestational diabetes, demonstrated an increase in placental leptin DNA methylation (suggesting decreased transcription) which may contribute to altered metabolic programming (Lesseur et al. 2014). Collectively, these results suggest that altered placental development combined with impaired maternal-to-fetal nutrient exchange in maternal obesity is a major contributor to the poor pregnancy outcome with increased fetal demise.

At the University of Southampton, the institution at which the late David James Purslove Barker (1938–2013) (Fig. 11.3) worked during the last decades of his life, the prospective-longitudinal Southampton Women's Survey was initiated in 1998 to explore the interrelations of maternal diet, BMI, and environmental factors on the offspring. Although the study is ongoing, preliminary results demonstrate the malignant effects of maternal high-fat diet pervade the genetic programming of the offspring (Barker et al. 2002; Crozier et al. 2006, 2010). Although gene variants associated with obesity cause only minor differences in body weight (Rampersaud et al. 2008), examples of fetal effects of such antenatal exposure include increased mRNA expression of gluconeogenic genes, elevated plasma glucose levels, and histone modifications on the *Pck1* gene (Strakovsky et al. 2011) and altered methylation of several genes involved in endocrine, metabolic, and vascular function (Lillycrop and Burdge 2011). The initial consensus from this survey supports the conclusion that epigenetic factors mediated altered the gene expression related to a collective metabolic syndrome initiating a cycle of susceptibility to obesity and metabolic disease in later life (Dyer and Rosenfeld 2011; Lillycrop 2011).

A vital issue that has become evident is the importance of prepregnancy weight in terms of glycemic control of the mother and its role in the development of macrosomia and other sequelae in the offspring (Cnattingius et al. 1998). For the populace in general, several randomized trials of lifestyle modification, medications, and bariatric surgery have shown that, at least for a short period of time, weight loss reduces morbidity (Jensen et al. 2014), modest loss of body weight of only 5–10% making medical issues more manageable (Douketis et al. 2005; Knowler et al. 2009). Strikingly, several interventional studies have failed to reduce

the prevalence of macrosomia. For instance, in an 8-year prospective study of perinatal risk factors associated with childhood obesity, the maternal BMI, independent of gestational weight gain or maternal glucose status, was the strongest predictor of childhood obesity and metabolic dysfunction (Catalano et al. 2009). In a Danish randomized, controlled "Lifestyle in Pregnancy" intervention trial, one group of 150 obese women with a BMI of 30–45 kg m^{-2} at 10–14 weeks' gestation was initiated into a program of dietary guidance, membership in a fitness center, and personal coaching (Vinter et al. 2011). Although this group experienced significantly less gestational weight gain (7.0 $\pm$ 2.2 kg) as compared to controls (8.6 $\pm$ 2.5 kg) (in part because this group included fewer smokers (11 versus 18)), paradoxically infants of the intervention group had a significantly higher birthweight as compared to controls (3.74 $\pm$ 3.10 versus 3.59 $\pm$ 3.00 kg) (Vinter et al. 2011). In addition, a prospective trial of a low glycemic index diet in pregnancy of women in Ireland who previously had delivered an infant weighing greater than 4000 g (coined the ROLO study) found that despite a decrease in the gestational weight gain in the intervention group, the offspring showed no reduction in incidence of macrosomic infants with no differences in birthweight, birthweight percentile, or ponderal index as compared to controls (Walsh et al. 2012). The Hyperglycemia and Adverse Pregnancy Outcome study demonstrated independent associations between exceeding the Institute of Medicine gestational weight gain recommendations and neonatal obesity in overweight women, when controlling for glucose tolerance results (Badon et al. 2014). According to an updated meta-analysis of 24 random controlled trials with over 7000 participants that focused on the validity of moderate exercise (walking, aerobic, dance), dietary restriction, or both to prevent excessive gestational weight gain, the women's excessive weight gain was reduced by about 20% (Muktabhant et al. 2015). The risks of maternal hypertension and prevalence of cesarean section also were reduced. Other interventions have been proposed (Nathanielsz et al. 2013).

In summary, human obesity is a complex phenomenon in which biological, cultural, economic, and behavioral factors interact (Galtier-Dereure et al. 2000; Garrison 2013). It is clear that obesity in pregnancy increases the risk of multiple metabolic and endocrinologic abnormalities such as gestational diabetes and related disorders, as well as that of the newborn infant being macrosomic and subject to many complications including that of congenital malformations and neurologic disorders (Bilbo and Tsang 2010). Recommendations for pregnant women who are overweight or obese include preconception assessment and counseling, strict antenatal care with nutritional counseling, careful anesthesia counseling for women undergoing cesarean section, and other considerations for fetal well-being (ACOG 2013). A challenge, however, is that there is no global consensus (Scott et al. 2014) with little concerted action among healthcare workers, politicians, or the media to address this problem. In addition, research projects on either understanding the scope of the problem on the mother herself or her offspring or on effective intervention research are either lacking or poorly coordinated and organized (Arabin and Stupin 2014).

11.4 Diabetes in Pregnancy

A disorder of pregnancy, maternal diabetes, has profound implications for the developing fetus. The etiology of this condition includes both preconceptual type 1 (juvenile, insulin dependent) and type 2 forms (adult onset, non-insulin dependent) (Eidem et al. 2010; Fetita et al. 2006), as well as pregnancy-associated gestational diabetes (Reece et al. 2009). To a great extent type 2 diabetes is associated with maternal excess weight and/or obesity and constitutes a major health problem because of increasing rates of obesity both driving up the number of patients affected and reducing the age of diagnosis (Biggio et al. 2010; Wallach and Rey 2009; Waller et al. 2007); hence, the term "diabesity" has been applied to this association (Desoye and van Poppel 2015; Dunstan et al. 2001). In 2011–2012, the prevalence of diabetes in the USA was 12–14%, with higher rates (about 20%) among Black, Hispanic, and Asian populations (Menke et al. 2015).

Infants of diabetic mothers (IDM) are macrosomic, thereby increasing the risk of shoulder dystocia and injury at the time of birth. Despite falling rates of perinatal mortality in the past century, fetal and neonatal mortality in mothers with type 1 or type 2 diabetes remain three- to fourfold higher than for those with normal glycemic controls (Moore et al. 2014). Congenital fetal anomalies, many of which are debilitating and/or life-threatening, also remain several times more common in infants of diabetic pregnancy than those that are nondiabetic. For instance, such fetuses also demonstrate delayed development of the lung thereby increasing the incidence of respiratory distress syndrome and congenital heart disease. In addition to a doubling of the risk of serious birth injury, the likelihood of cesarean section is tripled, and the incidence of newborn intensive care unit admission is quadrupled. Following delivery, these infants often experience significant hypoglycemia, polycythemia, and serum electrolyte disturbances which require careful surveillance. The IDM also may have delayed neurologic maturation with decreased muscular tone, which may lead to a delay in feeding competence. As with antenatal obesity and other stress, offspring of diabetic mothers have a much higher prevalence of high BMI in childhood (Philipps et al. 2011). Thus, an important component of optimal management of such newborn infants is rigid glycemic control of the mother, with maintenance of low hemoglobin A1C levels (Miller et al. 1981).

Prior to the first quarter of the twentieth century, with the discovery of insulin (Banting and Best 1921/1922) and its introduction into clinical practice, pregnancy for a woman with diabetes mellitus portended death of the mother and/or fetus from ketoacidosis or other complications (Gabbe 1993). Shortly after this discovery, Priscilla White (1900–1989), Fig. 11.2, of the Joslin Clinic (named for Elliott Proctor Joslin, 1869–1962), New England Deaconess Hospital, Boston, MA, introduced approaches to ameliorate some of the ravages of this condition (Dunn 2004; Joslin et al. 1952; White 1936). With her clinical classification of diabetes based on the age at onset of the disease, its duration, and the presence of atherosclerotic vascular disease and renal complications, White first brought some semblance of order into the reports of results. Her classes A through E refer to fetal risk and class

Fig. 11.2 Priscilla White

F to maternal risk (see below). In addition, this classification allowed a partial prediction of the course of an individual diabetic patient during pregnancy and the changes for survival of the newborn infant. White concluded:

> Thus diabetes through its disturbed metabolism, hormonal imbalance, the transmission of congenital defects and vascular disease does have a profound effect upon the course of pregnancy and the structure and behavior of the child. The disturbed metabolism and the hormonal imbalance are the correctible parts of our problem; and although the expected fetal survival in diabetes today is 90 per cent, only when the entire genetic and vascular problem of diabetes is solved will our experience be equal to the best in non-diabetic, obstetric and pediatric experience.
>
> (White 1949, p. 616)

In the 1935 fifth edition of Joslin's *The Treatment of Diabetes Mellitus*, a chapter by White first reviewed the 81 pregnant diabetic patients cared for by her, which included 23 pregnancies in 15 patients with the onset of diabetes in childhood (White 1935). In this early report, stillbirths accounted for 12% of infants born, and the 5% maternal mortality rate was associated with severe preeclampsia/eclampsia (White 1935). A decade and a half later, in the 1952 ninth edition of Joslin's *magnum opus*, White reviewed the outcome of 1269 cases reported in the world literature since 1940 (White 1952). In this series fetal survival averaged 62%, varying from 30 to 92%. These figures agreed with a previous review 5 years earlier (Henley 1947). Among White's 525 patients managed between January 1936 and June 1951, in 60% of women the onset was under 20 years of age. About one-third were in class C and, most importantly, fetal survival was 90%. Although 100% of the infants of class A patients were live-born, the survival rates of patients with clinically overt disease decreased with the rise in classification (see below) (White 1952). A decade later, of 1415 cases managed by White, maternal and fetal survival were 99.8 and 87%, respectively (White 1965). In this report, she also added class R for those patients with proliferating retinopathy (White 1965). Later, the classification was modified further and her figures for maternal and fetal survival were 99.5 and 90%, respectively, while the incidence of preeclampsia, previously at 50%, decreased to 3% (White 1974). In her Banting Memorial Lecture of 1960, White

also contributed to knowledge of the newborn infant of the diabetic mother and of juvenile diabetes by presenting the classic description of the natural course of insulin dependency and neuropathic and vascular complications (White 1960). With her leadership in the early diagnosis and rigorous management of prenatal care of women with this disease, to a great degree, White must be credited with the decrease in the perinatal mortality rate from about 50% at mid-twentieth century to the present 3 or 4%.

As an aside, in the early 1930s, a husband-wife team of Boston endocrinologists noted in diabetic patients elevated levels of serum gonadotrophins associated with low urinary excretion of estrogen and pregnanediol glucuronides. Thus was born their hormonal theory of the cause of late pregnancy preeclampsia, unexplained prematurity, and intrauterine fetal death (Smith and Smith 1933). On the basis of this report of the hormonal theory, Priscilla White used it in the management of her pregnancy diabetics. She reported that in 174 patients given prophylactic hormone therapy, only 5% of the mothers developed preeclampsia and 90% of the infants survived (White 1945). Unfortunately, this work was not replicated elsewhere, and one report of the Medical Research Council details a clinical trial of 76 hormone-treated and 71 control patients in the UK that gave negative results. The study demonstrated no difference in maternal blood pressure, albuminuria, edema, hydramnios, or toxemia, and there were similar rates of stillbirth and neonatal death (24 and 26%, respectively) (Anonymous 1955).

In response to my query for the "Classic Pages" section of the *American Journal of Obstetrics and Gynecology*, asking whether she would reflect upon how her classification of diabetes in obstetrical patients was developed, Priscilla White replied:

> In 1924, soon after I joined the Joslin group consisting at that time of Dr. Elliott P. Joslin and Dr. Howard Frank Root (1890–1967), the study of the diabetes in youth was assigned to me. With insulin treatment (available in 1922), the diabetic girls had begun to grow at normal rates, to mature and soon presented us with the problems of their pregnancies characterized by a high risk of fetal loss. Little was known about pregnancy and diabetes prior to the availability of insulin. Bouchardat, the most famous French clinician of the nineteenth century, had never treated, had never even seen a pregnant diabetic woman (Bouchardat 1875). In his extensive experience of 19 years, from 1898 to 1917, Dr. Elliott P. Joslin treated 1300 patients with diabetes (Joslin 1915; Krall 1971). In this group, the distribution by sex was equal. Only ten pregnancies were observed in a woman with overt diabetes. In this small series there were four live born surviving infants, two intrauterine fetal deaths, and three maternal deaths (Joslin 1915). Two of these women died undelivered and one [committed] suicide after both of her pregnancies were terminated. This suicide answers the question asked so frequently today. Why do you permit, even encourage young women with diabetes to bear children? To many, to nearly all of these women, life lacks meaning, may even be unendurable without successful child bearing.
>
> Because of the size of the Joslin Clinic [following the introduction of insulin], and the greater than average number of juvenile onset diabetic surviving many years of diabetes (White 1960, 1972), the abnormal course and outcome of pregnancy in diabetes became apparent (White and Hunt 1943). Although maternal survival was ... [almost always] assured, only half of these patients delivered live born surviving infants. The classical accident was the intrauterine fetal death, the occurrence of which peaked in the thirty-sixth week. This fetal loss was associated with symptoms of toxemia (gain in weight, rise of

blood pressure, proteinuria) which subsided soon after the fetal death. When the timing of the delivery was changed to anticipate this accident, a neonatal death replaced the intrauterine one. The survival rate of infants in 128 viable pregnancies treated between 1924 and 1938 was only 54% (White 1952, 1974):

By the nineteen fifties, over 1000 viable pregnancies had been followed in our clinic, and it was my privilege to receive invitations to be a guest speaker in many obstetrical meetings and conferences. As was proper, famous professors, heads of obstetrical departments, preceded me on these programs dealing with obstetrical diabetes. The subject of their discussions consisted of the details concerning the diagnosis of diabetes appearing during pregnancy. Their conclusions were that diabetes complicating pregnancy did not constitute a risk for fetal survival. My presentations which followed dealt with the prediction and prevention of the accidents of pregnancy and diabetes. My conclusion was that pregnancy complicating diabetes was at high risk for fetal loss.

How could these contradictions occur? Each presentation represented factual observations. The only possible answer involved differences in experiences. At that time, obstetricians were, for the most part, dealing with gestational, with chemical diabetes, and with maturity onset diabetes in multigravida. The majority of these patients did not have the vascular sequelae or concomitants of diabetes. The experience of the Joslin Clinic was almost exclusively with overt diabetes recognized long before the pregnancy and often in those whose diabetes had developed in childhood. Whereas maturity onset diabetics, for the most part, continue to produce some insulin endogenously, the childhood onset patient loses this capacity within 5 years of the diagnosis in 95% of the cases. Exogenous insulin is life saving, but it fails to mimic the normal response of the body's beta cells to the body's need at the moment. Many of the childhood onset patients in the course of their long duration disease at the time of their pregnancies have developed vascular lesions including both microangiopathies such as retinopathy and nephropathy and macroangiopathies—calcification of the large blood vessels of the feet and legs, pelvis and even the heart.

In 1949 I presented a classification of diabetes in pregnant women (White 1949) and in the 1950s presented the following results (Nelson et al. 1953; White et al. 1953) in 278 pregnant diabetic women based upon fetal survival for the whole period of pregnancy. [In the several classes these were as follows]:

	Fetal Survival %
Class A: Chemical Diabetes	100
Class B: Maturity onset (age over 20 years), duration under 10 years, no vascular lesions	67
Class C: Age at onset 10–19 years or duration 10–19 years, no vascular lesions	48
Class D: Under age 10 at onset or duration over 20 years or calcification of vessels of legs or hypertension or benign retinopathy	32
Class E: Calcification of pelvic arteries	13
Class F: Nephropathy	3

In 1965 I added a separate Class R for those patients with proliferating retinopathy (White 1965). [A later] . . . version of the classification (White 1974) follows:

Classes A and B the same

C_1 10–19 for age of onset
C_2 10–19 for duration
D_1 Onset under 10 years

D_2 Duration over 20 years
D_3 Benign retinopathy
D_4 Calcified vessels of legs
D_5 Hypertension
E No longer sought
F The same
G Many failures
H Cardiopathy
R Proliferating retinopathy
T Added by Tagatz [and colleagues] of the University of Minnesota (renal transplant) (Tagatz et al. 1975)

Medical and obstetrical management practices have improved fetal survival rates.

Other classifications of obstetrical diabetes are also in wide use. Pedersen's "Prognostically Bad Signs of Pregnancy" includes the late enrollers, the neglectors, the toxemic, and those with febrile pyelonephritis (Pedersen and Pedersen 1965). This classification is especially helpful to the internist or the generalist caring for their patients. The British simplify the classification proposing the gestational diabetes, diabetes without complications, and diabetes with complications (Brudenell 1975) practical, but perhaps somewhat over simplified. Microcapillary aneurysms of the retinae cannot be compared with diabetic nephropathy.

Tyson's classification of chemical diabetes revealed with glucose tolerance tests is designed to identify the pre-diabetic woman (Tyson and Felig 1971). To the usual glucose tolerance tests he adds immunoreactive insulin. A_1 is glycosuria with a positive glucose tolerance test and insulinopenia. A_2 is an abnormal glucose tolerance test with hyperinsulinism. A_3 is obesity with an abnormal glucose tolerance test and insulinopenia. There is hardly a subject of more importance in the study of diabetes than its prediction except its prevention or its cure.

If diabetes, as is generally conceded, is transmitted genetically, should reproduction be encouraged? On the basis of the present concept that diabetes is multifactorial and probably polygenic, the probability of the offspring of the two diabetics developing diabetes in only 12%.

Our studies of the children of diabetic mothers and of diabetic fathers have shown 9% [each] of the offspring of . . . mothers and . . . fathers have developed overt diabetes. Too many? Perhaps, but the treatments of diabetes and its sequelae improve, and the cure is being sought.

Obstetricians have been very gracious in their acceptance of my classification of diabetes in pregnancy. If it has proved to be helpful in their management of their challenging patients, I am very happy indeed.

Female sex hormonal therapy was given orally or administered parenterally to Joslin Clinic pregnant diabetic women between 1938 and 1975. Some of the daughters of these women participated in the study of Leavitt (personal communication) who found 23% showed such vaginal changes as adenosis and vaginal ridges. Some of these women who had received stilbestrol or estradiol were also studied by Yalom et al. (1973). They found that compared with sons of diabetic women who had not received sex endocrine therapy they were less aggressive. Without sex hormonal therapy fetal survival has risen to 97%. The fetal survival influenced by modern techniques in obstetrical and pediatric care after this omission has reached that of population of the hospital as a whole.

(White 1978, pp. 228–230)

In the mid-1950s, Jorgen Pedersen (1914–1978) of the *Rigshospitalet*, Copenhagen, reported that from 1946 to 1952 among women who attended clinic 53 days or more ("long term") before the expected date of delivery, the fetal mortality was

only 12%, whereas in women whose clinic attendance was less ("short term"), the rate was 36% with an overall rate of 27% (Pedersen 1954). During the same era, Robin Daniel Lawrence (1892–1968) of King's College Hospital, London, a severe diabetic himself, established one of the first diabetic clinics in the UK and led out in the study of many aspects of the disease. Among his other contributions, with his diabetologist colleague Wilfred Oakley (1906–1998), he maintained good control over a group of 44 diabetic women with 54 pregnancies and the delivery of 57 infants. In this cohort the maternal death rate was only 2%, as compared to the 20–40% figure for the pre-insulin age. Nonetheless, fetal mortality was 33%, only slightly lower than other reports, with mortality rates of 23, 50, and 70%, respectively, depending whether the mothers had complete, partial, or no antenatal supervision of the diabetes (Lawrence and Oakley 1942). For the newborn infant, these workers advised early glucose feeding to prevent hypoglycemia followed by breast feeding whenever possible (Lawrence and Oakley 1942). Also about this time, what we now know as gestational diabetes first was recognized (Hoet and Lukens 1954; Hurwitz and Jensen 1946).

Of importance, Lawrence challenged one of the young consulting staff members of the Department of Obstetrics and Gynecology at King's, John Peel (later Sir John, 1904–2005) (Anonymous 2006; Richmond 2006), to learn something about the disease. As Sir John has recorded, the major problem was the horrendous perinatal mortality due to macrosomia, coupled with congenital anomalies and neonatal hypoglycemia. At mid-century, in a presentation to the 12th British Congress of Obstetrics and Gynaecology, Peel and Oakley recorded 141 pregnant patients for which they cared from January 1942 to March 1949 with a maternal mortality of only 1.4% and rates of intrauterine death, stillbirth, and neonatal death of 8.5, 2.8, and 14.2%, respectively, for a total fetal-newborn loss of 24.9% (Peel and Oakley 1949).

This compares with results obtained from a questionnaire of 458 diabetic pregnancies that were cared for in 26 different centers in Great Britain and Ireland. Among this group of patients, 2.2% of mothers died, as did 40.3% of the fetuses and newborn infants (died in utero 18.7%, stillborn 10.7%, and neonatal death 10.7%) (Peel and Oakley 1949). Also among the King's College Hospital group, 44.2% of mothers experienced polyhydramnios and 10.7% preeclampsia (among which total fetal loss was 46.7%); 27% of the infants weight 4.5 kg (10 lbs) or more, among which group the fetal loss rate was 42%. Although presenting some data on the Smith and Smith hypothesis and regime, the authors concluded that evidence from their small study of this matter was inconclusive (Peel and Oakley 1949). In the discussion that followed this presentation, several academicians who had managed pregnant diabetic patients agreed with essentially all of the conclusions.

On the basis of several reports, in 1958 Peel and colleagues elected to hospitalize diabetic pregnant patients at 32 weeks' gestation or earlier in the presence of complications. With such management their perinatal mortality rate fell to 4–7% (Peel 1972). Sir John attributed this remarkable record to rigid control with measurement of blood sugar and ketones four times daily, glucose tolerance tests twice weekly, and newer, long acting insulin. He also credited these results to analysis of

fetal blood sampling and fetal electronic heart rate monitoring during the course of labor. He concluded noting:

> I feel that we have now come pretty well to the end of this particular road, and I do not believe that progress in these areas alone will solve all the problems of the effects of maternal diabetes on the fetus … There is still much fundamental research for the next generation to carry out. We do not know today any more than we did when the observation was first made, why the fetus may be affected in exactly the same way when the mother is a severe clinical diabetic and when she has only the potentiality to develop the disease. We have improved the fetus's hold on life by greatly improved diabetic control and other means, but we still do not understand fully why even very minor deviations from normal of the maternal blood sugar level may affect it so profoundly, and why there is an increased hazard of developmental abnormalities ... The final answer can only come with the defeat of diabetes itself—"a full life despite diabetes" … Pregnancy and obesity are great revealers of diabetes … For my part, however, I have nothing but profound admiration for the majority of these patients who have come to terms with their chronic and incurable disease. They so often show patience, courage, and a determination, at times in the face of repeated disasters, to live a full life despite diabetes and fulfil their maternal role.
>
> (Peel 1972, pp. 393–394)

In the mid-1960s, in a group of 752 women, John B. O'Sullivan (1926–2001) with a colleague of the Boston Lying-In Hospital demonstrated the virtues of the 3 h, 100 g oral glucose tolerance test, including that of predicting the risk to the mother of developing type 2 diabetes following delivery (O'Sullivan and Mahan 1964). At successive Aberdeen Colloquia O'Sullivan compared controlled management trials of gestational diabetes at both the Boston City Hospital and the Boston Lying-In (O'Sullivan 1975), predictor variables (O'Sullivan 1979), and the morbidity and mortality results (O'Sullivan 1984). Several years later, he reviewed not only the results from his own and seven other centers with high-quality care and excellent results, but criteria for diagnosis, exclusion criteria, and other factors (O'Sullivan 1989). O'Sullivan also reviewed other issues relating to the occurrence of gestational diabetes and performed a meta-analysis of 12 worldwide studies disclosing an incidence that varied from 6 to 62% (O'Sullivan 1991). Since that time, the International Association of Diabetes and Pregnancy Study Groups, the American Diabetes Association, and other organizations have refined methods of testing and established criteria for diagnosis and optimal treatment plans of the several types of diabetes. Along this line, prior to becoming pregnant, a combination of four risk factors (normal BMI, healthy diet, regular exercise, and not smoking) has been shown to reduce the prevalence of diabetes mellitus during pregnancy by 47–83% (Zhang et al. 2014).

In the early 1980s, in a study of 381 gravid women 25 years or older, Marshall Webb Carpenter and Donald Ross Coustan of Yale University performed a 50 g, 1 h glucose screening test. They classified the distribution of values, those below 135 mg/dl, those above 182 mg/dl, and a central zone of uncertainty which required further testing. The probability of diabetes in the low- and high-glucose groups was <1 and $>95\%$, respectively. On the basis of these results which required two or more abnormal values, the authors suggested that the threshold for further testing be lowered from 143 to 135 mg/dl of plasma glucose (Carpenter and Coustan 1982).

Using more strict criteria of a 50 g, 1 h glucose load with 1 h 140 mg/dl or greater, a subsequent study disclosed 50% more cases of gestational diabetes accompanied by macrosomia umbilical cord hyperinsulinemia and a similar increase in perinatal mortality (Magee et al. 1993). In 2011, the National Diabetes Data Group (NDDG) compared perinatal outcome among 1542 women with gestational diabetes defined by their criteria of a 50 g, 1 h glucose load of 140 mg/dl or greater (and 3117 controls) underwent a diagnostic 3 h glucose tolerance test. Those patients diagnosed by the Carpenter-Coustan criteria showed a greater incidence of preeclampsia and greater risk of cesarean delivery, with infants weighing more than 4 kg. The study disclosed 42.5% additional women by the strictest criteria as compared to that of the NDDG (Berggren et al. 2011). A further analysis of perinatal outcomes has been given by the International Association of the Diabetes and Pregnancy Study Groups, which required only one abnormal value following a 75 g oral glucose tolerance test. Among 281 women this cohort showed greater birthweight than those 338 women of the Carpenter-Coustan group or 3114 controls (Ethridge et al. 2014). Without doubt, it will be some years before consensus is reached on many aspects of the optimal diagnosis and management of gestational diabetes.

In response to my query regarding the origin and background of the "Carpenter-Coustan" criteria, Coustan replied,

> My interest in diabetes during pregnancy began in 1973 when, upon completing my residency in obstetrics and gynecology at Yale, I entered the Navy and was stationed at the Oakland Naval Regional Medical Center in the San Francisco Bay area. The base had a clinical research unit that was a series of World War 2 Quonset Huts equipped with laboratory equipment and a research nursing staff; it was not heavily used because so many of the regular Navy physicians had been sent to Vietnam. I befriended Dr. Stephen B. Lewis, an endocrinologist at the hospital, and collaborated on a number of studies. Among these was the first description of glucose and insulin metabolism during the third trimester of normal pregnancy (Lewis et al. 1976a), which informed calculation of a formula for insulin therapy in patients with pre-existing diabetes (Lewis et al. 1976b). The proportions for NPH insulin to short-acting insulin of 1:1 before breakfast and 2:1 before dinner, with the total morning dose being twice the total evening dose, was widely used as a formula for initiation of therapy for many years thereafter.
>
> The O'Sullivan and colleagues randomized trial of prophylactic insulin (10 units NPH each morning) for women with gestational diabetes (USPHS criteria) demonstrated a reduction in babies weighing more than 9 pounds, but not in perinatal mortality (O'Sullivan 1975). Dr. Lewis suggested that 10 units of NPH insulin was a very low dose, and that even nondiabetic pregnant women, because of their marked insulin resistance, could tolerate as much as 30 units of insulin per day. We carried out a randomized trial comparing prophylactic insulin (20 units NPH and 10 units regular) each morning plus diet, diet alone, and neither. Seventy-two patients with gestational diabetes mellitus (GDM) consented to participate and large babies (>8.5 pounds) resulted in 50% of controls, 36% of diet-treated subjects and 7% of insulin-treated subjects (Coustan and Lewis 1978). Our randomization scheme was subject to appropriate criticism in later years because we used the last digit of each subject's social security number as the basis for treatment assignment.
>
> In 1975, I returned to Yale as assistant professor and continued my interest in diabetes during pregnancy. Dr. Marshall W. Carpenter was a fellow maternal-fetal medicine, worked with me on a project looking at the function of the 50-g, 1-h screening test for gestational diabetes. When O'Sullivan and his colleagues described the screening test in

1973 (O'Sullivan et al. 1973), as well as the diagnostic criteria for gestational diabetes in 1964 (O'Sullivan and Mahan 1964), glucose was measured in venous whole blood using the Somogyi-Nelson method of analysis. In the intervening years most clinical chemistry laboratories had switched the medium for glucose analysis to plasma or serum, and the Somogyi-Nelson method (Nelson 1944; Somogyi 1945a, b) had been replaced by enzymatic analyses such as glucose oxidase or hexokinase. Dr. Carpenter spoke to our clinical pathologists and did a literature search, determining that plasma glucose levels were approximately 14% higher than whole blood measurements. Furthermore, the newer enzymatic methods were more specific for glucose than Somogyi-Nelson, which measured approximately 5 mg dL^{-1} of non-glucose reducing substances. By subtracting 5 mg dL^{-1} and then adding 14% we calculated that O'Sullivan's original cutoff of 130 mg dL^{-1} would be equivalent to 143 mg dL^{-1} using glucose oxidase analysis on plasma samples. Previous to the study we had been applying the original cutoff of 130 mg dL^{-1}, and by measuring glucose in plasma by glucokinase we had data on 381 gravidas who had been screened and tested using that cutoff. We concluded that the cutoff should be lowered to 135 mg dL^{-1} (later 130 mg dL^{-1} based on additional data) because gravidas with a screening test value between 135 and 143 mg dL^{-1} had a 13% likelihood of gestational diabetes (Carpenter and Coustan 1982). The change in methodology also prompted us to update the original O'Sullivan and Mahan GDM criteria to plasma and glucose oxidase methodology. In reviewing the original description of those criteria, Dr. Carpenter noted that O'Sullivan had rounded off the 2-h and 3-h cutoffs to the nearest 5 mg dL^{-1} for ease of use. I often joked that O'Sullivan, a trained internist, must have assumed that ObGyns would never be able to remember values not divisible by 5! The "Carpenter and Coustan criteria" are derived from O'Sullivan's original unrounded cutoffs, although we followed O'Sullivan's lead and rounded off the final numbers to the nearest 5 mg dL^{-1}. I have always found it ironic that the glucose tolerance test thresholds to which our names have been attached for the past 40 or so years were really a byproduct of a study of the screening test rather than the glucose tolerance test itself.

Prior to the publication of our 1982 paper, the National Diabetes Data Group (NDDG) proposed a modification of the original O'Sullivan criteria which added 15% to each of the four values, but did not correct for the more specific enzymatic methodology then in common use. The two sets of criteria, the NDDG's and ours, continued as alternative approaches for many years. Both were based on the same O'Sullivan and Mahan 1964 recommendation. In 1989 Sacks and colleagues ran parallel samples using the original O'Sullivan whole blood, Somogyi-Nelson methodology against current plasma, enzymatic analysis (Sacks et al. 1989). They determined that 3 of the 4 NDDG conversions were above 95% confidence limits compared to the O'Sullivan values, whereas all four of our cutoffs were within those limits. Subsequently the American Diabetes Association adopted our values while to this day ACOG continues to offer either alternative.

The O'Sullivan criteria for gestational diabetes, and the various conversions, were widely embraced in the United States because they were the only ones specifically based on pregnancy, a time when there are important changes in maternal glucose metabolism. However, there were numerous other sets of criteria in use around the world, utilizing glucose challenges ranging from 50 g to 75 g to 100 g, with multiple sets of cutoffs. Some, such as those of the WHO, were simply the same criteria used for diabetes and prediabetes in nonpregnant individuals. This variation made it impossible to compare data across regions and created a veritable "tower of Babel" when researchers gathered to discuss gestational diabetes. While we might have wished for the O'Sullivan criteria to have been universally embraced, they are not without drawbacks. The chief of these being that the cutoffs were based on their predictive value for subsequent maternal diabetes rather than on pregnancy outcomes. In the United States and many other parts of the world all or most pregnant women are tested for gestational diabetes out of concern for adverse pregnancy outcomes such as perinatal mortality, macrosomia, and other conditions. By the early

1990s, it was becoming clear that a set of diagnostic criteria based on pregnancy outcomes rather than maternal prognosis would be necessary to achieve universal acceptance. In 1991, a group of investigators from various countries met in Newport, RI to discuss how such a universally accepted set of criteria for diagnosing gestational diabetes might be achieved. Subsequently, we met on numerous occasions (at our own expense I might add) in London and determined that what was needed was a data set relating glucose tolerance in pregnancy to adverse perinatal outcomes; the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study was conceived as a multi-institutional, multi-national observational study of blinded 75 g OGTTs, at 24–32 weeks, conducted in enough different parts of the world that the results would not be exclusive to any single country or nationality. It took many years of hard work, particularly on the part of our leader Dr. Boyd Metzger, to achieve funding from NICHD and NIDDK as well as a number of local agencies in places where field centers were located. Data collection began in 2000 and was completed in 2006, with the main publication appearing in 2008 (HAPO Study Cooperative Research Group 2008). The overarching finding was a continuous significant relationship between each of the three 75 g, 2-h OGTT values and the main outcomes (fetal macrosomia, primary cesarean section, neonatal hypoglycemia and umbilical cord blood C-peptide as a marker for fetal insulin). Similar relationships were found with secondary outcomes such as preeclampsia, birth trauma and neonatal body fat. These relationships held even when adjusted for potential confounders such as gestational age at birth, maternal BMI, parity, race/ethnicity, and field center. They were applicable in all 15 participating field centers.

Because there were no detectable inflection points in the relationships there were not obvious diagnostic cutoffs. For this reason the International Association of Diabetes in Pregnancy Study Groups (IADPSG) convened a large multinational group with representatives of countries around the world to consider the available data. After a rather exhaustive process this group made recommendations for diagnostic criteria, published in 2010 (International Association of Diabetes and Pregnancy Study Groups 2010). These diagnostic criteria (known as the "IADPSG criteria") are currently being considered by various authoritative bodies around the world. They have been controversial, primarily because their use would increase the prevalence of gestational diabetes by 2- to 3-fold and there is great concern about the costs to health care systems. However, this increase is parallel to the worldwide epidemic of obesity, diabetes and prediabetes, and needs to be considered in that context. The diagnostic criteria are not too different from those for prediabetes in nonpregnant individuals. Almost one third of adult Americans currently are believed to have prediabetes, so a rate of gestational diabetes totaling about half that proportion may not be unreasonable.

It is my hope that sooner rather than later there will be universally accepted evidence-based criteria for gestational diabetes, just as there are for diabetes around the world. New approaches to management of gestational diabetes will need to be developed in order for health care systems to be able to cope, but the same issues are currently being confronted with regard to diabetes and prediabetes in nonpregnant individuals. Nothing would make me happier than to see the "Carpenter and Coustan criteria" retired in favor of evidence-based thresholds derived from pregnancy outcome data.

(Letter from DRC to LDL, 16 November 2014)

With an infant mortality rate of ~7 deaths per 1000 live births, the USA has a rate rivaling that of many developing countries (Save the Children 2011), a leading cause of which is congenital malformations (Heron and Tejada-Vera 2009). Worldwide, ~8 million infants are born with major birth defects (World Health Organization 2010), with close to 185,000 of these in the USA (Reece and Hobbins 2007). A growing body of evidence links this pandemic to diabetes in pregnancy, which is a major issue whether type 1, type 2, or of gestational origin. In one of her early

essays, Priscilla White noted, "Congenital defects [including heart defects, gastrointestinal atresia, microcephaly, and achondroplasia] are doubtless beyond our therapeutic control. They are, we believe, related to a disease which is genetic in origin" (White 1936, p. 159). Although this conclusion may be considered unfortunate, in the post-insulin era most women with diabetes and their offspring survive. However, White was amazingly prescient in emphasizing the importance of macrosomia, despite being inaccurate regarding a genetic origin. Advances in the glycemic control of pregnant patients have demonstrated the ability to reduce, but not eliminate entirely, the prevalence of teratologic embryopathy. In addition, considerable evidence demonstrates that while epigenetic alterations in molecular mechanisms play a major role in their genesis, these anomalies are not genetically derived.

In the mid-1970s Lewis Ball Holmes of the Medical Genetics Unit at the Massachusetts General and Brigham and Women's Hospitals, Harvard Medical School, stressed the importance of maternal diabetes in the genesis of meningomyelocele and other neural tube defects (Holmes et al. 1976) and syndromes with yet other congenital anomalies (Holmes 2010, 2011; Stoler 2009). The sobering reality is that these diabetic-associated congenital malformations can affect several organ systems: neural tube defects, including anencephaly, encephalocele, holoprosencephaly, and spina bifida, and cardiovascular defects being the most common (Barr et al. 1983; Correa et al. 2008; Kucera 1971; Mills 1982). Caudal regression syndrome (underdevelopment of the lower spine and sometimes bladder and bowel) is also seen (Kalter 1993; Mills 1982).

As may be anticipated, embryogenesis involves a number of regulatory pathways in pattern formation (Gurdon 1992), many of which are altered in diabetic teratogenesis (Fine et al. 1999; Freinkel et al. 1986; Singh et al. 2012; Zabihi and Loeken 2010). In the embryos of diabetic and obese women, the regulation of gene expression by epigenetic mechanisms is altered in major ways (Jiang et al. 2008; Pavlinkova et al. 2009). This involves changes in histone acetylation (Salbaum and Kappen 2012), DNA methylation, and the generation of reactive oxygen species which may inhibit the expression of developmental control genes such as *Pax 3* (Epstein et al. 1991, 1993; Pavlinkova et al. 2008, 2009; Phelan et al. 1997). Several authors have reviewed the role of epigenetics in diabetic-induced embryopathy (Salbaum and Kappen 2011; Zhao and Reece 2013), including neural tube defects (Werler et al. 1996).

With his colleagues, Edward Albert Reece (Fig. 11.3) of the University of Maryland has explored many aspects of the etiology/pathophysiology of the role of hyperglycemia in diabetes-induced birth defects. In response to numerous studies, the Reece group has concluded that such anomalies represent a combination of altered mechanism of arachidonic acid, myoinositol, reactive oxygen species, and other compounds (Reece 1996, 2012; Reece et al. 1996b, 2006, 2009). In response to my query regarding several aspects of his work, Reece responded:

Fig. 11.3 Edward Albert Reece

Obstetricians and gynecologists provide care for patients [with] a range of health complications which might affect reproductive health. One of the most challenging and increasingly prevalent chronic conditions in women of childbearing age is diabetes (Lawrence et al. 2008). Managing a pregnant patient with preexisting type 1 or 2 diabetes mellitus (T1DM or T2DM), or a patient who develops gestational diabetes mellitus (GDM), requires vigilance on the part of the mother and her physician to maintain maternal glucose levels within the normal, or euglycemic range (Hayslett and Reece 1987; Homko and Reece 1998; Reece et al. 1991, 1994, 2002a). Stringent glycemic control in the mother throughout gestation, especially during early pregnancy and, ideally, beginning prior to conception, is particularly important because women with pregestational diabetes mellitus (PGDM) have a much higher rate of having infants with birth defects (Correa et al. 2008; Reece 2008, 2012; Reece and Hobbins 1986). Among the most severe and devastating congenital anomalies associated with PGDM are neural tube defects (NTDs) and heart defects (Hayslett and Reece 1987; Naftolin et al. 1996; Reece 1996; Turan et al. 2011).

In addition to birth defects, diabetes can impact other aspects of the mother's and infant's lives. Individuals with diabetes can have complications, including heart, kidney, [and] eye disease. Maintaining maternal blood glucose within a normal range can help to halt the progression of these comorbidities during pregnancy (Reece et al. 1988a, b, 1994, 1996a, b, 1998). Both PGDM and GDM also are risk factors for abnormal fetal growth (Baxi et al. 1984, Reece and Homko 1994; Reece et al. 1990), and PGDM can increase the mother's risk for hypertension (Hayslett and Reece 1987; Leguizamon and Reece 2000; Reece 2010), and diabetic ketoacidosis (Cullen et al. 1996; Whiteman et al. 1996).

The introduction of insulin and insulin analogues to treat diabetes during pregnancy dramatically changed the infant morbidity and mortality rate in the United States (Cullen et al. 1996; Homko and Reece 2006; Taitelman et al. 1977). Clinical studies also have demonstrated that maternal diet, exercise and weight loss can improve the outcomes of pregnancies affected by diabetes (Murtaugh et al. 1998; Reece and Homko 1998a, b). However, euglycemia can be most difficult to achieve and maintain and most women have unplanned pregnancies, making it necessary to understand the underlying causes of diabetes-induced birth defects to identify possible therapeutic targets for treatment.

Through decades of research using animal models of diabetic embryopathy, we have delineated key steps that underlie the pathophysiology and biology (morphological, biochemical, molecular), and identified targets (biochemical and molecular) that can be

manipulated in animals to alter outcomes of diabetic pregnancies. Initial work focused on isolated embryos cultured under normal and hyperglycemic conditions. These studies have revealed that high levels of glucose negatively affect the yolk sac and damage embryo membranes (Reece et al. 1994, 2002b).

More recent work has focused on understanding the mechanisms behind these morphological changes. Using both in vitro and in vivo model systems, we have found that levels of hyperglycemia indicative of uncontrolled diabetes lead to aberrant signaling in embryonic cells particularly the progenitor cells which form the neural crest and neural tube and the heart (Cao et al. 2011, 2012; Yang et al. 2013). Specifically, maternal diabetes appears to cause abnormal cell death (Li et al. 2007, 2012; Yang et al. 2008), which can be blocked by pharmacologic agents (Zhao et al. 2008), as well as gene manipulation (Li et al. 2011a, b; Wang et al. 2015b) in animal models. In both animal and human studies, dietary supplements, such as *myo*-inositol and vitamins D and E, appear to play protective roles in preventing diabetes-induced birth defects (Correa et al. 2012; Khandelwal et al. 1998). Obviously, more work is needed to translate the basic science findings (Zhao and Reece 2013) into treatments used in the clinic to develop more effective preventions for diabetes-induced birth defects.

(Letter from EAR to LDL, 29 October 2014)

In a more recent study in diabetic embryopathy, the Reece group has demonstrated the role oxidative stress plays in its pathogenesis with activation of the downstream cascade apoptosis signal-regulating kinase 1, forkhead box O-3a, TNF receptor 1-associated death domain protein, and caspase-8 cleavage, with apoptosis (Wang et al. 2015a).

11.5 Hypertension in Pregnancy: Preeclampsia/Eclampsia

Hypertensive disorders and preeclampsia are pregnancy-associated polygenic, multifactorial disorders characterized by hypertension (and often proteinuria), which presents after 20 weeks of gestation, while eclampsia [Greek to shine forth] is that associated with convulsions (Chesley 1974, 1984, 1985; Roberts and Hubel 2009, Ward 2000; Zuspan 1978). Although some trace the history of this syndrome complex back to the early Egyptians and Greeks, the distinction between eclampsia and epilepsy was not made until the Renaissance that is rather unlikely (Chesley 1972, 1974). The "disease of theories" or of "multiple inconsistent therapy regimes" (Zuspan 1978, p. 595), preeclampsia occurs in about 5–7% of pregnancies, and the prevalence is much higher in some ethnic groups and in certain parts of the world. Importantly, this disorder of unknown etiology occurs in both a "mild" and "severe" form (see definitions below) and has been characterized into two different disease entities, e.g., early onset (prior to 34 weeks' gestation) and late onset (at or after 34 weeks). In the USA and many other countries, preeclampsia is the leading cause of pregnancy-related fetal and maternal morbidity and mortality (Reece and Hobbins 2007).

At the turn of the twentieth century, John William Ballantyne (1861–1923) (Fig. 11.4) of the University of Edinburgh noted:

Fig. 11.4 John William Ballantyne

> Eclampsia was one of the first of the gestational maladies which began to benefit by . . . a revolution in the management of pregnancy. Whereas it had been common for the urine of a pregnant patient never to be tested—indeed, in many cases it was not customary for the medical attendant to be told about the pregnancy or summoned to the patient till labour was in the first stage, now the doctor was engaged to look after his patient in the early weeks of her pregnancy . . . The obstetrician of 1940 finds it difficult to understand why his brethren of the early part of the century paid so much attention to the one month of the puerperal period and so little to the nine months of pregnancy.
>
> (Ballantyne 1906/1907, pp. 24–25)

It was only with the rise of prenatal care in the early twentieth century that the pregnant woman's blood pressure, blood constituents, and urinalysis began to be checked and regarded on a regular basis (Longo and Thompsen 1981). These routine examinations of the pregnant woman disclosed the possibility of being forewarned of an impending eclamptic attack by the presence of edema, weight increase, elevated blood pressure, and albuminuria. This led to the recognition of preeclampsia and the possibility of eliminating convulsive attacks in those patients who were properly observed and treated.

In the 1920s several monographs or extended reviews on the pregnancy toxemias including eclampsia appeared. The first of these *The Toxemias of Pregnancy* by George William Kosmak (1873–1954), of New York City and editor of the *American Journal of Obstetrics and Gynecology*, appeared in 1922 (Kosmak 1922). In six chapters covering 232 pages with 33 illustrations, Kosmak reviewed the history of the condition, its etiology and symptomology, pathology, therapeutic modalities, associated genitourinary conditions, and significant blood and hematologic changes. He concluded with summaries of 29 case reports from his practice. Two years later, this was reprinted with little update (Kosmak 1924); and another edition with minimal revision appeared in 1926 (Kosmak 1926). His revised edition

of 1931 consisted of seven chapters (the new chapter being the illustrative case reports) and 226 pages and included two coauthors. In his discussion, Kosmak suggested that eclampsia may occur equally commonly in impoverished and wealthy women (Kosmak 1931). This contribution by Kosmak was important in setting the stage for subsequent reviews.

The most expansive of these volumes on the toxemias, *Die Eklampsie*, was that by Hans Hinselmann (1884–1959) of the University of Bonn. In his 1924 volume with 22 chapters and 952 pages of 13 other authors, he analyzed 6498 cases gleaned from the literature (Hinselmann 1924a). Here, he reported that 74% of severe toxemia occurred in primigravid women, although these patients constituted only 25–30% of all pregnancies. He thus calculated that a primigravida was six times as likely to develop eclampsia as a multiparous woman (Hinselmann 1924b). In 1929, Henricus Johannes Stander (1894–1948) of Johns Hopkins University published *The Toxemias of Pregnancy* (Stander 1929), a multi-chapter (depending upon one's calculation) of 161 pages and over 400 bibliographic citations; Stander noted the challenge presented by the lack of a uniform classification of this syndrome which promoted confusion in the field. His own classification—vomiting of pregnancy, low reserve kidney, nephritis complicating pregnancy, preeclampsia, eclampsia, and acute yellow atrophy of the liver—served a valuable role for several decades (Stander 1929). Several other major reviews of eclampsia appeared during the following decades (McIlroy 1936)

In 1941, William Joseph Dieckmann (1897–1957), of the Chicago Lying-In Hospital and the University of Chicago, Illinois, published his monograph *The Toxemias of Pregnancy* (Dieckmann 1941). In 32 chapters, 521 pages, and an exhaustive list of references, Dieckmann reviewed the classification, etiology, incidence, pathology, clinical aspects, treatment, and prognosis of this condition. Also included in the volume is an extensive section on the maternal physiology of pregnancy. In his preface Dieckmann noted that his overall objectives were to acquaint obstetricians with the state of the art, as well as to stimulate investigators who may be unfamiliar with the disease, suggesting some challenging ideas for research. He also observed that "It will be apparent to even the casual reader that the physiology of pregnancy is almost a virgin field. The problems are innumerable" (Dieckmann 1941, p. 9). In regard to the management of patients with preeclampsia and eclampsia, following an historical review Dieckmann considered the pharmacodynamics of a number of chemical agents, specific procedures of value, pregnancy termination, and detailed aspects of the obstetric treatment of both eclampsia and preeclampsia. In his second edition a decade later, following a similar format, the text was expanded to 710 pages and even more references (Dieckmann 1952). In the preface to this revised edition, Dieckmann presented his rather wry confession, "I had hoped that by the time this edition was prepared, the cause of eclampsia would be known" (Dieckmann 1952, p. 7). For over two decades Dieckmann served on the editorial board of the *American Journal of Obstetrics and Gynecology*.

As Frederick Paul Zuspan (1922–2009), then of Ohio State University, pointed out a half century ago, despite an incomplete understanding of its etiology, successful rational therapy can be based upon the physiologic information available

(Zuspan 1978; Zuspan and Ward 1964). An example of an early, rational approach to the management of patients with eclampsia is that of the Stroganoff regimen (named for Vasili V. Stroganoff (1857–1938), of the State Institute for Obstetrics and Gynecology in St. Petersburg). In this approach patients were placed in a quiet, dimly lighted, airy room; covered with warm, but light, blankets; and surrounded by water bottles. All unnecessary manipulation or noise was eliminated. Seizures were treated with light chloroform anesthesia with supplemental morphine injections. When consciousness was regained, chloral hydrate was administered. This was repeated as necessary. Respiration was encouraged by frequent change in position, and vaginal delivery was effected as soon as possible (Stroganoff 1926). Later, this routine was modified (Stroganoff 1930, 1934). With this approach, in a series of 201 women with eclampsia, the maternal mortality was reduced to 3% (six patients) (Stroganoff and Davidovitch 1937). This compared to the usual mortality rate of 20–30%.

Despite decades of research, the biochemical, cellular, and molecular mechanisms by which pregnancy per se incites or aggravates hypertensive vascular disease remain unsolved. It is established that a dysfunctional uteroplacental unit plays a major role in the etiology of preeclampsia (Roberts and Redman 1993), as the disorder abates following placental delivery. Moreover, some studies indicate that incomplete placental trophoblastic cell invasion (perhaps due to reduced differentiation; Fu et al. 2013), associated with defective remodeling and penetration of the spiral arteries in the maternal decidua, leads to this disorder. Inadequate remodeling of the spiral arteries leads to their insufficient dilatation, which leads to reduced blood flow to the placenta and fetus. As a consequence of reduced blood flow to the feto-placental unit, and probable reduced syncytiotrophoblast function (Arkwright et al. 1993), unknown soluble chemical messengers are released into the maternal circulation. With the release of cytokines, chemokines, and other noxious compounds, endothelial dysfunction follows in multiple organs including the brain, kidney, and liver leading to the various manifestations of preeclampsia and/or eclampsia (Rao et al. 2014; Roberts and Hubel 1999). These chemokines probably also play an important role in the pathogenesis of the long-term sequelae in some of these women (Carty et al. 2010). As noted earlier, elevated levels of triglycerides, but not HDL, LDL, or total cholesterol, are associated with hypertensive disorders of pregnancy and preeclampsia (Ray et al. 2006; Vrijkotte et al. 2012; Wiznitzer et al. 2009).

Additionally, vascular dysfunction in the placenta can lead to impaired blood flow to fetus and growth restriction, although this may also occur de novo. In turn, fetal growth restriction is associated with increased morbidity and mortality during the perinatal period, as well as a significant increase in multiple severe diseases later in life (see below). Importantly, why the preeclamptic-placental trophoblastic cells fail to invade maternal decidua as they do during normal pregnancy is unknown. In the fetus and newborn infant, preeclampsia commonly is associated with growth restriction, accompanied by hypoglycemia, neurotrophin, thrombocytopenia, polycythemia, and serum electrolyte abnormalities such as hypocalcemia.

In large part, these pathologies result from placental "insufficiency" leading in turn to diminished tissue and O_2 and nutrient delivery.

Although many of the molecular biomarkers released by the placenta or decidua into the maternal circulation are not known, it is accepted that complex gene-environment interactions between the mother and the fetus play an important role in their origin. Recent studies of the pathophysiology of preeclampsia suggest that the cascade commences with the restricted and incomplete trophoblastic invasion of maternal spiral arteries leading to poor remodeling of these arteries and of the maternal decidua. This results in inadequate placental development and poor placental oxygenation, ultimately leading to a relatively hypoxic environment, which sends signals to increase maternal blood pressure and volume. In association with placental dysfunction and inflammation with the release of cytokines, chemokines, and other agents, C-reactive protein and other angiogenic and antiangiogenic peptides may initiate a cascade of reactions that result in endothelial dysfunction, renal and placental damage, and arterial hypertension (Parchim et al. 2015; Vest and Cho 2014). The prevailing hypoxic/nutrient-deficient environment also can accentuate endothelial dysfunction with its deleterious consequences (Roberts and Hubel 1999) and have profound effects on fetal development. Similarly in mice, defects in genes required for placental development and angiogenesis frequently lead to preeclampsia (Singh et al. 2011; Venkatesha et al. 2006). A new paradigm to understand trophoblast invasion as well as defects in placental morphology and angiogenesis comes from a recent study of ELABELA (ELA), an endogenous ligand of the apelin receptor (APLNR or APJ) that is secreted by the placenta (Ho et al. 2017). In humans, ELA is predominantly expressed in first trimester villous cytotrophoblasts and syncytiotrophoblasts and in term placentas. ELA appears to regulate trophoblasts differentiating into invasive extravillous trophoblasts and development of placental vasculature, the impairment of which contributes to the etiology of preeclampsia. These findings raise the possibility that the placental hormone ELA acts locally and systemically to regulate maternal and fetal cardiovascular integrity during pregnancy.

As promising as this new paradigm maybe, further efforts are needed to incorporate evidence from other studies on genetic and epigenetic factors in the etiology of preeclampsia. Specifically in the placenta, hypermethylated of the 14-33 (YWHAQ) gene promoter (Liu et al. 2014) and reduced expression of the transcription factor glial cells, missing gene (GCM1, mRNA, and protein) may also contribute to the etiology of preeclampsia (Chen et al. 2004). Moreover, meta-analysis of microarray data from blood and placenta samples indicates a differential expression of 925 genes in women with preeclampsia compared with normotensive controls. Many of these also were expressed in women with cardiovascular disease (Sitras et al. 2015). These findings suggest that women with these two conditions share common traits in their gene expression profile with common signal transduction pathway changes in their pathophysiology. In another study of early-onset preeclampsia, 26 genes were found to be upregulated, while 124 were downregulated; several miRNAs were also differentially expressed (Song et al.

2015). Clearly, the molecular mechanisms that contribute to pathogenesis and symptomology of this condition are complex and remain to be elucidated.

In the face of the confusion as a result of inconsistent terminology, interpretation of the literature and classification of these disorders has presented difficulty over the years. A classification of the hypertensive disorders of pregnancy adopted by a working group of the National High Blood Pressure Education Program (NHBPEP) (NHBPEP 2000) defined five aspects of the syndrome.

- Gestational Hypertension. BP $\geq$ 140/90 mm Hg for the first time during pregnancy, absence of proteinuria, BP return to normal by 12 weeks postpartum, final diagnosis only made postpartum.
- Preeclampsia. BP $\geq$ 140/90 mm Hg after 20 weeks' gestation (formerly proteinuria $\geq$ 300 mg·24 h^{-1} or $\geq$ 1+ on dipstick was required). This also may be associated with platelets <10^5 mm^{-3} microangiopathic hemolysis, elevated liver enzyme, persistent severe headache, and/or epigastric pain.
- Eclampsia [for Greek "flash" or "bursting forth"]. Grand mal seizures that cannot be attributed to other causes in a woman with preeclampsia.
- Superimposed Preeclampsia. Superimposed upon chronic hypertension, new-onset proteinuria $\geq$ 300 mg·24 h^{-1} in a hypertensive woman after 20 weeks' gestation.
- Chronic Hypertension. BP $\geq$ 140/90 mmHg prior to pregnancy or diagnosed before 20 weeks' gestation, not attributable to gestational trophoblastic disease, hypertension persistent after 12 weeks postpartum (NHBPEP 2000).

The late Leon Carey Commodore Chesley (1908–2000; born Carey Commodore, but early in life added the name Leon to honor his father, whom he admired greatly; http://agosonline.org/member-pages-in-memoriam/chesley-l-2000.html) helped to unravel some of the mystery and debunk some of the misconceptions regarding preeclampsia. Late in his career, he came to believe that the condition was limited to primiparous women and that the diagnosis is questionable in the absence of proteinuria (Chesley 1985). In an early report of 301 pregnancies in 218 women with "hypertensive toxemia" cared for from 1931 through 1944, the number of both stillbirths and neonatal deaths significantly increased as a function of systolic blood pressure in those patients >180 mm Hg. There were six immediate and seven late (6 weeks to 4 months) puerperal deaths (Chesley and Annitto 1947). In 1953, Chesley moved to the State University of New York, Downstate Medical Center in Brooklyn where he worked until retirement. Three decades after his 1953 report, Chesley with colleagues completed a classic long-term study of remote prognosis, by following to 1973–1974 99 % (all but three) of 270 women who survived eclampsia from 1931 through 1951 at the Margaret Hague Maternity Hospital (some of whom were followed for almost 44 years) (Chesley et al. 1976). This study, portions of which had been reported earlier (Chesley and Somers 1941; Chesley et al. 1941), established that neither pregnancy accompanied by hypertension subsequent to eclampsia nor eclampsia in primiparous women is a sign of latent chronic hypertension or is its cause. Chesley also emphasized that eclampsia and "true" preeclampsia are diseases chiefly of primiparous patients,

whereas in multiparous patients eclampsia is more likely associated with underlying chronic hypertension. Among 187 Caucasian primiparous patients who survived eclampsia, the number of remote deaths did not exceed the expected number, in contrast to the significantly greater number of expected deaths in Caucasian multiparous ($n = 59$) patients and both primiparous ($n = 19$) and multiparous ($n = 5$) Black patients. Chesley and his colleagues concluded that pregnancy following eclampsia constitutes a screening test for underlying hypertensive disease and that in all analyses of sequelae, eclampsia in multiparous patients should be grouped separately from that in primiparous patients. In several subsequent reports, Chesley reviewed the development of ideas in this field (Chesley 1974, 1978, 1979).

In his monumental 1978 volume *Hypertensive Disorders in Pregnancy*, with 18 chapters, 628 pages, and over 1100 references, Chesley summarized his and others' work in the field (Chesley 1978). Based on personal experience of over four decades of investigation in which he used relatively simple methods, objective observation, and critical analysis, combined with exegetic literary search and historical perspective, Chesley considered everything from definitions, an historical survey, epidemiologic data, the topics of disseminated intravascular coagulation, blood pressure and circulation, the kidney, fluid and electrolyte balance, the renin-angiotensin-aldosterone system, the liver, structural-morphologic pathophysiology, proper diagnosis, management, assessment of the fetus, short- and long-term sequelae, and more—truly, a thoughtful and provocative encyclopedic survey (Chesley 1978).

In addition to reversing his views on the relation of the duration of the syndrome to the likelihood of permanent chronic hypertension, Chesley reviewed in extenso the several hypotheses regarding etiology (Chesley 1978). Subsequently, Chesley and Cooper (1986) presented evidence from follow-up studies in sisters, daughters, granddaughters, and daughters-in-law of women with eclampsia of a genetic component to preeclampsia-eclampsia. More recent data has suggested that in primigravid women, preeclampsia may have a remote cardiovascular prognosis different from that of the population as a whole, as women with preeclampsia occurring before 36 weeks' gestation may have hypertension both in subsequent pregnancies and in later life (Adams and Macgillivray 1961; Bellamy et al. 2007; Carty et al. 2010; Ghossein-Doha et al. 2014). With preeclampsia occurring late in gestation in primigravid women, there is no evidence of remote cardiovascular risk, but subsequent pregnancies will help to define the risk more accurately.

In an expanded second edition of *Hypertensive Disorders in Pregnancy*, Chesley, with two coeditors and 20 other colleagues, in 19 chapters of 654 pages covered essentially every aspect of hypertension in pregnancy, from historical considerations, epidemiology, the clinical spectrum of disorders, the pathology and pathophysiology, etiology, prevention, and management. This analysis concluded that women with early-onset disease, preeclampsia, or only hypertension as a multipara, and those manifesting gestational hypertension in any pregnancy, are at increased risk, information of some importance for long-term healthcare strategies (Lindheimer et al. 1999). The following year, the volume appeared in the *Handbook*

of Hypertension series (Rubin 2000). In 2009, Lindheimer and colleagues published a third edition of Chesley's *opus*, now with 21 chapters, 422+ pages, two coeditors, and 33 other contributors (Lindheimer et al. 2009). A fourth edition appeared in 2015 (Taylor et al. 2015). This most recent edition includes 20 chapters of 489 pages, four coeditors, and 34 other contributors. In this fourth edition, the senior editor Robert Neal Taylor observed in regard to Chesley's tie in a photograph which displays kangaroos, "While making that specific sartorial selection for his pose on the frontispiece, Dr. Chesley might have been wondering: 'Had humans evolved from marsupials, with their transient embryonic attachment to a simple yolk-sac placenta, would our species have ever developed preeclampsia?' You may have to wait for the fifth edition to find out."

Toward the end of his career, Chesley had noted that 10% of patients present with eclamptic seizures prior to the development of overt proteinuria (Chesley 1985). In an exhaustive study of sisters, daughters, granddaughters, and daughters-in-law of women with eclampsia, Chesley with a colleague concluded that preeclampsia-eclampsia is highly heritable, a single gene model best accounting for their observations (Chesley and Cooper 1986). Other studies have supported the concept of polygenetic inheritance (Nilsson et al. 2004; Trogstad et al. 2004), the histocompatibility antigen HLA-DR4 possibly playing an important role in the genesis of this disorder (Kilpatrick et al. 1989). Of 54 nulliparous women with eclampsia in their first pregnancy, those who experienced hypertension in subsequent pregnancies were at increased risk for the development of hypertension later in life, as compared to those women who remained normotensive in subsequent pregnancies (Chesley et al. 1976). Thus, they concluded that pregnancy may serve as a screening test for the future development of hypertension, and preeclampsia does not cause chronic hypertension (Fisher et al. 1981). Disorders of coagulation also may play a role in this regard (Pritchard et al. 1954, 1976), although these changes may not be significant (Pritchard et al. 1984).

In several reviews, Chesley traced the history of eclampsia, noting that seizures during pregnancy were recorded by the early Greeks, although it was not until the sixteenth century that eclampsia was distinguished from seizure disorders (Chesley 1944, 1974, 1978, 1984; Chesley and Somers 1941; Chesley et al. 1976). At a 1975 workshop on hypertension, Chesley commented upon many of the "false steps" in the study of preeclampsia, including his own (Chesley 1976). Shortly before he died, in response to my questions regarding his career, Chesley penned the following:

> In November 1934 I first went to the Margaret Hague Maternity Hospital as a clinical chemist. It was the depths of the Depression. I had a PhD in Zoology and had never heard of preeclampsia. In the late 1930s the predominant obstetric opinion was that the hypertensive disorders in and of pregnancy were attributable to renal insufficiency. Chronic hypertension in women of childbearing age pointed to chronic nephritis because the women were not old enough to have *senile plethora*, the term that Sir Thomas Clifford Allbutt (1836–1925) had used in differentiating what we now call essential hypertension from primary renal disease (Allbutt 1896). Chronic hypertension in pregnant women was called *nephritic toxemia*. Mild preeclampsia and, especially, gestational (transient) hypertension were called *low reserve kidney*.

William W. Herrick (1878–1945) a renowned internist, insisted that many pregnant women did have essential hypertension and that it was the most common form of hypertension found at follow-up. He suggested that all of the hypertensive disorders in pregnancy, including eclampsia, were manifestations of latent essential hypertension or renal disease brought to light by pregnancy. He generalized too broadly but seems to have been correct in the case of transient hypertension (Herrick and Tillman 1936).

German authorities for many years had advocated the prompt termination of eclamptic pregnancies to prevent aggravation of the supposedly underlying nephritis, and that concept led to the idea that prolonged preeclampsia-eclampsia could precipitate premature chronic hypertension or even cause it.

My early research in the field focused on the evaluation of renal function. Thus my initial idea was to use tests of kidney function in a follow-up study of women whose pregnancies had been complicated by high blood pressure. Physical examinations of the women were performed by … [two] residents on the obstetric service, and Harold Gorenberg an extraordinary internist. The American Committee on Maternal Welfare published a classification of the hypertensive disorders in pregnancy in 1940 while our study was in progress, which we then used to classify the patients. In their scheme rises in blood pressure to ≥140/90 mm Hg sufficed to make the diagnosis of preeclampsia. Because many of the multiparous patients met that criterion, we labeled them *preeclamptic*, which we discovered often turned out to be a mistake. In essence, *multiparous preeclamptic* made up half of the women with the *preeclamptic* diagnosis, because, as determined in a later study, many of them actually had frank or incipient essential hypertension. We concluded, erroneously, that prolonged preeclampsia had caused the hypertension in some of the women.

Characteristically, the diastolic blood pressure decreases significantly during pregnancy and rises again in the third trimester. Many women with hypertension, even of a severe degree, become normotensive during much of gestation. When their pressure returns toward or to prepregnancy levels late in pregnancy, it is likely to be mistaken for an acute onset, and the erroneous diagnosis of preeclampsia may be made. Usually the hypertension is the only sign, the women are without symptoms, and the pregnancy is allowed to proceed, often for several weeks. Such women are hypertensive at follow-up because they were hypertensive before pregnancy, not because of prolonged preeclampsia, which they did not really have.

In the literature one finds that the prevalence of hypertension at follow-up is higher in multiparous women than in primiparous ones, higher after mild than after severe preeclampsia, and higher after severe preeclampsia than after eclampsia; that is, the more reliable the diagnosis, the lower the prevalence of later hypertension. I began to doubt that preeclampsia causes chronic hypertension and was convinced when Alvin J.B. Tillman published an article showing that neither normotensive nor hypertensive pregnancy changed blood pressure at follow-up significantly from what it had been before the first pregnancy (Tillman 1955). I retracted my earlier conclusion (Chesley 1956) and abandoned follow-up studies of women without convulsions.

In 1935, one of the patients with eclampsia told me that two of her sisters had died of eclampsia within 6 months of the time that she had had it. I quickly found a high incidence of a history of convulsive eclampsia in the mothers and sisters of the women with eclampsia whom we were seeing at follow-up.

I had started such a study of women with eclampsia in 1935 and continued it until 1974 in collaboration with … [several colleagues] with periodic reexaminations of the women. Of the 270 women surviving eclampsia from the opening of the Margaret Hague Maternity Hospital in 1931 through 1951, all but three were traced to 1974 (Chesley et al. 1976). One of the three had no follow-up, one was seen twice in a period of 2½ years, and the third was examined six times over 26½ years.

We were painfully slow in recognizing that, even among women with eclampsia, those who are multiparous are different and should be analyzed separately. Among 206 women who had eclampsia in their first pregnancy and carried the fetus to viability, the prevalence of hypertension an average of 33 years later was not increased over that in unselected women matched for age in 11 epidemiologic studies of blood pressure. Remote deaths, up to 42 years after eclampsia, did not exceed the number expected for all American women matched for age. Only 29% of the remote deaths were associated with hypertension. In marked contrast, the prevalence of chronic hypertension was increased in the 64 women surviving eclampsia as multiparas; three times the expected number had died, and 80% of the remote deaths were associated with chronic hypertension. Many of the multiparous women were hypertensive before the eclamptic pregnancy. In a few cases we found recordings of prepregnancy blood pressures, but more often we found cardiomegaly, retinal angiosclerosis, and exorbitant hypertension, suggesting that the eclampsia was superimposed on chronic hypertension (Chesley et al. 1976).

Among the primiparous eclamptic women, 54 had later pregnancies, at least one of which was complicated by hypertension; only 9 of the pregnancies were accompanied by significant proteinuria. Apparently, the others had transient hypertension rather than recurrent preeclampsia, and the prevalence of remote hypertension was increased in that group. In contrast, 100 of the primiparous eclamptic women had later pregnancies, all of which were normotensive. The cumulative prevalence of hypertension, up to age 70, was only 10%—far lower than that in unselected women matched for age. We concluded that pregnancy is a screening test for future hypertension. Normotensive pregnancies, especially after age 25, predict a high likelihood of future normotension. Transient hypertension in pregnancy predicts the probability of future chronic hypertension. Preeclampsia predicts nothing about future blood pressure (Chesley et al. 1976).

Another feature of our follow-up study was the discovery that a genetic factor, possibly a single recessive gene, may determine the development of preeclampsia-eclampsia (Chesley and Cooper 1986). Over many years we found that preeclampsia-eclampsia had occurred in 37% of 147 sisters, 25% of 248 daughters, and 16% of 74 granddaughters of our eclamptic patients. The incidence in 131 daughters-in-law was 6%. Seven daughters had eclampsia, or 1 in 35, versus a general rate of about 1 in 1200. These results were later confirmed by a study from Iceland (Arngrimsson et al. 1990).

For each and every one of the three aspects of [our] article, it seems to me that one of the most important publications in the field is that of Marshall David Lindheimer's group at the Chicago Lying-in Hospital (Fisher et al. 1981). First, they reexamined at follow-up 53 women whose renal biopsies had proved the diagnosis of preeclampsia. The prevalence of hypertension was slightly less than the 10% among women in the National Institutes of Health survey, matched for age and race (Herrick and Tillman 1936). Preeclampsia had not caused chronic hypertension. Second, they also reexamined a control series of women who were matched with their preeclamptic subjects except that their pregnancies had been normotensive. The prevalence of hypertension was 0.5% (i.e., one twentieth the expected rate). Normotensive pregnancies predict a reduced likelihood of future hypertension. Last, the Chicago Lying-in Hospital has been a leading center of research in the field for 60 years. A blind review of renal biopsies with anatomic diagnoses confirmed the clinical diagnoses of preeclampsia in only 55% of cases. Five percent had no renal lesion, and 40% had the lesions of nephrosclerosis or other chronic renal diseases alone that had stimulated preeclampsia. Another 19% of those with the preeclamptic lesion had it superimposed on other renal lesions. The clinical diagnosis of preeclampsia was confirmed in 84% of the primiparous women, but only 24% of the multiparous subjects had the pure lesion of preeclampsia and another 14% had it superimposed on a renal disease. Thus the clinical diagnosis of preeclampsia in multiparous women was erroneous in nearly two thirds of cases, and among those who did have it, it was superimposed in 38% (Fisher et al. 1981). How

characteristic of preeclampsia are observations made in multiparous subjects with the clinical diagnosis?

Preeclampsia-eclampsia is overwhelmingly a disease of the first pregnancy carried to viability. Hans Hinselmann (1884–1959) in a survey of the literature, found >7800 cases of eclampsia described with enough detail for his analysis, in which he found that eclampsia is six times more common in primiparous women than in multiparous ones. The clinical diagnosis of preeclampsia in multiparous patients was usually erroneous, and when correct, in many cases it was superimposed on renal disease (Hinselmann 1924b). In such cases were the phenomena observed attributable to renal disease or preeclampsia?

I would suggest that editors reject any study of preeclampsia that includes multiparous women, unless they are analyzed separately.

(Chesley 2000, pp. 249–250)

Marshall David Lindheimer, of the University of Chicago and an *éminence grise* in the field of preeclampsia, has reminisced regarding Leon Chesley:

In the early 1970s Ralph Wynn then chair of Obstetrics and Gynecology at the University of Illinois in Chicago, telephoned, noting that a visiting professor, Leon Chesley, was writing a book devoted to hypertension in pregnancy, and wished to meet with me regarding the chapter relating to the kidney. I excitedly agreed, for then as an early career investigator I had published some work relating to kidney function during gestation, while clinically I was being asked to see more and more women whose pregnancies had been complicated by high blood pressure, most with suspected preeclampsia. Thus, on hearing Dr. Chesley's name I became excited as preeclampsia was an area that seemed to have few research achievements, and of the few I had found his publications were those I had cherished. Said otherwise, although never having met him he was already my hero.

First, I devoured the chapter sent for review, and a bit ebullient at that time (some might disagree that trait has passed!), when he arrived to discuss it I literally locked him in my office keeping him there for over 4 h. He listened quietly as I excitingly "overtalked," taking it in stride and tolerated my long phone calls and discussions for the remainder of his life. He got his revenge though as a speaker at my surprise sixtieth birthday party when he told the audience he had considered me amongst the very bright young minds in the field, but now realized that I "was not so young any more".

Also, in the early 1970s, aware that research in the hypertensive disorders of pregnancy especially preeclampsia, was sporadic at best, and that the few individuals working in the field, especially those from different disciplines, rarely communicated, the late Frederick Zuspan and I arranged an invitational workshop during 1975 at the University of Chicago, the results of which were published (Lindheimer et al. 1976). The keynote talk "False steps in the history of preeclampsia" has been republished in editions 2–4 of Chesley's *Hypertensive Disorders in Pregnancy* (1999, 2009, 2014). The Chicago meeting also gave birth to the International Society for the Study of Hypertension in Pregnancy. This group meets biannually, and bestows the coveted Chesley Award to an outstanding investigator for their contributions to the area.

Leon Chesley published his text at the end of 1977; it was a singled authored compendium that was to remain the major resource book for investigator and clinician for two decades (often kept under lock and key to escape "pilferage"!). In 1996, Leon having retired and recuperating from a minor stroke, Gary Cunningham, then the chair of Obstetrics and Gynecology at the University of Texas Southwestern Medical Center in Dallas, and chief editor of *William's Obstetrics* approached me with the following idea. While Leon had contributed to his text, but was now retired, needed was a full text like his devoted to hypertension in pregnancy, to be called *Chesley's Hypertensive Disorders in Pregnancy*. Following Leon's original it would be the second edition. We then approached Jim Roberts, director of the Magee Women's Research Institute in Pittsburgh, also principal investigator, to my knowledge, of the only NIH funded program project grant devoted solely to studying

preeclampsia. His group was the leading contributor to published research in the field. Unfortunately, despite the progress energized by both Leon's book, and the 1975 Chicago meeting, and a growing new international society on the hypertensive disorder of pregnancy, one of the three leading causes of maternal/fetal demise worldwide, remained one of the lowest research funded problems in terms of DALYs (Disability Adjusted Lost YearS). Upon our approaching Jim, Leon became even more excited to learn that the conference room at Jim's Institute was dedicated to Leon and contained his framed photograph. Thus there were now three enthusiastic editors dedicated to a second edition of Chesley's classic text.

During the ensuing 2 years the editors labored, and almost every other month Drs. Roberts and Lindheimer flew to New York City, picked up by Dr. Phyllis August of Cornell Medical School to spend a day with Leon on the outskirts of the boroughs of Queens, to discuss ideas and progress with him. A stroke had affected his hearing and the conferences were mainly through pad writing, the exchanges memorable. At his age and with his infirmities he still managed to complete a double cross-tix puzzle daily, and of course his remarks and advice were priceless. Also unlike his first edition, the second, reflecting the progress of two decades, could no longer be written by a single author and contained 19 chapters, each with two authors, one the "super" expert and the second one of the three editors, a maneuver that hopefully insured continuity and prevented duplication (as much as possible!) of text, table and figures. The text appeared at the end of 1998 entitled *Chesley's Hypertensive Disorders in Pregnancy*, edition 2 (1999), with, of course, Leon's ecstatic approval. Unfortunately, his stroke progressed and he died in March 2000 at the age of 92.

Two more editions, of *Chesley's Hypertensive Disorders in Pregnancy* have appeared, the 3rd edition in 2009 and 4th at the end of 2014, each like the conference room at Pittsburgh, with a photo of Leon watching to ensure its content. In many of the chapters in the 3rd and all in the 4th editions the text commences with an "editors comment", many noting Chesley's contributions to the topic the chapter covers. Also as these areas have become more complex many chapters now have two "expert" contributors and an editor author. With edition 4, a new chief editor, Robert Taylor, was added to follow Dr. Lindheimer's 3rd edition preface noting that an important reference text should not be chief edited by an octogenarian (we cannot all be Leons!).

(Letter from MDL to LDL, 1 June 2015)

James Michael Roberts, another modern-day pioneer in the exploration of the etiology/pathogenesis of preeclampsia-eclampsia (Woelkers and Roberts 2000), has challenged us with the question, "if we know so much about the disease, why haven't we cured it?" (Roberts and Bell, 2013). Roberts has written:

As a resident I was encouraged to do research. I worked with Robert Jaffe and Uwe Goebelsmann and although it was profitable, the experience was compromised by my busy schedule as a resident, thus I concluded I did not like research and looked for another path to academia. I was interested in internal medicine and eventually trained in medical hypertension. For my first several years on faculty, I attended in the Internal Medicine Hypertension Clinic. My passion for research was awakened by my training in the Cardiovascular Research Institute at the University of San Francisco California. However, my research originally was in signal transduction and had nothing to do with hypertension.

After several years of successful fundamental research I was approached by Bob Jaffe to head a Program Project Grant (PPG) on a topic of my choosing. I decided to study preeclampsia, a decision that had major effects of my future research and my career in general. I was well funded and decided to approach the study with an organized strategy. I assembled colleagues with expertise about preeclampsia. Together we identified what we considered the important questions. The next step and one that had a great impact on my career was to assemble experts who could answer these questions, but who in most

instances knew nothing about preeclampsia. The results were astounding. We subsequently submitted the PPG, which was funded and had a major impact to modify research. We addressed the pathophysiology of preeclampsia, which we considered far more than hypertension in pregnancy. One of our first publications, "Preeclampsia: an endothelial cell disorder" (Roberts et al. 1989) was the third most cited publication in obstetric research for the last 50 years (Brandt et al. 2010). The success and excitement of encouraging a diverse group to merge their expertise to address questions eventually led me to take the job as Director of the Magee-Womens Research Institute at the University of Pittsburgh to facilitate this research approach.

I had two major influences on my career and life. The first was from my scientific mentor and best friend Alan Goldfien. Allen had a superbly sensible and ethical approach to research and a remarkable approach to life as an academic investigator. He was brilliant, but more importantly he was wise. The other influence was my hero, Leon C. Chesley. I knew Leon largely through his work until late in his life. From his work I was impressed by his clear thinking and use of several strategies to answer questions of interest and importance. He was the first to apply biochemical principals to the study of obstetric issues, but also authored some of the most complete epidemiological studies of outcomes in preeclampsia. In this setting it was most impressive that he has the integrity to admit that his original contention that preeclampsia caused later life hypertension was not correct (Chesley 1956). In his later years he visited the Magee-Womens Research Institute for the naming of a lecture room in his honor and to deliver a remarkable talk at age 87. With Marshall Lindheimer and Phyllis August I then began to visit him at his home a practice that continued until his death. It was a delight to visit him, and I had hoped to acquire an oral history of the early years of obstetric research. However, Leon reverted to his "salty" personality and most information was unprintable.

Currently my major effort is directed at facilitating collaborative research. I head a Bill and Melinda Gates Foundation funded project called the Global Pregnancy CoLaboratory (CoLab). The goal of this project reflects my current thinking about preeclampsia. As pointed out in the article that I did with Mandy Bell (Roberts and Bell 2013), there is virtually no question that preeclampsia is more than one disorder. With CoLab we have brought together 27 centers from around the world including over 300,000 pregnancies to share data and resources to attempt to resolve this complexity.

However, despite an exacting and rewarding career of discovery my greatest pride is my mentoring. I have mentored close to 100 MDs and PhD at several points in their careers and a gratifying number remain in academic careers. My proudest accomplishments are mentoring awards from the NICHD and the Society for Gynecologic Investigation, now the Society for Reproductive Investigation.

(Letter from JMR to LDL, 22 September 2014)

Another who is contributing to our understanding of the pathophysiology of the hypertensive disorders of pregnancy and their effects on the fetus is Ian Michael Bird of the University of Wisconsin. He recounts how he got there and what we believe.

As I finished my undergraduate training in Biochemistry in the 1980s the field was making a transition from traditional protein based enzymology, microscopy driven structural biology and tissue metabolism, to the new emerging world of molecular biology and cell biology. Protein purification and sequencing were still a means to identifying what we now consider the product of alternate splicing or gene families, and a PhD was awarded for isolating and sequencing a single cDNA. New areas in cell biology were beginning to look at extracellular matrix proteins and their effects of cell function. PCR became established and new variants such as asymmetric PCR were hot areas of study. Cell signaling was emerging from the post cyclic nucleotide era and since at that time, my undergraduate

teachers at University of Birmingham, UK included Robert H. (Bob) Michell, PhD, my generation appreciated the phosphoinositide signaling pathway and its intimate relationship to intracellular calcium (Ca^{2+}) signaling. In certain respects I was lucky to learn many older techniques and at the same time be exposed to newer ways of thinning as the pace of change was picking up. Just as with computers, actually having worked on every generation of PC ever made is a huge advantage to understanding technology (and far more than most will appreciate when looking back with disdain).

My PhD training was in the emerging field of phosphoinositol signaling in a 'lipid lab' of Tony Smith, PhD at the Middlesex Hospital (London UK). This was valuable experience because quite apart from understanding those new areas signaling, I also got an early appreciation of the fact membranes are subject to all kinds of regulation according to the proteins and lipids in them, and that the plasma membrane is far from a uniform structure. The fact that proteins and lipids influence each other means all kinds of structured microdomains exist within a single plasma membrane and they can each have different specialist function.

In the short time of my graduate training the 'basic' phosphoinositide and associated Ca^{2+} imaging field rapidly became very crowded. If I had stayed in just that one field I would have become one of very many competing to do the same work. I decided to follow more along the path of adrenal steroid endocrinology in a medical school setting rather than basic cell signaling in a biochemistry department. I accepted my first postdoctoral fellowship at the Royal Infirmary Edinburgh, working with Simon Walker, MB, BS, DM and Brent C. Williams, PhD. The goal was to extend my work on adrenal cells phosphoinositide metabolism and now to include Ca^{2+} imaging with Fura 2 to study their roles in the endocrine control of steroidogenesis. I did not realize then how important this would be later in my career. I also learned throughout my first Postdoc position that there were two levels of publications emerging, and the very best labs were not limited by reagents they could not buy or put off by complexity of methodology. They just grasped the nettle and did whatever was necessary to answer a question. As daunting as it was at the time, I committed to the same approach in the career. I learned that the best labs also mastered techniques, they did not just perform them, and I committed to do the same. It turned out to be a wise move.

In considering my second postdoctoral fellowship, I was offered the chance to visit The Green Center at University of Texas Southwestern Medical Center, Dallas to look at the longer term endocrine control of zonal steroid biosynthesis through changes in gene expression. I had seen first-hand how far the molecular field had come and realized this was an area I had to master as a part of my studies in endocrinology, or be left behind. Again this turned out to be a wise move. Quite apart from an exceptionally productive time working with Drs. J. Ian Mason and William E. (Bill) Rainey on control of P450 expression, I learned a whole new area of science and way of thinking. One additional skill I picked up from Bill was to really talk and plan a project, mapping out manuscript figures in principle even before we even did the experiments, and then fill them in one at a time. It was a really efficient way to make progress.

In considering a future career in independent research, I recognized I now had a very strong cross training in biochemistry, pharmacology, cell biology and molecular biology. Throughout my pre and postdoctoral positions a constant theme had been the action of Angiotensin II to control adrenal function. At that time the initial cloning of the first AT1-R cDNA was published and it was key in my deciding to stay beyond the 6 month planned. (It did not hurt that I had also met the person who was to become my wife!) One of the most important molecular studies I performed in Dallas included some of the first characterization of AT1-R mRNA expression and how it is regulated by multiple stimuli. It was our ability to study, AT1-R message, AT1R and AT2-R protein by antibody and ligand binding, phosphoinositol formation, Ca^{2+} imaging and steroid output that was key to these studies. As my work in the field grew and I worked on more and more physiologically relevant

questions of the role of Angiotensin II in the control of adrenal zonation, I did not realize this systems approach to signaling integration and control of cell function was laying the foundation for a future as yet unknown career.

Around the time my work was reaching a peak in this field I was also beginning to reach the ceiling of my salary path as a postdoc. I had gained my PhD at 24 and then spent 5 years in two highly productive postdoctoral positions. As I passed 28 I began looking at new positions on faculty. At that time I was offered two positions: one in a soft faculty position, and the other at University of Wisconsin, department of obstetrics and gynecology on tenure track. Both studied receptor expression and function but the Wisconsin position was also related to the possibility that in preeclampsia the core defect was indicated by a failure to alter endothelial response to infusions of Angiotensin II. The assumption Ronald R. Magness was making in seeking to appoint me at the time was this was a defect in AT1-R signaling and I was the most likely to work it out. As it turns out, there was indeed a normal change in expression of AT1-R in uterine artery endothelium in pregnancy, but my training outside the vascular field told me this alone was not really enough as a sole defect to cause preeclampsia. Far more agonist responses were also likely defective and it seemed to me it was far more likely there was a post receptor point of signaling convergence that was failing, rather than a receptor defect for each of the hormones in question. What was clear was we really needed to get the cells out of the sheep vessels and into cell culture so we could see what was really going on. Initial studies with inositol phosphates measures and Ca^{2+} imaging (at Loma Linda University with my now very good friend Lubo Zhang) showed very quickly that even in normal pregnancy the phosphoinositol and Ca^{2+} responses to Angiotensin II in uterine artery endothelium were not of the great magnitude we expected or had assumed, and certainly were far less than I previously had seen in adrenal cells. Ligand binding suggested just a few hundred per endothelial cell rather than thousands of receptors in adrenal cells. It was clear that other agonists must also be contributing to increased vasodilation in pregnancy, and while some were thought to do so through kinases acting on eNOS or cPLA2, I also guessed this was shifting Ca^{2+} sensitivity to agents such as ATP and bradykinin, rather than acting as a substitute for Ca^{2+} (and we stated that in our 2003 review (Bird et al. 2003)). In discussions with Lubo Zhang he informed me the common ligand many used in such endothelial studies was ATP and perhaps we should try that. It was in making this switch that it became clear the cells from nonpregnant and pregnant ewes showed clear differences even after being in culture for two weeks, and so we had an early assay for pregnancy adaptation of cell signaling. Not long after we began to see preliminary evidence that kinase signaling could also be pregnancy adapted.

From that point onwards I have been guided by my very earliest work on membrane biochemistry and an understanding of the cell as it sees hormone signaling in reality- from the inside out. A cell does not know what just arrived at the surface, it only knows the complexity of signaling it sees inside the plasma membrane and then the cytosol of the cell. From my adrenal work, I understood that complex cell endpoints can be regulated by multiple receptor types controlling competing signaling processing that then integrate to determine cell function. As the field of eNOS activation and regulation progressed we studied the many phosphorylation sites and how they changed with exposure of cells to ATP (Sullivan et al. 2006) or to a growth factor known to both drive angiogenesis in pregnancy and be elevated to excess in preeclampsia, namely VEGF (Grummer et al. 2009). Comparative work of these responses in nonpregnant and pregnant derived uterine artery endothelial cells in culture was incredibly successful in showing that while underlying eNOS phosphorylation by kinases was altered and indeed explained part of the story, it was really Ca^{2+} signaling that was key (Boeldt et al. 2011; Yi et al. 2011). An analogy we often use now is that phosphorylation is the music mixer (balance, treble and base), but the Ca^{2+} was the volume control.

One of the most important steps we took in the last 10 years was to attempt to show our work to be relevant in physiologic terms, and this was made possibly when FuXian Yi

joined our laboratory. He had developed the use of DAF-2 imaging to quantify NO in vascular endothelium and spent about 2 years setting up that work in our laboratory. It was this ability to now image both Ca^{2+} and NO in single cells while still on the luminal surface of the intact vessel (Yi et al. 2011) that gave our group such a huge edge in the field. We were able to now show that whatever interfered with Ca^{2+} signaling in these cells also interfered with NO output. As we switched from older style photometry measurement of Ca^{2+} output from uterine artery endothelial cells to video imaging of many cells at high density, cells from pregnant sheep showed a repeated burst pattern that was synchronized between cells, and this response in intact vessels always accompanied the prolonged and greater magnitude NO output of pregnancy in the uterine artery. Agents blocking Ca^{2+} burst always blunted NO (Boeldt et al. 2011; Yi et al. 2011).

It was also at this point, 16 years after starting to ask what is pregnancy adaptation, our thoughts turned to how that response could be lost in preeclampsia. One of the key observations in Dr. Yi's study was the fact the bursts were cell density dependent and it was highly likely this was due to cell-cell communication. It did not take us long to identify that the channel involved was Connexin 43 forming cell-cell gap junctions. While cell expression of Cx43 in nonpregnant and pregnant derived cells was constant, it was clear cell-cell communication was higher in cells from pregnant ewes and anything that interfered with that communication prevented Ca^{2+} bursting and NO output. We initially undertook a number of pharmacological and endocrine treatments of agents known to inhibit Cx43 by phosphorylation or direct disruption as a proof of this principle but as we continued to search the literature for such reagents it stuck me many such studies were about wounding, and particularly non healing wounds. As I read many studies and read particularly the reviews by Lampe and colleagues (Solan and Lampe 2009), I also started to notice that many of the hormones present in non healing wounds were familiar and often reported to be elevated in preeclampsia. Our most recent work in the past 5 years has focused on this theme, and we have found factors directly or indirectly leading to the activation of ERK and Src result in phosphorylation and closure of Cx43 gap junctions in pregnant adapted cells (Bird et al. 2013; Boeldt et al. 2015). We also recently confirmed in human umbilical cords of preeclamptic subjects that they do lack extended Ca^{2+} bursting and show blunted NO output compared to that seen in normal cords (Krupp et al. 2013). We have shown in uterine arteries and umbilical vein endothelium from normal pregnancies that even brief (<1 h) exposure to growth factors and cytokines blunt these responses down to a nonpregnancy or preeclamptic level of dysfunction. We are now extending the work in these areas to look at long-term effects of these same growth factors and cytokines over many hours, and shown they also promote breakdown of the membrane environment in which Cx43 exists through the same ERK and Src pathway (unpublished).

So we are back to my home ground, looking at the cell integrating complex signaling inputs on a more holistic scale rather than one agonist at a time. A picture now has emerged that in normal pregnancy cells respond not only to ATP but to a variety of phospholipase C Beta coupled agonists that can promote prolonged Ca^{2+} signaling and this normally favors vasodilation as needed. In the event of fine tuning being necessary, elevation of mild kinase activators such as growth factors can limit vasodilation as the cells consider moving to angiogenesis, but this is a reversible and indeed the normal choice. However, in the case of severe and prolonged hypoxia and in the presence of marked increases in cytokines (from endothelium, placenta and/or immune cells inappropriately activated) then a wounding phenotype begins that is likely co-amplified as time goes on and uterine perfusion falls. Even if each patient begins with a different tissue producing only a sub set of growth factors and cytokines to initiate the disease, in the secondary phase of endocrine co-amplification the full wounding cocktail emerges. Once this is clearly established the activation of ERK and particularly Src by these many factors becomes overwhelming and, if not kept in check, ultimately becomes destructive. As these wounding cocktails spill into the general circulation, enhanced vasodilation also is lost systemically, and a wounding phenotype

> dominates to misrepresent the pregnancy to the mother as a wound incapable of healing. The body's natural response in this case is to restrain blood to the wound as would then be expected, no matter how tragic. In some vascular beds monolayer integrity is lost and edema and proteinuria results, with possible seizures as the blood brain barrier also declines. Without removal of the placenta (which is now the focal wound site, even if it was not initially) there is no solution, or at least not yet. It now is our hope that this new wounding model of preeclampsia will guide us to new treatments targeting the inhibitory signaling pathways and restoring Cx43 function. To me this truly is an endocrine broad spectrum disease resulting in plasma membrane dysfunction and the answer is to stabilize and restore plasma membrane function. To date all treatments have been aimed at relaxing smooth muscle. If we succeed we will restore endothelial function for the first time in preeclamptic subjects and provide a brand new therapy potentially that can be used in addition to current therapy. If we succeed we will take a major step to combatting this devastating disease that has proven so resistant to treatment . . .. Finally—I have already seen many changes in research technology and approaches in the past 30 years. The incredible developments in systems biology, big data analysis and high throughput assays will continue to revolutionize the research world. Even if I started again now it would be a completely different path. But one thing remains true—training in one field and moving into another prevents you accepting the assumptions of your new discipline. You are much more likely to see the flaws in conventional thinking. You also have to see the big picture and the small detail you are working on at the same time. You must understand how greater processes of physiology (or even social biology) and the molecular sciences integrate at the same time. Without an understanding of normal health and disease there is no way to identify what the therapy needs to do in general terms. Without identifying a molecular target there is not therapy. To me research success both in the past and in the future still requires us to understand these opposite sides make the same coin.
>
> (Letter from IMB to LDL, 28 October 2015)

The presence of a placenta is both necessary and sufficient to result in the hypertensive disorders of pregnancy, and women with a hydatidiform molar pregnancy are at greater risk for developing this complication as compared to normal pregnant women (Page 1939). A major gap in our knowledge is to understand the role of oxidative stress (Hubel 1999; Redman and Sargent 2000), including that of hypoxia-reoxygenation (Burton and Hung 2003), inflammatory response (Burton and Jauniaux 2011) with tumor necrosis factor-α (Alexander et al. 2002; Hung et al. 2004), and other factors in endothelial dysfunction. A current challenge in the prediction and/or diagnosis of the hypertensive disorders of pregnancy is that of obtaining appropriate clinical data to combine with identifiable biomarkers (biochemical, cell free DNA, RNA) that play a role in the involved cellular mechanisms (Fu et al. 2013; Kenny et al. 2014).

11.6 HELLP Syndrome

Hemolysis, in conjunction with abnormal liver function tests and thrombocytopenia, has been recognized for many years as a complication of severe preeclampsia-eclampsia. Yet it remained until the early 1980s for Louis Weinstein (Fig 11.5) of the University of Arizona, Tucson, on the basis of 29 patients with these

Fig. 11.5 Louis Weinstein

complications, to coin the term HELLP syndrome ("H" for hemolysis, "EL" for elevated liver enzymes, and "LP" for low platelet count) (Weinstein 1982). Such patients often constitute a diagnostic dilemma for the physician, who may view the hematologic picture of crenated erythrocytes (Burr cells or spinocytes) and irregularly shaped schistocytes (split cells; also called helmet cells), typical of microangiopathic hemolytic and other anemias (Brain et al. 1962), hemolysis thrombocytopenia, hepatic disorder with possible hemorrhage, and/or uremic syndrome as separate and/or unrelated entities (many patients do not, in fact, have hypertension). Experimental evidence suggests the cell confirmation is a consequence of interaction between red cells and fibrin strands under arterial flow conditions (Bull and Kuhn 1970). Patients with this syndrome are subject to increased risk of adverse outcomes, including severe epigastric pain associated with hepatic subcapsular hemorrhage and an increase in disseminated intravascular coagulation. Another major consideration is that of death and/or death of her fetus/newborn infant, such as the one mother with severe preeclampsia and microangiopathic hemolytic anemia and three infants in Weinstein's original account. Since this first description, the natural history has been detailed (Martin et al. 1991), and there have appeared well over a thousand reports of many thousands of patients with this life-threatening disorder.

In response to my query about this discovery, so relatively late in the history of preeclampsia-eclampsia, Weinstein detailed his "great ride":

> It is circumstances and proper timing that give an action its character and make it either good or bad (Agesilaus, 444–400 BCE).
>
> This quote, written in approximately 400 BC, is as true today as it was when it was stated. Timing is everything. There are many examples of the value of timing and the importance of observation that are applicable to medicine. In 1846, Ignaz Philipp Semmelweis (1818–1865) was appointed first assistant (lecturer) in Division I (the lying-in hospital) of Vienna's *Allgemeines Krankenhaus* [general hospital], where the medical students practiced obstetrics. It was in the hospital's Division II, where obstetrics was

practiced by the midwives, that Semmelweis observed a much lower mortality rate among the patients who were delivered, as related to puerperal infection. The importance of observation, timing, and being in the right place presented itself when Semmelweis's colleague, Jakob Kolletschka (1803–1847) cut his hand while performing an autopsy on a post-partum patient who had died of childbed fever. Semmelweis observed that Jakob's friend's death was identical to that of the women who died from puerperal fever and that the cause was likely to be from the transmission of the putrid organic material from examiner to patient. By the initiation of the simple task that was instituted by Semmelweis of scrubbing one's hands in a chlorinated lime solution between patients, a marked decrease in the mortality rate was noted in the postpartum patient on the medical student ward (Semmelweis 1861). A similar story exists about the discovery of penicillin by Sir Alexander Fleming (1881–1955). It was his observation of the inhibition of the growth of bacteria around the site of a mold that was growing in an open glass dish that had been left in his laboratory that led to the discovery of the antibiotic penicillin, which revolutionized the treatment of bacterial infections (Fleming 1929).

Both of these examples demonstrate the power of being in the right place at the right time and making some simple observations. This principle presented itself during my career and led to my discovery of the variant of preeclampsia now called hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome (Weinstein 1982).

In 1979 after completing my obligatory military service, I was recruited to the University of Arizona to be their first maternal-fetal medicine fellow. At that time, fellows functioned in an independent manner and took call independently as a faculty member. In early 1980, I received a telephone call from an Indian Health Service physician in Tuba City, AZ, about transferring a patient to the University hospital in Tucson. The history of the patient was that she was at 29 weeks of gestation, had minimal elevation of blood pressure, 1 to 2+ proteinuria, blood sugar of 25 mg dl^{-1} (patient was lucid), platelet count of 52,000, hematocrit level of 30%, and abnormal liver function test results. It was being the attending physician on call that started my process of being in the right (or wrong) place at the right time.

Obviously, it was apparent to me on the telephone that the laboratory workup of the patient was in error and that I would correct this when she arrived. The patient, who was brought by air transport, arrived late in the afternoon. Her history and physical examination were unremarkable, except that she stated that she had felt sick for approximately 1 week and that she had experienced right upper quadrant pain for several days. Her blood pressure was 130/84 mm Hg, and she had 2+ proteinuria. She was lucid and able to answer all questions but was quite stoic, as were most of the Native American patients. As I was not able to determine the presentation of the fetus, an ultrasound examination was performed that revealed the fetus to be anencephalic. Laboratory testing was performed and revealed a blood sugar of 30 mg dl^{-1}, platelet count of 35,000, hematocrit level of 25% with schistocytes and Burr cells on peripheral smear, liver function test results that were ten times normal, and an elevated bilirubin level, with the majority being indirect. She had no evidence of any active bleeding or uterine activity.

The clinical picture was very confusing to me, so I consulted two of the best physicians I have ever known, my mentors, C. Donald Christian and William Droegemueller. Neither of them was sure of the clinical diagnosis; but, because of the concern for acute fatty liver, we all agreed to proceed with delivery and to support the patient metabolically. We administered intravenous fluids, and labor induction ensued. Six hours later she was delivered of an anencephalic infant. Over the next 18 h, the patient's platelet count continued to decrease; her hematocrit level decreased because of hemolysis with no active bleeding, and she remained profoundly hypoglycemic but lucid. A solution of 10% dextrose was administered intravenously with no change in blood sugar levels; the plan was to consider plasmapheresis. Approximately 24 after delivery, she became comatose, experienced cardiorespiratory arrest, and was unable to be resuscitated.

Postmortem examination revealed a massively swollen liver with multiple petechial hemorrhages, a large quantity of ascites, a pancreas with internal hemorrhage, and no

obvious cerebral findings. Microscopic examination revealed disruption of both liver and pancreas cells with no obvious necrosis. A small amount of fatty deposits were present in the liver, but these were not consistent with the diagnosis of acute fatty degeneration. Although the patient probably had preeclampsia, we had no adequate explanation for her death.

This had a terrible personal impact on me. I had never experienced a maternal death, and I felt that it was my fault that the patient died. I then decided to devote much of my time to determine the cause of her demise and to try to educate myself as to how to treat the next patient with similar findings.

My initial thought was that the patient had some variant of preeclampsia. I started by doing an extensive literature search and looking for any pregnancy that was complicated by hemolysis, abnormal liver functions, thrombocytopenia, and/or hypoglycemia that was associated with maternal death. I was amazed by the number of articles, many of which were not in the obstetric literature and many of which described patients similar to the one I had treated. The key article that described a similar entity was the report of three cases in the *New England Journal of Medicine* in 1954 by Pritchard et al from Texas. Their three patients all experienced eclampsia with 1 survivor. McKay in 1972, Kitzmiller et al in 1974, and Killam et al in 1975, all reported patients similar to the patient that I had treated, each being linked to preeclampsia. There were many other isolated reports in the medical literature of the presence of hemolysis uremic syndrome and thrombotic thrombocytopenic purpura. The surgical literature described the entity of spontaneous rupture of the liver that occurred in pregnant patients who all appeared to have findings that were compatible with preeclampsia. The paper that impacted me the most was by Goodlin in 1976, "Severe pre-eclampsia: Another great imitator" (Goodlin 1976). It was becoming more obvious to me, even early in my career, that preeclampsia was truly the great imitator. Now that I am a senior clinician, I am absolutely convinced of this.

After I had finished educating myself by my literature review, I sent a notice to the physicians who were practicing obstetrics in Arizona that I was looking for pregnant patients with unexplained thrombocytopenia, hemolysis, and elevated liver enzymes and that I would care for them if they were referred. Over the next 30 months, I had the opportunity to care for 29 patients and to learn much about this variant of preeclampsia. As I put the information that I gathered together, I appreciated that I was seeing a form of preeclampsia that often did not have hypertension of >140/90 mm Hg, proteinuria, or edema. I was able to determine that the most of the women who were affected had generalized malaise during the week before hospital admission that was out of proportion with her being in the late second or early third trimester. Many of the patients were experiencing nausea with or without vomiting and right upper quadrant pain. Looking at the natural progression of this entity, it appeared that the thrombocytopenia occurred first, elevated liver enzymes second, and hemolysis third. Also, the disease was progressive, and delivery was the only means of ending the process. However, approximately 25% of the patients had their worst manifestations during the postpartum period. What became apparent to me was that I was seeing a variant of preeclampsia.

As I started to put together the data for the 29 patients that would result in the 1982 *American Journal of Obstetrics and Gynecology* article, I wanted to help educate my fellow clinicians to assist them in recognizing these patients to prevent what happened to the first patient who I had seen with this entity. It was my belief that I needed to have a distinctive name for this "syndrome" so that most physicians would not forget it once they heard it. The problem with these patients, I realized, was not how to care for them when diagnosed but how to recognize that they were sick and needed care. It was then that the light dawned on me that what these women needed was what the entity should be called; therefore, the term HELLP syndrome was born. I was pleased that this term was both descriptive and reminded the clinician of what to give to the patient. I then collected data for 57 patients, which resulted in a second article in 1985 (Weinstein 1985). My colleague at the University

of Tennessee, Baha Sibai, went on to publish several larger series, because his patient volume was much greater than mine (Sibai et al. 1986; Sibai et al. 1993). What has been most interesting and gratifying since the original 1982 article is the plethora of papers that have been published about patients with HELLP syndrome. Little has changed in the description: The entity is still a variant of severe preeclampsia, and delivery remains the primer therapy.

Over my academic career, I have been called numerous times to discuss the treatment of patients with HELLP syndrome and have reviewed many medical records to determine whether negligent medical care occurred. Several observations that I have made are clearly repetitive in nature.

The first observation is that the disease is progressive, and often the patients do not look very sick. The second observation is that the major marker for death is the presence of hypoglycemia and that the blood sugar should be checked frequently during the labor process and that every attempt should be made to keep the blood sugar at >60 mg dl^{-1}. Often the patient will need an infusion of 10% dextrose or a push of 50% dextrose to maintain the blood sugar level. I believe that the hypoglycemia is related to decreased glycogen stores in the liver and increased levels of circulating insulin from disruption of the acini of the pancreas from cellular edema. The third observation is that I cannot tell from either the history or physical examination the levels of the patient's platelet count or liver enzymes. Therefore, I often order these tests with minimal clinical indications other than the possibility of preeclampsia. The fourth observation is that, of all the records that I have reviewed of patients who died from HELLP syndrome, the most common family of drugs that all patients have received in the days before death is not an antihypertensive or magnesium sulfate but an antacid. Most, if not all, patients with HELLP syndrome complain of "heartburn" and therefore have been advised to take an antacid. If a patient complains of heartburn or right upper quadrant pain during the third trimester, clinical and laboratory evaluations are warranted. The key to the treatment of any patient with suspected HELLP syndrome is simply recognition. Once the clinician has identified that the entity is present and that it is a variant of severe preeclampsia, delivery can be expedited, with the prolongation of pregnancy offered only to administer antenatal steroids for the benefits of the developing fetus.

I am somewhat pessimistic that any direct single cure will be found for preeclampsia. I believe that it is a disease of the placenta and microvascular system that has an immunologic basis. It is a form of rejection of the paternal half of the fetal genetic makeup and signifies that it is best for the baby to leave the hostile intrauterine environment and began life in a brave new world.

Currently, much research is being performed to evaluate the immunologic basis for the disease, but little progress in the clinical management has ensued. I believe that the cure for preeclampsia will remain elusive but that we shall improve in prolonging the pregnancy and safely inducing labor when necessary. I have done many things during my academic career and am proud of my accomplishments and articles. The question we all need to ask during and near the end of our careers is "Did we truly make a difference?" I believe that my experience with this patient who died allowed me to identify this entity and to make other health care providers aware of the serious nature of this disease so that proper medical treatment can be offered and morbidity and death can been avoided. I believe that the time and effort that I put into learning, writing, and teaching about HELLP syndrome have resulted in better care and possibly the saving of life for other patients. I am truly grateful to my teachers and mentors who planted within me the seed for scientific curiosity and the desire to make a difference. I can truly say that it has been a great ride.

(Weinstein 2005, pp. 860–863)

This is a remarkable illustration of the manner in which careful observation can contribute to clinical diagnosis and treatment, even in the present day.

11.7 Mental Health and Neuropsychiatric Issues

A growing recognition is that of the public health challenge of staggering proportions of the large number of individuals with mental health problems, constituting perhaps as much as 10–15% of the global burden of ill health. Of all medical causes, mental and substance abuse disorders lead in years lost to disability (Whiteford et al. 2013). Pregnancy constitutes a period of unparalleled change in the life of a woman, with satisfaction and hope for the future. Nonetheless, for many mothers to be and their families, the perinatal period can be a time of intermittent or chronic stress and challenge overshadowed by mental illness. Although until recently postpartum depression has attracted the chief focus of attention, depression also can be manifest during the antenatal period itself. A sobering reality is that of about 4 million births per annum in the USA, approximately 10% are complicated by antepartum and/or postpartum depression, and this figure is severalfold greater among teenage and low-income mothers (Kozhimannil and Kim 2014).

As is well appreciated, neuropsychiatric abnormalities constitute a wide spectrum of both psychotic and nonpsychotic disorders, and diagnostic criteria have been presented (Howard et al. 2014a). Bipolar disorder, affective psychosis, and schizophrenia are several of the major mental disorders, and affected patients require individualized diagnosis and care (Jones et al. 2014). Also of concern, depression is more common than previously believed and may have adverse effects on offspring in terms of brain development and subsequent social and emotional health (Kozhimannil and Kim 2014; Stein et al. 2014). Of note, pregnant women with depression face the dilemma and ambiguity of whether to take their selective serotonin reuptake inhibitors (SSRIs) or other medication and face the danger of injury to the fetus or to eschew the medication and attempt to deal with the horrors of the disorder "cold turkey." A New York Times essay by Andrew Solomon explores several case studies of the nuances of such decisions, pregnancy for these women being a state of "universal lent" and often living in a "penumbra of regret and guilt" (http://www.nytimes.com/2015/05/31/magazine/the-secret-sadness-of-pregnancy-with-depression.html).

Diagnosis in psychiatry, in contrast to most of medicine, remains restricted to subjective symptoms and observable signs. The pathogenesis of perinatal depression and associated disorders is poorly understood; however, studies in both humans and laboratory animals suggest possible interconnected factors, including hormonal dysregulation, particularly of the hypothalamic-pituitary-adrenal or ovarian axis, genetic vulnerability, as well as epigenetic mechanisms (Kozhimannil and Kim 2014). Recently, psychiatry has undergone a tectonic shift as the intellectual foundation of the discipline begins to incorporate concepts of contemporary biology with neuroimaging and physiology, especially cognitive, affective, and social neuroscience with the launch of the US National Institute of Mental Health "precision medicine for psychiatry" project (Insel et al. 2010), so that "mental disorders" may be redefined as specific "brain circuit disorders" (Rosa and Lisanby 2012).

Mental disorders, often hidden and underestimated in their effects are growing in recognition and prominence. The UK's Royal College of General Practitioners' report *Falling through the Gaps: Perinatal Health and General Practice* noted the description of "... a predominantly rushed, reactive and unreliable system of identification and support which often led them to fall through the gaps in this system of care" (Khan 2015, p. 23). The diagnosis and treatment of mental illness during the perinatal period is of vital importance, in part, because in developed countries suicide is a major cause of maternal deaths (Cantwell et al. 2011). Although in developing countries maternal suicide may be as common, its prevalence is not known with certainty because of problems with reporting (Fuhr et al. 2014). Treating the mother's disorder may not be sufficient to mitigate the impact on the child, and evidence suggests that depression in the mother raises the risk of depression in the offspring even into late adolescence (Pearson et al. 2013). In developing countries, perinatal depression is associated with poor infant growth and stunting, and this may be aggravated in those children with socioeconomic disadvantage (Stein et al. 2014). As a complication, comorbidity is more prevalent in these societies, thus making perinatal mental illness of even more consequence.

From an economic standpoint, in the UK, mental health disorders during the perinatal period are estimated to exceed $11 billion for each annual cohort (National Institute for Health and Care Excellence 2014). Unfortunately, these costs are a scant reflection of their effects and consequences of the well-being of affected parents and their families, particularly the children at a vulnerable age in their development. If the mother is receiving medication, this may impact the fetus, as it may the infant in cases of postnatal depression (Howard et al. 2014a; Stein et al. 2014).

In summary, the physical, emotional, and economic costs of perinatal mental disorders are great, not only for the mother, but for the offspring as well as family. In developing countries, this may be even more true. Evidence supports the maternal and child physical and mental health and economic benefits of early intervention for mothers and children in adversity. A major challenge in this regard is that governmental leaders, legislators, insurance providers, healthcare professionals, and others in both developed and developing countries need to dedicate themselves to improve mental healthcare throughout the globe. Such measures should be a critical aspect of any new sustainable development goals (Howard et al. 2014b; Kozhimannil and Kim 2014; Thornicroft and Patel 2014). For neuropsychiatric disorders as well as other clinical problems, with ever-increasing individualized and personalized precision medicine, and our ability to harness vast amounts of new knowledge, revised disease classification and more targeted treatment options will become the norm (Jameson and Longo 2015).

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Chapter 12
Fetal Growth and Its Restriction

We are too late for the gods and too early for the being.
Being's a poem, just begun, is man.
Martin Heidegger 1971

12.1 Early Studies

In his essay "On being the right size," the British geneticist, biometrician, and popularizer of science John Burdon Sanderson (JBS) Haldane (1892–1964) theorized on and presented some mathematical analysis for the optimal size of various organisms. In addition to consideration of body mass, he included surface area, weight-bearing bone structure, respiratory and circulating mechanisms, and other features of comparative anatomy and physiology (Haldane 1927). His analysis extended from insects to birds to a number of mammals which varied in size from mice to the rhinoceros. Haldane concluded ". . . that for every type of animal there is an optimal size" (Haldane 1927). A number of studies have disclosed that even for the fetus development follows clearly defined mathematical principles (Roberts 1906; see below). Prior to full maturity, timely growth, both physical and mental, is one of the best indicators of a fully functional physiological system and health. As is appreciated, optimal growth of the embryo-fetus and its various organs is a complex process that is a function of genetic makeup, state of maternal health, the availability of nutrients and oxygen, as well as a multitude of growth factors and hormones of maternal, placental, and fetal origin. In addition, a host of environmental factors that influence epigenetic programming play vital roles in this process. These factors are associated with physiologic, biochemical, and molecular changes, most of which are only poorly understood (Cheek 1975; Timiras 1972; Winick 1972). From mid-century onward, considerable emphasis has been placed on the mechanisms of cell division and multiplication (hyperplasia) and cell enlargement with cytoplasmic growth (hypertrophy) that result in the deposition of new tissue and change in anatomical form (Cheek 1975; Cockburn 1988; Fowden 1989; Winick 1972; Winick and Noble 1965).

In the late nineteenth century, the developmental embryologist/anatomist Charles S. Minot noted that from conception to maturity, guinea pigs grow at a

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rate of 1.8 g day^{-1}, rabbits at 6.3 g day^{-1}, and humans at 6.7 g day^{-1} (Minot 1891). Humans grow much larger than rabbits because they grow for a longer length of time; however, rabbits grow larger than guinea pigs because they grow at a more rapid rate. This was the first numerical demonstration of the differing manners by which one could attain a given mass and size. A philosopher of science, Minot stressed that, "The proper object, the final purpose of Biology, is the discovery of the nature and laws of life" (Minot 1891, p. 97). Along this line, he worked to discover the fundamental properties of living organisms and established the implausibility of vitalism (see below). Minot stated what he called the law of *cytomorphosis* (now termed morphogenesis), "... to designate comprehensively all of the structural alterations which cells, or successive generations of cells, may undergo from the earliest undifferentiated stage to their final destruction" (Minot 1901, p. 494). In his monograph *The Problem of Age*, *Growth*, *and Death*, Minot stressed the field of morphogenesis with an understanding of the fundamental mechanisms of cell growth and development and the systematic change in protoplasm from more elemental to highly differentiated cells and tissue, as a most promising field for research (Minot 1908). Since that time, longitudinal or serial studies of human growth have demonstrated relatively rapid growth during the perinatal period, continued prepubertal growth, and the rapid spurt with puberty (seen only in humans), with continued growth for a decade or more until the mid-third decade of life (Scammon 1927). In most mammals, when corrected with an appropriate time scale, growth follows a smooth curve that plateaus (Brody 1945). The relative roles of growth hormone, insulin, insulin-like growth hormones, leptin, cell cyclins, and other hormones in hyperplasia and hypertrophy are yet to be fully understood.

As articulated by Horatio Hackett Newman (1875–1957), professor of zoology at the University of Chicago, in regard to twins (whether monozygotic, e.g., identical, dizygotic, or fraternal), the biologic and physiologic considerations differ from that of a singleton infant (Newman 1917, 1923, 1940). Not only do twin infants tend to be born prematurely and therefore relatively small, but they have higher rates of mortality and morbidity than infants born at term (Benirschke 1961; Guttmacher and Kohl 1958; Stoch and Smythe 1963). Some references to the placental pathologic aspects of monochorionic twins and their consequences for fetal growth have been given earlier. For reference, a multitude of particulars with comprehensive data in relation to growth are given in one of the volumes of the Biological Handbook series published under the aegis of the Federation of American Societies for Experimental Biology (Altman and Dittmer 1962).

One of the first to study the quantitative aspects of growth of the fetus and newborn and the relation to nutrition and the environment was the Scottish biologist and classicist D'Arcy Wentworth Thompson (later Sir D'Arcy; 1860–1948) (Fig. 12.1a) of the University College, Dundee, and the University of St. Andrews, Scotland. Thompson's 1917 masterly *On Growth and Form* departed from the contemporary conventional consideration of anatomy and morphology, demonstrating that growth, from embryo to fetus to infant to maturity, encompasses an orderly and progressive change that follows mathematical principles. He

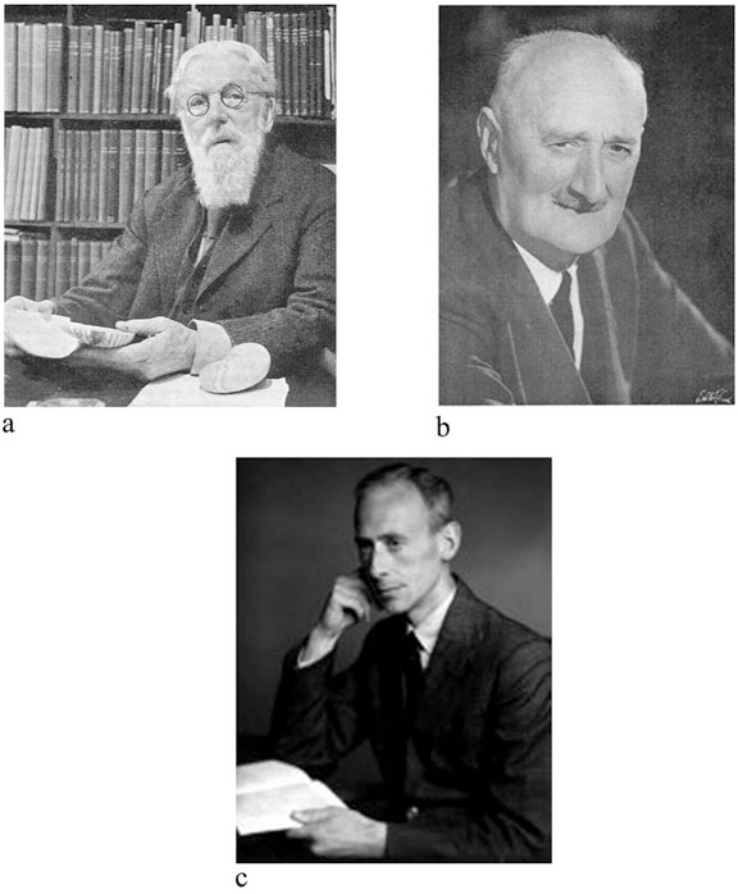

Fig. 12.1 (**a**) Sir D'Arcy Wentworth Thompson (1860–1948). (**b**) Sir John Hammond (1889–1964). (**c**) Robert Alexander McCance (1898–1993)

presented calculations of allometry, i.e., the determination of the relations of two varying dimensions for various structures from the shape of cells to the development of organs. Differences in forms of related animals he described by relatively straightforward mathematical transformations. With his heritage in the classics and strengths in biology, Thompson held that all learning in science constitutes a unity. In his analysis of life forms, he attempted to reduce biologic development as being subject to the laws of physics, mechanics, and mathematics, following varying rates and degrees along well-defined developmental "gradients." A central motif is his consideration of the logarithmic spiral in life forms. In speaking of growth, Thompson suggested a dimension to the scientific quest, an almost transcendental value beyond our ken. He stated:

> The search for differences or essential contrasts between the phenomena of . . . animate and inanimate things has occupied many mens' minds, while the search for community of

> principles, or essential similitudes has been followed by few . . . the physical phenomenon which meet us by the way have their manifestations of form, not less beautiful and scarce less varied than those which move us to admiration among living things. The waves of the sea, the little ripples on the shore, the sweeping curve of the sandy bay between its headlands, the outline of the hills, the shape of the clouds, all these are so many riddles of form, so many problems of morphology, and all of them the physicist can more or less easily read and adequately solve: solving them by reference to antecedent phenomena, in the material system of mechanical forces to which they belong, and to which we interpret them as being due. They have also, doubtless, their *immanent* teleological significance; but it is on another plane of thought from the physicist's [sic] that we contemplate their intrinsic harmony and perfection, and "see that they are good."
>
> (Thompson 1917, p. 7)

This "other plane of thought" for Thompson was of a somewhat platonic cast, but we need not imagine a metaphysical dimension to acknowledge that in our understanding there is a component that lies outside the "material system of mechanical forces." He stressed that the ". . . problems of growth are essentially physical problems; and the morphologist is, *ipso facto*, a student of physical science" (Thompson 1917, p. 8).

Strictly speaking, only two chapters of Thompson's tome *On Growth*. . . concern growth per se. In Chapter XVII, "On the Theory of Transformations, or the Comparison of Related Forms," he demonstrated the formation of related organic forms, presented graphically, and mapped out in accordance with Cartesian coordinates (Thompson 1917). A "descriptive" work sui generis, Thompson presented no fundamental thesis of causality between these emerging forms and the underlying laws of physics or biology. He admitted that this work was only a first approximation, having ". . . little need [for a] preface, for indeed it [the entire volume] is all preface from beginning to end" (Thompson 1917, p. v). An opponent of "vitalism," Thompson originally had discussed these concepts as an ". . . exploration of the borderline of morphology and physics." He noted that although contemporary chemistry had not reached a science of ". . . mathematical mechanics, nevertheless physiology is vastly strengthened and enlarged by making use of the chemistry . . . [and] physics, of the age. Little by little it draws nearer to our conception of a true science, with each branch of physical science which it brings into relation with itself: with every physical law and every mathematical theorem which it learns to take into its employ" (Thompson 1917, pp. 1–2). Thompson continued his credo, "My sole purpose is to correlate with mathematical statement and physical law certain of the simpler outward phenomena of organic growth and structure or form: while all the while regarding, *ex hypothesi*, for the purpose of this correlation, the fabric of the organism as a material and mechanical configuration" (Thompson 1917, p. 10). Remembered for striding around St. Andrews with a talking parrot on his shoulder, Thompson elucidated the rules that determine the shape of the chambered nautilus and other mollusk shells. He developed the concept of transformation, whereby small tweaks in geometry explain differences in the shapes of related animals. The concept of the diversity of body shape reflects variation on a common theme, was at core of Thompson's thinking. As an aside, Thompson's collection of shells can be seen in the Bell Pettigrew Museum of the

University of St. Andrews. In Chapter III, "The Rate of Growth," Thompson considered the role of varying growth rates on form and successive growth velocities moving through space and time.

In his 1911 presidential address to the British Association for the Advancement of Science, "*Magnalia Naturae*; or the Greater Problems of Biology," Thompson considered the "formidable complexity" of development, confessing, "... that we are utterly ignorant of the manner in which the substance of the germ-cell can so respond to the influence of the environment as to call forth an adaptive variation. ..." Thompson continued, considering the question of whether "... something other than the physical forces animates and sustains the dust of which we are made ... is rather the business of the philosopher than the biologist." He stressed that the structure of an object is a "diagram of forces" (Thompson 1911, pp. 422–423). In an address "Morphology and Mathematics" given to the Royal Society of Edinburgh, he expanded upon these ideas stressing the value of mathematical analysis and use of Cartesian coordinates in understanding proportion and developmental transformations (Thompson 1915). In 1942, Thompson published a second and greatly revised (over 1000 pages) edition of *On Growth and Form* (Thompson 1942). A scholar of the classics, in 1928 Thompson served as president of the Classical Association of England and Wales and in 1936 as president of the Scottish Classical Association (Thompson 1976). With his considerable influence on the fundamental principles of scientific discovery and thought, Thompson was knighted in 1937 (Dobell 1949; Thompson 1976).

A work noted in Chap. 7 on embryology that presents a quasi-mathematical approach to embryonic development, and which may have played a role in Thompson's analysis, was *Growth in Length*: *Embryological Essays* by Richard Assheton (1916). An earlier work devoted to growth of the human was that of Hastings Gilford (1861–1941), a surgeon in Reading, who published *The Disorders of Postnatal Growth and Development* (Gilford 1911). Previously, Gilford had described a case of progeria, a term he coined (Gilford 1897), and later was named "Hutchinson-Gilford progeria syndrome." In this volume, Gilford chiefly reviewed a number of disorders of growth and development including malignancies, which presciently he stressed constituted a vast group of diseases, rather than a single entity (Gilford 1911).

Another notable in establishing principles of growth and differentiating between the role of genetics and that of uterine environment was John Hammond (later Sir John; 1889–1964) (Fig. 12.1b) of Cambridge University. After initially studying agriculture, during the "Great War" as an officer in the British Expeditionary Force, he became interested in animal production to optimize the nutrition of troops under his command. Following the war, Hammond fell under the influence of Francis Hugh Adam Marshall (1878–1948) to work on veterinary physiologic issues such as "fertility, milk secretion, and growth." With limited funding, at Cambridge where he was a colleague of Barcroft, Hammond commenced his experimental studies in rabbits, developing strains for production of either meat or milk and for differing fertility rates (Hammond and Marshall 1925). Early, he sought evidence for the importance of antenatal environment and the role of the mother in

determining the size of her offspring at birth. Demonstrating this maternal role in the rabbit, but nonetheless with the continual problem of limited funding, he collaborated with Arthur Walton (1897–1959) of the School of Agriculture. Taking advantage of the huge size difference between breeds of horses, these investigators conducted their classic study of reciprocal crosses between the Shire and Shetland breeds. Hammond and Walton postulated that any difference in size between the offspring of the reciprocal crosses "would therefore be due not to chromosomal differences but to differences in the environment brought about by the difference in the size of the mother. In other words, we would have a controlled experiment in which "mother-size was the only or predominating variable" (Walton and Hammond 1938, p. 312).

Despite opposition from colleagues who thought the experiment cruel and/or unethical, these workers used the newly developed technique of artificial insemination, which Walton had learned on a visit to Russia (Walton 1936), to impregnate the mares with semen from stallions from the opposite breed. Strikingly, at term the three Shetland mares bore foals weighing ~18 $\pm$ 2 kg, as compared to the pure Shetland foals of ~20 $\pm$ 2 kg. In contrast, the Shire dams (weighing several times that of the Shetland mares) bore foals weighing 49 $\pm$ 2 kg, compared to purebred Shire foals of ~71 $\pm$ 5 kg (Walton and Hammond 1938). To the amazement of attendees of a Cambridge meeting of the British Society for Experimental Biology, Hammond displayed these dams and their offspring to establish his thesis "The contrast in weight and size [of the foals] was so enormous that it was difficult for the assembled scientists to believe the evidence of their eyes" (Slater and Edwards 1965, p. 104). Following weaning, the genetic differences in these foals became apparent; when on similar diets, the crosses from the Shire mares grew less rapidly than purebred Shire foals, while those from the Shetland dams grew more rapidly than purebred Shetlands. At about 18 months of age, the growth rates of the foals became constant; nonetheless, the differences between reciprocal crosses persisted into adulthood (Walton and Hammond 1938).

Hammond later followed this equine experiment, with similar studies in cattle, summarizing this work in major reviews (Hammond 1940; Hammond and Walton 1938). Although these results suggest that fetal growth is regulated entirely by uterine constraint, other maternal factors have been shown to play a major role. For instance, studies of mice of different strains suggest that intrauterine constraint is operative principally in late gestation (Aitken et al. 1977). The inverse correlation between birthweight and litter size in rodents and other mammals and the late-gestation slowing of growth rate in human twin pregnancy support this view. For many of his studies on growth and the development of the bone, muscle, and fat, Hammond often attended Stock Shows in surrounding communities (Slater and Edwards 1965). Importantly for animal husbandry throughout the world, Hammond's work contributed greatly to optimize the production of cattle (for beef and milk), sheep, and swine (Hammond 1940; Marshall and Hammond 1946). In the UK, Walton and Hamilton's introduction to and advocacy of the technique of artificial insemination contributed greatly to the improvement of animal breeding. In 1949, Hammond was appointed a commander of the Order of

the British Empire, and in 1960 he was knighted (Sanders 1965; Slater and Edwards 1965). The following year (1961), Hammond co-chaired the Ciba Foundation Symposium *Somatic Stability in the Newly Born* (see below) (Hammond 1961a, b).

12.2 Neonatal Birthweights, Fetal Growth Restriction, and the Small for Gestational Age Infant

Fetal growth restriction (FGR), defined as 10th percentile or less of estimated fetal weight, is a complication of 7–10% of pregnancies in the USA and throughout the world. In terms of fetal growth and development, perhaps surprisingly, reasonably correct birthweights of normal newborn infants were not determined until the mid-eighteenth century. Thomas E. Cone, Jr. (1915–1998) has presented a rather fascinating account of the vicissitudes in measurement of what one would regard as this rather straightforward variable (Cone 1961). As has been documented in many studies, prior to the mid-twentieth century, the outlook for newborn infants, regardless of size and gestational age, was fraught with hazards, particularly for those infants not nursed by their mothers. A significant factor in determining their fate was how they were fed. It was in late nineteenth and early twentieth centuries that weighing of the newborn became a relatively common practice and contributed to defining an index of viability. Nonetheless, at this time some recognized that "... tiny-puny infants could possess great vitality, and that gestational age was as important as birth weight *per se*" (Budin 1907). Weighing also was used to determine milk intake and, by the association of nutrition and body weight to health, was used as a quantitative measure of normality of growth and development (Weaver 2010).

Not until the mid-twentieth century did the concept arise that growth failure, rather than premature birth may be responsible for the small size of some neonates. Prior to that time, all infants with weights <2500 g were classified as "premature" (for instance, "Expert Group on Prematurity" of the World Health Organization defined premature on the basis of birthweight, e.g., <2500 g, alone; World Health Organization 1950). Thus, the idea of a fetus being small for gestational age (SGA) and failing to reach its full potential was pioneering. In 1947 from Los Angeles "... with much trepidation," a report of the SGA newborns was presented on 69 undernourished infants of a 6641 total at term (McBurney 1947). These infant weights ranged from ~1.5 to 2.5 kg, and the placentas tended to be smaller than normal. Based on the California State Board of Health statistical requirements, the infants were "premature" despite their being born at or near full-term (McBurney 1947). Thereafter, 20 small-for-dates infants were diagnosed in utero by both serial measurements of pubic symphysis to uterine fundus height and weight at the time of birth. Excluding stillborns and one mongoloid infant, the weight of the 12 surviving infants was 1702 ± 135 g (Rumbolz and McGoogan 1953). Since that time, it has become recognized that a number of such low-birthweight infants are a

consequence of decreased rate of intrauterine growth, e.g., shades of Minot, rather than their being delivered prior to term. The relevance of this concept was affirmed by subsequent studies that demonstrated correlations of stillbirth and fetal asphyxia to restricted growth (North 1966). To account for smaller than normal size and weight, the terms "small for gestational age," "intrauterine growth restriction" (IUGR; initially "retardation"; Gruenwald et al. 1967; Warkany et al. 1961), and "fetal growth restriction" (FGR) were introduced to describe these infants.

In an historic perspective, Joseph Dancis (1916–2010) (Fig 12.2a) of New York University and the Bellevue Hospital recalled that in regard to normal patterns of newborn growth, it was when he was a senior resident that his chief of pediatrics, Luther Emmett Holt Jr. (1895–1974), pointed out the lack of standard weight curves for premature infants. This made it difficult to judge the progress of a given infant following birth (Dancis 1983). Dancis enlisted a fellow resident to tabulate the weight gains for their first 50 days of life of a series of 100 infants that weighed from 1000 to 2500 g at the time of birth. On analyzing what appeared a "tangled mass of curves", Dr. Holt "... sketched in over them, at 250-g intervals, simple straight lines seeking to reproduce the summation of the slopes." Following some corrections, with the early weight losses, "... the rough outline of the premie weight grid emerged" (Dancis 1983, p. 3; Dancis et al. 1948). Here, Dancis admitted the unusual nature of their report. There was no section on methods (including the population surveyed, criteria for inclusion or exclusion), no careful analysis of results, no regression lines or statistics, and no "... extensive and penetrating discussion" (Dancis 1983, p. 2). Of note, the shape of the grid demonstrates that less mature preemies reach their weight nadir later than those that are more mature, and their rate of weight gain is slower so that they require more time to regain their birthweight. These observations on the significant decrease in weight during the first days to week of life confirmed an earlier study of the weight changes of almost 3000 newborns, which separated males and females and white and blacks (Dunham et al. 1939).

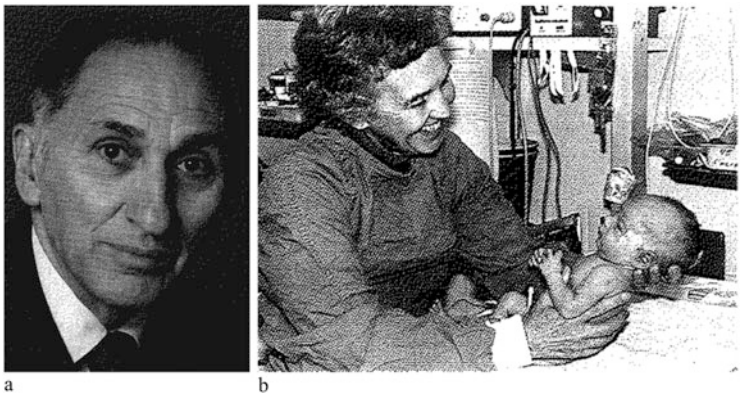

Fig. 12.2 (**a**) Joseph Dancis (1916–2010). (**b**) Lula O. Lubchenco (1915–2001)

A decade later, Lula O. Lubchenco (1915–2001) (Fig. 12.2b) and colleagues at the University of Colorado, in part motivated by those conclusions, first described the relation of birthweight to gestational age (Lubchenco et al. 1963). Although their study was conducted at moderate altitude (Denver, CO, 1609 m, 5280 ft.), it set a new standard for evaluation of the newborn, and the "Lulagram" developmental growth chart became used worldwide. For infants whose growth patterns lie outside normal developmental profiles, the terms small-, appropriate-, and large-for gestational age (SGA, AGA, and LGA) were introduced on the basis of birthweight (both in absolute terms and centiles), length, head circumference, and weight-length ratio (ponderal index) (Battaglia and Lubchenco 1967; Lubchenco 1970; Lubchenco et al. 1966, 1972). Although the term "low birthweight" was introduced in the early 1960s to replace the word "premature" for infants under 2500 g at birth, its acceptance was markedly facilitated by Lubchenco's popularization of matching birthweight with gestation. Too long had the designation for term and premature been based on birthweight above and below 2500 g without awareness of two distinct populations for each weight group. Subsequently, she demonstrated that for those infants below the 10th percentile, the risk of neonatal death increased at every gestational age (Lubchenco et al. 1972).

Recognition of the concept of fetal growth restriction (Battaglia and Lubchenco 1967; Battaglia et al. 1992) led to dramatic advances in prenatal care, as well as vast improvements in the management of the newborn infant. The Denver population-based growth curves became used widely, setting a normal range of fetal weight between two standard deviations of the mean or between the 10th and 90th centiles for a given gestational age. Initially, the term small for gestational age (SGA) was used to describe newborn infants whose birthweight (BW) was below the 10th centile. Later, this term was used interchangeably with intrauterine growth restriction (IUGR) (Wollmann 1998). Although, later, IUGR was distinguished from the SGA infant, however this distinction led to considerable confusion (Saenger et al. 2007). More recently with the development of refined serial ultrasonographic techniques to establish with accuracy fetal body size and its growth velocity in utero, the term fetal growth restriction has become the preferred terminology. A study by the University of California, Berkeley, biostatistician Jacob Yerushalmy (1904–1973) and coworkers showed that there were as many term as preterm infants born weighing under 2500 g in the USA and surprisingly more preterm infants weighing above than below that figure (Yerushalmy et al. 1965).

In her monograph *The High-Risk Infant*, Lubchenco summarized standards of intrauterine growth from the 24th to the 42nd week of gestation. She emphasized the importance of the use of gestational age along with birthweight, as a dimension to understanding not only pre- and perinatal risk factors but also in the consideration of long-term outcome (Lubchenco 1976). Her work became a classic. The studies of Lubchenco and others helped to replace the pediatric concept of "failure to thrive" with that of the extent to which the yet-to-be-born infant grows in an optimal manner. Nonetheless, the Denver standards differed from those at sea level and did not reflect the increase in median birthweight that has occurred during recent decades. Subsequently, several investigators have reported

birthweight-gestational age data for infants born at sea level among different communities and ethnic groups (Altman and Coles 1980; Arbuckle et al. 1993; Ballard et al. 1979; Brenner et al. 1976; Dunn and Wharton 1985; Gardosi et al. 1992; Ott 1993; Usher 1970; Usher and McLean 1969; Usher et al. 1966; Williams et al. 1982). The introduction of diagnostic ultrasound, with measurements of both head and abdominal circumference and femur length, has revolutionized the determination of fetal body size in utero. Although continuous throughout pregnancy, fetal growth follows a biphasic curve, with the period of ~32 weeks being most rapid for acceleration in mass (weight) gain and ~18 weeks for the peak rate of increase in body length (Tanner 1978; Villar and Belizan 1982). Although many instances of IUGR have been attributed to "placental insufficiency" (Gruenwald 1963, 1970), the term has caused confusion in the literature and been defined in various ways. In addition, and importantly, although FGR is defined as a birthweight below the 10th percentile for gestational age, many such infants are normal and simply "constitutionally" small. Further, an adverse outcome is increased for those infants whose birthweights are between the 10th and 20th percentile, but have failed to achieve their full growth potential (Acharya et al. 2015).

As noted, for several decades, neonatologists attempted to distinguish fetuses with FGR from those that were SGA. While FGR referred to a pattern of fetal growth below the expected norm, the classification of SGA was based on birthweight alone. Related terms are low birthweight (LBW), defined as a newborn infant of less than 2500 g regardless of gestational age, very low birthweight (<1500 g), and extremely low BW (<1000 g). In terms of morphology, and as a consequence of ultrasonic measurement of both head and abdominal circumference, fetal growth restriction has been categorized as asymmetric (or asynchronous) in which the head grows at a near normal rate (head sparing) or symmetric (synchronous or global) with growth restriction of the head as well as the body (Campbell and Thoms 1977). The former, accounting for about 70% of cases, is commonly caused by extrinsic factors limiting fetal growth during the third trimester of gestation. Chiefly these include hypoxia, inadequate maternal nutrition, or other maternal or placental dysfunctions. The symmetric growth pattern commences much earlier in pregnancy. A variety of causes including genetic and chromosomal abnormalities, infection, and drugs contribute to the genesis of FGR (Table 12.1). These results in a higher incidence of preterm delivery and higher rate of neonatal morbidity (ACOG 2001; Brodsky and Christou 2004; Lin et al. 1991; Lin and Santolaya-Forgas 1998; Manning 1995; Nardozza et al. 2012; Resnik 2002; Seeds 1984). Further, an adverse outcome is increased for those infants whose birthweights are between the 10th and 20th percentile, but have failed to achieve their full growth potential (Acharya et al. 2015). Nonetheless, caution is required as the trajectory of fetal growth can vary widely with organ-specific differences and yet result in a given birthweight. A major factor for these differences is that of growth velocity profiles. Because previous to the advances in ultrasonic technology, the FGR infant could be diagnosed with certainty only following birth, a significant number of whom are constitutionally healthy have

Table 12.1 The "mosaic" of maternal, placental, and fetal factors in the genesis of fetal growth restriction[a]

Maternal	Uteroplacental	Fetal
• Persistent hypoxia (e.g., high altitude, pulmonary disease, severe anemia; moderate to heavy smoking; hemoglobinopathies, heart diseases) • Cardiovascular disorders Hypertension (>140/90) Cyanotic heart disease Diabetes mellitus Renal disease Collagen vascular disease • Hypercoagulable disease Thrombophilia Antiphospholipid antibody syndrome • Undernutrition (caloric and/or protein deprivation) • Toxins and teratogens (e.g., tobacco, alcohol, illicit drugs, medications, irradiation) • Age <15, >45 • History of IUGR	• Uterine malformation or masses • Placental insufficiency • Idiopathic Preeclampsia • Abnormal placentation Abnormal trophoblast invasion Placental infarcts Placenta previa Circumvallate placenta Chorioangioma Velamentous insertion of umbilical cord Umbilical-placental vascular anomalies (twin-to-twin transfusion syndrome)	• Genetic Chromosomal abnormalities Trisomy 18, 13, 21 Congenital malformations Turner's and other syndromes • Epigenetic Hypoxia Undernutrition • Multiple gestation • Intrauterine viral infections Rubella, cytomegalovirus, parvovirus, herpes virus, human immunodeficiency virus • Other infections Syphilis, malaria, toxoplasmosis

[a]Fetal growth restriction encompasses an extremely heterogeneous group that can be categorized into maternal, placental, and fetal factors. A subgroup of infants includes overlapping etiologies. In addition, there are also a significant number of infants with unexplained etiologies
Table modified from several that are in the literature

been and will be subjected to high-risk management resulting in iatrogenic prematurity.

Although differences in growth profiles have been thought to occur chiefly during the third trimester (27+ weeks), by means of contemporary ultrasonography those for head circumference, femur diaphysis length, and abdominal circumference are evident by 18, 20, and 22 weeks gestation, respectively (Milani et al. 2005), and growth velocity differences may become evident at 16–17 weeks gestation (Milani et al. 2005). Of great importance to the advancement of perinatology and neonatology, the dramatic consequence of refinements in ultrasonography has led to more rational care of the developing organism with the saving of lives. For instance, these advances allow assessment of blood flow/velocity in vessels including the uterine artery (Albaiges et al. 2003; Axt-Fliedner 2004; Hershkovitz et al. 2005; Thaler et al. 1990), the umbilical artery and vein (Chen et al. 1986; Gill et al. 1981; Sutton et al. 1990), and other vessels including the cerebral arteries (Fong et al. 1999; Hershkovitz et al. 2000; Mari et al. 2007) and the ductus venosus (Baschat et al. 2001). A caveat of these studies is that sonography measures the velocity of blood flow, while flow per se is the product of velocity and

vascular diameter. Critical aspects of many of these studies will not be rereviewed here but have been surveyed (Battaglia 2011).

As noted, although many instances of FGR have been attributed to "placental insufficiency" (Gruenwald 1963, 1967, 1970), the term has caused confusion in the literature and been defined in various ways. A skeptic, Nicholas Salem Assali (1916–2004) of the University of California Los Angeles, stated the term "placental insufficiency" to be:

> . . . an umbrella to cover our ignorance of the etiology and pathogenesis of chronic uteroplacental-fetal disturbances; . . . a waste basket to dump a variety of disorders interfering with maternal supply of nutrients to the fetus or with fetal metabolism or with disorders related to abnormal placental functions . . . in my opinion, there is no adequate physiological method to test placental sufficiency or insufficiency.
>
> (Assali et al. 1975, p. 88)

Nonetheless, others dispute this view, noting not only altered function of the placenta but many circulatory and metabolic changes of the fetus (Battaglia 2011). The ability to distinguish between an infant born "too soon" (preterm) versus that which is small for gestational age (whether due to nutritional deficiency as noted above or the fact that the parents are small) has important biological implications and determines their clinical care, as infant rates of morbidity and mortality vary considerably (Abramowicz and Kass 1966; Battaglia 2011; Battaglia and Simmons 1978; Low et al. 1972; see below).

Also at mid-century, a number of other concerns became evident. One was that of neurological development and the use of neurological testing to determine gestational age. Initially in these studies, the examiner attempted to characterize the newborn's reflexes and tone (Saint-Anne Dargassies 1955; Thomas and Saint-Anne Dargassies 1952). Subsequently, these were quantified, in part, for seven groups from 28 to 40 weeks gestational age (Amiel-Tison 1968; Amiel-Tison and Grenier 1980). Soon these criteria were refined further (Dubowitz et al. 1970; Fitzhardinge and Steven 1972; Robinson 1966).

Another issue was the question of the optimal nutrients to feed infants, particularly those that were premature. Nutritional balance studies of Harry H. Gordon (1906–1988) and Samuel Zachary Levine (1895–1970) of Cornell University Medical Center in New York City were the first to establish an evidence-based scientific basis for infant feeding (Gordon and Levine 1944). Soon it became evident that mother's milk, with its rich content of carbohydrates, proteins including immunoglobulins, calcium, and other constituents, is the most desirable food for premature and term infants. In the late 1970s, Frank Tardrew Falkner (1918–2003) of the Fels Research Institute and Wright State University, Ohio, and James Mourilyan Tanner (1920–2010) of the Institute of Child Health, University of London, edited a three-volume *arbeit* on human growth. In 21 chapters, Volume 1, *Principles and Prenatal Growth*, addressed aspects of developmental biology, the mechanisms of regulation of growth, biometrical methods of interpreting human growth data, the relation of genetics to prenatal and postnatal growth, and the process of maturation (Falkner and Tanner 1978a). Volume 2, *Postnatal Growth*, in 19 chapters considered methods of auxological anthropology,

growth of the various body tissues and organs, and problems of growth of the low-weight infant (Falkner and Tanner 1978b). The 17 chapters of Volume 3, *Neurobiology and Nutrition*, focused on neurobiological aspects of growth, growth of the brain and nervous system, and the overall role of nutrition in growth and maturation (Falkner and Tanner 1979). The final chapter presents a history of scientific growth studies, from that of Georges-Louis Leclerc, *Comte* de Buffon (1707–1788) in the eighteenth century to D'Arcy Thompson and others in the early twentieth century (Tanner 1979). In addition to normal standards for intrauterine growth (Tanner 1970), Tanner is also widely regarded for his studies of children in the Harpenden orphanage, north of London, documenting the series of steps that define physical maturation, growth pattern, and their variations, the "Tanner stages" or "Tanner scale" (Tanner 1978, 1981, 1990).

In an attempt to expand the recognized number of atypical fetal growth patterns, to provide anthropomorphic data (crown-heel lengths, ponderal index, weight-height ratios, and others) on newborn infants who were free from all known growth-limiting influences in utero, and to use newborn body measurements collected by a single individual to construct standards of presumed normal fetal growth, in 1973 a study of fetal growth in all infants (primarily Caucasian) born at the University of Kansas Medical Center was begun (Miller and Merritt 1979). This fetal growth research program, conducted on about 6000 pregnancies over a 5-year period, led to the identification of a number of factors leading to fetal growth restriction and/or associated with premature birth. These included poorer outcomes for teenage mothers, those mothers over the age of 35, those who smoked, suffered from substance abuse, or lacked prenatal care, and numerous other variables. In addition to providing growth standards, the investigators stressed the need for studies to determine the extent and degree to which postnatal growth is a continuum of the different patterns of prenatal growth (Miller and Merritt 1979).

In 1988, Forrester Cockburn of the Royal Hospital for Sick Children, Glasgow, edited a volume on *Fetal and Neonatal Growth*. Exploring early and critical phases of human development, a dozen essayists considered what was known about the role of genetics, growth factors, enzymes, hormones, and other antenatal and postnatal aspects in growth and its developmental regulation (Cockburn 1988). Another volume, *Fetal Growth and Development* edited by Richard Harding of Monash University, Victoria, Australia, and Alan D. Bocking of the University of Western Ontario, Canada, in 12 chapters considers a number of aspects of this topic for different physiological systems and their relevance (Harding and Bocking 2001). During the past several decades, a number of other investigators have contributed to studies on the role of both placental and fetal hormones and other factors that influence growth and metabolism of the fetus and newborn infant (for instance see Fowden 1989; Fowden et al. 1996, 1998; Robinson 1977; Robinson et al. 2000; Ward et al. 2004; Winick 1972). Because normal fetal growth is such a critical component of a healthy pregnancy, in clinical obstetrics considerable effort has been dedicated to develop appropriate references and standards for its evaluation (Alexander et al. 1996; Gruenwald 1966; Bottoms et al. 1999; Hoffman et al. 1974; Kramer 1987; Acharya et al. 2015; Williams 1975; Zhang et al. 2010).

12.3 Further Perspectives on Fetal Growth Restriction

As noted, fetal growth restriction is a failure of the fetus to achieve its genetically determined growth potential. Strictly defined by being in the lower 10th percentile of estimated body weight, because of its many associated complications, fetal growth restriction is a health issue of vital importance. In the USA, as well as the entire globe, FGR occurs in 7–10% of pregnancies and accounts for a significant increase in the rates of stillbirth (Gardosi et al. 2013), as well as infant morbidity and mortality (Kramer et al. 1990; Lin et al. 1991; Manning 1995; Marsál 2002; Resnik 2002; Acharya et al. 2015). Worldwide, FGR is believed to be associated with, and perhaps account for, ~60% of the four million neonatal deaths that occur annually (Lawn et al. 2005; Bellamy and UNICEF 2003). The perinatal morbidity and mortality in these cases often are associated with hypoxic-ischemic encephalopathy, intraventricular hemorrhage, pulmonary hypertension, necrotizing enterocolitis, and related complications (Amon et al. 1987; Baschat et al. 2000; Batalle et al. 2012; Bernstein et al. 2000; Gilbert and Danielsen 2003; Kramer et al. 1990; Resnik 2002; Schauseil-Zipf et al. 1989). Based on a number of studies, a related problem is that of the wide variations seen in growth profiles (Bloomfield et al. 2006). An additional factor of importance is the failure to detect FGR prior to birth. For instance, in the UK, the antenatal detection rate varies from 12 to 50% (West Midlands Perinatal KPI Report 2011), and such figures are similar in other studies (Gardosi and Francis 1999; Gardosi et al. 2013) including Australia (Roex et al. 2012). Finally, an economic consideration for these infants is that their required prolonged stay in hospital results in enormous healthcare costs (Bernstein et al. 2000; Gilbert and Danielsen 2003).

In a broader perspective and despite years of study, the pathophysiology of fetal growth restriction is incompletely understood. A large number of epidemiologic studies have identified many clinical associations with the FGR infant (Table 12.1). Of the preventable, environmental causative factors, probably the most common is smoking by the mother (Wollmann 1998). Some have ascribed the increased prevalence of FGR to the delay in childbearing in contemporary society (Balasch and Gratacos 2011). Ultimately, the independent role of many specific factors and some strong statistical correlations do not definitively establish causality.

The complexity of understanding a mechanism for fetal growth restriction is highlighted by evidence for typically normal growth of the brain and heart in fetuses/newborns with bodies thin for their length. These individuals are described as having a low ponderal index ($100 \times$ weight in grams·crown-heel length^{-3}), a measure of leanness (both weight in g^{-2} and $^{-3}$ are used), although this is not always the case (Sweeting 2007). Several decades ago it was suggested that custom- rather than population-based growth standards are more likely to discriminate between the fetus/newborn that is constitutionally small but normal from a biologic-physiologic standpoint, from that with true FGR (Gardosi 2004; Gardosi and Francis 1999; Gardosi et al. 1992, 2011; Resnik 2007). These custom-based standards, with consideration of variables such as maternal ethnicity, parity, height and weight in

early pregnancy, as well as fetal sex, utilize optimal birthweight as the proper endpoint of a growth curve and are based upon the fetus' ability to achieve its full growth potential independent of maternal/placental pathology. As may be obvious, each of these variables plays an important role in the determination of fetal potential. Of considerable relevance is the distinction of the FGR infant from that which is born prematurely and preventative methods that may be of value in this regard (Iams 2014). In a large study from New Zealand, the use of customized birthweight centiles identified more preterm infants as FGR. This contrasted with population-based studies that reported more near-term infants as FGR (Groom et al. 2007). In the customized studies, perinatal death among preterm infants occurred only in those classified as FGR. In contrast, no perinatal deaths and low rates of preterm birth occurred for FGR infants classified by population percentiles (Groom et al. 2007).

Other investigators have noted that customized birthweight standards also more accurately predict stillbirth, neonatal deaths, and neurological sequelae (Clausson et al. 2001; McCowan et al. 2005). Nonetheless, not all epidemiologists agree on the utility of customized growth curves (Hutcheon et al. 2011a, b). Also in comparison with customized and population-based standards from Sweden, the rates of stillbirth and neonatal mortality (FGR) did not differ significantly (Hutcheon et al. 2008). To place these views in perspective, Robert Resnik of the University of California San Diego observed, "one size does not fit all, and it would seem . . . time for . . . obstetricians to adopt the use of customized fetal growth standards" (Resnik 2007, p. 221). Further, it has been emphasized that these issues require further internationally recognized and integrated definitions (Zhang et al. 2010). Nonetheless, others argue for a so-called integrative definition of normal and abnormal fetal growth, based upon fetal size, placental health measured by maternal and fetal Doppler velocimetry, biochemical biomarkers, and genetics (Zhang et al. 2010). Along this line, Jay D. Iams of Ohio State University, Columbus, has proposed that the conflation of definitions be resolved by restricting the term SGA to those fetuses/infants weighing $<$10th percentile for ". . . gestational age (population-based) and by limiting the FGR designation to infants and fetuses whose growth is suspected to be less than optimal, recognizing that SGA infants are not all FGR and that FGR infants are not all SGA. SGA would be based on growth percentiles, and FGR would be based on evidence of pathologic growth . . . this integration of obstetric and pediatric terminology could improve the antenatal, intrapartum, and neonatal care of small babies" (Iams 2010, p. 513).

Implicit in all of these studies is the requirement to monitor serially a number of functions of fetal physiology and well-being of the growth-restricted fetus. Some of these have been noted above, but in addition to the amniotic fluid index, fetal electrocardiography, and head and abdominal circumference, they include laser Doppler of blood velocity waveform profiles (aorta, umbilical artery, middle cerebral artery, ductus venosus pulsatility). The relative importance of these varies with gestational age, e.g., early versus late FGR (Baschat 2003; Hecher et al. 2001; Hershkovitz et al. 2000). For optimal fetal and newborn care, these issues need to be better defined and established.

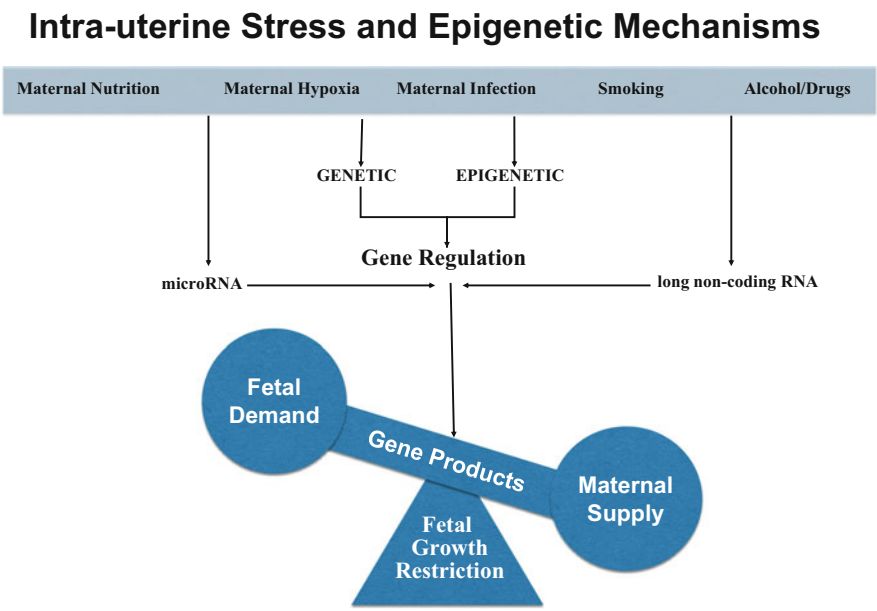

Fig. 12.3 Some intra-uterine stressors and the proposed mechanisms of gene regulation that determine fetal growth

In a broader perspective, two major factors/processes determine fetal growth and development are genetic and epigenetic. In contrast to the genetic fixed nucleotide sequences of the DNA, nutritional, metabolic, and other environmental influences operate through epigenetic mechanisms to affect gene expression. A multiplicity of interrelationships with some associated variables may characterize fetal demand and maternal supply to affect the course of fetal development (Fig. 12.3). Moreover, the fetus does not develop in isolation. Rather, in concert with the placenta and maternal organism, the fetus is an essential element of the maternal-placental-fetal complex or unit (Diczfalusy 1964). Thus, whether considered from the perspective of its general physiology, endocrinology, metabolism, circulation, immunology, or other elements, the fetus is part of this integrated organic unit, a dynamic module in the matrix of reproductive development. It should be evident that in the maternal-placental-fetal complex, a host of biochemical factors at the cellular and molecular level can interact with physiologic variables at the tissue and organ levels including that of inadequate trophoblast invasion and/or dysfunction (see below) to result in growth restriction. Rather than existing as a distinct pathological entity, FGR, in fact, is a symptom complex resulting from many causes. One may posit, therefore, that as in the case of hypertension, metabolic syndrome, and other disorders, the FGR phenotype is a mosaic resulting from many underlying conditions. Of note, in comparison with a stillbirth rate of 2.4 per 1000 deliveries in pregnancies without FGR, this rate is increased to 9.7 when FGR was defected antenatally and increased

further to 19.8 when it was not diagnosed (Gardosi et al. 2013). This finding emphasizes the need to improve antenatal detection of FGR as early detection can lead to close monitoring and optimal management and timing of delivery (Unterscheider et al. 2013).

12.4 Fetal Growth Restriction in Laboratory Animals

From an experimental standpoint, fetal hypoxia with resultant FGR can be induced by several means. These include maternal hypoxia (Gheorghe et al. 2007; Giussani et al. 1994; Goyal et al. 2011a, b; Kitanaka et al. 1989), maternal hyperthermia (Thureen et al. 1992) restricting maternal uterine blood flow (Baserga et al. 2009, 2010; Challis et al. 1989; Phillips et al. 1996; Wilkening and Meschia 1983), reducing fetal umbilical blood flow (Giussani et al. 1997; Unno et al. 1997), embolization of the placenta (Boyle et al. 1984; Bubb et al. 2007; Clapp et al. 1980; Gagnon et al. 1997), and maternal uterine carunclectomy prior to mating (which restricts the number of placentomes; Alexander 1964; Dyer et al. 2009; Phillips et al. 1996; Robinson et al. 1979). Details of many aspects of these approaches with their strengths and weaknesses, as well as similarities and differences in fetal physiologic responses, have been reviewed by others (Morrison 2008). As a caveat, despite many similarities in the responses to these stresses to the fetus, these several methodologies are associated with differing degrees of acidemia, hypoglycemia, and/or nutrient deprivation.

A recent review considers several aspects of laboratory animal "models" of FGR, including justification for their use, special considerations in terms of number of fetuses, the length of gestation, the placentas, the creation of such "models," and considerations for translational medicine (Swanson and David 2015). As a "model" for almost every aspect of reproduction, rodents have been used to explore antenatal maternal stress and its sequelae for the mother, fetus, and offspring as an adult. These include the most vulnerable period of gestation, the degree of hypoxia, and its duration. Based on an analysis of 22 studies in rats and 6 in mice that met criteria of having appropriate controls and stringent statistical analysis (Jang et al. 2015), it is clear that, in addition to the factors given above, the species/breed studied is important and these variables interact. For instance, in the rat 7 days or more and in the mouse 3 days or more of hypoxia at 14% or less O_2 concentration during the third (in some cases second) trimester of pregnancy are required to produce FGR. Also in the rodent, overall analysis suggests that a newborn pup weight reduction of 22% is required to meet the human FGR definitions of below the 10th percentile (Jang et al. 2015). In the rabbit, uteroplacental ischemia produced by ligation of 50% of blood vessels on day 25 (of a 30-day gestation) produced a number of fetal cerebral neurostructural abnormalities associated with functional impairments (Illa et al. 2013).

Endothelial nitric oxide synthase knockout ($eNOS^{-/-}$) mouse dams exhibited dysregulation of vascular adaptations to pregnancy, and the $eNOS^{-/-}$ fetus of such

dams had FGR. This was manifested with increased constriction and decreased relaxation of isolated uterine arteries, reduced (endothelium-dependent) maternal to fetal amino acid transport, and elevated placental superoxide levels (Kusinski et al. 2012). This mouse model demonstrates yet another variety of uteroplacental hypoxia with free radical formation leading to reduced placental nutrient transport and FGR. As noted, prolonged fetal hypoxia, as a consequence of any of the factors noted above, can result in considerable changes in cardiovascular function (Kamitomo et al. 1993) with FGR (Giussani et al. 2001; Moore et al. 2011). In high-altitude acclimatized long-term hypoxia in fetal sheep, these include significant reduction (–24%) of combined right and left ventricular cardiac output compared to the normoxic fetuses (Kamitomo et al. 1992, 1993).

A number of studies have reported that in developed nations, FGR is associated with consequential neurodevelopmental/neurocognitive outcomes later in life (Frisk et al. 2002; Leitner et al. 2007; Paz et al. 1995; Strauss 2000; Sung et al. 1993; Zubrick et al. 2000). In an attempt to discover the mechanistic basis for this association, Robert H. Lane and colleagues formerly at the University of Utah and currently at the Medical College of Wisconsin have explored several aspects of developmental neurogenesis in laboratory animals. In addition to significant changes in composition of the neuronal *N*-methyl-D-aspartate receptor subunits, which are critical for synaptogenesis, the alterations showed gender specificity (Schober et al. 2009). Also, FGR newborn infants, as well as those 30 and 60 days of age, showed a significant decrease in the N1/P2 component of the auditory evoked potential to be associated with increased free, and decreased bound, fractions (but no change in total) of plasma L-tryptophan levels (Manjarrez et al. 2005). This suggests impairment of brain serotonergic transmission and probably that of other cerebral cortical sensory mechanisms as well (Manjarrez et al. 2005).

12.5 Cardiovascular Function with Fetal Growth Restriction

In human FGR infants, several studies have demonstrated significant alterations in cardiovascular function. For instance, such newborns have increased thickening and stiffness of the aorta with reduced distensibility (Cosmi et al. 2009; Koklu et al. 2006; Skilton et al. 2005), key components of cardiovascular disease in adults (Arnett et al. 1994). Associated pathologies in FGR infants are ventricular hypertrophy with increased heart weight (Veille et al. 1993), decreased myocyte size and ventricular volume (Mayhew et al. 1999), and ventricular ejection force (Rizzo et al. 1995). In a report using ultrasonic measurements to compare myocardial function in the late second and throughout the third-trimester FGR fetuses, a number of parameters in these fetuses differed from AGA controls. For instance, both isovolumetric contraction and relaxation times were prolonged, ejection time

was reduced, and the calculated myocardial performance index was increased (Hassan et al. 2013). These indices of altered myocardial function appeared prior to the observed arterial and venous umbilical and other arterial Doppler abnormalities that characterize hypoxia (Hassan et al. 2013). In view of the evidence in animal models that LTH results in these alterations, it would seem reasonable that hypoxia is the culprit for mediating these changes in the FGR newborn infant.

In response to acute hypoxia, the fetus experiences redistribution of cardiac output from the peripheral circulation to maintain circulation of the brain, heart, and adrenal glands (Cohn et al. 1974; Lorijn and Longo 1980; Peeters et al. 1979). Such redistribution has been shown to be mediated by a carotid body chemoreflex (Giussani et al. 1993) in concert with the release of catecholamines (Jones and Robinson 1975), arginine vasopressin (Perez et al. 1989), neuropeptide Y (Fletcher et al. 2000), nitric oxide (NO) (Morrison et al. 2003), and other vasoactive factors. Reactive oxygen species also play a role in this response (Thakor et al. 2010).

Fetal asymmetric growth restriction and cardiovascular dysfunction also have been demonstrated in the rat (Herrera et al. 2012; Williams et al. 2005a, b). In the guinea pig, Thompson and his group have demonstrated several cardiovascular sequelae including increased cardiac production of endothelial nitric oxide synthase (NOS) (Dong and Thompson 2006; Thompson et al. 2000) with increased NO in such dysfunction (Thompson et al. 2009). Other investigators also have demonstrated NO-mediated endothelial dysfunction following antenatal hypoxia in the adult rat (Hemmings et al. 2005; Morton et al. 2010, 2011; Williams et al. 2005a, b) and sheep (Giussani et al. 2012). Along this line, FGR offspring shows evidence of endothelial dysfunction as manifested by high-resolution ultrasound in both 9–11-year-olds (Leeson et al. 1997), as well as in early adult life (Leeson et al. 2001).

In a rat, bilateral uterine artery ligation FGR "model," the renal expression of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) mRNA and protein were significantly decreased (Baserga et al. 2007). This occurred in association with increased corticosteroid levels at the time of birth as well as at day 21 of life (Baserga et al. 2005). This enzyme plays a key role in the regulation of renal steroid sensitivity, catabolizing glucocorticoids to an inactive form in the kidney and other aldosterone target tissues, its deficiency being an important mechanism that can lead to hypertension. This FGR "model" also has been demonstrated to be associated with decreased binding of the transcription enhancers specificity protein 1 and NF-κB p65, with increased transcriptional repressors early growth response factor NF-κB p 50 to the 11β-HSD2 promoter in males. Some of these changes were more predominant in females. In addition, DNA CpG methylation occurred in a sex-specific manner (Baserga et al. 2010).

In the rat, antenatal chronic hypoxia also has been shown to affect fetal myocardial expression of cardioprotective enzymes such as protein kinase C epsilon (PKCε) with programming of an increase in cardiac susceptibility to ischemia and reperfusion injury in male offspring (Li et al. 2003; Patterson et al. 2010; Xue and Zhang 2009). Presumably the result of ROS, studies also have demonstrated increased methylation of the promoter of the *PKCε* gene, an epigenetic change which could be prevented by administration of an inhibitor of DNA methylation

(Patterson et al. 2012). The adult offspring of dams subjected to antenatal hypoxia also displayed cardiac structural and functional changes including increased expression of collagen type I and III and the ratio of beta- to alpha-myosin heavy chains (Xu et al. 2006), as well as increased left ventricular end diastolic pressure (Rueda-Clausen et al. 2009), and decreased myocardial metabolism (Rueda-Clausen et al. 2011). Further studies have demonstrated decreased beta 1 receptor and increased muscarinic receptor responses in adult offspring of rats so subjected to LTH (Giussani et al. 2012). In the guinea pig, intrauterine FGR followed by overnutrition afterbirth is associated with vascular dysfunction, including stiffening of the aortic wall and a number of related dysfunctions (Thompson et al. 2014). Also of clinical relevance, cardiovascular disease in adults has been associated with elevated sympathetic and decreased parasympathetic reactivity. Thus, overall studies from several species support the idea of antenatal hypoxia as well as nutritional deficiency resulting in cardiomyopathy in the adult offspring. Provocatively, many of these antenatal hypoxic-mediated (Hemmings et al. 2005; Kuzawa 2004) changes show gender specificity for many complications, with males being more susceptible than females.

To explore aspects of growth and the developing cardiovascular system independently from those influences of maternal physiologic responses, the chicken embryo also has been used to advantage. Several groups have demonstrated the effect of hypoxia in causing asymmetric embryonic/fetal growth as well as the growth of specific organs (Giussani et al. 2007; Lindgren and Altimiras 2011; Miller et al. 2002; Ruijtenbeek et al. 2003a, b; Sharma et al. 2006). The reported changes include aortic hypertrophy and left ventricular dysfunction (Rouwet et al. 2002), with enlargement of both ventricles (Villamor et al. 2004), and cardiomyopathy (Salinas et al. 2010). Such cardiovascular changes are associated with altered endothelial reactivity (Ruijtenbeek et al. 2003a, b) and sympathetic hyperinnervation of peripheral arteries (Rouwet et al. 2002; Ruijtenbeek et al. 2000). The prolonged hypoxic-induced asymmetric growth restriction and cardiac remodeling were not seen when supplemental O_2 was administered to those chick embryos at low barometric pressure (Giussani et al. 2007; Salinas et al. 2010). As in the fetal lamb, in the chick embryo, long-term hypoxia demonstrated decreased ventricular $+dT \cdot dt_{max}^{-1}$, peak pressure, and ventricular ejection fraction (Sharma et al. 2006). Other studies have reported adult sequelae of antenatal hypoxia. For instance, following hypoxic incubation as chicks, the femoral arteries of adult chickens showed increased sensitivity to both pharmacologic and electrical stimulation of periarterial sympathetic nerves, as well as decreased NO-dependent vasodilatation (Ruijtenbeek et al. 2003a).

12.6 Fetal Growth Restriction and Neuropsychological Correlates

The correlation of cerebral neuroanatomical and neuropsychological changes with fetal growth restriction has been described by numerous investigators (Bhide 2011). Chronic hypoxia per se or hypoxia-ischemia as a consequence of prolonged reduction in uteroplacental blood flow can have invidious short-term and long-term consequences for the developing brain (Rees et al. 2008). For instance, an association of FGR with poor neurobehavioral and cognitive performance has been reported in neonates and 1-year-olds (Batalle et al. 2012; Figueras et al. 2009). Some neurobehavioral impairment appears to be even more pronounced in preterm, as compared to near-term FGR infants (Rees et al. 2008), although these differences were not observed over a long-time period of life (Bassan et al. 2011). Nonetheless, long-term follow-up studies have demonstrated significant neurodevelopmental delays in childhood (the GRIT Study Group 2004) persisting into adolescence (Aarnoudse-Moens et al. 2009; Feldman and Eidelman 2006). Other investigators have reported cognitive impairment and learning deficiencies observed in school being related to a characteristic pattern of altered short-term memory, attention span, and anxiety (Feldman and Eidelman 2006; Geva et al. 2006a, b; Leitner et al. 2007), with in some cases an increased risk of overt attention deficit disorders (Geva et al. 2006a, b; Heinonen et al. 2010). A European randomized multicenter Growth Restriction Intervention Trial disclosed a significant increase in disability at 2 years of age in infants <31 weeks gestation, as compared to near term; however, differences were not apparent between the immediate and delayed groups (GRIT Study Group 2004).

These behavioral changes have been suggested to serve as indices of specific neurological changes such as the anterior hippocampal-prefrontal cortical network, the parahippocampal complex, the striatum thalamus, and other structures (Cubillo et al. 2012; Eichenbaum et al. 2007; Geva et al. 2006a, b). Evaluated at 40 ± 1 weeks, preterm growth-restricted fetuses with evidence of placental dysfunction (abnormal umbilical artery pulsatility index) and abnormal middle cerebral artery pulsatility showed markedly longer neurobehavioral scores and outcome (Neonatal Behavioral Assessment Scale: attention span, motor, and social interactive) (Figueras et al. 2011). In a major multi-study review of neurodevelopment endpoints from birth up to 34 weeks of gestation, fetal growth restriction was associated with lagging prenatal size of head and other parameters (Baschat 2011), as well as motor dysfunction and neurological delay within the first 2 years of life. The role of placental dysfunction to increase the risk of neurodevelopmental delays has become apparent (Baschat 2014). Long-term follow-up is required to appreciate the impact of prenatal growth on behavioral, cognitive, speech and health liabilities. Abnormal middle cerebral artery Doppler findings have been shown to be predictive of neurobehavioral impairment among preterm (<34 weeks gestation) FGR infants by others (Figueras et al. 2011). Cerebral palsy was increased manifold in FGR infants, particularly those with a major birth defect and born near term to

normotensive mothers (this association was not observed in hypertensive mothers) (Blair and Nelson 2015).

Magnetic resonance imaging (MRI) has demonstrated a number of structural changes in the brain of the FGR fetus and newborn infant (Batalle et al. 2012; Sanz-Cortés et al. 2013), including changes seen in the cerebral cortex (Dubois et al. 2008) and hippocampus (Lodygensky et al. 2008). Rather than gross tissue destruction, FGR is believed to be associated with more subtle disruption of normal neurodevelopment (Rees et al. 2011). To detect such changes in structure and organization requires modifications of MRI. To date however, we know of no long-term studies that have evaluated the correlation of functional impairments with the underlying neurological anomalies, however. A challenge for the future is to clarify the relation of antenatal and postnatal stress to the developing organism to neuropsychologic and new behavioral capabilities in early and later life.

12.7 Fetal Growth Restriction and the Placenta

Because of the intimate relations of the placenta to growth of the fetus and the genesis of its restriction (Baschat 2004; Bell et al. 1999; Cetin and Alvino 2009; Cetin and Antonazzo 2009; Mellor 1983; Myatt 2006; Sibley et al. 2005; Wallace et al. 2005), it may be of value to consider several aspects of these under conditions of long-term hypoxia (Charnock-Jones et al. 2004; Tissot van Patot et al. 2012) or other stress. Designed for efficient exchange of O_2 and nutrients between the maternal and fetal circulations, as noted in Chap. 8, the placenta elaborates hormones that determine fetal and, in some respects, maternal metabolism. Among other considerations, the efficiency of placental exchange also is a function of morphology, which varies considerably among species (Amoroso 1952; Leiser and Kaufmann 1994; Mossman 1987).

In addition to its role in serving as the fetal lung in supplying oxygen to the developing organism, to support its own energy demands, the placenta consumes a considerable fraction of the O_2 exchanged. During the third trimester, with exponential growth of the fetus and proportionally modest enlargement of the placenta, O_2 availability may be limited, thus fetal O_2 demands may not be fully met and a risk for growth restriction may result (Longo 1987). In patients with preeclampsia or other vascular disorders, this may be aggravated to be more than an issue of unbalanced supply and demand (Corso and Thomson 2001; Murray 2012; Postigo et al. 2009; Singla et al. 1997; Soleymanlou et al. 2005; Zamudio et al. 2007).

Of relevance, in a relatively recent retrospective report, in the near-term placenta of FGR human newborns, several measures of growth of villi and fetal capillaries have been shown to be abnormal, including a significant 24% decrease in placental weight (from 470 ± 52 to 357 ± 41 gm) (Calvert et al. 2013). Others report reduced villous and capillary growth, without changes in measures of lumen caliber or shape (Mayhew et al. 2004). Perhaps of relevance in FGR placentas, fibrocyte-like cells demonstrate a reduced ability to promote angiogenesis (Riddell et al. 2013). As

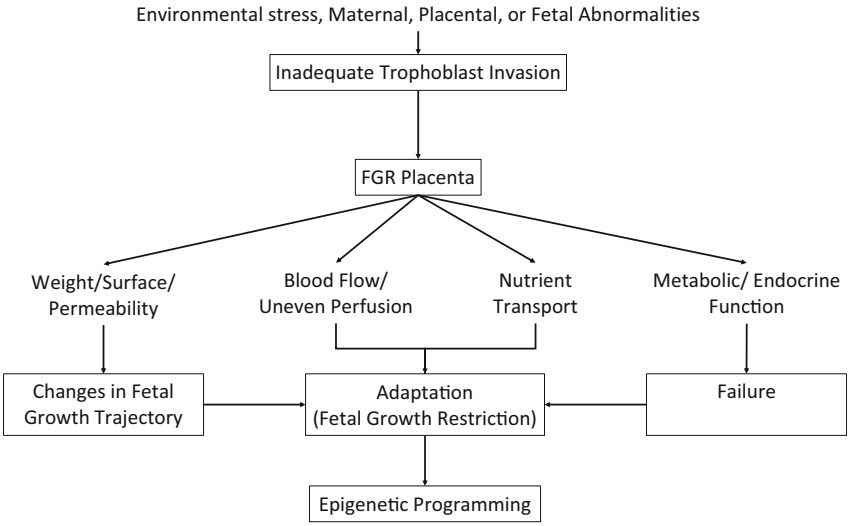

Fig. 12.4 Some of the possible mechanisms involved in environmental stress with maternal, placental, and/or fetal abnormalities that result in inadequate trophoblast invasion and epigenetic programming of diseases

depicted in Fig. 12.4, inadequate trophoblast invasion may play a key role in the genesis of FGR. As an aside, although it has been suggested that asymmetric growth of the FGR fetus is a consequence of altered trophoblast apoptotic activity, the evidence does not support this thesis (Roje et al. 2014).

In a further attempt to understand the mechanistic basis of some of the prolonged hypoxia-associated FGR changes noted above, in a mouse "model" of FGR, we tested the hypothesis that the placental response to hypoxic stress is associated with important gene expression changes. We quantified such expression in response to 48 h of hypoxia near term (Gheorghe et al. 2007). Pregnant mice at 15.5 DPC were exposed to 48 h of hypoxia (10.5% O_2), after which the Affymetrix Mouse 430A_2.0 array was used to measure gene expression changes (Gheorghe et al. 2007). 171 probe sets, corresponding to 163 genes, were regulated by hypoxia ($P < 0.01$). Ninety of these genes were upregulated, and 73 were downregulated. We annotated the regulated genes and observed several overrepresented functional categories. Upregulated genes included those involved in metabolism, oxygen transport, proteolysis, cell death, metabolism of reactive oxygen species, and DNA methylation. Genes involved in transcription, cell cycle regulation, and cell structure were downregulated. The observation that hypoxia upregulates ROS metabolism, in conjunction with DNA methylation enzymes, suggests that in addition to the placenta, hypoxia may contribute to long-term epigenetic changes in stressed fetal tissues and organs (Gheorghe et al. 2007).

In the human FGR placenta, microarray gene expression confirmed by real-time polymerase chain reaction studies demonstrated several-fold increased expression

of a number of genes, including those for soluble endothelial growth factor receptor, human chorionic gonadotropin, HIF-2α, follistatin-like 3, and leptin. These changes suggest active placental angiogenesis (McCarthy et al. 2007). In the placenta of FGR infants, studies also demonstrate significant differences in gene expression patterns of insulin-like growth factor 1 and 2 (IGF 1, 2) and IGF-binding protein 3 (Börzsönyi et al. 2011). In addition, underexpression of the *11β-HSD2* gene with impaired feto-maternal glucocorticoid metabolism was seen (Börzsönyi et al. 2012).

Additionally, in women diagnosed as having FGR with preeclampsia, a number of placental vascular lesions were noted, as compared to controls (Kovo et al. 2015). Furthermore, in the human FGR placenta, the ratio of mRNA from the maternally expressed gene *Phlda2* to that of the paternally expressed gene *Mest* has been shown to be increased. No changes in DNA methylation were observed, however. Four other imprinted genes were differentially expressed, as were a number of non-imprinted genes (McMinn et al. 2006). In another study of gene expression in the FGR placenta, signaling pathway analysis disclosed upregulation of 47 genes including those of the inflammation-mediated cytokine and chemokine pathways, as well as those for angiogenesis, with downregulation of genes that encode for ribosomal proteins (the latter suggesting reduced translation) (Sitras et al. 2009). Of interest, in this report none of the known imprinted placental genes were expressed differentially, and those FGR gene expression changes were similar to that seen with preeclampsia (Sitras et al. 2009). Also of more than passing intent, material plasma RNA levels of fms-like tyrosine kinase 1 (FH-1), the VEGF receptor 1, were elevated significantly in each trimester in those women destined to give birth to a FGR infant (Takenaka et al. 2015). Use of this marker, presumably elevated because of placental dysfunction with altered angiogenesis, may prove of value in the prediction and early diagnosis of FGR.

Of relevance, in cultured human trophoblastic cells, hypoxia increased the expression of glucose transporters (Esterman et al. 1997) while inducing the expression and activity of system A amino acid transporters (Nelson et al. 2003). Also of importance, expression of the ATP binding cassette (ABC) superfamily member G2 (ABCG2), a major membrane transporter for xenobiotics, was reduced significantly in the placenta of infants showing growth restriction (Evseenko et al. 2007). Further, in the human FGR placenta the mitochondrial DNA content was increased significantly (35%), and this change was correlated inversely with umbilical venous PO_2 (Lattuada et al. 2008). These changes all suggest placental adaptation to O_2 and/or nutritional restriction. In pregnancies complicated by FGR, an examination of microRNAs, short noncoding RNAs that regulate gene expression at the posttranscriptional level, disclosed slightly higher levels of several species in maternal plasma with lower levels in the placental tissue (Mouillet et al. 2010). This finding has great implications for dysregulation of protein synthesis in the placenta and fetus. FGR infants, born at high altitude on the *altiplano* of Bolivia, demonstrated inhibition of the peroxisome proliferator-activated antigen gamma, a potential link between long-term hypoxia and growth restriction (Julian et al. 2014).

A unique feature of placental mammals in the existence of so-called imprinted genes is that they exhibit parent-of-origin monoallelic differences in expression. Not found in other animals, these play important roles in development of the placenta as well as in embryonic and fetal growth (Angiolini et al. 2006; Fowden et al. 2006a, b; Reik et al. 2003). Disruption of any of the 60 or so imprinted genes expressed in the placenta can result in abnormal placental development and FGR (Morgan et al. 2005; Piedrahita 2011). In humans, over 50 imprinted genes have been identified. These are distributed in distinct clusters that are regulated by a common imprinting control region (Reik and Walter 2001). In regard to placental and fetal development, two clusters of imprinted genes lie within chromosome 11p15.5, each regulated by a separate imprinting control region, -1 and -2. Decreased expression of the paternally expressed *IGF-2* gene has been reported in the placenta of the FGA infant (Guo et al. 2008).

Among imprinted genes, those for the cullins code for hydrophobic proteins that provide a scaffold for ubiquitin ligases and combine with RING proteins to form cullin-RING ubiquitin ligases have been appreciated to be of importance. As a family of seven distinct genes, cullins not only are involved in targeting proteins for ubiquitin-mediated destruction as in embryonic limb patterning but also serve as a docking site for ubiquitin-conjugating enzymes involved in cell cycle control. The placentas of patients with FGR, preeclampsia, and several other conditions show significant elevation of cullin7, 1, 4A, and 4B, and these may serve as biomarkers for several varieties of trophoblastic disease (Gascoin-Lachambre et al. 2010). Emphasis should be given to the identification of targets of cullin-mediated protein degeneration in association with FGR. Along this line, homogenized umbilical cord samples from a population of Chinese women showed significant inverse relation of expression of the imprinted gene pleckstrin homology domain, family A, member 2 (PHLDA2) and BW, with downregulation of paternally expressed gene 10 (PEG10). The latter was associated with concomitant methylation patterns of the PEG10 promoter as a biomarker for FGR (Lim et al. 2012).

In a knockout mouse model in which the paternal allele placental-specific transcript of the imprinted *Igf-2* gene was deleted, a placental phenotype similar to that seen in FGR, with a decrement in fetal growth, was observed (Constância et al. 2002; Sibley et al. 2004). These studies have led to speculation that alterations in the patterns or phenotype of the human placenta can be associated with specific patterns of fetal development under conditions of growth restriction (Sibley et al. 2005). Further studies in the FGR placenta reported upregulation of leptin, corticotropin-releasing hormone IGF-binding protein 1 (Struwe et al. 2010), with differences noted for site of placental sampling (Tzschoppe et al. 2010). In addition, mutations of Tgf-1 and its receptor IGF^{-2} may be associated with severe FGR (Begermann et al. 2015). Investigation also reported increased placental leptin mRNA and protein, as well as in venous umbilical cord blood-elevated leptin binding capacity with reduced leptin levels (Tzschoppe et al. 2011; Lepsch et al. 2016). The authors suggest that in the infant this may play an important role in induced dysregulation of appetite regulatory mechanisms which may lead to further growth restriction (Tzschoppe et al. 2011; Lepsch et al. 2016). Reduced methylation (associated with increased

gene expression) of *Icr-1* is associated with FGR in normotensive patients (Bourque et al. 2010). In an analysis of 74 "putatively" imprinted genes in placental tissue of FGR and normal control pregnancies of 52 (70%) genes so expressed, five were upregulated and four downregulated; but loss of imprinting gain of function did not play a major role in these changes (Diplas et al. 2009). One must caution that although perturbation in genomic imprinting has been associated with FGR, no correlation of differentially methylation regions with a specific biological function has been demonstrated. Rather the correlation was with gene length (Lambertini et al. 2011). A caveat to differential expression in all of these placental genome studies is that the cell of origin, i.e., syncytiotrophoblast versus cytotrophoblast or other cell types, was not defined.

A related consideration for the growth restricted fetus is that of decreased amniotic volume (oligohydramnios) which may aggravate a nonhospitable environment. The association of low values of amniotic fluid index (an estimate of the amount of amniotic fluid and part of the biophysical profile (Griffin et al. 2009) with a growth-restricted fetus has been reported by several groups (Banks and Miller 1999; Chamberlain et al. 1984; Chauhan et al. 1999).

12.8 Fetal Growth Restriction and the Developmental Origins of Adult Health and Disease

A major cause of death in the USA and the world, heart disease imposes an enormous burden on patient health as well as the economy in terms of lives, lost productivity, and medical costs (Heidenreich et al. 2011; World Health Organization 2012). Although risk factors in adulthood, such as cigarette smoking, high body mass index, and lack of exercise, are important contributors to this pandemic of cardiovascular and other diseases, a significant percentage of affected individuals do not have these risk factors. Evidence has accumulated to recognize that early in life, well before birth, environmental stresses such as maternal hypoxia, dietary imbalance, and other maternal physiological considerations can affect specific gene expression patterns in the fetus to "program" cardiac or other disease in later life (Barker 1994; Barker et al. 1989; Dessì et al. 2012; Fowden et al. 2005, 2006a, b; Hales and Ozanne 2003; Leon et al. 1998). A number of these factors have been tabulated (Table 12.2). Increasingly appreciated is that the factors responsible for FGR may have profound influences beyond childhood in one's life course, including the developmental origins of adult health and disease with intrauterine programming during critical periods of vulnerability, the failure to meet certain developmental milestones, and the permanent nature of specific sequelae (Barker 1994) (Table 12.2). Despite the demonstration of strong associations in this regard, little is understood about the underlying mechanisms.

During the past several decades, the concept of the developmental origins of adult disease (DOHaD) has become a focus of studies for understanding the genesis

Table 12.2 Adolescent and/or adult long-term sequelae of fetal growth restriction in offspring

• Effects on growth and puberty
Born with low birthweight
Poor postnatal growth
Short stature in adolescents and adults
Premature adrenarche
Premature pubarche in females
Body composition: decrease in fat mass at birth, accelerated gain in fat mass during adolescence and later
• Metabolic conditions
Glucose tolerance impaired
Mild-to-moderate insulin resistance
Resetting of IGF/insulin systems, circulating concentrations of IGF-1 below average for age and sex
Metabolic syndrome
Dyslipidemia
Type 2 diabetes mellitus
Obesity
• Other endocrinopathies
Mild hyperthyrotropinemia in absence of overt hypothyroidism
Decreased adiponectin and follistatin in children
Increased fetal/neonatal glucocorticoid exposure
Polycystic ovary syndrome
Early menopause
• Cardiovascular disease
Hypertension
Coronary artery disease
Cyanotic heart disease
Atherosclerosis
Cardiomyopathy with heart failure
Cerebrovascular accident (stroke)
Coagulation disorders
• Chronic pulmonary disease
Obstructive lung disease
Asthma
• Renal insufficiency
• Immunodeficiency
• Neuropsychiatric sequelae
Neurodevelopmental delay
Multiple syndromes
Schizophrenia
• Skeletal system
Osteoporosis

Table modified from several that are in the literature

of long-term sequelae of the offspring when they become adolescents and adults (see Chap. 16). Among conditions so identified in children and adolescents are short stature and premature adrenarche. In adults, the host of conditions so identified include cardiovascular disease (hypertension, coronary artery disease, cardiomyopathy, and cerebral vascular accident), metabolic syndrome and type 2 diabetes, some malignancies, and several neuropsychiatric disorders (Table 12.2). In addition

to the implications of these diseases for the lives of individuals, their social and public health considerations are far from trivial (see below).

The lack of understanding of this complex syndrome of growth restriction is not unlike a similar situation in the seventeenth century. Until the latter part of that century, specific infectious diseases such as typhus, typhoid, malaria, etc., were not recognized as such. Rather, these and other febrile illnesses were classified as a single disorder—fever, i.e., distinguished as continual, intermittent, hectic, recurrent, or remittent. There was little or no understanding of the underlying etiology for the febrile condition (King 1958). It was Thomas Sydenham (1624–1689), the "English Hippocrates," who first differentiated between typhoid fever and typhus and defined other febrile conditions such as measles and scarlet fever (Payne 1900). Today, over three centuries later, we need a contemporary Sydenham to cut through the Gordian knot[1] of the various mechanisms and their interactions which produce the fetal growth restriction phenotype.

While falling far short of that goal, the present chapter attempts to synthesize into a coherent whole some of the stress-induced mechanisms which interact in several key organ systems to result in abnormal fetal growth and development. One may thus consider the manner(s) by which failure of the normal mechanisms might result in growth restriction of the fetus/newborn infant in those instances when the etiology is not known. For the most part, these mechanistic-based experimental studies have been performed in species suitable for investigation of physiologic (sheep) and cellular and/or molecular (rodents) mechanisms. Therefore, one may view this as an exercise in exploring what is known and what is unknown of the mosaic of interdigitating and complementary factors and mechanisms that eventuate in the developing fetus experiencing growth restriction (Longo 1984).

The past several decades have witnessed considerable advance in our understanding the role of prolonged antenatal hypoxia, not only in the genesis of FGR and associated disease in the fetus/newborn (Kramer et al. 1990) but in establishing the long-term consequences of this stress in adolescent and adult offspring. These conditions in the adult include the major causes of morbidity and death, cardiovascular and cerebrovascular disease (Barker 1994; Leon et al. 1998; World Health Organization 2012), as well as metabolic syndrome (Gluckman et al. 2008), some malignancies, and a number of neuropsychiatric disorders (Table 12.2). In an attempt to understand the association between stress experienced as a fetus and/or infant and disease as a young adult or later in life, the "thrifty phenotype" hypothesis was proposed (Hales and Barker 1992). That is, epigenetic changes in gene transcription alter cellular metabolic functions to affect receptor and/or enzyme activation and downstream events such as insulin resistance, vascular contractility, and other functions that extend into later life. (The cellular/subcellular mechanisms by which this is affected remain unknown, however). Along this line, the

[1] An intricate knot tied by King Gordius of Phrygia and cut by Alexander the Great (356 BCE-323 BCE) with his sword after hearing an oracle promise that whoever could undo it would be the next ruler of Asia.

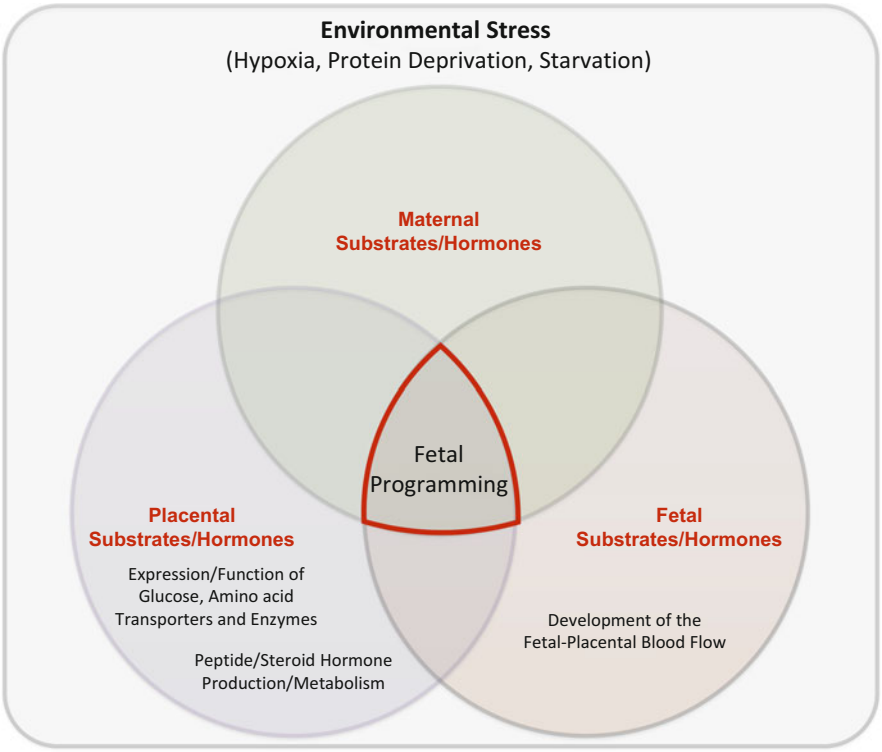

Fig. 12.5 Illustration of the convergence of various environmental stressors and thier interaction in the mother, placenta, and/or fetus that interact to effect fetal programming

"predictive adaptive response" suggests metabolic responses such as insulin resistance that emerges in anticipation of poor-quality adult environment (Wells 2011). Also to be considered is the competing and "maternal capital" hypothesis which considers thrift to involve reductions in lean body mass and organ phenotype arising from constrains on maternal phenotype (Wells 2009a, b, 2010, 2011). A major issue in this regard is to move beyond the phenomenology of occurrence and possible relations to that of causative mechanisms. Some relevant environmental stresses are diagramed in Fig. 12.5. In turn, some of the epigenetic-induced molecular mechanisms are illustrated in Fig. 12.6.

Although a consideration of the nuances of antenatal hypoxia and the fetal programming hypothesis is beyond the scope of this chapter, evidence from both epidemiologic studies in humans and experimental studies in laboratory animals about the genesis of FGR has raised a number of questions in regard to the underlying mechanisms of signaling for the development of these major long-term sequelae. Additional questions pertain to means of possible preventative intervention. With the evidence of NO oxidative stress and many epigenetic factors playing a role in the pathophysiology of this syndrome, such interventions have

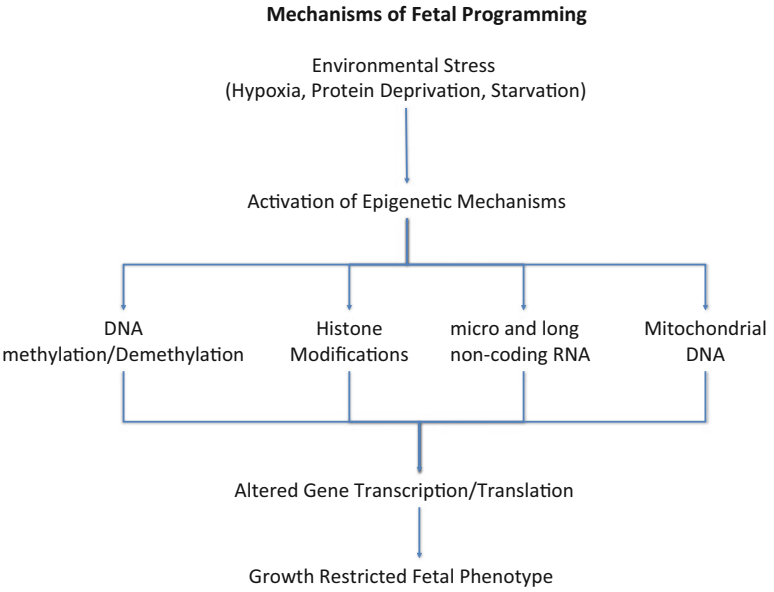

Fig. 12.6 Overview of different epigenetic mechanisms responsible for fetal growth restriction and programming

been explored by several groups. These pursuits raise the possibility that postnatal treatment may rescue the FGR newborn from long-term sequelae. Many of these recent studies, with their promise and problems, have been reviewed (Giussani and Davidge 2013).

12.9 Conclusions with Perspective

The syndrome of FGR, a complex interplay of confounding variables, presents a variety of phenotypes. Beyond the phenomenology of having a birthweight at or below the 10th percentile, and beyond genetic- and environmental-epigenetic-mediated factors, a seemingly infinite mosaic of cellular, subcellular, and molecular alterations and mechanisms are involved. That said, we remain in a circular argument. Despite increasing evidence to support maternal stress in the pathogenesis of FGR, at present we have little or no knowledge of the initiating event and the multiplex of signaling networks involved.

Oxygen being an essential requirement for aerobic metabolism and life, from the earliest stages of development, cellular hypoxia poses significant challenges for survival. Fortunately, cardiovascular, endocrinologic, metabolic, and other acclimatization responses mitigate that risk and work to preserve oxygen homeostasis at the organismal level. Hypobaric hypoxic-induced physiologic responses in the

mother include increased ventilation and O_2 transport capacity and compensatory changes in uteroplacental blood flow. Placental function also is optimized with greater capillary blood volume and shorter transcapillary diffusion distance. For the fetus, a number of metabolites, growth factors, and other molecules may influence its tissues to constrain protein synthesis and growth in an attempt to survive limited O_2 availability. In this regard, because of its potent influence on central nervous system function, the pathophysiology of cerebral hypoxia, the regulation of cerebral blood flow, and the mechanisms involved in the development of and responses to cerebral edema and ischemia are of profound importance. Whereas cellular hypoxia and caloric/protein deprivation are perhaps the most important primary stimulus to homeostatic responses, multiple secondary responses in levels of circulating catecholamines, cortisol or other stress-related hormones, the multiple growth factors and cytokines involved in protein synthesis, and other cellular responses are clearly critical to the process.

As noted, despite explorations into fundamental mechanisms that account for FGR, there remain enormous gaps in our understanding. Whereas, to some degree, the changes in the fetal environment are "buffered" by maternal homeostatic mechanisms, it also is clear that under conditions of prolonged hypoxia, caloric/protein restriction or other invidious environmental factors, the fetus constitutes a stress for the mother. From this perspective, it would enhance the survival mechanisms for both the mother and conceptus if the latter were to mature more rapidly. Whereas this hypothesis has some attraction, it remains highly speculative and in need of definitive experimental verification.

In particular, identification of the signals whereby cells sense limited O_2 or other nutrient availability remains a critical unknown. How, and by what cells and tissues, the "prime mover" responses are mounted is a fascinating question that has been pursued for decades. Recent advances are promising in that they point the direction for the focus of more cellular and molecular investigation of gene regulation to elucidate their role in this process and the mechanisms by which they are activated to effect protein synthesis in the developing organism. The rapidly growing diversity and power of new and powerful technology offers an unprecedented opportunity and great promise for furthering our understanding. Our challenge today, however, is to promote a new generation of studies of the mosaic of hypoxic- and other mediator-induced gene regulation and/or metabolomics that will yield the key clues to the origins of FGR and to develop possible therapies to correct/alleviate the mechanistic dysfunction. These are discoveries for which this field has for so long been in search.

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Chapter 13
Fetal-Neonatal Growth and Metabolism

13.1 Robert A. McCance, Elsie May Widdowson, and Continued Studies of Growth and Metabolism

Several previous chapters include aspects of metabolism of the fetus and newborn; consideration of fetal neonatal growth and metabolism also must reference Robert A. McCance and his long-time associate Elsie May Widdowson (1906–2000). Both of these workers' contributions to the chemical composition of foods and nutrition changed the understanding of metabolic energy balance in newborn infants and children. Following his service as a pilot with the Royal Naval Air Service during World War I, McCance obtained his Cambridge doctorate in biochemistry under Sir Frederick Gowland Hopkins. He also studied physiology under Joseph Barcroft, "who became one of his heroes". Because of his interest in clinical chemistry, McCance then enrolled in King's College Hospital, London, to complete his medical education (MB, 1927; MD, 1929). Although acknowledged widely for his contributions to infant nutrition, McCance investigated a variety of problems in metabolism, the composition of foods, and that of body tissues, and the body's responses to cold and heat. His early studies on the chemical composition of raw and cooked foods, performed under the aegis of a small subvention from the Medical Research Council, obtained by his mentor and early specialist in diabetes Robert Daniel Lawrence (1892–1968), lead to a special MRC report *The carbohydrate content of foods* (McCance and Lawrence 1929). This was followed by three other such special MRC reports on various types of foods (McCance and Shipp 1933; McCance and Widdowson 1940; McCance et al. 1936). During these years, McCance also involved himself in studies of mineral metabolism and salt balance (McCance 1936).

Because of the influence of the newly appointed (1935) Regius Professor of Physic, John Alfred Ryle (1889–1950), in 1938 McCance was asked to return to Cambridge as a Reader in Medicine. Later (1945), the Medical Research Council created a personal chair for McCance, and he chose the title Professor of

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Experimental Medicine. This unit was the first such in the UK. As at mid-century, Ryle played a critical role in the development of clinical research in Great Britain, it may be of value to consider in brief his contributions. Previously at Guy's Hospital, London, Ryle came to believe strongly that clinical science in medicine constituted an important aspect of academics and the profession (Ryle 1930). In part, this concept and commitment was due to the influence of Sir Thomas Lewis of University College Hospital. As noted earlier, Lewis was a UK pioneer on the role of scientific investigation to the advancement of medicine (Lewis 1930). In a 1931 address to the Cambridge University Medical Society, "The Physician as Naturalist", Ryle summarized his views (Ryle 1931). In opening his address he quoted Francis Bacon's *Advancement of Learning*, "Only there is one thing remaining, which is of more consequence than all the rest: namely, a true and active Natural Philosophy for the Science of Medicine to be built upon" (Ryle 1931, p. 278). Upon moving to Cambridge, and assuming his Regius Professorship, in his inaugural lecture "The aims and methods of medical science" Ryle elaborated on his views of the physician-scientist. He observed, "The aims of medical science ... are to increase and perfect our knowledge (with a view to its control) of disease in man, and equally our knowledge of man in disease, by every legitimate means of science and art at our disposal" (Ryle 1935, p. 8). Stressing the role of the University in this regard, Ryle called for "... a renaissance in Medicine, which will be marked by an eagerness on the part of the younger men ... to turn more frequently to the study of problems at the bed-side and with a greater care and accuracy than they have ever before been studied ... the ward, with its associate out-patient clinic and laboratories, must become once more both the starting and the rallying point of many researches" (Ryle 1935, pp. 40–43). To implement his ideals, Ryle sought to establish at Cambridge's Addenbrooke Hospital a strong program of clinical-translational research (Weatherall 2000). In attracting him to this newly created post, Ryle held that McCance represented a new variety of physician investigator who specialized in that most fruitful branch of science—biochemistry in its application to medicine.

In the mid-1930s, a chance observation by one of McCance's former house physicians, Winifred Ferguson Young (1909–1969), who had worked with him on experimental salt deficiency in adults, changed the course of their lives. Young had moved to the Children's Hospital in Birmingham to work under Sir Leonard Gregory Parsons (1879–1950), a UK leader in the development of pediatrics as a medical specialty. An advocate of "antenatal pediatrics" (Neale 1956; Parsons 1946), Parsons had worked with infants to call attention to rickets (vitamin D deficiency, Parsons 1928) and scurvy (vitamin C deficiency, Parsons 1933). In the course of testing the urine of newborns for sugar and albumin, Young also had measured the extent to which these infants excreted "proper amounts" of chloride. To her astonishment, she discovered none of this anion, raising the question of whether these newborns were salt deficient. After reporting this to McCance, they decided to commence renal function studies in newborn infants. Later, McCance recorded that:

> ... we decided therefore, albeit with some trepidation, to make as satisfactory an investigation of renal function of newborn infants as we felt would be ethically correct. We did not feel justified in catheterising normal newborn infants and making multiple venous punctures for the injection of solutions of inulin and the collection of timed specimens of urine and blood, but someone ... suggested that we should use infants born with inoperable meningomyeloceles for such investigations. After considering very carefully ... we decided to make the studies ... and we did the infants no harm by doing so.
>
> (McCance 1978, p. 2)

With a battery of tests (glomerular filtration rate, inulin clearance, and others), McCance and Young discovered that, compared to the adult, the newborn kidney in many respects was inefficient, forming hypotonic urine (McCance and Young 1941).

> When we came to consider our results it was clear that the kidneys of the newborn infant had very low clearances of urea, chloride, and sodium by adult standards, and also very low powers of concentration. The serum potassiums were on the whole higher than those of adults, occasionally twice as high, and the clearances low. In short, the kidney of the newborn infant was revealed to be a relatively ineffective organ. Yet it was apparently able to maintain the internal environment in a healthy state.
>
> (McCance 1978, p. 2)

This phenomenon was even more pronounced in the premature newborn (Young et al. 1941). McCance extended these studies to newborn laboratory animals: rats, puppies, and piglets (McCance 1948). He demonstrated in humans and other species that the renal system does not acquire the capacity to regulate these functions (fluid, electrolyte, and acid–base balance) until the newborn period and suggested that "... mother's milk was such a perfectly constituted food for the newborn, and the rate of growth at that time was so great ... that for a period after birth the kidney had little to do. Subsequent work revealed [this to be] correct" (McCance 1978, p. 3). Later, Young was appointed research clinician to London's Queen Elizabeth Hospital for Children (1948) and was a driving force in founding (1962) the Research Appeal Trust (Anonymous 1969). Findings by these workers led to the appreciation of growth during infancy in maintaining stability of the *milieu interior*, as defined by Claude Bernard (1865).

When fed on mother's milk, the newborn's rapid growth required so much of the ingested nutrients, with the nitrogen being incorporated into growing tissue, that the infant "... could almost do without kidneys at all" (McCance 1948; Widdowson 1993, p. 385). During the following decade, understanding of renal function in the newborn infant made great progress, including comparisons and contrasts of humans with other mammals, and aspects of the relation of renal function to body growth and metabolism (Alexander et al. 1958; Smith 1959 (3rd Ed)). As an example, Gertrude Falk (1925–2008), working with Adolph at the University of Rochester, reported studies on mechanisms of postnatal renal development. In the rat, Falk examined renal responses to water excess, water deprivation, hypertonic solutions, and the effect of posterior pituitary and adrenal hormones, in an attempt to determine the basis of impaired water excretion, reduced glomerular filtration rate, and inability to concentrate urine in the newborn (Falk 1955). With the support of a Guggenheim Fellowship, in 1961 Falk moved to University College, London, where she remained to explore the mysteries of photoreceptor cells in the retina (Anonymous 2008).

As is well known, the onset of World War II necessitated food rationing. As a consequence, the threat of undernutrition was appreciated as being a potentially important clinical problem. With their background in nutrition (see Widdowson and Shackleton 1936), McCance and Widdowson, members of the Medical Research Council (MRC) staff, prepared their classic monograph *The chemical composition of foods* (McCance and Widdowson 1940). At the end of the war, and aware of the impending critical food shortage in Germany, McCance persuaded Sir Edward Mellanby, Secretary of the MRC, of the need for a team of experts to study the effects of undernutrition on metabolism and well-being on the continent. Following a preliminary survey, the small town of Wuppertal, Germany, an area in which adult rations were only about 1000 kilo calories per day, and in which "hunger edema" was prevalent, was selected as the site for these studies. The series of studies on this population were published as a MRC Special Report, *Studies of undernutrition, Wuppertal, 1946–1949* (McCance and Widdowson 1951). In these, McCance focused his efforts chiefly on malnutrition in the adult population, while Widdowson followed these effects in children (see below and Widdowson 1995). One study of this series examined the effects of undernutrition and size at birth and the yield of maternal breast milk (Dean 1951).

These studies in Germany were followed by a series of experiments on nutrition and metabolism in the newborn. Upon understanding that in the newborn infant, growth, rather than the kidneys, played a critical role in maintaining *homeostasis*, as defined by Walter B. Cannon (Cannon 1932), McCance summarized these studies on aspects of newborn metabolism and its regulation in his 1959 Sir Leonard Parsons Lectures at Birmingham, in tribute to the individual who had done so much to develop the field of infant nutrition (McCance 1959b). He also reviewed this work in his 1962 Lumleian Lecture to the Royal College of Physicians, "Food, growth, and time" (McCance 1962). In addition, McCance reviewed his and others' studies on thermal stability in the newborn (McCance 1959a). As an aside, McCance resided in Bartlow, a village about 20 km from Cambridge, from which he daily cycled to and from his office. Widdowson has recorded that ". . . he used his cycling time to think up new ideas for research or to solve problems arising from those already underway" (Widdowson 1993, p. 386). In a 1980 tribute to McCance, Widdowson recorded that in the 1950s and 1960s, McCance, ". . . paid many visits to Oxford to learn all he could about the work of Geoffrey Dawes and his group were doing, and to London to see Kenneth Cross and [others] . . . He always came back full of enthusiasm about the discoveries these people were making, and his lectures covered their pioneering work as well as his own" (Widdowson 1980, p. 3).

Several years later, McCance updated this synthesis in his contribution "Characteristics of the newly born" for the 1961 *Ciba Foundation Symposium on Somatic Stability in the Newly Born*, in which Dawes participated. In this review, McCance stressed that despite "instability" of many metabolic functions in the newborn, its "tolerance" and plasticity is considerable (McCance 1961). In two further recapitulations a decade following his "retirement" in 1966, McCance addressed the issue of "Critical periods of growth," and the thesis that, for the undernourished newborn, its ability or non-ability for "catch-up" growth was a function of the temporal

relation to the period of undernutrition to the time of maturation of the hypothalamic food satiety centers (McCance 1976, 1977). In an effort to study further several aspects of fetal metabolism, McCance experimented with development of an artificial placenta, which he used on piglets and several nonviable human fetuses (Lawn and McCance 1962). Several decades later, in recalling his interactions with McCance, Dawes stated, "Perinatal physiology owes a great debt to the pioneer work of Professor McCance and his distinguished colleagues on renal function and nutrition ... My own interests were in an area somewhat different to that which Mac had already established. He had set us the excellent example of combining basic and applied research without letting one dilute the other." Dawes also praised McCance's "... initiative... and interdisciplinary cooperation on a friendly and informed intellectual scale" (Dawes 1980, p. 4).

Having joined McCance in Cambridge at the time of his 1938 move, Elsie May Widdowson continued their collaboration on renal function, metabolism, and thermal regulation of the newborn. As noted, following World War II, this was interrupted by a 3-year "sabbatical" (1946–1949) working in Wuppertal on nutrition with emphasis on that of children. With several orphanages nearby, she determined the optimal constituents for a loaf of bread for the growth of children who were undernourished (Widdowson and McCance 1954). In these studies, Widdowson serendipitously discovered the importance to growth and development of emotional contentment and mental well-being, despite adequate nutrition *per se* (Widdowson 1951). She wrote, "Tender loving care of children and careful handling of animals may make all the difference to the successful outcome of a carefully planned experiment." In reference to this conclusion, one of her associates commented, "love must be the best diet" (Ashwell 2002, p. 495).

Following her return to Cambridge in 1949, Widdowson commenced a study in which she compared body composition of human fetuses and stillborn infants, with that of an older child and several adults (Widdowson and Dickerson 1960). She extended this study, examining body fat in several species, showing that in comparison to the newborn of many species with 1–2% body fat, at birth the human infant has ~16% body fat. These studies also demonstrated some general principles, such as the long-term impact of early nutrition on development (Widdowson 1963), as well as important species differences relating to maturity. To compare chemical development in the various tissues, Widdowson noted that one must calculate the constituents per unit of fat-free body mass. In other studies, with a herd of pigs McCance raised at his home in Cambridgeshire, she followed up her postwar Germany studies on the relation of severe undernutrition to subsequent reproductive function in the adult (Widdowson 1974). In the 1961 Ciba Foundation Symposium *Somatic Stability in the Newly Born*, Widdowson summarized many aspects of newborn metabolism, with comparison of the human to that of the piglet, calf, and kitten (Widdowson 1961). Almost two decades later, in a manner reminiscent of Minot (Minot 1891), McCance and Widdowson collated data from a number of reports on rates of growth of 17 species and their length of gestation. Their graphical representation suggested that these growth profiles could be classified into three groups: those that grow at a quite rapid rate (mouse, rat, rabbit, blue

whale), those species that grow at a more modest rate (cat, dog, guinea pig, goat, pig, sheep, porpoise, hippopotamus, horse), and those that grew at a still slower rate (macaque, man, ox, elephant) (McCance and Widdowson 1978). In addition, McCance and Widdowson graphically depicted the species differences in percentage of solids versus water in lean body tissues. They compared the human fetus during gestation, with that for nine other species at the time of birth. Not only do these other species grow in size more rapidly than the human, but the percentage of body solids increases much more rapidly. In considering the biochemistry of growth, these workers stressed the challenge of understanding the mechanisms by which the various cell types of different species change in a relatively consistent manner (see also McCance and Widdowson 1974; McCance et al. 1978). They concluded with the observation:

> The search for an animal model to further our knowledge of man has become fashionable, and the phrase itself has become a modern cliché. It is safe to say that the search will make little difference to the work being done on animals, which is frequently undertaken for its own sake. It is interesting, however, to speculate how much would be known about human growth requirements and the physiology of growth had animals not been studied. It is indeed the value of a comparative approach that we have tried to show, for it is the one way which enables one to realize the importance of species differences and hence of picking the appropriate animal with which to solve the problem at hand or perhaps initiate some widespread biological principle. If those interested only in human nutrition want an animal model, they are crying for the moon unless they have the patience and the ability to find it for the particular purpose they have in mind.
>
> (McCance and Widdowson 1978, pp. 161–162)

In 1968 at the time of McCance's retirement, Widdowson became head of the Infant Nutrition Research Division of the MRC's Dunn Nutrition Laboratory. Here, she compared the composition of infant milk formula from several European countries. With the formula in Holland, in which the cow's milk fat was replaced with maize oil consisting of 60% fatty acids in the form of polyunsaturated linoleic acid (as compared to 8% in human breast milk and 1% in the cow), she demonstrated significant effects on infant body fat (Widdowson et al. 1975). To ascertain the difference this markedly elevated level of unsaturated fatty acid made on the composition of various tissues, she also studied the effect of altered milk formula in newborn guinea pigs (which have ~10% body fat at birth). In this latter study, she demonstrated major compositional differences in several organs, including that of myelin in the brain (Pavey and Widdowson 1980). She also performed comparative studies of nutrition and neonatal growth in rats, guinea pigs, and pigs (Widdowson 1974). Following her 1973 move to Addenbrooke Hospital's renamed Department of Investigative Medicine (the successor to McCance's Department of Experimental Medicine, because several hospital authorities chaffed at the word "Experimental"), she collaborated on a study of infant milk composition and the composition of tissues in two species of Labrador Seals (Oftedal et al. 1989). Widdowson was elected to the presidency of the British Neonatal Society (1978–1979) and the British Nutrition Foundation (1986–1987). In 1979, Queen Elizabeth II (Elizabeth Alexandria Mary) awarded her Commander of the Order of the British Empire, and in 1993, Companion of Honour (Ashwell 2002).

13.2 Metabolic Rate

An additional consideration in relation to that of growth of the fetus and newborn is that of energy expenditure and metabolic rate, these being functions of many factors including the endocrine and nervous systems. In his *Foetal and neonatal physiology* . . ., Dawes stressed the importance of the larger body surface area to weight in the newborn human infant and that of other mammals, compared to the adult, in terms of regulating their body temperature, and their rate of O_2 consumption and basal metabolic rate under varying conditions (see Dawes 1968, pp. 191 ff). For almost a century, it has been assumed that the basal metabolic rate of an organism increases as a function of body mass. In 1916, the Nobel Prize-winning physiologist August Krogh, of the University of Copenhagen, suggested that the relation of metabolic rate to body mass is described best by a power function. That is, rather than metabolic rate being proportional to mass *per se*, it is a function of mass raised to some power (Krogh 1916). In addition to proposing a power law for the relation of metabolic rate to body mass, Krogh suggested that for endothermic organisms (mammals and birds) the value of "*p*" equals 2/3, similar to the law of metabolic rate being proportional to body surface area, as proposed earlier by Max Rubner (1854–1932) (Rubner 1902). When the power function "*p*" equals unity, this relationship is a straight line, and metabolic rate is said to scale isometrically (in proportion) to mass. When the power function does not equal one, the relationship is curvilinear, and metabolic rate is said to scale allometrically (in divergence or by other).

In the early 1930s, Max Kleiber (1893–1976), of the University of California, Davis, (Kleiber 1932), and independently Samuel Brody (1890–1956), of the University of Missouri (Brody et al. 1934), reported that across a wide range of mammalian species, metabolic rate scales are a nonlinear function of body mass. Kleiber's "mouse to elephant" curves for metabolic rate suggested the value of the scaling parameter "*p*" to equal 0.75. This power scaling of metabolic rate, "The Fire of Life" (Kleiber and Rogers 1961), to body mass became known as Kleiber's Law and has been extended to a wide range of organisms (Hemmingsen 1960). Since the mid-twentieth century, this line of investigation has spawned a number of studies, with investigators seeking to determine with accuracy and to explain this apparently universal exponent and its importance (Bartels 1982; Feldman and McMahon 1983). In fact, the debate has included those who reject the concept of a single power value to describe this relationship, stating that the value varies with environment, taxonomy, body temperature, and other factors (Dodds et al. 2001; McNab 2008; Schmidt-Nielsen 1984; White 2011; White and Seymour 2003). Recent reports have suggested that the standard power equation may not be appropriate to describe the relation of basal metabolic rate to body mass, and that, in fact, the value of "*p*" increases with body size, e.g., the metabolic rate increases more rapidly with mass for large animals, than for those that are small. In addition, deviations from the exponent 0.75, which some have suggested fits a "fractal distribution network model" based on geometry of vascular and other nutrient supply networks (West et al. 1997), are real, as originally

suggested by Krogh (Kolokotrones et al. 2010). As noted earlier, this issue has been of great interest to fetal-neonatal physiologists, and the question of the extent to which metabolic rate scales allometrically and its influence on life history remain (McNab 2012).

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Chapter 14
Fetal Growth Restriction at High Altitude: Clinical Observations

14.1 High-Altitude Long-Term Hypoxia and the Human Condition

In terms of the optimal conditions for fetal development, numerous investigators have noted that fetal growth and development occurs in an environment of maternal homeostasis and well-being, and with the mother's ability to respond appropriately to a particular stress. One such stress is that of long-term hypoxia (LTH). Because fetal growth critically depends upon adequate maternal oxygenation, conditions such as residence at high altitude (>2500 m) or that of mothers who are moderate to heavy smokers or with cyanotic congenital heart disease, lung disease, severe anemia, and other conditions that cause prolonged hypoxia may be associated with fetal growth restriction (FGR) (Hutter et al. 2010; Longo 1984; Longo and Goyal 2014; Neerhof and Thaete 2008). The impact of hypoxia on embryonic/fetal biology is a function of factors such as the stage of gestation and development, severity of the hypoxic event, its duration, and its association with other confounders including acidemia, hypercapnia, and/or ischemia. In addition, and as noted, fetal growth and development critically depend upon adequate placental substrate transport and metabolism.

In contrast to acute hypoxia, the fetal responses to chronic or long-term hypoxia such as that at high altitude are less fully known. A related problem is that one must attempt to assess the adaptive value of a given response in terms of its relative benefit to the individual or the population, that is, the extent to which it is a physiological response as opposed to being maladaptive. Of interest along this line is a consideration of those reports of pregnant women who live at high altitude and experience LTH. Worldwide, over 100 million people are believed to be permanent residents at altitudes >2500 m (8208 ft). For example, in the mountain states of the USA, on the *altiplano* [high plane] of the South American Andes Mountains, and in the Himalayas, many individuals live at elevations of 3000 m, some as high as 4600 m (West 2002). This hypobaric hypoxia presents a challenge

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for two groups; permanent residents who have lived at high altitude for generations show evidence of genetic adaptations to optimize cellular oxygenation. In addition, short-term sojourners, individuals who spend weeks to a month or more as visitors or mountain climbers, must undergo the process of acclimatization of tissues and cellular responses to accomplish the same goal. As documented in many studies, acclimatization demonstrates its highest degree of efficiency in those born and raised at high altitude (Beall 2007; Wang et al. 2011). For the pregnant woman at high altitude, whether permanent resident or sojourner, the long-term hypoxia is a not an uncommon cause of FGR (Giussani et al. 2001; Keyes et al. 2003; Soria et al. 2013).

14.2 The Colorado and Mountain States Studies

As noted, in a 1950 study by the United States Public Health Service of a reported total of 837,736 live births, neonatal mortality was considerably higher in the mountain states than that of other areas of the country (Shapiro et al. 1954). For the USA as a whole, these were: mean birth weight = 3320 g; neonatal mortality = 20 per 1000 live births; infants who weighed 4000 g or more were 9.8 %; and infants who weighed 2500 g or less were 7.4 %. For all mountain states (~1790 m), the values were: mean birth weight = 3240 g; neonatal mortality = 24 per 1000 live births; infants who weighed 4000 g or more were 6.3 %; and infants who weighed less than 2500 g were 9.1%. For Denver, Colorado (1609 m), the comparable values were: mean birth weight = 3035 g; neonatal mortality was not available; infants that weighed 4000 g or more were 3.3%; and infants who weighed less than 2500 g were 11.7%. In comparison, the values for Lake County (Leadville), Colorado (3100 m) were: mean birth weight = 2655 g; neonatal mortality = 49 per 1000 live births; infants who weighed 4000 g or more were 0%; and infants who weighed 2500 g or less were 48.3% (McClung 1969; Shapiro et al. 1954). Thus, in comparison to the USA as a whole, the mean birth weights for live born infants at Leadville were 20% less, the neonatal mortality was over twice greater, and the percent of infants who weighed 2500 g or less was many fold greater. It is a sobering reflection.

In light of its relatively large population that lives at high elevations, and with its careful maintenance of health records, the earliest controlled studies that established the inverse relation of birth weight to altitude originated in Colorado. Stimulated by the observation that its Lake County had a several-fold higher incidence of “prematurity” than the state average, in the mid-1950s the Colorado State Department of Public Health initiated a study to explore the reasons for this anomaly. Further analysis of this unexplained dilemma was conducted by a combined effort of the departments of pediatrics and obstetrics and gynecology at the

University of Colorado, School of Medicine and the State Department of Public Health, and other groups, and was headed by John A. Lichty. This report compared several obstetrical and newborn indices at Leadville in Lake County with those at Denver. For live births of 2500 g or less, the comparative numerical values were as follows: "Prematurity" 31% versus 10%, neonatal death rate (deaths under 1 month of age per 1000 live births), 42 versus 23 per 1000. The Lake County birth weights were 380 g less than Denver controls, with a significant shift to the left of birth weight distribution curves, as compared to controls (Lichty et al. 1957). The authors also quoted a personal communication from Dr. Elena Boder of Mexico stating that, in a comparison of live birth weights, those in Mexico City (2134 m) were 8.7% less than those in Mazatlan (sea level). Of critical importance, the authors concluded that these findings "... support the statement of practicing physicians in Lake County that the babies are often small but not otherwise abnormal. Possibly the generally accepted birth weight of 2,500 gm is not appropriate for distinguishing between full term and premature infants in this community" (Lichty et al. 1957, p. 669). This then was the first study that clearly demonstrated the fact that low birth weight was a consequence of high altitude, not a de facto index of prematurity. In related reports, the authors confirmed these findings and showed that newborn crown-heel length and head length and width were appropriate for gestational age (Howard et al. 1957b). These authors also reported the surprising paradox that in Lake County newborns neither the oxyhemoglobin saturation nor hematocrit values differed from lowland controls (Howard et al. 1957a). Of vital importance, further studies from 1969 to 1973 in Colorado confirmed these conclusions and demonstrated that high-altitude hypoxia, rather than decreased gestational age with preterm delivery, accounted for the altitude-associated lessened birth weight and increase in neonatal mortality. Birthweight was inversely associated with altitude at each gestational age beyond 35 weeks (McCullough and Reeves 1977; McCullough et al. 1977).

In a follow-up study for the years 1950–1957 of almost one million live births in the USA, again the mountain states and Lake County stood out for both their high rates of "prematurity" and neonatal deaths (Grahn and Kratchman 1963) (see http://www.cdc.gov/nchs/products/vsus/vsus_1939_1964.htm for Vital Statistics of the USA). Notable in this review was the association of these variables at high altitude, with minimal association with exposure to terrestrial irradiation. The authors concluded, "The weight of the evidence—historical, experimental, and clinical—strongly suggests that the reduced partial pressure of oxygen is responsible for the reduced fetal growth and subsequently increased neonatal death rate" (Grahn and Kratchman 1963, p. 350).

A more recent analysis was conducted of all live births and infant deaths in the state of Colorado for the years 1978–1981 (Yip 1987). After adjusting for socioeconomic factors, a modest association was noted between altitude and the percentage of preterm births of infants weighing <2500 g. This association was much

stronger, however, for term births with birth weight <2500 g (Yip 1987). Among comparable subpopulations of infants with gestational ages of at least 37 weeks, the lower birth weight at high altitude was similar for each birth weight distribution at differing altitudes (Yip 1987). In terms of birth weight, one might ask about the extent to which it really matters, and what is its relation to morbidity rates. I am unaware of comparative morbidity rates, a fruitful area to explore. In terms of neonatal mortality, the studies from Lake County, CO, have been highly valuable.

14.3 Clinical Studies from the *altiplano* of South America

Anecdotal evidence by Peruvian doctors had suggested that newborn infants in the highlands were relatively small, while their placentas were "very large." In the mid-1960s, a rather serendipitous encounter occurred between Jean McClung, a Radcliffe College graduate student interested in anthropological aspects of human adaptations to high altitude, and Professor Paul Thornell Baker (1927–2007) (later a member of the National Academy of Sciences) of Pennsylvania State University, who headed an investigation of the physical anthropology of high-altitude populations in the highlands of Peru (Garruto et al. 2009). With his wide knowledge of various anthropological relationships, Professor Baker suggested to Ms. McClung that this might be a fruitful area to explore. This was at the time that Richard B. Mazess, one of Baker's graduate students at Penn State, published his survey of neonatal mortality in Peru (Mazess 1965). In an analysis of neonatal mortality from the Peruvian census of 1958 and 1959, Mazess had discovered that neonatal mortality in the highland departments was ~52, about double that in the lowland, 28.4 (Mazess 1965). These values were 40–70% higher comparable to figures in the USA. In this report, the post-neonatal death rates (deaths from 29 days to 1 year per 1000 live births) also were much greater in the highlands (Mazess 1965). Mazess has reviewed in extenso aspects of the human adaptations to high altitude (Mazess 1975).

Subsequently, McClung was awarded a fellowship for high-altitude studies from the Committee on Latin American Studies of Harvard University. Following proper education in examination of the placenta and training in some of the fine points of measuring features of the placenta and newborn infant, McClung embarked for Peru to conduct her studies at the *Hospital Regional* in Cuzco (3416 m) and the *Maternidad* in Lima (203 m), in an attempt to differentiate the premature infant from that which is strictly growth restricted, and hopefully to test the hypothesis that the growth restriction seen at high altitude is a consequence of hypoxia per se, rather than other factors.

Indeed, as anticipated at Cuzco, with the decrease in mean birth weights of 213 g (6.4%), the placental to fetal weight ratio increased 13% (from 0.15 to 0.17 and

0.18) (McClung 1969). No significant changes were noted in infant total length or crown-rump length, head circumference, thoracic circumference, arm length, skinfold thickness, or other measures of nutrition. This decrease in mean birth weight in the Cuzco high-altitude group was highly significant statistically, showing no interaction with other factors. A minor caveat is that the patient numbers were only 73 in Cuzco and 88 in Lima, although elsewhere she gives values of 100 each (McClung 1969).

An additional feature of the McClung studies showed that when Cuzco-born women gave birth in Lima the infant's birth weight did not differ significantly from those women native to Lima. Also of importance, this study confirmed the almost 50% increase in neonatal mortality, as well as mortality during the first 2 years of life, in the hypoxic high-altitude environment, and the need for further investigation to deepen our understanding of the fundamental causes of hypoxia-induced FGR (McClung 1969).

Although her data on infant mortality is not as definitive as one might like, McClung noted:

> . . . the data showed a significant increase in child mortality at high altitude. Thirty-three (16.7 percent) of the 198 children live-born to Cuzco women had died before age two; the percentage for Lima women was 12.5 percent (37/295). The difference between these percentages is highly significant (P=.01). This . . .. agrees with Mazess' finding[s] based on vital statistics, of significantly higher neonatal mortality in the highland[s] of Peru. [Mazess, 1966] . . . Within the study samples themselves, three of the 100 Cuzco infants died before leaving the hospital (birth weights: 700, 1,720, 2,750) and one of the Lima infants died (birth weight: 1,000). No information on mortality was available from hospital records in Lima, but Cuzco records showed that 27 (5.7 percent) of the 470 lower class infants born in 1965 had died before leaving the hospital. Seventeen of these 27 infants were of low birth weight; thus, 46 percent (17/37) of all low birth weight infants born in 1965 died before leaving the hospital. Of the 27 infants who died in the hospital, 21 (78 percent) were males . . . the data indicate that the increased neonatal mortality at high altitude is significant and is associated with very high mortality among the increased percentage of infants of low birth weight.
>
> (McClung 1969, p. 102)

In her 150-page thesis with several hundred references, McClung presented her studies including a historical review of the literature (McClung 1969). Although not commonly cited, it is clear that this seminal report laid the groundwork for many of the studies during the following several decades. In a subsequent report, McClung reviewed many of her findings (Goodwin 1974).

In accounting for the significant decreases in birth weight observed at high altitude, several questions have arisen in regard to confounding variables. In an attempt to address these, a group at Cornell University, Ithaca, New York, examined the role of ethnic background, nutritional status, and sex of newborn infant in the highlands and lowlands of Peru (Haas et al. 1977), Bolivia (Hass et al. 1980; Haas et al. 1982), and Mexico (Haas et al. 1987). In their original study from Puno (3870 m), Arequipa (2363 m), and Tacna (568 m), Southern Peru, infants delivered

in hospital at ~2.5 days of age weighed 3175, 3263, and 3443 g, respectively. No mortality rates were given, but the infant weight data followed the trend of previous workers (Haas et al. 1977). In the indigenous group at La Paz, the birth weights of both sexes exceeded those for non-Indians, for both groups these were significantly less than that of Santa Cruz (400 m), and at high altitude male newborns were affected to a greater degree than females. Neither socioeconomic nor nutritional status differed significantly among specific groups (Hass et al. 1980). In a subsequent study in Bolivia, Haas and his coworkers continued to document the effects of high altitude on birth weight, as well as on the rate of growth during the first year of life. Despite their lighter birth weight, the highland infants showed significantly greater triceps and subscapular skin-fold thickness and fat accumulation. The possible causes of the greater fat accumulation in the highland group (Haas et al. 1982) may relate to significant alterations in leptin metabolism (see below). Further, in a study in which they compared anthropomorphic measurements in two distinct Latin American populations, Mexico City (elevation 2134 m) and Santa Cruz, Bolivia, the Haas group found similar high-altitude effects on birth weight and other parameters (Haas et al. 1987).

In a study from Peru of the critical barometric pressure below which birth weight at high altitude decreases, the authors concluded that 590 mmHg is that value, corresponding to ~2500 m elevation (Mortola et al. 2000). From Bolivia shortly thereafter, Dino A. Giussani's group reported that in contrast to the effect of altitude, socioeconomic status played no significant role in the lower infant birth weights at La Paz (3649 m), as compared to those born in the lowlands (<500 m), and that the FGR phenotype was associated with high-altitude LTH per se (Giussani et al. 2001).

Overall in humans, several studies have documented that, on average, above 2500 m the weight of near-term newborns decreases ~100 g per 1000 m of elevation, independently from other factors, and that several times as many newborns show FGR for their gestational age, compared to low-altitude controls (Jensen and Moore 1997; Julian et al. 2007; Krampl 2002; Moore 2003; Moore et al. 2011). Of interest, this slowing of growth commenced at the beginning of the third trimester (after about 31 week) of gestation (Unger et al. 1988). At altitudes at or above 4300 m, this declining growth rate began at 25–29 weeks gestation (Krampl et al. 2000; Mortola et al. 2000).

14.4 The Relation of Birthweight to Gestational Age

A decade and a half following the early Colorado studies, and, in part, motivated by those findings, the pediatrician Lula O. Lubchenco (1915–2001) and colleagues at the University of Colorado first described the relation of birth weight to gestational

age during the third trimester (Lubchenco et al. 1963). Although this study was conducted at moderate altitude (Denver, CO, 1609 m, 5280 ft), it set a new standard for evaluation of the newborn infant, and the "Lulagram" developmental growth chart became used worldwide. For infants whose growth patterns lie outside normal developmental profiles, the terms small, appropriate, and large for gestational age were introduced based on weight, length, head circumference, and weight–length ratio (ponderal index) (Battaglia and Lubchenco 1967; Lubchenco 1970; Lubchenco et al. 1966, 1972). Although the term "low birth weight" was introduced in the early 1960s to replace the word "premature" for infants under 2500 g at birth, its acceptance was markedly facilitated by Lubchenco's popularization of matching birth weight with gestational age. Too long had the designation for term and premature been based on birth weight above and below 2500 g without awareness of two distinct populations for each weight group. A study by the University of California Berkeley biostatistician Jacob Yerushalmy (1904–1973) and coworkers showed that there were as many term as preterm infants born weighing under 2500 g in the USA, and surprisingly more preterm infants weighing above than below that figure (Yerushalmy et al. 1965).

14.5 Translational Studies of Pregnancy at High Altitude

Continuing in the Colorado tradition, in a series of enlightened studies, the physical anthropologist Lorna Grindlay Moore, of the University of Colorado, has described several important variables and adaptive mechanisms in both the pregnant mother and their fetus at high altitude. These include studies in Leadville (3100 m), the Andean *altiplano* of Peru (4300 m), and the Tibetan plateau (3658 m) (Moore 1990). Among other parameters these reports compare the infant birth weights and mortality rates among several groups (Moore 1990, 2003; Moore et al. 1998, 2001, 2004). Other studies from this group suggested that the decrease in neonatal mortality seen at altitude reflects, in part, regionalization of healthcare delivery, with more infants at risk of being born and cared for in tertiary care centers (Unger et al. 1988).

On the Bolivian *altiplano*, birth records by ethnic group of Andean, Mestizo (mixed ethnicity), or European ancestry, the duration of highland ancestry partially protected against the effects of altitude (Julian et al. 2007). In a subsequent study by this group, this data was confirmed (Andean $= 3148 \pm 15$ g, Mestizo $= 3081 \pm 6$ g, European $= 2957 \pm 32$ g, $p < 0.001$ for trend) (Soria et al. 2013). As noted, for reasons that remain to be elucidated, the degree of growth restriction may reflect, in part, in an inverse fashion the number of generations of ancestors who lived in a given location (Zamudio et al. 1993). Additionally, birth weight correlates positively with the vigor of the maternal ventilatory response to the hypoxic stress (Moore et al. 1986). Ethnic ancestry (Julian et al. 2007) also may serve as a fetal protective mechanism in this regard.

For unknown reasons, the decrements in birth weight and increases in infant mortality are less in individuals of the Himalayas than in those in the Peruvian Andes. Specifically, as compared to several near sea level studies that give control birth weights of ~3.4 ± 0.3 kg, the birth weights at Leadville, Cerro de Pasco, Peru, and Lhasa, Tibet, were 3.2 ± 0.1, 2.9 ± 0.1, and 3.3 ± 0.1 kg, respectively (Moore 1990). Also in Lhasa, Tibet, in comparing indigenous Tibetan versus Han Chinese at 3000–4000 m, the Han experienced a much greater birth weight reduction and their postnatal mortality rate was almost twice that of indigenous Tibetans (Moore et al. 2001). In another study, which compared birth weight and infant mortality between native Tibetans with non-Tibetans (Han Chinese and Hui Muslims) in Lhasa, birth weights were significantly lower (5.5%) and neonatal mortality was significantly decreased (~35%, a change the authors deemed to be nonsignificant) in the non-Tibetans (Yangzom et al. 2008). In separate studies, genome-wide scans showed that Tibetans positively selected haplotypes of *Egln1* and *PPPARA* that were associated with decreased hemoglobin phenotype unique to this highland population (Simonson et al. 2010).

Overall, because of its implications for pregnant women, high altitude must be included in the list of risk factors to be taken into account when considering pregnancy, or when comparing birth weight with gestational age distributions, or in preparing so-called "standard" birth weight for gestational age charts. Others have challenged the validity of birth weight for gestational age charts, this in view of the wide variation in race/ethnicity, socioeconomic status, parity, altitude, and other factors (Macfarlane 1987). As noted below, some of the maternal acclimatization responses to high-altitude associated prolonged hypoxia include adaptive responses such as hyperventilation, increased red cell mass and hemoglobin concentration, changes in the placenta to promote transplacental oxygen flux, and others. Maternal maladaptive changes that may result in fetal growth restriction include reduced diameter of iliac and uterine arteries, increased blood viscosity, and perhaps reduced uteroplacental blood flow.

14.6 High Altitude and the Placenta

As noted, in considering the hypoxia-mediated correlates of FGR, an obvious condition to consider is that of the pregnant mother herself at high altitude. In human high-altitude pregnancy, reports vary as to the size of the placenta; however, almost all such studies report a modest increase in relation to that of the fetus. For instance, in her review "The Placenta at High Altitude," Stacy Zamudio tabulated ten reports of placental and fetal weights and placental index (the ratio of placental to fetal weight) at altitudes ranging from 2000 to 4300 m (Zamudio 2003). For normoxic control subjects at low altitude (347 ± 60 m), the placental index equaled 0.15 ± 0.01. In contrast, at high altitude (3550 ± 620 m) this value was 0.17 ± 0.01. For the most part, this increased ratio with high altitude reflects the decrease in fetal weight rather than an increase in weight of the placenta (which although greater in

some reports was not significantly so). As the report noted, a *caveat* is that the total sample size for this comparison was less than 100 subjects (Zamudio 2003). A later study from her group substantiated these findings among both Andean natives and those of European ancestry in Bolivia (Zamudio et al. 2007). Based on other studies, it is apparent that these placental index ratios of 0.15 and 0.17 for pregnancies from low and high altitude, respectively, are reasonable (Hass et al. 1980; Khalid et al. 1997; Krüger and Arias-Stella 1970). In concert with placental morphometric (Chabes et al. 1968) and other vascular changes, this increased ratio must help to optimize the exchange of O_2 and other nutrients under the stress of pregnancy at altitude (Mayhew et al. 1990). Thus, a fetal to placental weight ratio of >0.15 may serve as an index of growth restriction independent of fetal weight. In addition, Zamudio has emphasized that in the human placenta at altitude the most consistent morphometric findings were increased villous terminal capillary branching with increased villous vascularization/angiogenesis (capillary density and capillary diameter), thinning of the villous membranes, and proliferation of the villous cytotrophoblast with trophoblastic bridges and syncytial knots (Alia et al. 1996; Espinoza et al. 2001; Khalid et al. 1997; Zamudio 2003). Along this line, in 13 patients with normal pregnancies at Leadville, CO, stereological analysis disclosed significant remodeling of the distal decidual ends of the more than twofold increased numbers of uteroplacental arteries, as well as an increase in fetal capillary density (Tissot van Patot et al. 2003). This fits with the well-known finding that angiogenesis with neovascularization is a robust response to chronic hypoxia.

14.7 Conclusions with Perspectives

As is evident, pregnancy at high altitude is fraught with problems beyond those of the fetus/newborn showing growth restriction. Chiefly a function of altitude per se, this FGR can vary with ethnicity and geographic location, the latter of which reflects the number of generations that a given group has lived at that environment. Also as is evident, the syndrome of fetal growth restriction, as seen at high altitude, is a complex interplay of confounding variables that can present a variety of phenotypes. Beyond the phenomenology of having a birth weight below the 10th percentile, as well as, genetic- and environmental-epigenetic-mediated factors noted in Chap. 16, a seemingly infinite mosaic of cellular, subcellular, and molecular alterations and mechanisms may result in moderate to severe hypoxia.

Under normal conditions, the developing fetus has an arterial O_2 tension (but not that for CO_2) simulating "Mt Everest in utero" (Eastman 1954; Longo 1987). Thus, severe hypoxia of more than momentary duration can pose particular peril. In turn, the mechanisms by which the fetus "acclimatizes" to long-term hypoxia are of more than passing interest. Understanding adaptations of basic hypoxic-mediated cardiovascular, metabolic, neuropsychiatric, and other cellular and subcellular signal transduction mechanisms, and their role in the dysregulation of protein synthesis

in the pathogenesis of growth restriction and functional dysregulation in the developing fetus, is likely of great clinical relevance to reduce the risk of prematurity, preterm birth, and neonatal morbidity.

Whereas, to some degree, the changes in the fetal environment are "buffered" by maternal homeostatic mechanisms, it is also clear that under conditions of prolonged hypoxia or other invidious environmental factors, the fetus constitutes a stress for the mother. From this perspective, it would enhance the survival mechanisms for both mother and conceptus if the latter were to mature more quickly. Although attractive, this hypothesis remains highly speculative and in need of experimental verification. Our challenge today is to promote a new generation of studies of the mosaic of hypoxic- and other mediator-induced gene regulation and/or metabolomics that will yield the key clues to the origins of FGR at high altitude. Discovery of the genetic and/or epigenetic mechanisms that promote successful acclimatization and/or prevent high-altitude associated disease may have profound implications for human well-being under a variety of circumstances. Among our challenges is that of not delaying to make these advances.

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Chapter 15
Fetal Growth Restriction at High Altitude: Basic Cellular and Subcellular Physiologic Considerations

15.1 Pregnancy and High-Altitude, Long-Term Hypoxia

Based on considerable epidemiologic and laboratory based experimental evidence, it is clear that the optimal conditions for fetal growth and development require an environment of maternal homeostasis and well-being, and with the mother's ability to respond appropriately to a particular stress. In view of its association with increased morbidity and mortality, fetal growth restriction (FGR) is to be avoided at all costs. Chapter 14 presents the clinical aspects of one not uncommon stress, that of high altitude or other [causes of] long-term hypoxia (LTH). Because fetal growth critically depends upon adequate maternal oxygenation, in addition to residence at high altitude (>2500 m), FGR may be associated with conditions such as that of mothers who are moderate to heavy smokers or with cyanotic heart disease, lung disease, severe anemia, and other conditions that cause prolonged hypoxia (Giussani et al. 2001; Hutter et al. 2010; Longo 1984, 1987; Longo and Goyal 2014; Neerhof and Thaete 2008). The importance of oxygen for fetal physiology was a foundation for my research interests and the development of surgical approaches to directly investigate the fetus during pregnancy (methodology described in Yellon and Apostolakis 1994). The chapter reviews the development of ideas in terms of the many physiologic, biochemical, cellular, and molecular aspects of LTH for the fetus and newborn.

As a two-edged sword, O_2 is a Januslike gas (Burton 2009). While essential for oxidative phosphorylation and metabolism, in either excess or deficiency, O_2 can be metabolized into reactive O_2 species (ROS) such as superoxide anions, hydrogen peroxide, lipid peroxides, hydroxyl radicals, and other malevolent ions (Burton 2009; Burton and Jauniaux 2011; Maiti et al. 2006; Maulik et al. 1998; Pringle et al. 2010). Beyond its cellular antioxidant capacity in mMolar (mM) concentrations, ROS generation may have a number of invidious effects including disruption of plasma membranes, oxidation of cellular proteins, DNA fragmentation, disruption of RNA metabolism, and other alterations (Giordano 2005; Longo and

L.D. Longo, *The Rise of Fetal and Neonatal Physiology*, Perspectives in Physiology,
https://doi.org/10.1007/978-1-4939-7483-2_15

Packianathan 1997). At the systemic, local, cellular, and molecular levels, mammals respond to reduced O_2 level (hypoxia) in many different ways. In general terms, these responses are designed to decrease cellular O_2 dependence and to increase tissue O_2 availability (Hochachka 1986, 1992). The impact of hypoxia on embryonic/fetal biology is a function of factors such as the stage of development, the severity of the hypoxic event, its duration, and its association with other confounders including acidemia, hypercapnia, ischemia, and/or hypoglycemia. In addition, fetal growth and development critically depend upon adequate placental substrate transport and metabolism (see below).

Thus, quite obviously for the developing fetus in utero, its ability to respond to hypoxia in a manner to optimize tissue cellular oxygenation is of critical importance. Under normoxic physiologic conditions, fetal arterial O_2 tensions are low by adult standards (fetal arterial PO_2 ~25 ± 3 Torr vs. ~95 ± 5 Torr in an adult; e.g., the fetal arterial O_2 tension equals about one-quarter that of the adult). Nonetheless, with its higher O_2 affinity and its relative greater hemoglobin concentration, fetal blood helps to maintain circulating O_2 content at levels near that of the adult. That the normal fetus at near sea level is not hypoxic is suggested by its lack of elevated circulating lactate concentrations, and that increasing fetal arterial O_2 levels does not increase its rate of O_2 consumption (Battaglia and Meschia 1978). In response to acute hypoxia (often a decrease in arterial O_2 tension of 50% for minutes to several hours), many of the cardiovascular, hemodynamic, endocrinologic, metabolic, and other responses are reasonably well defined (Giussani et al. 1994a, b).

In contrast, alterations of cellular and subcellular mechanisms in response to chronic or long-term hypoxia, such as that at high altitude are less fully known. Several problems confound consideration of adaptations to environmental stress such as acclimatization to high altitude. One is the challenge of delineating in a rigorous manner the pattern of cellular, structural, functional, and behavioral responses, so that in measuring a given variable, one does not alter that function (such as intracellular O_2 tension) being measured. In addition, one must attempt to assess the value of a given response in terms of its relative benefit to the individual or the population, that is, the extent to which it is adaptive as opposed to being maladaptive. As with many environmental stresses, the description and defining of responses to high altitude have advanced to a much greater extent than has sophistication in the evaluation of specific adaptive values. Because of it being a not uncommon experience for humans, including women who are pregnant, such understanding of the lessons of high altitude are of vital importance. Here, I consider some of the cardiovascular, cerebrovascular, metabolic, and related adjustments or acclimatization responses of the developing fetus to hypoxia of prolonged duration that play a major role in the genesis of FGR, and attempt to gain an understanding of the adaptive value of these responses. Of course, a critical question in this regard is the extent to which this information will provide clues to improve the outcome for women who deliver at high altitude.

From an experimental standpoint, in the different laboratory species, fetal hypoxia with resultant FGR can be induced by several means. These include maternal hypoxia (Giussani et al. 1994a, b; Kitanaka et al. 1989b), maternal

hyperthermia (Thureen et al. 1992), restricting maternal uterine blood flow (Baserga et al. 2009, 2010; Challis et al. 1989; Phillips et al. 1996; Wilkening and Meschia 1983), reducing fetal umbilical blood flow (Giussani et al. 1997; Unno et al. 1997), placental embolization (Boyle et al. 1984; Bubb et al. 2007; Clapp et al. 1980; Gagnon et al. 1997), and maternal uterine carunclectomy prior to mating (which restricts the number of placentomes; Alexander 1964; Dyer et al. 2009; Phillips et al. 1996; Robinson et al. 1979). Details of many aspects of these approaches with their strengths and weaknesses, as well as similarities and differences in fetal physiologic responses, have been reviewed by others (Morrison 2008). As a caveat, despite many similarities in the responses to these stresses, these several methodologies in producing uteroplacental ischemia/hypoxia are associated with differing degrees of acidemia, hypoglycemia, and/or nutrient deprivation.

In the previous chapter, I presented the clinical examples in the mountain states of the USA, on the *altiplano* of the South American Andes Mountains, and in the Tibetan Plateau of the Himalayas; many individuals live at elevations of 3000 m, some as high as 4600 m. It was in the latter part of the nineteenth century with the publication of Paul Bert's (1833–1886) *La pression barométrique: Recherches de physiologie expérimentale* [Barometric pressure: researches in experimental physiology] (Bert 1878) that established the role of reduced partial pressure of oxygen, rather than the hypobaric pressure per se, that results in erythropoiesis as well as the principal signs and symptoms of high-altitude sickness. In this work, Bert was the first to investigate the conditions of ascent to high altitude in a hypobaric chamber. As an aside, he credited his colleague Denis Jourdanet (1815–1892) for collaboration in studies of high-altitude effects in remote areas of Latin America and mid-Southeast Asia (Jourdanet 1875). Continuing to the early twentieth century, a number of investigators explored various aspects of the physiologic effects of high-altitude acclimatization in the adult (Barcroft 1927; Barcroft et al. 1923; Douglas et al. 1913; Haldane 1922; Hurtado 1964; Longo 2016; Monge 1948; Monge and Monge 1966; Mosso 1897, 1899; Weihe 1964; West 2002). Essentially none of these studies concerned the physiology of pregnancy at altitude, however. (Quite obviously, this is not the place to review in extenso the physiology of high-altitude acclimatization per se.)

It was the Peruvian physiologist Carlos Monge Medrano (1884–1970) who recounted the early years following the Spanish subjugation of the Incas in the highlands of Peru, during the initial period of their occupation in which fertility of the Spanish woman was quite low. Those infants born died shortly thereafter, it being over 50 years before a newborn infant survived (Monge 1948). Commencing in the decade or so following World War II, investigators in South American and the USA first studied the effects of altitude on the pregnant woman and her infant (Hurtado 1955, 1960, 1964; Monge 1948, 1960; Sobrevilla et al. 1967). These investigations were criticized, however, on grounds that the observed differences in birthweight were a consequence of factors other than altitude. Those confounding variables included small sample size, racial or nutritional differences, socioeconomic status, the presence of disease, exposure to cosmic rays and/or cold

temperatures, maternal parity and age, the amount of prenatal care, the degree to which mothers smoked cigarettes, multiple gestation, the extent of placental pathology, and sex of infant.

The fundamental problem of acclimatization to high altitude is that, in contrast to an ambient O_2 partial pressure of about 154 Torr (20.9% of 760 Torr) at sea level, at high altitude the hypobaric partial pressure is only a fraction of that value (Fig. 15.1). For instance, at 5000 m [16,404 ft, somewhat less than the one-half atmosphere of 5486 m (18,000 ft)], the ambient partial pressure is only about 85 Torr. As noted below, one would anticipate that at this elevation, the arterial O_2 tension of the fetus would be much lower than normal. Surprisingly, however, and as illustrated in Table 1 and as discussed in greater detail in Chap. 14, such is not the case (Longo 1987). In essence, the mean birthweights for live born infants at Leadville, CO, and on the *altiplano* of Peru were 20% less than near sea level controls. The neonatal mortality was over twice greater, and the percent of infants who weighed 2500 g or less was manyfold greater.

Overall in humans, several studies have documented that, on average, above ~2500 m the weight of near-term newborns decreases ~100 g per 1000 m of elevation, independently from other factors; and several times as many newborns show FGR for their gestational age, compared to low-altitude controls (Jensen and Moore 1997; Julian et al. 2007; Krampl 2002; Moore 2003; Moore et al. 2011). This slowing of growth commences after about 31 weeks' gestation (Unger et al. 1988), although at altitudes at or above 4300 m, this declining growth rate begins at 25–29 weeks' gestation (Krampl et al. 2000; Mortola et al. 2000). As noted above, a decade and a half following the early Colorado studies, and, in part, motivated by those findings, Lula O. Lubchenco and colleagues at the University of Colorado first described the relation of birthweight to gestational age during the third trimester (Battaglia and Lubchenco 1967; Lubchenco 1970; Lubchenco et al. 1963, 1966, 1972).

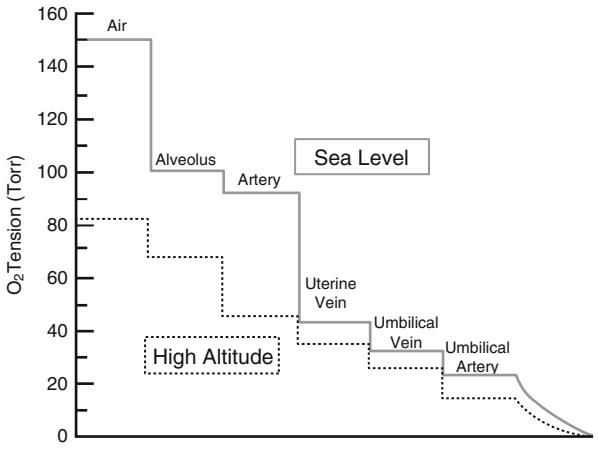

Fig. 15.1 Changes in oxygen partial pressure with altitude: sea level compared to high altitude

Table 15.1 Fetal physiologic responses to high altitude, long-term hypoxia

Physiologic variable	Normoxic control	High altitude hypoxic	% Change	References
Arterial blood gas and other				
PO_2 (Torr)	23 ± 1	19 ± 1*	−17.2	Kamitomo et al. (1993) Kitanaka et al. (1989)
$[HbO_2]$ (%)	59 ± 3	50 ± 3†	−15.9	Kamitomo et al. (1993) Kitanaka et al. (1989)
[Hb] (g dl^{-1})	10.1 ± 0.7	12.6 ± 0.6*	24.7	Kamitomo et al. (1993) Kitanaka et al. (1989)
O_2 content (ml dl^{-1})	7.7 ± 0.5	7.8 ± 0.5	1.0	Kamitomo et al. (1993)
P_{CO2} (Torr)	42 ± 1	38 ± 1*	−9.5	Kamitomo et al. (1993)
pH	7.36 ± 0.01	7.37 ± 0.01	0.1	Kamitomo et al. (1993)
Lactate (mg dl^{-1})	13.1 ± 0.7	14.4 ± 1	9.9	Kamitomo et al. (1993)
Breathing incidence (min h^{-1})	25	25	–	Koos et al. (1988)
Uterine artery blood flow				
Human uterine artery blood flow at 36 weeks gestation (ml min^{-1})	312 ± 22	203 ± 48	−34.9	Zamudio et al. (1995)
Uterine artery fraction of common iliac blood flow (% at 36 weeks gestation)	74 ± 6	46 ± 7	−36.3	Zamudio et al. (1995)
Cardiac function				
Heart rate (beats min^{-1})	168 ± 5	165 ± 5	−1.6	Kamitomo et al. (1992, 1993)
Arterial pressure (mmHg)	44 ± 1	52 ± 1*	18.1	Kamitomo et al. (1992, 1993)
Right ventricular output (ml min^{-1} kg^{-1})	276 ± 10	183 ± 10*	−33.6	Kamitomo et al. (1992, 1993)
Left ventricular output (ml min^{-1} kg^{-1})	166 ± 16	142 ± 16	−14.5	Kamitomo et al. (1992, 1993)
Right stroke volume (ml kg^{-1})	1.66 ± 0.05	1.11 ± 0.05*	−33.1	Kamitomo et al. (1992, 1993)
Left stroke volume (ml kg^{-1})	0.97 ± 0.09	0.84 ± 0.08	−13.4	Kamitomo et al. (1992, 1993)
Combined ventricular output (ml min^{-1} kg^{-1})	441 ± 23	335 ± 28†	−24.1	Kamitomo et al. (1992, 1993)

(continued)

Table 15.1 (continued)

Physiologic variable	Normoxic control	High altitude hypoxic	% Change	References
Cerebral artery structure and composition				
Vessel resting inside luminal diameter (mm)	0.94 ± 0.07	1.30 ± 0.04	39*	Henderson and Longo (2003)
Media thickness (μm)	21 ± 1	30 ± 6	42*	Henderson and Longo (2003)
Media cross-sectional area ($\mu m^2\ 10^{-3}$)	67 ± 7	124 ± 22	85*	Henderson and Longo (2003)
Number of layers of SMC in media	5.6 ± 0.2	11.5 ± 2.1	105†	Henderson and Longo (2003)
Base-soluble protein (%dry wt)	24.5 ± 2.2	30.0 ± 4.1	22*	Longo et al. (1993)
Cranial artery contractile proteins				
Flk-1 (protein 10^{-6} total protein)	2.0 ± 0.6	5 ± 1	170↑	Adeoye et al. (2013)
Flt-1 (protein 10^{-6} total protein)	0.4 ± 0.1	3.0 ± 1.0	780↑	Adeoye et al. (2013)
Myosin light chain kinase (protein 10^{-6} total protein)	0.6 ± 0.1	0.07 ± 0.02	90↓	Adeoye et al. (2013)
Myosin light $chain_{20}$ (protein 10^{-6} total protein)	0.4 ± 0.1	0.63 ± 0.10	61↑	Adeoye et al. (2013)
Cerebral artery electromechanical coupling				
K^+ max tension (g)	1.0 ± 0.06	1.1 ± 0.1	10	Long et al. (2002)
K^+ stress (10^{-6} dyn cm^{-2})	0.41 ± 0.05	0.44 ± 0.05	7	Longo et al. (1993)
L-type Ca^{2+} channel (% Inhibition tension by nifedipine)	100 ± 10	100 ± 10	–	Zhao et al. (2004)
K_{ATP} channel (%inhibition tension by pinacidil)	5.2 ± 0.1	4.7 ± 0.1	10†	Long et al. (2002)
K_{ca} channel (%inhibition tension by NS-1619)	100 ± 10	41 ± 5	−59†	Long et al. (2002)
Membrane potential (mV)	−26.1 ± 1.4	−42.0 ± 5.2	61†	Tao et al. (2015)
BK channel current density ($pA\ pF^{-1}$)	57.9 ± 6.6	75.1 ± 4.8	30*	Lin et al. (2005) Tao et al. (2014)
Calcium set point (10^{-6} M)	4.7	3.0	−36*	Lin et al. (2006); Tao et al. (2014)
Right shift by dephosphorylation state (mV)	52.3 ± 8.1	11.1 ± 5.6	−79†	Lin et al. (2005), Tao et al. (2014)
$V_{1/2}$ of dephosphorylated channels (mV)	64.1 ± 4.8	23.6 ± 5.2	−63†	Lin et al. (2006), Tao et al. (2014)

Endogenous PKA shift (mV)	30.9 ± 4.7	23.0 ± 3.7	−26*	Lin et al. (2005), Tao et al. (2014)
Cerebral artery pharmacomechanical coupling				
Amine-induced tension, g (10^{-5} M serotonin and 2 10^{-5} M histamine)	2.0 ± 0.2	1.5 ± 0.2	−26	Longo et al. (1993)
Amine/K^+ max (%max)	85 ± 5	75 ± 5	−12	Longo et al. (1993)
NE-induced tension (g)	1.4 ± 0.1	1.4 ± 0.1		Long et al. (2002)
NE/K^+ max (%max)	140 ± 12	127 ± 10	−9	
Total α_1-AR density (fmol mg protein^{-1})	47 ± 4	11 ± 1	−77†	Goyal et al. In Review
Ins(1,4,5)P_3 basal	40 ± 10	42 ± 7		Ueno et al. (1997)
Ins(1,4,5)P_3 response (%basal)	345 ± 27	225 ± 30	−35*	Ueno et al. (1997)
Ins(1,4,5)P_3[a] α_1-AR^{-1}	5.2 ± 1	11.7 ± 1	125†	Ueno et al. (1997)
Ins(1,4,5)P_3-receptor density (fmol mg protein^{-1})	115 ± 15	22 ± 3	−80†	Zhou et al. (1997)
ERK1/2–total (%control)	1.0	0.9 ± 0.1	−10	Zhao et al. (2004)
–Phosphorylated	1.0	2.5 ± 0.4	150†	
MLC_{20}–total (%control)	1.0	5.5 ± 1.0	450†	Zhao et al. (2004)
–Phosphorylated	1.0	0.8 ± 0.2	−20	
CPI-17–total (%control)	1.0	1.9 ± 0.2	90†	Zhao et al. (2004)
–Phosphorylated	1.0	1.1 ± 0.2	10	
Cerebral artery perivascular innervation				
Basal NE release (10^{-9} g 10^{-9} g content^{-1})	4.7 ± 0.5	8.2 ± 0.7	74*	Buchholz and Duckles (2001)
Stimulation-evoked fractional NE release after uptake blockade	0.52 ± 0.05	0.69 ± .10	33*	Buchholz and Duckles (2001)
Stimulation-induced adrenergic contractions (%K_{max} at 8 Hz)	0.5 ± 0.1	8 ± 2	>1,500†	Pearce (1995)
Cerebral vasorelaxation pathways				
A-23187-induced relaxation (%)	35	24	−31	Longo et al. (1993)
sGC (10^{-9} M GMP·10^{-3} M protein min^{-1})	0.43 ± 0.03	0.13 ± 0.02	−70	Williams et al. (2006)
sGC (10^{-12} g·10^{-3} protein)	7.1 ± 0.9	6.0 ± 0.5	−15	Williams et al. (2006)
cGMP (10^{-9} M·10^{-3} g protein min^{-1})	29.5 ± 5.6	84.5 ± 16.4	186	Williams et al. (2006)

(continued)

Table 15.1 (continued)

Physiologic variable	Normoxic control	High altitude hypoxic	% Change	References
cGMP (10^{-9} M · 10^{-3} g soluble protein^{-1})	0.35 ± 0.1	0.25 ± 0.1	−28*	Williams et al. (2006)
cGMP [10^{-9} M·sGC^{-1} (10^{-6} g) min^{-1}]	145 ± 20	105 ± 20	−28*	Williams et al. (2006)
Max eNOS specific activity slope, NO (10^{-12} M)·eNOS^{-1} 10^{-6} g min^{-1}	3.8 ± 0.5	1.5 ± 0.3	−61†	Williams et al. (2006)
Cranial artery gene regulation				
38 genes upregulated	>2-fold			Goyal et al. (2013)
9 genes downregulated	>2-fold			Goyal et al. (2013)
Fetal HPA axis regulation				
CYP11A and CYP17				Myers and Ducsay (2012)
ACTH-receptor				Myers et al. (2005)
ACTH response to AVP increased				Myers et al. (2005)
Hormonal				
Norepinephrine (pg ml^{-1})	553 ± 55	635 ± 65	14.8	
Epinephrine (pg ml^{-1})	81 ± 19	113 ± 12	39.5†	
ACTH (pg ml^{-1})	66 ± 8	60 ± 9	−10	
Cortisol (ng ml^{-1})	47 ± 3	50 ± 1	6.4	
Erythropoietin (mU ml^{-1})	23 ± 2	31 ± 17	35	
Some placental cellular parameters				
Placental nuclear functions				
90 genes upregulated		–		Gheorghe et al. (2007)
73 genes downregulated		–		Gheorghe et al. (2007)
Mitochondrial DNA content[a]	455	698	35*	Lattuada et al. (2008)

Values are mean ± SE
*$p < 0.01$
†$p < 0.05$ from normoxic control values
[a]Human FGR studies

Again, as noted in Chap. 14, Lorna Grindlay Moore and her group have described a number of important variables and adaptive mechanisms in both the pregnant mother and their fetus at high altitude in Leadville (3100 m), the Andean *altiplano* of Peru (4300 m), and the Tibetan Plateau (3658 m) (Moore 1990). For instance, using pulsed-wave gated Doppler ultrasound compared birthweight to the uterine blood flow in Denver and Leadville measured a month earlier at 36 weeks' gestation. At 40 weeks' gestation, BWs of the high-altitude group were reduced 8%, in part reflecting the decrease in the uterine artery fraction of common iliac blood flow seen at 36 weeks' gestation. The authors concluded that this decrease in birthweights was a consequence of the combined effect of the smaller uterine artery diameter (27%) seen at 36 weeks' gestation, a smaller percentage of common iliac flow (36%), and reduced volumetric flow to the placenta (35%). This is despite the high-altitude-associated increase in blood flow of the uterine artery per se (Zamudio et al. 1995) (Table 1).

In a subsequent study of 150 women of Andean or European ancestry in La Paz (>3600 m) and Santa Cruz, Bolivia, Zamudio and colleagues demonstrated in both ancestry groups slight to modest decreases in both uterine artery diameter and mean blood flow velocity at high altitude, but with the increase in hematocrit slightly greater increase in uterine O_2 delivery in those of Andean ancestry (Zamudio et al. 2007). The altitude-associated decrement in BW was 418 g in European, as opposed to 236 g in the Andean women. The authors conclude that a deficit in maternal O_2 transport to the placenta is not likely to be causally related to the decreased birthweight (Zamudio et al. 2007). In an editorial commentary on this report, Giussani referred to these and related findings as "the Andean curse on the Conquistadors" (Giussani 2007, p. 472). As a corollary, these investigators showed that DNA synthesis decreased significantly in the guinea pig uteroplacental vasculature at high altitude (Rockwell et al. 2000). Another of their studies from the highlands of Bolivia (3600–4100 m) demonstrated that uterine vascular high-end arteriolar resistance restricts fetal growth in those patients with preeclampsia, this incidence of which is higher at high altitude (Browne et al. 2011).

In regard to diet, it has been suggested that impaired transplacental glucose transport also plays a role in high-altitude-associated FGR (Zamudio 2003; see below). In humans, Doppler ultrasonic measurements of fetal arterial circulatory responses at 4300 m have shown a lack of flow redistribution, as would be seen in acute hypoxia (Krampl et al. 2001a). Paradoxically, in some of these fetuses, the velocity of arterial blood flow was significantly less than that measured at low altitude (Krampl et al. 2001a), presumably because of the LTH increase in blood viscosity associated with increased erythrocyte concentration (Ballew and Haas 1986). In light of the above, with the use of ultrasonography, to avoid the overdiagnosis of FGR, it probably is appropriate to use altitude-specific biometry charts to assess fetal dimensions during the third trimester of pregnancy (Krampl et al. 2000, 2002).

In terms of brain metabolism at high altitude, indigenous Quechua natives of the Peruvian *altiplano* (3700–4900 m; 12,139–16,076 ft altitude) by proton nuclear magnetic resonance displayed normal spectra with no evidence of unusual lactate accumulation. This was in contrast to many hypoxia-tolerant species (fresh-water

turtles and deep-diving seals) in which a large fraction of glucose taken up by the brain is released as lactate (Hochachka et al. 1994). In contrast, positron emission tomographic measurements showed systematically lower region-by-region rates of glucose metabolism, particularly in frontal cortex (Hochachka et al. 1994). The differences between those individuals with lifetime exposure to hypoxia and lowlander controls may indicate a defense adaptation to chronic hypoxia. Of related interest, in separate studies, genome-wide scans showed that Tibetans positively selected haplotypes of *Egln1* and *PPPARA* were associated with decreased hemoglobin phenotype unique to this highland population (Simonson et al. 2010). In a related study, the *Egln1* gene was identified as contributing to natural selection in Andeans as well as Tibetans (Bigham et al. 2010), and a number of hypoxia-inducible factor-1α (HIFα) pathway genes (but not HIF1α itself) were upregulated in the Andeans (Bigham et al. 2009). A timely review on "Altitude adaptations: a glimpse through various lenses" analyzes over two dozen reports of altitude-associated gene expression in human Andean, Tibetan, and Ethiopian highlanders (Simonson 2015).

15.2 Interrelations of Fetal Blood O_2 Affinity and Capacity with that of the Mother

The role of fetal hemoglobin in cellular respiration and the manner in which it differs from that of the adult have intrigued investigators since the mid-nineteenth century (van Korber 1866). Nonetheless, the discovery of the existence of a distinct fetal hemoglobin awaited almost a century (Brinkman and Jonxis 1935; Kleihauer et al. 1957). Despite the attention given to hemoglobin in the human fetus, many mammalian species lack such a specific moiety (see Longo 1987 for review). As noted in Chap. 2, in the goat, Arthur St. George Joseph McCarthy Huggett (1897–1968) first demonstrated that the oxyhemoglobin saturation curve of the fetus differs from that of the mother (although Huggett mistakenly reported that the O_2 affinity of the fetal blood was less than that of the adult, Huggett 1927). In terms of placental respiratory gas exchange and fetal oxygenation, the O_2 capacity and affinity of fetal hemoglobin are of critical importance (Longo 1987).

A major factor in the determination of the tolerance to hypoxia in mammals would appear to be the affinity of hemoglobin for oxygen. Many reports have explored the role of fetal hypoxia in increasing hemoglobin concentration and some of the cellular and molecular mechanisms by which this occurs (Longo 1987). For instance, in a number of species fetal and neonatal mice, rats, rabbits, and puppies exposed up to 20 h·day^{-1} to a degree of hypoxia that did not inhibit growth increased their production of hemoglobin F and tolerance to hypoxia, as determined by righting reflex times (Barker 1957). Also, when incubated in vitro with 0% O_2 for 1–4 h reticulocyte-rich suspension of human umbilical cord erythrocytes showed a striking increase in Hgb F synthesis (Allen and Jandl 1960).

In some early studies, it was noted that the blood O_2 affinity studies also "... appear to involve a plasma transported substance, its production or activity being regulated by O_2 tension" (Barker 1957, p. 289). Later this led to the recognition of 2,3-diphosphoglycerate (2,3-DPG) being the organic phosphate in greatest concentration in the erythrocyte (Benesch and Benesch 1967; Chanutin and Curnish 1967; also called 2,3-bisphosphoglycerate). By cross-linking the beta chains with stabilization of the quaternary structure of deoxyhemogblin, 2,3-DPG decreases hemoglobin O_2 affinity. In fetal erythrocytes with alpha/gamma chain dimers, such binding is minimized, resulting in increased O_2 affinity (Bauer et al. 1968, 1969; Delivoria-Papadopoulos et al. 1971a; Salhany et al. 1971). During the latter third of gestation, the fetal erythrocyte concentration of 2,3-DPG rises to adult levels at term. During the first year of life, this increase continues, in concert with a decrease in hemoglobin F, before decreasing again to adult levels (Delivoria-Papadopoulos and McGowan 2011; Delivoria-Papadopoulos et al. 1971b).

15.3 Initial High-Altitude Studies on the Peruvian *Altiplano*

Based on increasing interest in the effects of high-altitude hypoxia on various aspects of mammalian reproduction, and the fact that under control conditions at sea level the fetal arterial O_2 tension is much lower than that of the adult and similar to that of a mountaineer at altitude, shortly after mid-century, Donald Henry Barron (1905–1993) and the Yale University group commenced a series of classic studies at the *Instituto de Biologia Andina*, Morococha, Peru (~4270 m, 14,009 ft) (Barron et al. 1964). Phrased another way, the fundamental issue revolved around the question "If the fetus at sea level enjoys only a small margin of safety with respect to its oxygen supply, how does one at altitude manage to survive?" (Barron et al. 1964, p. 116). Thus, with the assistance of Alberto Hurtado, Barron and colleagues spent part of the summer and autumn 1958 exploring this question. In Merino sheep, they tested several hypotheses relating to the biology and physiology of maternal and fetal acclimatization responses. For example, although plasma volume was similar in the two groups of ewes, red cell mass and thus blood O_2 capacity were significantly greater in the high-altitude animals (Prystowsky et al. 1960), while arterial oxyhemoglobin saturation values were considerably less (~67% as compared to ~95% at sea level) (Barron et al. 1964; Metcalfe et al. 1962a), and the high-altitude animals showed no significant difference in blood O_2 affinity (Meschia et al. 1961). To the investigator's surprise, high-altitude newborn weight at birth and crown-rump length showed a little significant difference from Dorset sheep near sea level in New Haven, Connecticut (Metcalfe et al. 1962b). This similarity in growth rate was even more remarkable when one considers that the body weights of the ewes at high altitude were significantly less than those of their ewes at sea level. Also of interest, their measurement of umbilical blood gas values did not differ significantly from sea level controls (Metcalfe et al. 1962a). This

may have been the combined result of variance in values and small sample size, however. As an index of the work required to maintain the circulation of the fetus at high altitude, compared to that of sea level controls, the relation of fetal heart weight to body weight was similar in the two groups. This despite the fact that for the ewes, heart weight and that weight in relation to body weight were significantly greater in the high-altitude animals (Metcalfe et al. 1962b). Also of importance, the group measured uterine blood flow, reporting a 35% increase in the high-altitude ewes, although their calculated rates of O_2 consumption were similar.

As striking as these studies were, and indeed they represent a milestone in both the physiology of high altitude and fetal physiology in general, they present a problem. Unfortunately, this series of investigations were conducted prior to the Barron group's introduction of the technique of chronic catheterization of the fetus in utero (Meschia 2006; Meschia et al. 1965, 1969–1970). Thus, the experiments had the drawback of all such studies in acutely anesthetized preparations. That is, the effect of anesthesia per se, acute stress of a surgical procedure, and availability of single measurements in a given fetus, so that the altitude effects may have been too subtle or liable to have been detected under their experimental conditions.

Along this line, under standard conditions (38 °C, $PCO_2 = 40$ Torr, pH = 7.4), Meschia and his colleagues observed a significant difference in the O_2 affinity and electrophoretic mobility and chromatographic separation of sheep blood (Naughton et al. 1963). In essence, in the adult sheep, two hemoglobin types could be distinguished: a fast moving (type A) and a slow moving (type B) with PO_2 half-saturation values of 27.7 and 37.8 Torr, respectively (Naughton et al. 1963). These differences also were evident in the high-altitude animals, and the Barron group used these in an attempt to calculate maternal to fetal transplacental PO_2 gradients (Barron et al. 1964). Further, in a study of the maternal and fetal llama, the blood of which also showed a high O_2 affinity (21.9 and 18.0 Torr, respectively), these workers concluded that the transplacental PO_2 gradients were similar despite the llama placenta having an additional membrane layer to that of the sheep (Table 2). These findings added to the evidence that the transplacental O_2 gradient was not a function of the number of cell membrane layers and/or thickness [that is, with increasing number of placental cell layers and thickness, the fetal umbilical venous (arterialized blood) O_2 tension would be lower] (Meschia et al. 1960).

In response to my query regarding some aspects of these studies at Morococha, Peru, Giacomo Meschia responded:

Table 15.2 Fetal sheep and fetal llama: a comparison of some cardiovascular functions and responses to acute hypoxia

Physiologic function	Sheep	Reference	Relative value	Llama	Reference
Normoxia control					
Gestational length (days)	147 ± 3		<	350 ± 5*	Fowler (1989)
Fetal weight (kg)	4.4 ± 3		<	11 ± 1*	Fowler (1989)
Mean arterial blood pressure (mmHg)	44 ± 1	Kamitomo et al. (1992, 1993)	=	48 ± 2[a]	Llanos et al. (1995)
Heart rate (beats min^{-1})	168 ± 5 156 ± 5	Kamitomo et al. (1992, 1993)	>	124 ± 5 113 ± 6 122 ± 6	Benavides et al. (1989) Llanos et al. (1995, 1998) Giussani et al. (1996)
Hemoglobin concentration (gm dl)	11 ± 1 12 ± 1	Benavides et al. (1989)	>	10 ± 1* 16 ± 1	Benavides et al. (1989) [a]Moraga et al. (2011)
P_{50} (Torr)	17.0	Meschia et al. (1961) Longo (1987)		18.0	Meschia et al. (1960)
Arterial PO_2 (Torr)	23 ± 1 24 ± 1	Kamitomo et al. (1993) Kitanaka et al. (1989) Giussani et al. (1999) Moraga et al. (1996)	=	25 ± 1	Llanos et al. (1995)
Combined ventricular output (ml min^{-1} kg^{-1})	465 ± 17	Benavides et al. (1989)	>	238 ± 18 250 ± 13 113 ± 11	Benavides et al. (1989) Giussani et al. (1996) Llanos et al. (2002)
Umbilical blood flow (ml min^{-1} kg^{-1})				82 ± 10	
Carotid blood flow (ml min^{-1} kg^{-1})				22.8 ± 4	Giussani et al. (1996)
Femoral blood flow (ml min^{-1} kg^{-1})	6.1 ± 0.4	[a]Moraga et al. (2011)	>	4.6 ± 0.6*	[a]Moraga et al. (2011)

(continued)

Table 15.2 (continued)

Physiologic function	Sheep	Reference	Relative value	Llama	Reference
Oxygen consumption (ml O_2 min^{-1} kg^{-1})	~8		>	~4.2	Llanos et al. (2003)
Acute hypoxia *Cardiac output and blood flows*					
Arterial blood pressure (mmHg)	81 ± 1		<	51 ± 3	Llanos et al. (1995, 1998)
Arterial PO_2 (Torr)	17 ± 2	Hunter et al. (2003) Peña et al. (2007)		12–16	Giussani et al. (1999) Llanos et al. (1998, 2002)
Heart rate (beats min^{-1})	120 ± 5	Peña et al. (2007)		95 ± 7	Llanos et al. (1998)
Combined ventricular output (ml min^{-1} kg^{-1})					
Umbilical blood flow (ml min^{-1} kg^{-1})				82 ± 10	?
Cerebral blood flow	~40%↑	Hunter et al. (2003) Peña et al. (2007) Llanos et al. (2002, 2003)	>	0–5%↑	
Coronary blood flow (percent change)	~60%↑	Llanos et al. (2002, 2003)	<	2 to 3-fold↑	Llanos et al. (1995, 1998, 2002, 2003)
Adrenal blood flow (percent change)	~160%↑	Llanos et al. (2002, 2003)	>	~100%↑	Llanos et al. (1995, 1998, 2002, 2003)
Kidney and other blood flow (percent change)	~20%↓	Llanos et al. (2002, 2003)	<	~80%↓	Llanos et al. (1995, 1998, 2003
Femoral blood flow (ml min^{-1} kg^{-1})	5.2 ± 0.4		>	3.7 ± 0.8	Moraga et al. (2011)
Circulating hormones					
Cortisol					Llanos et al. (2003) Riquelme et al. (1998, 2002)
ACTH				% ↑	Llanos et al. (2003)

(continued)

Table 15.2 (continued)

Physiologic function	Sheep	Reference	Relative value	Llama	Reference
					Riquelme et al. (1998, 2002)
Catecholamines	2-fold↑				Llanos et al. (2003) Riquelme et al. (1998, 2002)
Arginine vasopressin	2-fold↑		<	2 to 3-fold↑	Herrera et al. (2000)
Neuropeptide Y			<	2 to 3-fold↑	Giussani et al. (1996, 1999)
Angiotensin II			>	–	Giussani et al. (1996, 1999)
Interventions					
α1-AR blockade	Vascular collapse		=	Vascular collapse	Giussani et al. (1999) Moraga et al. (2011) Reuss et al. (1982)
AVP-R blockade					Giussani et al. (1993, 1999) Llanos et al. (2003) Perez et al. (1989)
Nitric oxide synthase blockade			<		Giussani et al. (1999) Llanos et al. (2003)
Carotid sinus denervation			>	–	Giussani et al. (1994a, b, 1996)
Other changes					
Electrocorticogram	ECoG flattens, may develop seizure			ECOG flattens, but no seizure activity	Llanos et al. (2003)

*$p < 0.05$
[a]Newborn

Our work at Yale was briefly interrupted by an expedition to the Peruvian Andes from July to November of 1958. The members of the expedition were, in addition to Dr. Barron, James Metcalfe, William Huckabee and me from New Haven, Andre Hellegers and Harry Prystowsky joined us from the Obstetrics Department at the Johns Hopkins. It is my

understanding that the idea for the expedition came to Barron while he was considering Barcroft's view of fetal development. According to Barcroft, the oxygen saturation of fetal blood becomes progressively lower during pregnancy because the ability of the placenta to supply oxygen does not keep pace with the increasing oxygen demand of the growing fetus. To emphasize this concept, Barcroft compared the fetus to a mountain climber who is exposed to a progressively decreasing atmospheric oxygen pressure and coined the colorful expression "Mount Everest in utero". In Barcroft's view, the oxygenation of the term fetus has virtually no safety margin left. However, many mammals, humans and sheep included, reproduce at high altitude. If Barcroft was correct, what kind of adaptation made possible fetal survival at high altitudes? The main objective of the expedition was to study pregnant ewes living at high altitudes using the facilities of the laboratory of Andean Biology of the University of San Marco. The laboratory is located at 4,270 m (14,000 ft) on the Morococha altiplano east of Lima, in proximity of a copper mine.

The expedition was a major undertaking, for which Barron assumed virtually the entire burden. I remember going with him to New York to order a long list of chemicals and instruments. Included in the list were two big nitrogen tanks, because someone had informed Barron that in Peru one could find tanks of oxygen and CO_2, but not nitrogen. We needed all of these gases to construct the oxyhemoglobin dissociation curves of maternal and fetal blood. We were also told that if we wanted our supplies to reach their destination, we had to travel with them by ship. So, it was arranged that Barron and I would travel on a cargo ship from New York to Callao (the harbor next to Lima), while our colleagues would come later by airplane. I had been on ships before, but this was by far the longest trip. It had some interesting aspects, like the day we went through the Panama Canal, and many more monotonous days on the open sea. Fortunately, I was constantly reminded of the important reason why we were traveling by ship. Chained on the deck, visible from about any angle, were our nitrogen tanks.

When we arrived in Callao, we experienced a delay of one day in getting the supplies through customs. First, we could not find the tanks. After a frantic search, we found them in an enclosure named "Flammables". Second, I had to translate the list of supplies into Spanish. My translation was into the comical Spanish of an Italian who does not know the language. After a stop in Lima to pay a visit to Alberto Hurtado, the head of Physiology at San Marco University, Barron and I took a quick trip across the Andes to the western edge of the Amazonian jungle. In a single day, we saw llamas and snow-capped mountains, tropical birds, and banana trees.

Then, we joined our colleagues in Morococha and started unpacking the supplies. There was a tense moment when we discovered that both nitrogen tanks were completely empty. However, we had among our supplies large quantities of a reagent that binds oxygen and so we made our own nitrogen by passing air through the reagent. We all started working at a fast pace, knowing that we had not much time and wishing to gather as much information as possible. In about seventeen sheep we used Huckabee's method to measure uterine blood flow and sampled maternal and fetal blood in a larger number of ewes. We even found time for sampling maternal and fetal blood in a few pregnant llamas that we had purchased from the indigenous population. In addition, Hellegers and Metcalfe were able to perform a study of alveolar CO_2 pressure in the wives of the mining engineers at Morococha. They found that at high altitude, the alveolar PCO_2 of pregnant women is extremely low due to the combined effect of progesterone and hypoxia on the respiratory center.

Toward the end of our expedition, we took time off for some sightseeing. I flew to Cusco and visited the Inca ruins. Back in Lima, we returned the oxygen and CO_2 tanks that we had rented locally from a manufacturing plant. In chatting with one of the engineers at the plant, I mentioned all the trouble we had because one could not purchase nitrogen in Peru. He laughed. We extract oxygen from the air like anybody else, he said. What do you think we do with the nitrogen?

> With respect to its main objective, the Peruvian expedition was inconclusive. It showed that pregnant ewes pastured at 14,000 feet were able to produce fetuses of normal weight, but it could not establish whether there was a significant decrease in the level of fetal oxygenation with respect to sea level conditions. Our basis for comparison, fetuses sampled under various degrees of surgical stress was unreliable. An unforeseen difficulty was that in adult sheep two co-dominant genes control the expression of two types of hemoglobin with substantially different oxygen affinities. At the time of the expedition, we were unaware of this important source of variability.
>
> (Letter from GM to LDL 16 December 2014)

In a further communication, Meschia recalled:

> The New York-Lima trip lasted about a month. The Morococha lab was adequate for our purpose. No Peruvian physiologist worked with us . . .We did not have any major technical problems, and no one got sick. We worked hard, and were even able to sample a fetal llama . . . as you probably know, Barron wrote an account of the scientific findings.
>
> (Letter from GM to LDL 21 January 2015)

15.4 Studies in Sheep Subjected to Hypobaric Hypoxia

As is well documented, for an understanding of the biology and physiology of the fetus, that of the sheep *Ovis aries* is widely used. This is in large part because of its relatively large size, accessibility and tranquility of the ewe, reliability of chronic catheterization and instrumentation for studies under physiologic conditions, and modest cost. As noted, because the arterial O_2 tension of the normoxic fetus at sea level approximates that of an adult at ~5000 m, one would anticipate that the PO_2 values of a fetus at high altitude would be much lower than sea level control. As has been demonstrated in a number of studies, surprisingly, such is not the case (Barron et al. 1964; Kamitomo et al. 1993; Kitanaka et al. 1989a, b; Longo 1987). Nonetheless, it is instructive to examine these blood gas values at different barometric pressures, in part, because those values for the unacclimatized animal differ considerably from those acclimatized for 6 weeks or more.

A question of relevance at this time was the extent to which the fetus produces hemoglobin in a maximal manner or whether hemoglobin synthesis might be increased in response to hypoxia or other stresses. In an effort to address this issue, from the University of Illinois and Minnesota, in one of the first studies to make use of a high-altitude decompression chamber, at about 0.64 gestation (95 days vs. 149 days term), for 10 days 4 ewes were exposed to diminished ambient pressure at 385 mmHg (5400 m simulated altitude), and 4 ewes were exposed to 345 mmHg (6100 m). Then under analgesia and local anesthesia, the ewes' uterine arteries and veins were isolated, as were the fetal umbilical vein and artery. At the higher barometric pressure-lower equivalent altitude, all fetuses survived. This was in contrast to findings in the lower pressure-higher altitude group in which one ewe and two fetuses died. In the one surviving fetus, umbilical PO_2 plummeted to ~7 Torr in this extremely diminished O_2 state (Kaiser et al. 1958).

The authors contended that the hematologic response observed in this study demonstrated that hypoxia as that observed at lower barometric pressure is of importance in this regard. The mean hemoglobin values in the ewe and fetus at the lower simulated altitude were 11.1 and 10.3 g dl^{-1}, respectively. In turn, these values at the higher "elevation" were 17.4 and 9.4 g dl^{-1}, respectively (Kaiser et al. 1958). Normoxic control values for the various parameters were not included, although these values (except that of the single fetus at high altitude) appear to be greater than that reported by ourselves and others (Longo 1987). Also of interest, lyophilized maternal plasma from several of these ewes showed marked erythropoietic activity (Kaiser et al. 1958).

In a further study along this line at the University of Florida, Gainesville, one pregnant Nubian goat and three Dorset sheep were chronically catheterized in one uterine vein and one each femoral artery and vein (Cotter et al. 1967). Then following several weeks for recovery from surgery, decompression was initiated (rate = 1000 ft min^{-1}) to 5000 and 10,000 ft (three animals) and 15,000 (two animals) at which levels they were maintained for 30 min before withdrawing blood for hemoglobin, hematocrit, respiratory gases, and pH. At 10,000 and 15,000 ft, arterial oxyhemoglobin saturation values fell from sea level control of 96 ± 3% to 83 ± 3% and 53 ± 10%, respectively. Comparable oxyhemoglobin saturation values for the uterine venous blood were 79 ± 4% to 68 ± 6% and 44 ± 5%, respectively. No obvious trends were noted in either arterial CO_2 tension or bicarbonate levels, and pH values did not change significantly (Cotter et al. 1967). In the goat, PO_2 (the only value reported) fell from sea level control of 29–24 Torr; again, there were no significant changes in CO_2, bicarbonate, or pH values. The authors concluded that despite the acute fall in uterine venous O_2 values at 10,000 ft, the decrease in umbilical venous (and presumably transplacental O_2 tension gradient) was far less (Cotter et al. 1967). In a follow-up study using this facility with near-term ungulates (five goats and one sheep), blood gas values were measured at 1524 m (5000 ft), 3048 m (10,000 ft), and 4592 m (15,000 ft) (Blechner et al. 1968). At these simulated elevations, compared to sea level values, maternal arterial PO_2 values were ~75 (control), 64, 47, and 34 Torr, respectively. Uterine venous PO_2 values averaged 39 (control), 36, 31, and 26 Torr, respectively. Those values for fetal umbilical venous (arterialized) blood were 20 (control), 18, 15, and 13 Torr, respectively. Finally, for the umbilical arterial (venous) blood, the values were 16 (control), 17, 11, and 9 Torr, respectively. This was the first such study to demonstrate that at high altitude the decreases in fetal blood respiratory gas values were considerably less than one would expect based on decrements of PO_2 values in the mother (Blechner et al. 1968).

Then the animals were returned to Denver for an additional 18 days of investigation (Makowski et al. 1968). Although the duration of time at high altitude was insufficient for full acclimatization to occur, the investigators witnessed the beginnings of a hematologic response. This was evidenced by increased blood O_2 capacity (hemoglobin and hematocrit values were not reported) in response to decreased PO_2 and oxyhemoglobin saturation values and a response in these variables upon return to Denver (Makowski et al. 1968). As a high-altitude comparison,

in acclimatized humans at Cerro de Pasco, Peru, (4200 m) fetal scalp PO_2 averaged 19 Torr, a value only slightly less than sea level control of 22 Torr (Sobrevilla et al. 1971). Thus, from mid-century onward, with the use of both hypobaric chambers and studies at altitude, an understanding of certain aspects of fetal oxygenation at high altitude was achieved, albeit slowly.

15.5 Cardiovascular Studies in the Chick Embryo

To explore aspects of growth and the developing cardiovascular system independently from those influences of maternal physiologic responses, the chicken embryo also has been used to advantage. Several groups, including that of Carlos E. Blanco of Maastricht University, the Netherlands, have demonstrated the effect of hypoxia in causing asymmetric embryonic/fetal growth as well as the growth of specific organs and many other effects (Giussani et al. 2007; Lindgren and Altimiras 2011; Miller et al. 2002; Mulder et al. 2001, 2002; Ruijtenbeek et al. 2003a, b; Sharma et al. 2006). The reported changes include aortic hypertrophy and left ventricular dysfunction (Rouwet et al. 2002), with enlargement of both ventricles (Villamor et al. 2004), and cardiomyopathy (Salinas et al. 2010). Such cardiovascular changes are associated with altered endothelial reactivity (Ruijtenbeek et al. 2003a, b) and sympathetic hyperinnervation of peripheral arteries (Rouwet et al. 2002; Ruijtenbeek et al. 2000). For instance, alpha adrenergic receptor blockade abolished the acute hypoxic-mediated redistribution of cardiac output to the brain and heart (Mulder et al. 2001); nonetheless non-neurogenic mechanisms also participated in this response (Mulder et al. 2002). As in the fetal lamb, in the chick embryo, LTH decreased ventricular $+dT \cdot dt_{max}^{-1}$, peak pressure, and ventricular ejection fraction (Sharma et al. 2006).

In a critical study of fertilized eggs from hens acclimatized to either sea level or high altitude (3600 m) and incubated at either condition of oxygenation (five groups: eggs laid at sea level and incubated at either sea level or high altitude, eggs laid at high altitude and incubated at either sea level or high altitude, and eggs laid at sea level but incubated at high altitude with O_2 supplementation to mimic sea level values, percent O_2 not given). As expected, embryo weight was restricted in the high-altitude incubated groups (45% in the eggs from sea level and 22% in those from high altitude) (Giussani et al. 2007). In the group of chick embryos at high altitude in which supplemental O_2 was administered, the LTH-induced asymmetric growth restriction and cardiac remodeling were not observed (Giussani et al. 2007; Salinas et al. 2010). Of importance, these studies isolated the effects of alterations in embryonic/fetal oxygenation on growth and development independent of maternal nutrition or other physiologic variables associated with high altitude. Other studies have reported on the adult sequelae of antenatal hypoxia. For instance, following hypoxic incubation as chicks the femoral arteries of adult chickens showed increased sensitivity to both pharmacologic and electrical stimulation of periarterial sympathetic nerves, as well as decreased NO-dependent vasodilatation (Ruijtenbeek et al. 2003a, b). In a follow-up review, this group studied blood

pressure regulation in adult (6 months of age) chickens that had been incubated, hatched, and raised at high altitude (3600 m, feet elevation), as compared with a similar group from near sea level. Independent of sex, the high-altitude chickens had significantly lower blood pressure; however, the gain in arterial baroreflex activity was decreased in high-altitude males, but increased in the females (Herrera et al. 2013). Thus, in adulthood high altitude altered baroreflex sensitivity in sex-dependent manner.

15.6 Studies of Prolonged Hypoxia in Rodents

As a laboratory "model" for almost every aspect of reproduction, rodents also have been used to explore antenatal maternal hypoxia and its sequelae for the mother, fetus, and offspring as an adult. These include the most vulnerable period of gestation, the degree of hypoxia, and its duration. Based on an analysis of 22 studies in rats and six in mice that met criteria of having appropriate controls and stringent statistical analysis (Jang et al. 2015), it is clear that, in addition to the factors given above, the species/breed studied is important and that these variables interact. For instance, in the rat 7 days or more and in the mouse 3 days or more of hypoxia at 14% or less O_2 concentration during the third (in some cases second) trimester of pregnancy was required to produce FGR. In both rodent species, the overall analysis suggests that a newborn pup weight reduction of 31% is required to meet the human FGR definitions of below the 10th percentile (Jang et al. 2015). In the rabbit, uteroplacental ischemia produced by ligation of 50% of blood vessels on day 25 (of a 30 days' gestation) produced a number of fetal cerebral neurostructural abnormalities associated with functional impairments (Illa et al. 2013). In addition, Wistar rats exposed to 13% O_2 from day 6 to 20 gestation, although fetal body weight was not decreased, aortic wall thickness and the vascular wall to lumen area increased 176% and 170%, respectively (Herrera et al. 2010). In a similar rat preparation, this group demonstrated the role of antenatal hypoxia-induced oxidative stress in producing aortic thickening with enhanced nitrotyrosine staining (indicative of peroxynitrite generation) and an increase in HSP70 expression. In adulthood (4 months) a cohort of these animals displayed impaired NO-dependent relaxation of femoral resistance arteries and increased myocardial contractility with sympathetic dominance (Giussani et al. 2012). These invidious effects were not observed when vitamin C was provided in the maternal drinking water during the antenatal period (Giussani et al. 2012).

Fetal asymmetric growth restriction and cardiovascular dysfunction also have been demonstrated in the rat (Herrera et al. 2012; Williams et al. 2005a, b). In the guinea pig as well, Thompson and his group have demonstrated several cardiovascular sequelae including increased cardiac production of endothelial nitric oxide synthase (NOS) (Dong and Thompson 2006; Thompson et al. 2000) with increased NO in such dysfunction (Thompson et al. 2009). Antenatal chronic hypoxia also has been shown to affect fetal myocardial expression of cardioprotective enzymes such

as protein kinase C epsilon (PKCε) with programming of an increase in cardiac susceptibility to ischemia and reperfusion injury in male offspring (Li et al. 2003; Patterson et al. 2010; Xue and Zhang 2009). Presumably the result of ROS studies also has demonstrated increased methylation of the promoter of the *PKCε* gene, an epigenetic change which could be prevented by administration of an inhibitor of DNA methylation (Patterson et al. 2012). The adult offspring of dams subjected to antenatal hypoxia also displayed cardiac structural and functional changes including increased expression of collagen types I and III, and the ratio of beta to alpha myosin heavy chains (Xu et al. 2006), as well as increased left ventricular end-diastolic pressure (Rueda-Clausen et al. 2009) and decreased myocardial metabolism (Rueda-Clausen et al. 2011b). Further studies have demonstrated decreased beta 1 receptor and increased muscarinic receptor responses in adult offspring of rats so subjected to LTH (Giussani et al. 2012). Also of clinical relevance, cardiovascular disease in adults has been associated with elevated sympathetic and decreased parasympathetic reactivity. Thus, overall studies from several species support the idea of antenatal hypoxia resulting in cardiomyopathy in the adult offspring. Provocatively, many of these antenatal hypoxic-mediated changes show gender specificity for many complications, with males being more susceptible than females (Hemmings et al. 2005). Other investigators also have demonstrated NO-mediated endothelial dysfunction in the adult rat following antenatal hypoxia (Hemmings et al. 2005; Morton et al. 2010, 2011; Williams et al. 2005a, b), and sheep (Giussani et al. 2012).

15.7 Studies in Sheep Acclimatized to High Altitude at the White Mountain Research Station

The White Mountain Research Station (WMRS), Bishop, CA, was established following World War II when the US Naval Ordnance Test Station built a road in 1948 along the crest of the mountain range east of the Owens Valley and erected a laboratory at an elevation of 3230 m (10,600 ft). Initially, the building was used for classified astrophysical investigation by the Navy. In 1950 the installation was transferred to the Office of Naval Research (ONR), with the operation of the facility delegated to the University of California. Although up to this time the Station had been used for research in astrophysics and related matters, one of the architects in its operation, Nello Pace (1916–1995), an environmental physiologist at the University of California, Berkeley, recognized its potential for studies of the effect of high altitude in humans and animals. In an effort to extend the stress of elevation for their studies, in 1951 a building which was to house the Barcroft laboratory was erected at 3801 m (12,470 ft), somewhat below the summit of White Mountain itself at 4343 m (14,250 ft) (Cook and Pace 1952).

In an early series of studies at the White Mountain Research Station with Professor Pace, Paola S. Timiras (1923–2008), also of the University of California,

Berkeley, explored the altitude effects in Long Evans rats in an effort to develop "... strains that over many generations will have become thoroughly adapted to high altitude" (Cook and Pace 1952, p. 699). Although the majority of this work was in adult males, the F_1 offspring and F_2 descendants were also included (Timiras 1964). Compared to sea level controls, birthweight and body weight of F_2 generation offspring were and remained lower throughout life (up to 8 months). The F_2 rats showed marked cardiac hypertrophy, and their mortality rate was considerably increased, with only 27% of these reaching 8 months of age (Timiras et al. 1957). The investigators' hypothesis that metabolic disturbances account for this growth restriction was supported by the observation that following descent to sea level the F_2 young adult body weights remained below normal over a 3-month period (Timiras et al. 1957). Of the organs studied in the F_2 descendants, the heart showed the most change with pronounced hypertrophy (90%) as a constant and predominant finding (Vaughan and Pace 1956). The adrenal glands also demonstrated considerable enlargement, while weights of the thymus and spleen decreased. These changes also were associated with an increase in skeletal muscle myoglobin and a decrease in glycogen content of the liver and heart (Vaughan and Pace 1956). As an aside, over a period of one-half a century, studies by investigators from a number of universities at WMRS have contributed to an understanding of acclimatization responses in many species including birds, other rodents, sheep, horse, and humans.

In an effort to understand some of the fundamental cellular and molecular mechanisms associated with the fetal and adult physiologic acclimatization responses to prolonged hypoxia, almost three decades ago, our group at Loma Linda University developed a "model" using both pregnant and nonpregnant sheep. The ewes are transported to high altitude at the White Mountain Research Station at ~30 days' gestation where they are kept until the latter stages of gestation near term, e.g., ~140 days. At this altitude, the adult arterial PO_2 falls from a normoxic control value of 95 ± 5 Torr to 60 ± 3 Torr, and fetal arterial PO_2 falls from 25 ± 3 to 19 ± 2 Torr (Kitanaka et al. 1989a, b). Although arterial PCO_2 falls slightly in both fetus (from 35 to 29 Torr) and adult (from 49 to 40 Torr), arterial pH remains unchanged. As may be clear, sheep are of great value for such studies in light of their relatively low cost, the large size of the fetus, and relative ease of chronic instrumentation for prolonged periods of time and the similarity of physiologic organ systems in terms of their tissue, cellular, and subcellular response mechanisms.

Of note, in the pregnant sheep acclimatized to altitude at the WMRS, despite numerous studies over almost three decades with long-term reduction in arterial PO_2, we have observed no reduction in body or organ weights of the term fetus, ~4.1 ± 0.7 kg compared to sea level control 3.9 ± 0.6 kg (Harvey et al. 1993; Kamitomo et al. 1992) (also our unpublished data for 5 years, *n* ~230 each group). This agrees with data from the studies at Morococha (~4270 m) on the *altiplano* of Peru of Donald H. Barron (1905–1993) and colleagues (Metcalfe et al. 1962a, b; Prystowsky et al. 1960). Even at that level of high-altitude-induced hypoxemia, despite compensatory acclimatization responses in a number of systems (see below), the ability of the sheep fetus to defend itself in terms of growth restriction

is remarkable and may be of value in considering the basic physiologic mechanisms involved.

15.8 Further Sheep Studies on the *Altiplano*

In contrast to these observations at the WMRS, in a series of studies at about 3600 m (11,811 ft) on the Chilean *altiplano*, an international group led by Dino Giussani of Cambridge University, Anìbal J. Llanos of the University of Chile, and Julian T. "Bill" Parer of the University of California San Francisco has explored several aspects of pulmonary and systemic vascular function in sheep at high altitude, as compared to low-altitude (LA, 580 m) controls. In newborn lambs (8–12 days of age) the pulmonary artery pressure was elevated significantly (20.2 ± 2.4 vs. 13.6 ± 0.5 mmHg), as was cardiac output (342 ± 23 vs. 279 ± 13 ml min^{-1} kg^{-1}). The HA small pulmonary arteries displayed both greater contractile capacity and higher sensitivity to NO (Herrera et al. 2007). HA newborn small femoral arteries showed lower maximal contraction with higher maximal response and sensitivity to the sympathetic agonists norepinephrine and phenylephrine. With superimposed acute hypoxia HA newborns reached higher pulmonary pressure and greater femoral vascular resistance (Herrera et al. 2007). With graded acute hypoxia, pulmonary arterial pressure also was elevated, as was their contractile status.

Again, in newborns from high-altitude ewes, but delivered at low altitude, at 6–11 days following birth, the lamb weights were reported to be 3.8 ± 0.3 kg and 7.0 ± 0.4 kg, respectively, a 43% difference (Herrera et al. 2010). Their pulmonary arterial pressure was elevated and increased more following NO inhibition. Small pulmonary arteries from these newborns showed greater maximal contraction to K^+, higher sensitivity to both endothelin-1 and NO (nitroprusside), and persistence of dilatation following soluble guanylate cyclase blockade (Herrera et al. 2010). These newborns also showed an elevated ratio of right ventricle to left ventricle plus septum. Small pulmonary artery media cross-sectional area was significantly elevated, and the vessels showed increased expression of endothelial NO synthase, phosphodiesterase, and a Ca^{2+}-activated K^+ channel. In contrast, heme oxidase-1 expression and CO production were decreased (Herrera et al. 2010). Thus, pulmonary hypertension persisted in the newborn following its gestation at high altitude. Supporting the idea that store-operated channels contribute to the development of neonatal pulmonary hypertension was demonstrated in HA newborn by increased pulmonary expression of two store-operated channel-forming subunits TRPC4 and STIM1 and a greater decrease in pulmonary artery pressure in following channel blockade (Parrau et al. 2013). In yet another contribution, these authors demonstrated that chronic hypoxia is associated with blunting of several cardiovascular responses such as an increased cerebrovascular contractile capacity with diminished responses to catecholamines, in contrast to the decreased femoral artery contractile capacity with increased adrenergic contractility (Herrera et al. 2016). In sheep born

and raised on the *altiplano* of Chile, these investigators reported considerable (26%) fetal growth restriction (2680 ± 168 vs. 3613 ± 176 g) with reduced brain and liver weights (but the greater brain to liver weight ratio, 0.59 ± 0.06 vs. 0.44 ± 0.02) as compared to low-altitude controls (Herrera et al. 2016). The high-altitude animals showed a markedly blunted increase in superimposed hypoxia cardiovascular responses. These workers also have reported increased oxidative stress with reactive oxygen species in the placenta and fetus (Giussani et al. 2012; Herrera et al. 2014).

Other comparisons by another group at the University of Chile have been made of fetal growth parameters in ewes which had resided at high altitude for several generations (HH group), as opposed to those native to low elevation but moved to the highlands (LH) upon confirmation of pregnancy by ultrasound (18 ± 2 dpc). Compared to the birthweight of sea level controls, the near-term lamb weights were 4.2 ± 0.03 kg; BWs in the LH and HH groups were decreased almost 30% to 3.0 ± 0.5 and 3.2 ± 0.8 kg, respectively (Parraguez et al. 2005). In a subsequent report, the BWs were 3.0 ± 0.1 and 3.3 ± 0.2 kg, respectively, for the LH and HH groups as compared to normoxic sea level controls (4.3 ± 0.2) (Parraguez et al. 2011). Of interest but of unknown significance, in the HH group, the length of gestation was increased, being 153 ± 4 days as compared to (146 ± 5) at altitude and 145 ± 3 in the LH and HH groups (Parraguez et al. 2005). Also in these animals, the administration of vitamin C (500 mg) and vitamin E (350 IU) during mating and gestation increased both lamb BW and placental cotyledon number, while reducing placental weight, suggesting that pregnancy at high altitudes is associated with oxidative stress (Parraguez et al. 2011). These changes were associated with normal maternal plasma levels of carbonyls and malondialdehyde (as contrasted with the non-supplemented ewe) suggesting oxidative stress at HA. In addition, antenatal administration of vitamins C and E to HA ewes restored relatively normal values for plasma progesterone and estradiol-17β concentrations (Parraguez et al. 2013). The newborn lambs in the HA-vitamin supplemented group also had higher weights than in any other group (Parraguez et al. 2013). This suggests a broad spectrum of effects caused by oxidative stress (Parraguez et al. 2011). Whether the prolonged hypoxemia during fetal development that is associated with elevated pulmonary arterial pressure, other vascular changes, and pulmonary hypertension in the newborn infant (Herrera et al. 2007, 2008, 2010; Llanos et al. 2012) is due to prenatal oxidative is beyond the limits of this chapter.

15.9 Fetal Cardiovascular Responses to Long-Term Hypoxia

In response to acute hypoxia, the fetus experiences redistribution of cardiac output from the peripheral circulation to maintain circulation of the brain, heart, and adrenal cortex in association with increased vascular resistance and decreased

blood flow to the trunk and lower limbs (Cohn et al. 1974; Lorijn and Longo 1980; Peeters et al. 1979). Such redistribution has been shown to be mediated by a carotid body chemoreflex (Giussani et al. 1993) in concert with the release of catecholamines (Jones and Robinson 1975), arginine vasopressin (Peréz et al. 1989), neuropeptide Y (Fletcher et al. 2000), nitric oxide (NO) (Morrison et al. 2003), and other vasoactive factors. Reactive oxygen species also play a role in this response (Thakor et al. 2010).

As noted, prolonged fetal hypoxia, as a consequence of any of the factors noted above can result in multiple cardiovascular changes (Kamitomo et al. 1993) with significant FGR (Giussani et al. 2001; Moore et al. 2011). In our "model" of high-altitude acclimatized LTH in fetal sheep, combined right and left ventricular cardiac output was significantly reduced (−24%) compared to the normoxic fetuses (Kamitomo et al. 1992, 1993) (Table 1). Although heart rate was unchanged, fetal arterial blood pressure increased 18%. This decrease in ventricular output was similar to that observed following only 2 weeks of normobaric hypoxia in the near-term sheep fetus (Alonso et al. 1989; Kamitomo et al. 1994). Despite prolonged hypoxia in these fetuses, blood flow to the brain and heart was maintained at near normal low-altitude levels. This, coupled with an increase in hemoglobin concentration (from 10.1 ± 0.7 to 12.6 ± 0.6 g dl^{-1}), resulted in normal oxygen delivery to these organs (Kamitomo et al. 1993). Nonetheless, in a striking manner, the distribution of cardiac output was altered, with significantly decreased blood flow and oxygen delivery to the kidneys, gastrointestinal tract, and carcass (skin, muscle, and bone) (Kamitomo et al. 1993). Of importance to the LTH fetus, despite cardiac output already being redistributed to favor the brain and heart, when subjected to a bout of superimposed hypoxia (with further reduction of fetal arterial PO_2 from 19 ± 2 Torr to 11 ± 1 Torr), cardiac output was redistributed to even a greater extent to maintain oxygen delivery to the brain and heart at the expense of organs in the trunk and extremities (Kamitomo et al. 1993). In addition to alterations in cardiac contractility, the mechanisms for these cardiovascular changes include those of structure and function in the several vascular beds.

Mechanisms potentially responsible for the reduction in cardiac output in the LTH sheep fetus appear to involve extrinsic factors that alter cardiac output, such as afterload (mean arterial blood pressure), preload (atrial pressure), and heart rate. Although the LTH fetuses showed a significant 18% elevation of arterial blood pressure, compared to control (from 44 ± 2 mmHg to 52 ± 1 mmHg), this increase in afterload could account for only a small fraction of the reduction in cardiac output (Kamitomo et al. 1992). No differences were observed in right or left atrial pressures (preload) between the hypoxic and control values (4.1 ± 0.1 mmHg for each ventricle), nor in heart rate (165 ± 6 bpm for each) that could account for a decrease in cardiac output. Thus, mechanisms responsible for the reduction in cardiac contraction appear to be intrinsic to the heart. In fetal sheep at low altitude subjected to 1 h of hypoxemia (9% O_2 with 5% CO_2) at several ages during the third trimester of pregnancy (125–130, 135–140, and >140 days gestational age), cardiac chemoreflex function and a battery of vasoconstrictor hormones were measured. Fetal bradycardia increased in magnitude and persistence, as did the increase in

femoral vascular resistance in concert with the near-term rise in plasma cortisol concentration, as well as that for catecholamines, NPY, AVP, and ACTH (Fletcher et al. 2006). These findings demonstrate the near-term changes in pattern and magnitude of fetal cardiovascular defense to hypoxemia and some of their in parallel neural and endocrinologic mechanisms. In another parallel series of studies, Giussani and colleagues have demonstrated the vital role of sympathetic (adrenergic) nerves in mediating the cardiovascular reflex responses. For instance, induction of acute hypoxia in fetuses in which the superior cervical ganglion had been removed resulted in rapid demise (Giussani et al. 1993).

Papillary muscles isolated from WMRS-LTH fetal sheep, studied in vitro in a well-oxygenated (95% O_2 + 5% CO_2) bath system in which electrical stimulation parameters could be optimized, demonstrated development of reduced maximum tension (T_{max}) in response to increasing concentrations of external calcium, reduced maximum rate of tension development ($+dT \cdot dt_{max}^{-1}$), and reduced rate of relaxation ($-dT \cdot dt_{max}^{-1}$) compared to controls (Browne et al. 1997a). Although the reduction in T_{max} was the same in papillary muscle from both ventricles, the reduction in $+dT \cdot dt_{max}^{-1}$ was larger in the right than in the left ventricle. Of interest, when acute hypoxia was imposed in the bath (20% or 40% O_2, PO_2 ~3 and 7 Torr, respectively), papillary muscles from LTH fetal hearts maintained T_{max}, $+dT \cdot dt_{max}^{-1}$, and $-dT \cdot dt_{max}^{-1}$ at significantly higher values than papillary muscle from control fetuses (Ohtsuka et al. 1997). In addition, LTH fetal hearts displayed elevated levels of lactate dehydrogenase and citrate synthase (but not pyruvate kinase) activities, changes that may enhance aerobic energy production during hypoxia (Ohtsuka and Gilbert 1995). Overall, there appeared to be no decrement in the calcium-induced-calcium-release mechanism in the LTH fetal heart. Rather, at least in the right ventricle, there was a greater dependence on SR released Ca^{2+} for contraction. This suggests a potential adaptive process to increase contractile function.

In addition to the reduction in cardiac output in the LTH fetus compared to normoxic control, there was also a reduced augmentation of cardiac output by stimulation of cardiac β_1-adrenergic receptors (β_1-AR) with the agonist isoproterenol (Kamitomo et al. 1995). Isoproterenol stimulation of isolated, well-oxygenated papillary muscles resulted in significantly less augmentation of T_{max}, $+dT \cdot dt_{max}^{-1}$, and $-dT \cdot dt_{max}^{-1}$ in papillary muscles from both ventricles of LTH fetal hearts, compared to normoxic controls (Browne et al. 1997b). Although in the LTH fetal heart the β_1-adrenergic receptor pathways β_1-AR number (B_{max}) did not change in the left ventricle, the right ventricle showed a 55% increase in β_1-AR number (Browne et al. 1997a). These findings contrast with the downregulation of β_1-adrenergic receptors found in the hearts of the adult rat exposed to LTH (Kacimi et al. 1992) and, in addition to species, may result from differences in circulating catecholamine levels in the hypoxic adult versus the fetus (Harvey et al. 1993).

For the intracellular cyclic AMP second messenger system, although resting levels in either right or left ventricle of LTH ventricles showed no difference compared to controls, stimulation with a maximal dose of the agonist isoproterenol increased cAMP severalfold higher in the ventricles than in controls. Stimulation

with forskolin, which acts directly on adenylyl cyclase to increase cAMP levels, resulted in reduced contractile responses in both ventricles of LTH fetuses, but threefold higher cAMP levels (Browne et al. 1997b). Thus, the reduced augmentation of contraction in both ventricles of the LTH fetal sheep heart cannot be explained by a downregulation of β_1-adrenergic receptors nor of decreased adenylyl cyclase nor cAMP production. The elevated levels of cAMP generated by β_1-adrenergic receptor stimulation may have resulted in a higher than normal phosphorylation of troponin I by protein kinase A. This would result in decreased Ca^{2+} binding to troponin C which would reduce the β_1-AR stimulation responses, as has been reported in the spontaneously hypertensive adult rat (McConnell et al. 1998). In other studies, in the placental embolized FGR sheep fetus, the incidence of left ventricular cardiomyocyte binucleation was significantly less, suggestive of retarded cardiomyocyte maturation (Bubb et al. 2007).

At the level of the contractile proteins, the isoform composition of myosin, actin, troponin T, or troponin I did not change in the LTH fetal heart (Kamitomo et al. 2002). In the hypoxic right ventricle (but not the left), however, there was a significant reduction in the activity of Mg^{2+}-activated myofibrillar ATPase (a - contractile-associated enzyme involved in regulation of both the rate and strength of contraction) with no change in Ca^{2+}-activated ATPase activity (Kamitomo et al. 2002). The fall in myofibrillar Mg^{2+}-activated ATPase activity may be partially responsible for the decrease in contractility in the right ventricle of LTH fetal hearts. Overall, these findings contribute to the concept of FGR myocardial changes playing a major role in decreased contractility and, in some instances, having lifelong consequences.

15.10 Fetal Coronary Vascular Responses

In terms of histomorphometry of the LTH fetal heart, no change was observed in ventricular capillary volume density, capillary-to-fiber ratio, or capillary anisotropy coefficient (Lewis et al. 1999). Nonetheless, the LTH heart showed significant changes in coronary artery reactivity and responses (Table 1). The maximum tension (T_{max}) response to 90 mM KCl was significantly lowered about 50% in isolated segments of left circumflex, left anterior descending, and right coronary arteries, compared to controls (Garcia et al. 2000a). Nitric oxide (NO) played no role in these responses. The sensitivity to U-46619 (a thromboxane A_2 receptor agonist), but not the T_{max} response, was significantly reduced in these artery segments. Relaxation responses to adenosine also were unaltered. In permeabilized coronary artery segments, the maximum Ca^{2+}-activated T_{max} (determined from a dose-response curve to Ca^{2+}) was significantly decreased in the left circumflex and left anterior descending coronary arteries of these hearts (Garcia et al. 2000b).

The reduced vascular Ca^{2+} responsiveness may partially explain the reduced contractile response of the coronary artery segments to KCl. However, the effects of LTH on the intracellular Ca^{2+} regulatory mechanisms involved in coronary artery

smooth muscle contraction required examination, as these reduced contractile responses may play a role in the maintenance of coronary flow at normal levels in the LTH fetal heart. In other studies, in the near-term placental embolized FGR ovine fetus, the coronary arteries showed enhanced contractility to the vasoconstrictors angiotensin II and the thromboxane analog U-46619 (Bubb et al. 2007). In contrast, endothelin-dependent and endothelin-independent relaxation did not differ between the FGR fetus and control (Bubb et al. 2007). These investigators also reported increased glutathione levels in the liver and γ-glytamyl cysteine synthetase in the lung, liver, and kidney (Oh et al. 2008). The guinea pig coronary vasculature showed an LTH-mediated increase in eNOS and NO (Thompson et al. 2000). Thus, characterization of contractile responses in all microdomains of the coronary vasculature is required for a comprehensive understanding of coronary vascular regulation during long-term hypoxia, its relation to FGR, and lifelong cardiovascular consequences. Along this line, FGR offspring show evidence of endothelial dysfunction as manifest by high-resolution ultrasound in both 9- and 11-year-old children (Leeson et al. 1997) and in early adult life (Leeson et al. 2001).

15.11 Fetal Cerebrovascular Responses to Long-Term Hypoxia

In the fetus, acute hypoxia can increase cerebral blood flow (CBF) severalfold (Ashwal et al. 1980; Peeters et al. 1979). In contrast, following acclimatization to long-term CBF has returned to near normal, despite significant decreases in cardiac output and blood flow to the abdominal viscera and skeletal muscles (Kamitomo et al. 1993; Peña et al. 2007; Tomimatsu et al. 2006). Similarly, in adults who have been acclimatized to high altitude, CBF also is relatively normal (Huang et al. 1987; Severinghaus et al. 1966). These findings support the concept for the cerebral circulation that while a number of physiologic changes, in part mediated by HIF-1α and cascade of events that follow, produce profound responses in the signal transduction pathways, when that stress is prolonged, as in LTH, cerebrovascular compensatory alterations act to return CBF and O_2 delivery to a relatively normal state of homeostasis. The identity of these mechanisms remains unclear; however, as does the extent to which, in response to LTH, vascular resistance is altered in the brain or other organs. With the brain being one of the most important organs in the body, and because the maintenance of the homeostasis of the cerebrovascular system is so critical in both health and disease, we have used this in the sense of a "case study" to explore a number of facets of vascular biology.

As noted, with hypoxic acclimatization, CBF is maintained despite a steady-state decrease in cardiac output; thus, basal cerebrovascular resistance would appear to decrease. One possible contribution to such a decrease in cerebrovascular resistance could be a shift in the structure and/or composition of the cerebral arteries favoring larger diameters and reduced hydraulic resistance. Consistent

with this possibility in terms of composition and structure, the unstressed inside diameters of the LTH acclimatized middle cerebral arteries (MCA) showed a 39% increase as compared to vessels from the normoxic control fetuses. Chronic hypoxia also was associated with a 42% increase in artery wall (media) thicknesses and an 85% increase in media cross-sectional area with a doubling of the number of layers of smooth muscle cells in the media (from 5.6 ± 0.2 to 11.5 ± 2.1) (Henderson and Longo 2003) (Table 1). In addition, in both carotid and cerebral arteries LTH significantly increased base soluble protein content, which includes cytosolic and enzymatic (but not structural) proteins (Longo and Pearce 1998, 2005). Together, these results demonstrate that LTH had a significant effect on artery size and structure with an increase in protein composition that can affect cerebrovascular contractility. This response appears to be mediated, in part, by vascular endothelial growth factor (VEGF) (Pearce et al. 2011; Silpanisong and Pearce 2013).

In addition, cerebral arteries from the LTH fetus showed a large (2–3×) increase in perivascular innervation, the nature of which remains to be defined (Henderson and Longo 2003; Pearce 1995). These changes were accompanied by a modest decrease in cerebral artery NE content (Pearce 1995). Evidence from a study of cocaine-sensitive NE uptake, a sensitive measure of NE containing nerve terminals, supports the idea of hypoxia-induced sympathetic nerve density in the common carotid and cerebral arteries (Pearce 1995). As is appreciated, neuronal NE content is the product of both the tissue nerve density and mean NE content in each nerve fiber. Of course, NE content may be decreased in those nerves that fire frequently, as opposed to those that are quiescent. In other studies, while basal norepinephrine (NE) release increased, LTH was associated with decreased stimulation-evoked release and a 20–30% decrease in vessel NE content (Buchholz et al. 1999). Because inhibition of NO synthesis with 10 μM L-NAME (N-nitro-L-arginine methyl ester) significantly depressed electrical stimulation-induced NE release in normoxic fetal MCA, basal NO release appears to facilitate NE release. In arteries from hypoxic acclimatized animals, this effect was abolished, possibly due to the ability of LTH to significantly reduce the relative abundance of neuronal NO synthase in these vessels (Mbaku et al. 2003), as well as by a large increase in stimulation-evoked fractional NE release following blockade of reuptake mechanisms (33%; Buchholz and Duckles 2001). Together with the huge increase (>1500) in hypoxia-induced transmural stimulation-induced adrenergic mechanisms (Pearce 1995), these findings suggest intense neurogenic-mediated cerebrovascular vasomotion in the regulation of blood supply to the acclimatized fetal brain. This concept is supported further by the evidence of increased co-release of NPY with NE in the hypoxic fetal cranial arteries (Pearce 1995). Regarding the effects of chronic hypoxia on perivascular peptidergic influences, the only transmitter studied to date is neuropeptide Y. Following depletion of NE from fetal MCA adrenergic nerve terminal by 1 μM guanethidine, stimulation-induced contractions were dramatically enhanced in LTH acclimatized vessels (Pearce 1995). Whereas this result may be explained by reduced synaptic cleft widths and/or generalized acceleration of maturation of the neuromuscular apparatus, other mechanisms also are possible. These include changes in the intravesicular ratio of

neuropeptide Y to NE, the postsynaptic density and coupling of neuropeptide Y receptors, and pathways for disposition of NPY. Each of these mechanisms remains potentially fruitful topics of investigation.

Hypoxia also may attenuate presynaptic inhibition of electrical stimulation-induced NE release. Blockade of prejunctional alpha-2 adrenoceptors with idazoxan increased electrical stimulation-induced NE release by blocking presynaptic inhibition, this increase being attenuated by LTH. Long-term hypoxia attenuated both NO-mediated facilitation of NE release (see below), as well as alpha-2 adrenoceptor mediated inhibition of NE release through mechanisms that were prominent in adult, but not in fetal, cranial arteries. These effects of LTH in fetal arteries cannot be explained by the modest effects of chronic hypoxia on fetal artery NE release and instead suggest that hypoxia may accelerate maturation of the neuromuscular junction, perhaps by decreasing synaptic cleft width (Pearce et al. 1999).

In terms of adrenergic regulation of fetal cerebral vascular tone, LTH acclimatized sheep cranial arteries show significant increases in basal levels of both norepinephrine and epinephrine (Longo and Pearce 1998, 2005). Paradoxically, despite these increased catecholamine levels, we recorded a 20% reduction in MCA contractile response to either NE or phenylephrine (PHE; Goyal et al. 2014). In LTH CA, we examined the effects of specific α_1-AR subtype blockade on contractility responses. Whereas the α_{1A}-AR protein expression was unchanged in response to LTH acclimatization, the α_{1B}-AR increased 20–30%, and the α_{1D}-AR subtype expression decreased ~30% (Goyal et al. 2014). In addition to LTH effects on the fetal CA α1-AR subtype per se, we have shown that the α_{1B}-AR subtype plays a major role in the regulation of the mitogen-activated protein kinase (MAPK)-extracellular regulated kinase (ERK) negative feedback regulation of phenylephrine-induced contractility (Goyal et al. 2014). Overall, in ovine fetal cerebrovasculature, acclimatization to prolonged hypoxia was accompanied by a profound effect on α_1-AR subtype expression and function. Fetal LTH acclimatized superior cervical ganglia sympathetic neurons (which govern Ca^{2+} release) also demonstrated a loss of Ca^{2+}-induced Ca^{2+} release, an important component of their function (Behringer et al. 2009).

A related influence on cerebrovascular resistance is the release of other vasoactive neurohormones from perivascular nerves. In addition to perivascular innervation from the adrenergic system, cranial arteries receive abundant innervation from cholinergic and peptidergic components (MacKenzie and Scatton 1987). Consistent with the observations above, and despite the fact that nitric oxide plays a prominent role in hypoxic-mediated cerebral vasodilatation (Hunter et al. 2001), preliminary measurements of shear-stress-induced NO release suggest that it is elevated in chronically hypoxic adult carotid arteries. On the other hand, responses to exogenous NO released from s-nitroso-N-acetylpenicilamine (an NO donor) were not significantly different in normoxic and hypoxic MCA from adult sheep, suggesting that the enhanced vasodilator responses to A-23187 observed in hypoxic adult arteries reflect the greater release of, but not greater sensitivity to, endothelium-derived NO. In addition, although in fetal MCA LTH had no significant effect on

vasodilator responses to A-23187, on shear-stress-induced NO release, or on responses to exogenous NO, it is clear that the acclimatization-associated depressed cerebrovascular resistance must involve other mechanisms.

In terms of cerebrovascular vasorelaxation pathways, increased vasodilator release potentially could contribute to the decreased resistance characteristic of hypoxic acclimatization. Most prominent among possible vasodilator influences are the endothelium-dependent vasorelaxant factors, which include nitric oxide, endothelium-derived hyperpolarization factor, prostacyclin, and numerous vasoactive growth factors such as vascular endothelial growth factor (Zachary 2001). In experiments designed to assess maximum endothelial vasodilator capacity, although the receptor-independent calcium ionophore A-23187 at 1 μM produced endothelium-dependent relaxation in cranial arteries from LTH adult sheep, this had no effect in the fetus (Longo et al. 1993; Pearce 1995). Other studies by the Pearce group reveal that long-term hypoxic inhibition by NO-induced vasodilation can be attributed to attenuation of soluble guanylate cyclase activity, but does not involve significant changes in its abundance or the activity of cyclic GMP (Pearce et al. 2009).

When all perivascular neuronal peptides are released by treatment with capsaicin, the net effect in normoxic arteries is vasodilatation, the magnitude of which is much greater in fetal than in adult arteries (Longo and Pearce 2005). This observation indicates that perivascular peptidergic nerves are predominantly vasodilator in nature. Following LTH acclimatization, the responses to capsaicin are modestly attenuated, which suggests that hypoxic enhancement of vasodilator peptide release probably contributes little to the observed reduced cerebrovascular resistance. The effects of chronic hypoxia on perivascular cholinergic innervations remain unexamined. In the adult, however, the available evidence indicates that NE release is attenuated by perivascular nerves. This is not the case for fetal arteries. Although this effect is significant, it is probably not sufficient to explain the bulk of the effect of chronic hypoxia on basal cerebrovascular resistance, which in turn indicates that by other mechanisms hypoxia must directly affect the cerebral artery smooth muscle per se.

Electromechanical coupling describes the relation between membrane potential and contractile tone that is an intrinsic feature of all excitable smooth muscle. The single most important component of this coupling is the L-type calcium channel. By virtue of its voltage-dependent conductivity, this channel directly couples changes in membrane potential to changes in the rate of Ca^{2+} influx (Xiong and Sperelakis 1995). For many artery types, including those of the brain (McCalden and Bevan 1981), Ca^{2+} entry through calcium channels constitutes the main fraction of contractile Ca^{2+}, owing to sparse or poorly developed sarcoplasmic reticulum. This is particularly true for immature cerebral arteries, which are almost completely dependent upon Ca^{2+} influx through the calcium channels for contraction (Akopov et al. 1998; Long et al. 1999, 2000).

A common approach to assess electromechanical coupling is to monitor the contractions produced by depolarization with high concentrations of extracellular potassium (K^+). With this method, K^+-induced tensions were generally decreased

by chronic hypoxia (Longo et al. 1993), these measurements possibly reflecting changes in L-channel function, however. In fetal arteries, when tensions were normalized relative to the artery wall cross-sectional area, potassium-induced stress was decreased significantly, suggesting a hypoxia-induced change in L-type Ca^{2+} channel function. Because the L-type Ca^{2+} channel density is much greater in immature than mature cerebral arteries (Blood et al. 2002), LTH may depress L-type Ca^{2+} channel density, consistent with the accelerated maturation effect of hypoxia discussed above. Arguing against this possibility in fetal MCA, however, is the finding that LTH had no effect on potassium-induced increases in cytosolic Ca^{2+} (Long et al. 2002). This latter observation suggests that L-type Ca^{2+} channel function may be preserved in hypoxia acclimatized fetuses and that hypoxic changes in K^+-induced contractile force are a function of decreased calcium sensitivity. Compared to the adult, myofilament Ca^{2+} sensitivity is greatly upregulated in fetal cerebral arteries (Akopov et al. 1998; Geary et al. 2004; Long et al. 2000; Longo and Goyal 2013); thus, hypoxic downregulation of myofilament Ca^{2+} sensitivity would be consistent with hypoxia-induced accelerated maturation.

Yet another important component of electromechanical coupling includes the plasma membrane K^+ channels that largely determine the smooth muscle electrical responses to physiological stimuli, including a basal stretch that governs myogenic tone (Nelson and Quayle 1995). Hypoxia appears to modulate the activity of at least some types of K^+ channels, as suggested by the finding that sensitivity to the ATP-sensitive potassium channel opener pinacidil was decreased significantly in hypoxic fetal MCA (Long et al. 2002). This effect suggests that LTH decreases either the density of these channels or their affinity for pinacidil. Similarly, activation of the big Ca^{2+}-sensitive K^+ (BK) channels with NS-1619 also yielded less inhibition of tension in the fetal, compared to adult, arteries. Again, this suggests that LTH decreased either the density of these channels or their Ca^{2+} sensitivity (Long et al. 2002). Certainly, a clear interpretation of these results requires detailed measurements of potassium channel densities and their current-voltage relations.

In this regard, we tested the hypothesis that during LTH acclimatization, basilar arteries (BA) in the near-term fetus would show increased smooth myocyte BK channel activity. In isolated fetal BA myocytes, we used both whole-cell and inside-out patch-clamp techniques, flow cytometry, and confocal microscopy to study BK channel activity, expression, and cell surface distribution. We identified several functional features that distinguish BK channels of LTH acclimatized vessels from normoxic controls (Tao et al. 2015). These included (1) BK channel Ca^{2+} set points for fetal LTH BA were significantly lower. (2) Fetal BA BK channels were relatively dephosphorylated in LTH compared to normoxic controls, presumably as a result of increased Ca^{2+} BK channel affinity. (3) BK channel half-activating voltages of LTH animals were left shifted 30–40 mV independently of phosphorylation state. (4) BK channel open or "dwell" times from LTH animals were generally longer and more sensitive to changes in phosphorylation state. In addition, in the perforated-patch mode, the LTH fetus exhibited two- to threefold upregulation of the BK β-1 subunit surface expression and twofold increased BK channel clustering

with increased "coupling" to Ca^{2+} sparks and increased BK current density (Tao et al. 2015). These findings suggest increased BK channel to Ca^{2+} spark coupling in the LTH fetus to maintain decreased cerebral vascular resistance.

As opposed to electromechanical coupling, which governs the relations between membrane potential and contraction, pharmacomechanical coupling involves the relations between membrane receptor activation and changes in contractile tone. This coupling, in turn, is highly specialized for each of the many receptor types present in the vasculature. One pharmacomechanical pathway modulated by chronic hypoxia is that activated by the binding of the sympathetic neurotransmitter norepinephrine to adrenergic receptor. As indicated by studies of NE-induced contractility, in fetal MCA LTH only slightly attenuated (3%) NE's ability to induce contraction, as compared to a greater extent (18%) in adult (Long et al. 2002). Some of this effect in the LTH acclimatized fetus may be attributable to a 39% downregulation of alpha-2 adrenergic receptor density (Ueno et al. 1997), although, as noted, the α_{1B}-AR was upregulated 20 to 30% (Goyal et al. 2014). Because the magnitudes of the decreases in alpha-1 receptor density were much greater than the corresponding decrease in contractility, these results suggest either a substantial receptor reserve (Zhu 1993) that is ablated by LTH and/or that this stress also affects components of the pharmacomechanical coupling pathway downstream from the receptor. Consistent with the first possibility, NE-induced responses of inositol 1,4,5-trisphosphate (IP3) mobilization following alpha-1 receptor activation were decreased only 35% in fetal LTH arteries (Ueno et al. 1997). Given that the ratio of the NE-induced IP3 signals to alpha-1 receptor density was increased by chronic hypoxia in fetal but not adult arteries, the results suggest further that the size of the receptor reserve for alpha-1 adrenoceptors must be relatively large in these vessels. It is also clear, however, that chronic hypoxia affects multiple components of the alpha-1 adrenergic receptor and other signal transduction pathways in a developmental age-dependent manner. In addition, whereas LTH decreased basal IP3 levels 30% in the adult, they remain unchanged in the fetus (Ueno et al. 1997). Nonetheless, in fetal arteries, LTH significantly decreased (80%) IP3 receptor density (Zhou et al. 1997).

The net effect of these fetal vascular changes was that NE-induced no significant increase in cytosolic Ca^{2+} (despite being decreased 21% in adult arteries). Here again, the finding that LTH decreased the magnitudes of both the NE-induced inositol 1,4,5-trisphosphate (IP3) signal and IP3 receptor density to a much greater extent than it affected contractility suggests that hypoxia probably eliminated receptor reserve for alpha-1 adrenergic and IP3 receptors. In the fetus, some of the effects of reduced receptor densities may have been offset by corresponding relatively high Ca^{2+} sensitivity (Long et al. 2002). The increase in LTH myofilament Ca^{2+} sensitivity is associated with an increased ability of phosphorylated myosin to increase this sensitivity (Nauli et al. 2005). Altogether, these findings indicate that LTH reduces fetal artery densities for these receptors. Because receptor reserve appears to be high for both receptor types in the fetal arteries, hypoxia affects have relatively little influence on NE-induced fetal artery contractile responses. This complex pattern of hypoxic effects reveals that alpha-1 adrenergic

receptor pharmacomechanical coupling is regulated closely by multiple physiological mechanisms.

Although in carotid arteries acclimatization to long-term hypoxia is accompanied by no significant increases in vascular endothelial growth factor (VEGF) per se, its receptors Flk-1 and Flt-1 increase 171% and 786%, respectively (Adeoye et al. 2013). In turn, while increasing the abundance of myosin light $chain_{20}$ ~60% LTH decreased myosin light chain kinase ~90%. In this study, LTH also increased the colocalization of myosin light chain kinase with myosin light $chain_{20}$ with several other intracellular proteins. These results support the idea that VEGF plays a critical role in hypoxic-mediated vascular remodeling. Whether by changes in gene expression, in the efficiency of message translation, through posttranslational modifications, or by turnover of the key signaling proteins, these mechanisms remain unknown. Obviously, this constitutes a promising target for future investigation, given that these pathways and proteins are critical for several aspects of cardiovascular development and regulation in the embryo and fetus of both mammalian (Crossley and Altimiras 2000) and nonmammalian species (Mulder et al. 2001).

As noted, fetal cerebral arteries show striking differences in signal transduction mechanisms compared to the adult, and these differences are magnified in response to high-altitude LTH. Because PKC plays a key role in regulating CA contractility, we tested the hypothesis that LTH differentially regulates the PKC-mediated Ca^{2+} sensitization pathways and contractility (Goyal et al. 2010). In fetal normoxic and hypoxic sheep, we examined several hypotheses in relation to responses of CA tension and intracellular Ca^{2+} concentration and measured levels of several cellular proteins. In both oxygenation groups, the PKC activator phorbol 12,13-dibutyrate (PDBu) produced robust CA contractions. In the presence of mitogen-activated extracellular kinase inhibitor (U-0126), the PDBu-induced contractions were increased a further 20–30%. Furthermore, in fetal CA PDBu lead to increased phosphorylation of extracellular regulated kinase (ERK2) but not ERK1. PDBu-stimulated ERK2 phosphorylation also was significantly greater in hypoxic than normoxic CA. Although RhoA/Rho kinase played a significant role in PDBu-mediated contractions of the normoxic fetal vessels, this was not the case in the LTH group. In addition, in contrast to adult, the 17 kDa, PKC-potentiated myosin phosphatase inhibitor (CPI-17) played no significant role in fetal CA contractility (Goyal et al. 2010). Overall, this study demonstrated several important maturational and LTH acclimatization changes in PKC-induced contractile responses and downstream pathways. The latter may play a key role in pathophysiologic disorders associated with prolonged hypoxia.

Another important pharmacomechanical coupling pathway in CA is that activated by the binding of serotonin (5-hydroxytryptamine, 5HT) to 5HT2a receptors. In ovine cranial arteries, the 5HT2a subtype is the dominant 5HT receptor, and hypoxia has no effect on the subtype expression for serotonergic receptors in this model (Teng et al. 1998). Nonetheless, hypoxia appears to modulate the signaling pathway initiated by the 5HT2a receptor in a manner distinct from that observed for the alpha-1 adrenergic pathway. Most importantly, in fetal ovine MCA, LTH does

not appear to depress 5HT-induced contractions, despite hypoxia-induced decreases in 5HT2a receptor density of 49%. Correspondingly in fetal (but not adult) carotid arteries, LTH decreased the size of the 5HT-induced IP3 signal. Thus, as for the adrenergic system, the findings that LTH has little effect of 5HT-induced contractility, despite large age-dependent decreases in receptor density and IP3 signal, again suggest that this stress eliminated receptor reserve for serotonergic receptors and that hypoxia affects multiple components of the serotonergic pathway. Among these, LTH reduces the ability of protein kinase G (PKG) to phosphorylate its target proteins, which attenuates its ability to induce vasorelaxation (Thorpe et al. 2013). Also, although the abundance of PKG is relatively high in fetal cranial arteries, in 5-HT contracted vessels, pretreatment with an inhibitor of the BK channel failed to attenuate the vasorelaxation induced by a PKG agonist as occurs in normoxic arteries (Thorpe et al. 2013). These findings support the idea that LTH attenuates the vasorelaxant effects of PKG by suppression of its ability to activate the BK channel. Given that this pathway plays a critical role in hemostasis, it also may be involved in the development of cerebral vasospasm following intracranial hemorrhage (Szabo et al. 1992) and also may play a role in coupling perfusion and metabolism via serotonergic perivascular innervation (Bonvento et al. 1991).

With the vital importance of gene regulation, in an attempt to identify the signal transduction pathways and critical molecules which may be involved in acclimatization to high altitude, we conducted microarray with advanced bioinformatics analysis on carotid arteries from the normoxic near-term ovine fetus at sea level and those acclimatized to high altitude for 110+ days. In response to LTH acclimatization, in fetal CA we identified mRNA from 38 genes upregulated >twofold and 9 genes downregulated >twofold ($P < 0.05$ for each) (Goyal et al. 2013a, b). The major genes with upregulated mRNA were SLC1A3, insulin-like growth factor (IGF) binding protein 3, IGF type 2 receptor, transforming growth factor (TGF) beta-3, and genes involved in the AKT and BCL2 signal transduction networks. Most genes with upregulated mRNA have a common motif for Pbx/Knotted homeobox in the promoter region, and Sox family binding sites in the 3′ untranslated region. Genes with downregulated mRNA included those involved in the P53 pathway and 5-lipoxygenase activating proteins. The promoter region of all genes, with downregulated mRNA, had a common 49 bp region with a binding site for DOT6 and TOD6, components of the RPD3 histone deacetylase complex RPD3C(L). We also identified miRNA complementary to a number of the altered genes. Thus, this study identified molecules in the ovine fetus which may play an important role in the acclimatization response to high-altitude-associated LTH (Goyal et al. 2013a, b). In some respects, these results in the LTH fetus suggest that stress-induced changes can be regarded as advancing developmental maturation. Such a concept has been described in human FGR newborns that show decreased perinatal mortality, compared with those that are age-matched normally grown, although this concept has been negated by others (Bernstein et al. 2000). Figure 15.2 presents some of the chief molecular acclimatization responses to high-altitude long-term hypoxia in the near-term fetal sheep, with emphasis on

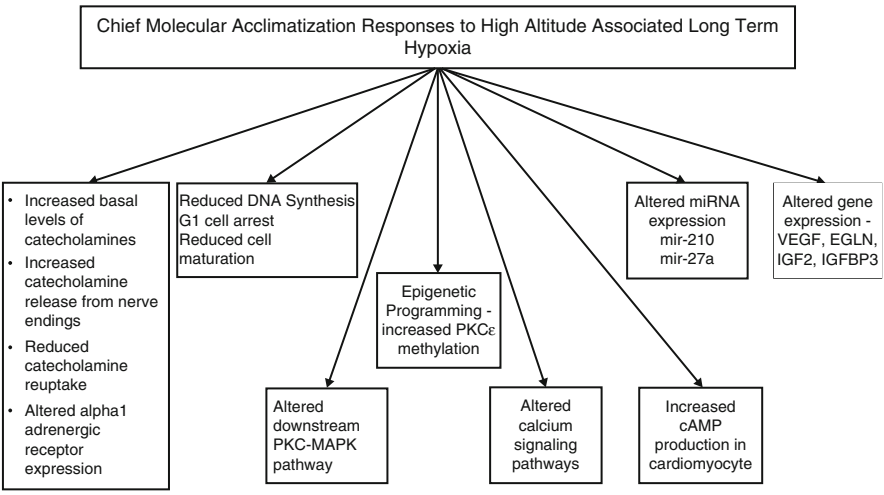

Fig. 15.2 Chief molecular mechanisms of high altitude acclimatization

epigenetic mechanisms. Of great interest, and a challenge to our understanding, is the fact that in the adult sheep carotid artery, these gene responses to high-altitude acclimatization differed in major ways from the changes demonstrated in the fetus (Goyal et al. 2014).

15.12 Some Aspects of Cardiovascular Function in the Llama Fetus

As a species, native to high altitude, several South American investigators have conducted investigations in the llama (*Lama glama*) on the *altiplano*, which illustrates successful adaptation to a rather inhospitable environment. As with other species at altitude, the llama fetus faces the inherent double threat of a relatively low arterial PO_2 as well as the stress of superimposed high-altitude hypobaric hypoxemia. For reasons unknown, the llama employs a number of differing cardiovascular mechanisms than the sheep and other lowland species in response to both acute and prolonged hypoxemia. Many of these have been reviewed (Giussani et al. 1994b, 1996, 1999; Llanos et al. 2003). For instance, under basal control conditions, the fetal llama has lower cardiac output and organ blood flows, with greater peripheral resistance than the sheep, and shows more efficient O_2 extraction (Benavides et al. 1989; Llanos et al. 1995, 1998; Peréz et al. 1989). Also in contrast to the sheep, acute continuous or graded hypoxemia (arterial PO_2 = 12–16 Torr) in the llama fetus is accompanied by intense peripheral vasoconstriction with no or little increase in cerebral blood flow (Giussani et al. 1996, 1999; Llanos et al. 1995, 2002). This lack of increase in CBF with no increase in brain O_2 extraction is accompanied by a decrease in O_2 consumption with

hypometabolism (Llanos et al. 2002, 2003). As in the sheep fetus, hypoxia in the llama was accompanied by a decrease in the high-voltage, low-frequency electroencephalographic state, as compared to the low-voltage, high-frequency state (Blanco et al. 1997). Also, in contrast to the sheep fetus which develops seizure activity with flattening of the ECoG activity, seizures do not occur in the fetal llama (Llanos et al. 2003). Despite its role in chemoreflex activity, carotid sinus denervation failed to modify the fetal llama vasoconstriction. Nonetheless, the increase in plasma AVP concentration was severalfold greater than that of sheep (Giussani et al. 1996). Surprisingly, administration of a V_1 AVP receptor antagonist did not modify this vasoconstriction (Giussani et al. 1999; Herrera et al. 2000).

In marked contrast, the alpha-1 AR blockade in the hypoxemic fetal llama produced a profound decrease in systemic arterial pressure as well as carotid and femoral blood flows. In both the llama and sheep, this blockade led to cardiovascular collapse with fetal death (Giussani et al. 1999), indicating the vital role of the α_1-AR-mediated responses in maintaining homeostasis in response to hypoxia. This response supports evidence from other studies that the α_1-adrenergic system plays a critical role in fetal survival in response to hypoxia (Airede and Weerasinghe 1995; Block et al. 1984; Giussani et al. 1999). In addition, an increase in femoral vascular resistance in the newborn llama was associated with increased expression of mRNA for the α_{1B}-AR, but lower expression for that of the α_{1A}-AR (although confirmatory Western immunoblots were not presented) (Moraga et al. 2011). This robust vasoconstrictor activity probably, in part, occurred in association with significantly higher plasma concentration of catecholamines (Fletcher et al. 2003).

Subsequently, these workers demonstrated the vital role of NO in the maintenance of fetal llama cerebral and femoral artery tone in both normoxia and hypoxia (Giussani et al. 1999; Llanos et al. 2003). In addition, other vasodilator factors play an important role in response to hypoxemia (Riquelme et al. 1998; Sanhueza et al. 2005). The relative increase in NO and its vasodilatory effects may serve as a mechanism to counteract the robust α-adrenergic mechanisms. Strikingly, and in agreement with the absence of an acute hypoxic-induced increase in CBF in the llama fetus, nitric oxide synthase levels in both cerebral cortex and cerebellum (chiefly in mitochondria, but also in microsomes and cytosol) did not increase significantly in response to 24 h of superimposed hypoxia (~15 Torr) (Galleguillos et al. 2001). In addition, during acute hypoxemia, blockade of endothelin A receptor ablated the hypoxic-induced increase in peripheral vascular resistance (Llanos et al. 2003).

Overall, the responses to acute hypoxemia in the llama fetus suggest either a stronger endocrine/autocrine vasomotor tone/vasoconstrictor response and/or weaker vasodilator mechanisms (Llanos et al. 2003). Evidence also suggests that, as with the Quechua Indians, who over many generations have acclimatized to their relatively hypoxic environment (Hochachka et al. 1994), the fetal llama can employ a state of hypometabolism with a rate of O_2 consumption that is ~50% of control, with decreased Na^+ K^+ pump activity and no increase in cell death (Giussani et al. 2001) in its defense against hypoxia (Llanos et al. 2003, 2007). Of great importance would be to discover the genes that have been selected in the llama to express the

enzymes and other proteins that allow its cardiovascular system to withstand hypoxic conditions. One can only imagine the implications of such knowledge for the prevention/treatment of human disease.

15.13 Long-Term Hypoxia and the Fetal Hypothalamic-Pituitary-Adrenal Axis

In light of its response to acute hypoxia, one might predict that the fetal HPA axis would mount a substantial response to prolonged hypoxia. Perhaps surprisingly, in early studies this was not evident. In the near-term LTH sheep fetus, basal levels of immunoreactive (IR) adrenocorticotropic hormone (ACTH) and cortisol were similar to that of normoxic controls (Adachi et al. 2004; Ducsay et al. 2009; Imamura et al. 2004). Nonetheless, LTH was associated with an increase in the ACTH precursor proopiomelanocortin (POMC) (Myers et al. 2005a, b). Significantly, in response to the superimposed stress of hypotension (Adachi et al. 2004) or umbilical cord occlusion (Imamura et al. 2004), cortisol concentrations became elevated despite IR-ACTH remaining normal. In addition, in contrast to normoxic controls adrenal denervation of the LTH fetus failed to alter cortisol production (Kato et al. 2003). In response to the secondary stress of umbilical cord occlusion, both ACTH (and to a lesser extent its precursors) were elevated (Myers et al. 2004). Also, despite elevated IR-ACTH expression in the LTH fetus, the expression of two enzymes that mediate cortisol synthesis, cytochrome P450 (CYP11A1 and CYP17), as well as monooxygenases (localized to the mitochondrial inner membrane), were paradoxically decreased significantly, as was the ACTH receptor (Myers et al. 2005a, b) (Table 1). In addition, in the LTH fetus, corticotropin-releasing hormone (CRH) mRNA and arginine vasopressin (AVP) mRNA were unregulated in the hypothalamic parvocellular division of the paraventricular nucleus, as were levels of anterior pituitary AVP receptor expression (both mRNA and protein) and the ACTH release response to AVP (Myers and Ducsay 2012). These complex findings suggest a LTH-mediated shift from CRH to AVP in regulation of the fetal HPA axis.

In further studies, the Ducsay-Myers group has demonstrated the intra-adrenal regulatory mechanisms in the LTH fetal lamb have increased expression of endothelial nitric oxide synthase (Monau et al. 2009). These workers also showed increased NO release which inhibits ACTH-induced cortisol production (Monau et al. 2010). In addition, in LTH fetal perirenal adipose tissue, these investigators demonstrated increased expression of leptin, associated with markedly elevated plasma leptin concentrations (Ducsay et al. 2006). Additionally, a 4-day infusion of leptin antagonist resulted in restored expression of both CYP11A1 and CYP17 to normoxic control values (Ducsay et al. 2013a, b). These findings emphasize an important role of leptin in mediating the LTH adrenal response with CYP expression.

As observed by Myers and Ducsay, the functional changes in the HPA axis in response to LTH may be considered "adaptive" (Myers and Ducsay 2012). Such changes are of critical importance in terms of LTH not precipitating prematurely the normal late gestation cortisol rise associated with the initiation of parturition. That is, in view of its normal relatively low fetal arterial PO_2, which is exacerbated by LTH, with elevated leptin levels modulating the HPA response, the organism can grow and develop in an optimal manner to prepare it for its neonatal existence. As noted, acute secondary stress superimposed on that of LTH can result in premature maturation of the adrenal cortex with elevated production of cortisol and other stress hormones that contribute to the genesis of FGR.

15.14 Fetal Metabolic Responses to LTH

Perhaps surprisingly, despite the plethora of studies on the systems noted above, and despite what is known about the programming of the fetus to develop the cardiovascular, metabolic, and other diseases later in life, relatively little investigation has addressed metabolic and endocrinologic issues (Styne 2011). Relatively early studies in several systems have demonstrated the role of mitochondrial oxidative metabolism with alteration in enzymatic pathways in preferentially producing the high-energy phosphate bonds of adenosine triphosphate (ATP; Reynafarje 1962). As noted, the hypoxic-mediated redistribution of cardiac output to the brain and heart occurs at the expense of the liver and other organs (Kiserud et al. 2006; Nathanielsz and Hanson 2003; Peeters et al. 1979). Early work in the mid-twentieth century established the role of hematopoiesis in the acclimatization responses for both adults and infants (Reynafarje 1957, 1959).

Chronic hypoxia also has been shown to impair carbohydrate metabolism (Regnault et al. 2010), and insulin signaling in the fetal liver (Thorn et al. 2009), and program insulin resistance in the offspring (Camm et al. 2011). In the rat liver, FGR modifies the epigenetic histone code of the *IGF2* gene (Fu et al. 2009). In livers of the near-term guinea pig, prolonged hypoxia (10.5% O_2 for 14 days; term = 65 days) produced evidence of severe oxidative stress, as evidenced by cellular damage, generation of elevated malondialdehyde (an index of peroxidation) and DNA fragmentation. These changes were attenuated by administration of the antioxidant *N*-acetylcysteine (Hashimoto et al. 2012). In humans at 4300 m on the *altiplano* of Peru, maternal serum insulin-like growth factor binding proteins were increased significantly during the second half of pregnancy (Krampl et al. 2002). By decreasing the circulatory levels of insulin-like growth factor, this could contribute significantly to FGR. In cultured bovine aortic and pulmonary artery endothelial cells, hypoxia (0% O_2 for 24–72 h) has been shown to lower IGF-1 protein but to upregulate several of the IGF binding proteins. This finding suggests that IGF-1 may be dominant paracrine regulators of endothelial cell proliferation (Tucci et al. 1998).

In the adult rat, both hepatic and skeletal muscle insulin signaling have been shown to be impaired following antenatal hypoxia (Camm et al. 2011). Such stress combined with a postnatal lipid obesogenic diet also has been shown to result in adult increases in intra-abdominal fat and adipocyte size with elevated levels of leptin, triglycerides, and free fatty acids, associated with elevated triglycerides and ceramides in liver and skeletal muscle, and evidence of insulin resistance (Rueda-Clausen et al. 2011a). To a large extent, these changes can be ameliorated by postnatal administration of the mammalian target of rapamycin (mTOR) antagonist resveratrol (Dolinsky et al. 2011).

As noted above, in addition to antenatal maternal hypoxia leading to FGR, studies suggest that LTH can play an important role in the genesis of idiopathic pulmonary hypertension of both newborn infant and adult. Because these also may be a consequence of alterations in the local pulmonary renin-angiotensin system (RAS), we tested the hypothesis that LTH is associated with alterations in gene and protein expression of the pulmonary renin-angiotensin system. In mice, we studied messenger RNA (mRNA) and protein expression, as well as promoter DNA methylation and microRNA (miRNA) levels in response to 48 h hypoxia (10.5% O_2) at 15.5 days post-conception (DPC) (Goyal et al. 2011a). In response to hypoxia, the pulmonary mRNA levels of angiotensin-converting enzyme (ACE) 1.2, ACE-2, and angiotensin II type 1b (AT-1b) receptors were increased significantly, as compared to controls. In addition, pulmonary protein levels of renin and ACE-2 were increased, whereas ACE-1 protein expression was reduced. In fetal lungs, we also observed reduced expression of the miRNAs: mmu-mir-199b, -27b, -200b, and -468 that putatively increase the translation of renin, ACE-1, ACE-2, and AT-1 receptors, respectively. Of note, promoter methylation of ACE was unchanged. We conclude that antenatal maternal hypoxia leads to significant changes in expression of pulmonary RAS of fetal mice (Goyal et al. 2011a). The possible implications of these changes for the regulation of pulmonary vascular contractility in later life remain to be explored. Many other aspects of metabolic changes in the growth-restricted fetus (Battaglia 2011) and sequelae in the adult such as metabolic syndrome, type 2 diabetes, and related disorders (Chernausek 2012) have been reviewed.

In both the human and sheep fetus, perirenal adipose tissue is prominent (Poissonnet et al. 1984), with the brown fat phenotype abundance greater than that for white fat (Pope et al. 2014). Following birth the brown fat transitions to the white fat phenotype, although some brown adipocytes persist into adulthood (Clarke et al. 1997). Following birth brown perirenal fat serves to provide effective thermoregulation to the newborn by expressing high levels of uncoupling protein 1 (UCP1), a mitochondrial protein that catalyzes thermogenesis. This occurs by its increase of proton conductance of the inner mitochondrial membrane (Cannon and Nedergaard 2004; Nicholls and Locke 1984). Perirenal fat also provides the neonate with an essential component of energy homeostasis and storage.

In response to moderate long-term hypoxia in fetal sheep, perirenal UCP1 expression has been shown to increase significantly (Myers et al. 2008). This was associated with an increase in genes that regulate brown adipocyte differentiation,

including UCP1, PPARγ, PGC1α, and PRDM16. Also evident was an increase in proteins that regulate local tissue concentrations of hormones essential for brown fat differentiation and function, such as 11β hydroxysteroid dehydrogenase type 1 for cortisol production, deiodinase type II for T_3 production, and the beta 3 adrenergic receptor for brown fat activation following birth (Ducsay et al. 2013a, b; Myers et al. 2008). Thus, the stress of moderately prolonged hypoxia can play a key role in the regulation of fetal perirenal fat differentiation. These investigators also have reported the effect of LTH increasing perirenal fat concentrations of eNOS, phospho-eNOS, and the mRNA for a number of enzymes (Myers et al. 2015). Thus, moderate, prolonged hypoxia would appear to be an important factor in supporting increased mitochondrial function and adaptive thermogenesis.

15.15 Hypoxia-Mediated FGR and Neuropsychological Correlates

The correlation of cerebral neuroanatomical and neuropsychological changes with fetal growth restriction has been described by numerous investigators. Chronic hypoxia per se or hypoxia-ischemia as a consequence of a prolonged reduction in uteroplacental blood flow can have invidious short-term and long-term consequences for the developing brain (Rees et al. 2008). For instance, an association of FGR with poor neurobehavioral and cognitive performance has been reported in neonates (Figueras et al. 2009). Some neurobehavioral impairment appears to be even more pronounced in preterm, as compared to near term, FGR infants (Rees et al. 2008), although these differences were not observed over a long-time period of life (Bassan et al. 2011). Nonetheless, other long-term follow-up studies have demonstrated significant neurodevelopmental delays persisting into adolescence (Aarnoudse-Moens et al. 2009; Feldman and Eidelman 2006). Investigators also have reported cognitive impairment and learning deficiencies observed in school being related to a characteristic pattern of altered short-term memory, attention span and anxiety (Feldman and Eidelman 2006; Geva et al. 2006a, b; Leitner et al. 2007), and in some cases an increased risk of outright attention deficit disorders (Geva et al. 2006a, b; Heinonen et al. 2010). These behavioral disturbances have been suggested to serve as indices of specific neurological changes such as in the anterior hippocampal-prefrontal cortical network, the parahippocampal complex, the striatum thalamus, and other structures (Cubillo et al. 2012; Eichenbaum et al. 2007; Geva et al. 2006a, b).

A number of studies have reported that FGR is associated with consequential neurodevelopmental/neurocognitive outcomes later in life (Frisk et al. 2002; Leitner et al. 2007; Paz et al. 1995; Strauss 2000; Sung et al. 1993; Zubrick et al. 2000). In an attempt to discover the mechanistic basis for this association, Robert H. Lane and colleagues at the University of Utah have explored several aspects of developmental neurogenesis in laboratory animals. In addition to significant

changes in composition of the neuronal *N*-methyl-D-aspartate receptor subunits, which are critical for synaptogenesis, the alterations showed gender specificity (Schober et al. 2009).

In terms of structural changes in the brain of the FGR fetus and newborn infant, magnetic resonance imaging has demonstrated a number of such changes (Sanz-Cortés et al. 2013), including the cerebral cortex (Dubois et al. 2008) and hippocampus (Lodygensky et al. 2008). Rather than gross tissue destruction, FGR is believed to be associated with more subtle disruption of normal synaptic and related neurodevelopment (Rees et al. 2011). To date, we know of no long-term studies that have evaluated the correlation of functional impairments with the underlying neurological anomalies, however.

15.16 High Altitude and the Placenta

In considering the hypoxia-mediated correlates of FGR at high altitude, reports vary as to the size of the placenta; however, almost all such studies report a modest increase in relation to that of the fetus. For instance, in her turn of the century review "The Placenta at High Altitude," Stacy Zamudio tabulated ten reports of placental and fetal weights and placental index (the ratio of placental to fetal weight) at altitudes ranging from 2000 to 4300 m (Zamudio 2003). For normoxic control subjects at low altitude (347 $\pm$ 60 m), the placental index equaled 0.15 $\pm$ 0.01. In contrast, at high altitude (3550 $\pm$ 620 m) this value was 0.17 $\pm$ 0.01, with for the most part this reflecting the hypoxia-induced decrease in fetal weight (Chap. 14 and Zamudio 2003). This, in concert with placental morphometric (Chabes et al. 1968) and other vascular changes, must work to optimize the exchange of O_2 and other nutrients under the stress of pregnancy at altitude (Mayhew et al. 1990). Thus, a fetal to placental weight ratio of >0.15 may serve as an index of growth restriction independent of fetal weight. In addition, Zamudio has emphasized that in the human placenta at altitude, the most consistent morphometric findings were increased villous terminal capillary branching with increased villous vascularization/angiogenesis (capillary density and capillary diameter), thinning of the villous membranes, and proliferation of the villous cytotrophoblast with trophoblastic bridges and syncytial knots (Ali et al. 1996; Espinoza et al. 2001; Khalid et al. 1997; Zamudio 2003). Along this line, in 13 patients with normal pregnancies at Leadville, CO, stereological analysis disclosed significant remodeling of the decidual ends of the more than twofold increase numbers of uteroplacental arteries, as well as an increase in fetal capillary density (Tissot van Patot et al. 2003). Similarly, in the Wistar rat exposed to 13% O_2 from day 6 to 20 of gestation, placental weight increased slightly (~4%) (Herrera et al. 2010). These findings fit with the well-known observations that angiogenesis with neovascularization is a robust response to chronic hypoxia.

A related response to high-altitude hypoxia is decreased impedance of uteroplacental arteries (Krampl et al. 2001b). Along this line, circulating plasma

nitrate, a stable metabolite of nitric oxide, is significantly elevated in pregnant sheep acclimatized to high altitude (Zhang et al. 1998). As noted above, in pregnant women at high altitude, both uterine artery diameter and blood flow were decreased significantly, and the levels of NO metabolites were lower, so that the ratio of endothelin-1, which remained unchanged, to NO metabolites was greater. At 20 and 30 weeks of gestation, this change accounted for 45% and 32%, respectively, of the BW decrement at altitude (Julian et al. 2008). Yet to be understood are the factors that account for uteroplacental blood flow being decreased significantly in normal non-high-altitude cases of FGR. For instance, in a study of 8 patients with FGR, 113 indium measured flow was reduced a profound 30–60% (Lunell et al. 1979).

To explore various mechanistic facets of these findings in the placenta at high altitude, a number of studies have examined aspects of this organ's structure and function in laboratory animals. To test the hypothesis that placental structural anatomy and diffusing capacity were altered in response to LTH, we subjected guinea pigs to 12–14% O_2 from DPC 15 to near term (64 days). Placental blood vascular volume increased 12 ± 3%, and despite a small decrease in tissue volume, the mean diffusion distance between maternal and fetal circulations decreased 18±4%, with an increase in diffusing capacity of 27 ± 7% (Bacon et al. 1984). Again, teleologically these changes suggest changes to maintain or increase the efficiency of transplacental exchange. Nonetheless, in regard to such exchange, despite the ability of the FGR infant to increase glucose uptake (Marconi et al. 1996), impaired transplacental glucose transport may play an important role in high-altitude-associated FGR (Zamudio 2003). Also in guinea pigs, as demonstrated by quantifying DNA synthesis, uterine artery growth was shown to be decreased significantly at high altitude (Rockwell et al. 2000).

In sheep, we quantified the gross morphology of ovine placental cotyledons to examine the extent to which these are altered in response to LTH acclimatization. In comparison to the distribution of the four cotyledon types in sea level singleton controls (type A = 76 ± 4%; B = 22 ± 3%; C = 1 ± 2%; D = 1 ± 1%), those at high altitude showed a markedly different distribution (type A = 33 ± 4%; B = 50 ± 3%; C = 10 ± 7%; D = 7 ± 1%) (Penninga and Longo 1998). Further, by the use of corrosion casts and histological sections of these placentas, marked differences were seen in both the size and arrangement of maternal and fetal vessels. Specifically, in comparison to controls, the LTH group demonstrated significant increases in the percentage of vessels of the fetal placentome and luminal size per cross section, with a decrease in number of vascular cross sections and frequent branching of stem vessels (Krebs et al. 1997), changes similar to those seen in the human. (Unfortunately, as yet we do not know the correlation of vascular changes with cotyledon type.) Overall with FGR, severe villous maldevelopment may represent the extreme of a spectrum of distorted angiogenesis.

In keeping with the observations on the human placenta at high altitude, on the *altiplano* of Chile, in conjunction with a somewhat longer gestation (see above), ovine placental weight was increased 40% (396 ± 80 g vs. 280 ± 40 g) in ewes bred at high altitude (Parraguez et al. 2005). Of interest, among lowland sheep

transported to high altitude (as in our studies), this placental weight increase was only 8% (303 ± 64 g vs. 280 ± 40 g) (Parraguez et al. 2005). Again, these findings suggest that the high-altitude, acclimatized placenta develops a significant increase in materno-fetal contiguous area to optimize O_2 and nutrient exchange. As a caveat, recent evidence suggests that even among those individuals living at low altitudes, variations in placental morphology can predict a wide range of disorders in the adult offspring (Barker and Thornburg 2013).

In a further attempt to understand the mechanistic basis of some of the LTH-associated FGR changes noted above, in a mouse "model" of FGR, we tested the hypothesis that the placental response to hypoxic stress is associated with important gene expression changes. We quantified such expression in response to 48 h of hypoxia near term (Gheorghe et al. 2007). Pregnant mice at 15.5 DPC were exposed to 48 h of hypoxia (10.5% O_2), after which the Affymetrix Mouse 430A_2.0 array was used to measure gene expression changes (Gheorghe et al. 2007). One hundred and one probe sets, corresponding to 163 genes, were regulated by hypoxia ($P < 0.01$). Ninety of these genes were upregulated, and 73 were downregulated. We annotated the regulated genes and examined overrepresented functional categories. Among these we observed several overrepresented functional categories. Upregulated genes included those involved in metabolism, oxygen transport, proteolysis, cell death, metabolism of reactive oxygen species, and DNA methylation. Genes involved in transcription, cell cycle regulation, and cell structure, were downregulated. The observation that hypoxia upregulates ROS metabolism, in conjunction with DNA methylation enzymes, suggests that in addition to the placenta, hypoxia may contribute to long-term epigenetic changes in stressed fetal tissues and organs (Gheorghe et al. 2007). In the human FGR placenta, microarray gene expression confirmed by real-time polymerase chain reaction studies demonstrated severalfold increased expression of a number of genes, including those for soluble endothelial growth factor receptor, human chorionic gonadotrophin, HIF-2α, follistatin-like 3, and leptin. These changes suggest active placental angiogenesis (McCarthy et al. 2007).

We also tested the hypothesis that antenatal maternal hypoxic stress leads to alterations in the placental renin-angiotensin system genes (Goyal et al. 2011b). In this same murine "model", we observed: (1) angiotensinogen (AT) mRNA was undetectable; however, AGT protein increased significantly. (2) Although renin mRNA was reduced, protein expression increased in association with decreased microRNA (miRNA) 199b, which can lead to increased renin translation. (3) Also, angiotensin-converting enzyme (ACE)-1 mRNA was unaltered; however, protein expression increased significantly, in association with decreased miRNA 27a, which can result in increased ACE-1 translation. (4) ACE-2 mRNA was reduced significantly, whereas protein expression was significantly greater, in association with reduced miRNA 429. (5) Angiotensin II type (AT)-1a receptor mRNA expression was unaltered, while AT-1b receptor mRNA was undetectable in both groups. Moreover, AT-1 receptor protein expression was unchanged. (6) AT-2 receptor mRNA and proteins were undetectable in both groups. We conclude that the normal murine placenta not only possesses several components of RAS, but that in

response to antenatal maternal stress several of these elements undergo important changes. In addition, differential expression of RAS mRNA, miRNA, and protein indicates posttranscriptional regulatory mechanisms involved with hypoxic stress and necessitates further investigation (Goyal et al. 2011b). More broadly, marked deviations in the pattern of gene expression in the placenta results from 48 h of hypoxia at 15–17 dpc results in (Gheorghe et al. 2007, 2010), as well as significant alterations in the placental renin-angiotensin system (Goyal et al. 2011b), fetal lung (Goyal et al. 2011a), and pancreas (Goyal et al. 2013a, b). Thus, prenatal hypoxic insults set the stage for more long-term postnatal consequences in a variety of critical organ systems.

In a rat, bilateral uterine artery ligation FGR "model," the renal expression of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) mRNA and protein were significantly decreased (Baserga et al. 2007). This occurred in association with increased corticosteroid levels at the time of birth as well as at day 21 of life (Baserga et al. 2005). This enzyme plays a key role in the regulation of renal steroid sensitivity, catabolizing glucocorticoids to an inactive form in the kidney and other aldosterone target tissues, its deficiency being an important mechanism that can lead to hypertension. This FGR "model" also has been demonstrated to be associated with decreased binding of the transcription enhancers specificity protein 1 and NF-κB p65, with increased transcriptional repressors early growth response factor NF-κB p50 to the 11β-HSD2 promoter in males. Some of these changes were more predominant in females. In addition, DNA CpG methylation occurred in a sex-specific manner (Baserga et al. 2010).

In a series of studies attempting to uncover aspects of the mechanistic basis for high-altitude FGR-associated placental and energy-demanding activities such as alterations in protein synthesis, compared to sea level controls in the placentas of women residing at Leadville, CO (3100 m), Burton's group demonstrated marked alterations in endoplasmic reticulum (ER) cisternae with increased phosphorylation of eukaryotic initiation factor 2 subunit α, reduced phosphorylation of AKT, and increased 4E-binding protein (Yung et al. 2012). As noted by the authors, these findings suggest ER stress with inhibition of protein synthesis. In these studies hypoxia (1% O_2) also reduced the proliferation of several placental cell types, changes that suggest reduced villous volume and the likelihood of similar changes in fetal cells (Yung et al. 2012). In further studies in high-altitude placental cells, this group demonstrated hypoxia altered mitochondrial function with suppression and compromise of electron transport chain complexes I and IV and thus energy metabolism, as well as increased expression of the HIF-responsive microRNA-210 (Colleoni et al. 2013).

15.17 Conclusions with Perspectives

As is evident, pregnancy at high altitude is fraught with problems beyond those of the fetus/newborn showing growth restriction. While chiefly being a function of altitude per se, this FGR can vary with ethnicity and geographic location, the latter of which reflects the number of generations that a given group has lived in that environment. Also evident, the syndrome of fetal growth restriction, as seen at high altitude, is a complex interplay of confounding variables that can present a variety of phenotypes. Beyond the phenomenology of having a birthweight below the 10th percentile, and beyond genetic- and environmental-epigenetic-mediated factors, a seemingly infinite mosaic of cellular, subcellular, and molecular mechanisms result from moderate to severe hypoxia. Long-term hypoxia can serve as a useful "model" to explore the physiologic mechanisms of acclimatization, as well as to study the genetic adaptations borne out over multiple generations. Thus, an understanding of the mechanisms by which the developing fetus and newborn "acclimatize" to prolonged hypoxia may prove to be of value in gaining insights into the optimal prevention and treatment of FGR.

Striking are the pronounced differences in response to hypoxia in the sheep and humans acclimatized to life at near sea level. In turn, these acclimatization responses differ in many regards from those in the llama and to a certain extent among human ethnic groups acclimatized to high altitude for generations. In light of the host of evidence from both epidemiologic studies in humans and experimental studies in laboratory animals, the role of antenatal hypoxia in the genesis of FGR has raised a number of questions in regard to the underlying mechanisms of signaling for the development of these major long-term sequelae. Additional questions pertain to means of possible preventative intervention. With the evidence of altered levels of many hormones, receptor-mediated signaling, NO-mediated oxidative stress, and many epigenetic factors playing a role in the pathophysiology of this syndrome, such interventions have been explored by several groups. Additionally, several investigators have exposed the possibility of postnatal treatment to rescue the FGR newborn from long-term sequelae (Giussani and Davidge 2013). A further consideration is the use of potential biomarkers to identify those pregnancies characterized by adaptive as opposed to maladaptive acclimatization responses.

Oxygen being an essential requirement for aerobic metabolism and life, prolonged residence at high altitude, or other causes of hypoxemia with cellular hypoxia pose significant challenges for survival. Fortunately, cardiovascular, endocrinologic, metabolic, and other acclimatization responses to LTH mitigate that risk and work to preserve oxygen homeostasis at the organismal level. Nonetheless, the responses to hypobaric hypoxia of pregnancy at high altitude or as a consequence of other conditions are complex.

The acclimatization responses may be adaptive or maladaptive in terms of their role in leading to appropriate fetal growth and tissue and cellular function as opposed to fetal growth restriction. Figure 15.3 presents several of the fetal acclimatization responses to high-altitude-associated long-term hypoxia. As

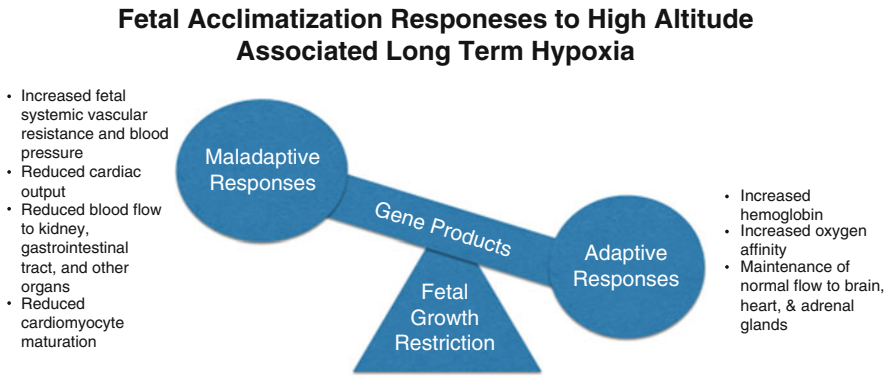

Fig. 15.3 Fetal physiological adaptations to high altitude and chronic hypoxia

detailed, adaptive responses include increased hemoglobin with the maintenance of blood flow to the brain, heart, and adrenal glands. Maladaptive responses that may lead to fetal growth restriction include generalized increase in systemic vascular resistance with an increase in blood pressure in association with decreased cardiac output and blood flow to the kidneys, gastrointestinal tract, and other organs and reduced cardiomyocyte maturation. Physiologic responses in the mother include increased ventilation and O_2 transport capacity and compensatory changes in uteroplacental blood flow. Placental function is optimized with greater capillary blood volume and shorter transcapillary diffusion distance. For the fetus, a number of metabolites, growth factors, and other molecules may influence its tissue protein synthesis to constrain growth in an attempt to survive limited O_2 availability. In this regard, because of its potent influence on central nervous system function, the pathophysiology of cerebral hypoxia, the regulation of cerebral blood flow, and the mechanisms involved in the development of and responses to cerebral edema and ischemia are of profound importance. As noted by Andrew J. Murray, when the oxygen supply becomes restricted, the fetus and placenta respond by altering its blood flow delivery and metabolism to optimize its allocation between the competing demands; thus, these issues lie beyond a question of supply and demand (Murray 2012).

Nonetheless, several caveats are in order. In terms of studies in LTH high-altitude sheep, while some showed significant fetal weight decrements, others report no significant decrease in the high-altitude animals. Nonetheless, although perhaps not constituting a strict "model" for FGR, the studies may be of value in consideration of successful acclimatization responses to LTH. Also, the significant differences in cellular responses of the fetus to LTH, in contrast to the adult (Longo and Goyal 2014), reflect much more than maturational differences. In part, this is because the degrees of both relative and absolute hypoxia differ in the fetus as compared to the adult. Presently, one can only speculate to what extent this may have compounded the maturational differences reported.

Under normal conditions, the developing fetus has an arterial O_2 tension (but not that for CO_2) simulating "Mt Everest in utero" (Eastman 1954; Longo 1987), and severe hypoxia of more than momentary duration can pose particular peril. In turn, the mechanisms by which the fetus "acclimatizes" in response to LTH are of more than passing interest. Thus, the basic hypoxic-mediated cardiovascular, metabolic, neuropsychiatric, and other cellular and subcellular signal transduction mechanisms, and their role in the dysregulation of protein synthesis in the pathogenesis of growth restriction and functional dysregulation in the developing fetus, are of great clinical relevance.

In the present chapter, in part, I have used the fetal cerebrovasculature as a case study for these responses. That is, that different elements of the adrenergic and other mediated perivascular contraction and relaxation mechanisms should be independently regulated and show differing responses to LTH should come as no surprise. This truism is even more so in comparing responses in the fetus and adult and only furthers the view that physiological stresses differ considerably in immature and mature animals, as are the homeostatic responses to these stresses. Whereas cellular hypoxia is perhaps the most important primary stimulus, multiple secondary responses in levels of circulating catecholamines, cortisol or other stress-related hormones, the multiple growth factors, cytokines, and other cellular responses including the synthesis of proteins are clearly critical to the hypoxic acclimatization process. Clearly, many of these involve epigenetic-mediated molecular mechanisms.

As noted, despite a number of studies to explore the fundamental hypoxic-mediated mechanisms that account for FGR at high altitude, there remain enormous gaps in our understanding. Whereas to some degree, the changes in the fetal environment are "buffered" by maternal homeostatic mechanisms, it also is clear that under conditions of prolonged hypoxia or other invidious environmental factors, the fetus constitutes a stress for the mother. From this perspective, it would enhance the survival mechanisms for both the mother and conceptus if the latter were to mature more quickly. Whereas this hypothesis has some attraction, it remains highly speculative and in need of experimental verification.

The current understanding of physiologic responses to prolonged hypoxia is incomplete. For instance (of great importance), identification of the signals whereby cells sense limited O_2 or other nutrient availability remains a critical unknown. How, and by what tissues, the "prime mover" responses to hypoxia are mounted is a fascinating question that has been pursued for decades. Recent advances are promising in that they point the direction for the focus of the molecular investigation of gene regulation to elucidate their role in cellular protein synthesis and related process and the mechanisms by which they are activated. The rapidly growing diversity and power of new powerful technology offers unprecedented opportunity and great promise for furthering our understanding.

Clearly, it is imperative for future research to fill these many gaps in our knowledge. Our challenge today is to promote a new generation of studies of the mosaic of hypoxic- and related mediator-induced gene regulation and/or metabolomics that will yield the key clues to the origins of FGR at high altitude.

Discovery of the genes that promote successful acclimatization and/or prevent high-altitude-associated disease undoubtedly will have profound implications for human well-being.

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Chapter 16
Epigenetics and the Fetal Origins of Adult Health and Disease

> *In physiology, as in all other sciences, no discovery is useless, no curiosity misplaced or too ambitious, and we may be certain that every advance achieved in the quest of pure knowledge will sooner or later play its part in the service of man.*
>
> (Earnest Henry Starling 1918, p. 147)

16.1 Overview

Physiology explores life and its complexity as it interacts with its environment. Presently, we are only beginning to understand the complex interactions between one's genetic inheritance and environmental influences on gene expression and the manners in which this impacts our lives. Events that occur early in our lives, both in utero and following birth, can mold our development and determine how we respond to environmental stress and our susceptibility to disease. Growth and development of the embryo and fetus, once the interest of only a minority of clinicians and public health officials, currently commands the attention of all concerned with the prevalence of a wide array of medical disorders in the adult. These include diabetes and related metabolic syndrome, coronary artery disease, hypertension, schizophrenia, and other neuropsychiatric diseases, as well as cancer and other conditions. As knowledge of human development has increased, evidence has amassed that the foundations for much of our life as adults are established in our mother's womb prior to birth. During the past several decades, both a number of epidemiologic studies in humans and mechanistic-based experiments in laboratory animals have given rise to the hypothesis of the "Developmental Origins of Health and Disease" (DOHaD) (Barker et al. 1993; Gluckman et al. 2008). Although much of the evidence is compelling, nonetheless controversy exists as to the basis for many of the associations drawn (Ben-Shlomo and Kuh 2002; Joseph and Kramer 1996; Kramer and Joseph 1996). Of particular relevance in this regard is nutrition. Commonly, we think of famine as a topic of the distant past or of malnutrition as being of limited scope (Delisle 2008). However, with the ever-increasing population on the planet, limited resources, rise in commodity prices, and the role of

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politics in human well-being, the issue of proper nutrition or the lack thereof is a worldwide problem, and its relation to health and disease is of considerable relevance to biomedical scientists and members of the healing profession.

In the Western world, despite remarkable advances in the biological sciences and other areas of life, systemic metabolic and cardiovascular disease, as well as cognitive and neuropsychiatric disturbances, are far too common and represent a virtual pandemic of morbidity and mortality. With increasing human life-span, the number of individuals who experience these disorders is escalating. Although associated with a number of risk factors, as yet unrecognized elements must be considered in the genesis of their pathology. An expanding body of evidence has highlighted the interaction of various factors with the genetic code to modulate gene expression of individual cells – so defining the field of epigenetics. The implications of understanding epigenetic mechanisms are that morphologic, biochemical, and molecular development, with both short- and long-term consequences for systemic and neurobehavioral functions, is a consequence of numerous interacting genetic and extra-genetic considerations.

16.2 A Brief Introduction to Epigenetics and Development

Classically, biologists have traditionally viewed inheritance and development to be a consequence of our chromosomal heritage, with genotypic specific genes determining the manner in which phenotypic traits and diseases are handed down from one generation to another. We now know that this mechanism is only partially correct. An expanding body of evidence has highlighted the effects of prenatal experiences on embryonic and fetal gene expression and epigenetic mechanisms on neonatal physiology. Thus, for the developing fetus, the role of epigenetic influences mediated by maternal stress (whether diet or a number of other factors) may have lifelong phenotypic and behavioral consequences (Paul 2010). In his monumental volume *The Physiology and Pathology of Exposure to Stress*, the McGill University and Universitó de Montréal endocrinologist, Hans Selye (1907–1982), observed that stress to the organism, in essentially any of its forms – dietary, environmental, disease, and others – could result in cellular, hormonal, and related damage, with the body mounting a response he termed the "general adaptation syndrome" (Selye 1950). Writing years before many of the nuances of biochemical and molecular mechanisms were established, and before the phenomenon of epigenesis was appreciated, Selye envisioned an orchestrated brain to tissue system-wide biological defensive response to the challenge of stress of whatever origin. A classic concept of "stress" and our ability to adapt to it was championed by Walter of Harvard Medical School in the early twentieth century with a "flight or fight" response (Cannon 1915). Although effects of stress during the course of gestation were not a specific part of Cannon's original proposition, the principles he describes are highly applicable to perinatal considerations. Subsequent investigation has clarified that while stress of relatively short duration is often followed by

successful adaptation of longer duration, repeated insult may result in cell damage and death (Bale et al. 2010; McEwen 2004). Moreover, adaptations by the young may give the impression of being particularly resilient to stress and the long-term consequences of various stressors. However, the ability to adapt has yet to be fully understood and is likely to be determined by both genetic heritage (nature) and antenatal/early-life experience (nurture). Such early adaptive stress responses or allostasis may be crucial to surmount postnatal challenges to health and survival. Some argue that aging and cumulative allosteric load may result in maladaptive responses due to metabolic imbalances that with time lead to vulnerability in physiological function (McEwen 2012). Others defend that stress during early-life experiences results in epigenetic changes that underline a "predictive" adaptive response. It is conceivable that accrual of stressors during critical epochs of development can have greater and more enduring effects. Many examples of this are cited below.

In its broadest sense, epigenetic [from Greek, above, upon, over, or beyond conventional genetic] mechanisms constitute an aspect of cellular/molecular "memory" of stable, heritable, self-perpetuating, and reversible modifications of chromatin that, without alterations in the genomic DNA sequence per se, modulate the on-off state of gene expression (Bonasio et al. 2010; Devaskar and Raychaudhuri 2007). Discovery of the phenomenon of epigenetics has added several layers of complexity to our understanding of the regulation of transcription and has transformed the way in which we consider gene expression and inheritance (Berger et al. 2009). For the most part, the "memory" of heritable genetic information is encoded in the sequence of nucleic acids that comprise the DNA of the genome, which provides stability and accurate heritability from generation to generation. Genetics and the environment can interact in germline mutations of the coding and promoter regions of genes. In addition, by epigenetic mechanisms, cells can inherit and transmit information that is not a part of the genomic sequence. This "hidden" heritability of epigenetic processes acts in a cell-specific, temporally regulated manner to direct development, differentiation, organogenesis, and related processes. As a metaphor, some have compared epigenetic mechanisms to the software that orchestrates and/or modulates expression of the genomic DNA hardware.

The pioneer University of Edinburgh geneticist and developmental biologist Conrad Hal Waddington (1905–1975) proposed that organismal development and response to the environment are regulated by an "epigenetic system" that sculpts the pathway of embryogenesis. He coined the term epigenetics for the study of how a wide variety of individual cellular phenotypes arise from a given genotype, e.g., those events seen in embryonic development that could not be explained from the principles of genetics (Waddington 1939, 1940, 1942, 1946). Waddington developed this concept with the metaphor of an "epigenetic landscape" to represent the process of phenotypic decision making during development from a single embryonic cell via the alternative pathways to a large number of distinct and differentiated cell types (Waddington 1957, 1959). More recently, epigenesis has been defined as the study of mitotically and/or meiotically heritable changes in gene

function without a change in DNA sequence (Allis et al. 2007; Dolinoy et al. 2007a, b; Russo et al. 1996) or more specifically "the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states" (Bird 2007). The contemporary field of "epigenetic epidemiology" has been defined ". . . as the study of the associations between epigenetic variation and risk of disease" (Waterland and Michels 2007, p. 368). These definitions encompass aspects such as DNA repair, cell cycle phases, and those stable changes maintained from generation to generation (Anway et al. 2005; Anway and Skinner 2006; Berger et al. 2009; Crews et al. 2007; Lane et al. 2003; Morgan et al. 1999; Pembrey et al. 2006; Rakyan et al. 2003).

As noted, environmental influences may have profound effects on gene regulation. Many differences in gene expression arise during the course of development and are retained throughout mitosis. Such stable, epigenetic changes, which not only affect development, but can regulate cellular responses, appear to be a consequence of a "mosaic" of mechanisms, including DNA methylation; the acetylation, methylation, and other modifications of histones; the regulation of short RNAs including microRNAs (miRNA) and long noncoding RNAs (lcRNA); methylation of mitochondrial DNA; telomere shortening; alternative splicing; and other mechanisms (Borrelli et al. 2008; Cooney et al. 2002; Franklin and Mansuy 2010; Nanney 1958; Sweatt 2009). In addition, some have applied the term "epigenomics" to the study of altered chromatin structure, such as complex folding, altered nucleosome configuration, and related phenomena (Murrell et al. 2005). In turn, the term "nutri(epi)genomics" has been applied to those sequelae following nutritional alteration (Tost 2008). Now widely appreciated, even early embryonic development has been established as a crucial period during which time epigenetic marks are established (Reik et al. 2001) and a time when the nutritional environment can affect the earliest stages of mammalian development (Doherty et al. 2000; Morgan et al. 2008). Thus, the epigenome, the overall epigenetic state of a cell, constitutes an interface between genes and the environment, allowing nutritional and a wide variety of other factors to affect transgenerational adaptation. Several reviews have detailed some of the vital issues and questions regarding the regulatory mechanisms in this rapidly evolving field (for instance, see Bernstein et al. 2007; Bird 2007; Fagiolini et al. 2009; Feng et al. 2007; Goldberg et al. 2007; Petronis 2010; Reik et al. 2001; Wadhwa et al. 2009).

Of relevance to developmental physiology, one may ask what is the role of epigenetics in placental, fetal, and newborn gene expression? During the course of life and reproduction, cells store information that has been handed down from their ancestors and that will be transmitted to their descendants. For the most part, this cellular "memory" is encoded in the sequence of nucleic acids that comprise the genomic DNA, the genotype, or the entire compliment of genes that provides the stability and accurate heritability from generation to generation. Much traditional research has explored the combined effects of genetics and the environment in germline mutations of the coding and promoter regions of genes. One major class of epigenetic mechanisms termed "cytoplasmic" is determined by *cis*-acting factors associated with DNA methylation, histone modifications, and/or the other changes

noted above. DNA with accompanying histones is packaged in nucleosomes, the core of which contains an octamer of histone proteins. Four basic forms of histones (H2A, H2B, H3, and H4, as well as minor variants) are encircled by 146 or 147 base pairs of DNA (Finch et al. 1977); a fifth histone, H1, serves as a linker protein (Bernstein et al. 2007). The histone modifications noted, DNA methylation, and other modifications confer a great increase in the regulatory capacity of each nucleosome, allowing specific functions such as DNA repair and gene activation to be modulated in the appropriate manner (Sarma and Reinberg 2005). Enzymes critically associated with these nucleosomal modifications include DNA methyltransferases, histone acetyltransferases, histone methyltransferases, histone deacetylases, histone demethylases, and others (Dodd et al. 2007; Klose et al. 2006). It is by these nucleosomal modifications of epigenetic memory, and with their influence on proximate genes, that genes may be regulated to affect phenotype, without changes in the nucleic acid code per se (Martin and Zhang 2005; Wolffe and Matzke 1999).

As is becoming increasingly apparent, epigenetic changes play a vital role in normal cellular function, as well as the development and differentiation of various cell types (Drake and Walker 2004; Jablonka and Lamb 2002; Monk 1988; Murrell et al. 2005; Rahnama et al. 2006; Reik 2007). Examples include X-chromosome inactivation in female mammals and genomic imprinting in which one parental allele is altered resulting in parent-of-origin or random modification of gene transcription (Willard et al. 1993). As noted, the embryonic/fetal epigenetic state can be disrupted by maternal environmental influences such as antenatal protein deprivation, caloric excess, hypoxia, and so forth. Also, a wide variety of environmental toxins, including low-dose radiation and psychological stress, have been demonstrated to be important in epigenetic mechanisms (Dolinoy et al. 2007a, b; Feinberg 2007; Hertz-Picciotto et al. 2008; Jirtle and Skinner 2007; Pryce et al. 2002; Szyf et al. 2007). Also increasingly, epigenetic changes are being recognized to be of importance in aging and the development of cancer and other diseases. Despite the general understanding that DNA and/or histone modifications constitute a major factor in the pathogenesis of epigenesis, a number of questions remain regarding this phenomenon. For instance, little is known of the molecular mechanisms whereby these chemical reactions/changes are regulated, the signal transduction mechanisms involved, the basis for many manifestations not becoming apparent until adult life, the mechanisms by which they are transmitted between generations, and others. A plethora of reviews are accessible on this rapidly expanding subject (For instance see Aagaard-Tillery et al. 2008; Bird 2007; Gheorghe et al. 2010; Hanson and Gluckman 2014).

16.3 The Dutch "Hunger Winter" of 1944–1945: A Case Study

As a case study of epigenetics, it may be appropriate to survey the role of antenatal maternal nutritional restriction with its effects on the fetus and newborn and the lifelong phenotypic and behavioral consequences in the developing organism, as experienced during the Dutch "Hunger Winter" of 1944–1945. Of monumental historic importance, a shocking human "experiment" is that which occurred during World War II, *de Hongerwinter* in German-occupied western Holland. This mainly urbanized region of its six largest cities included four million people, one-half the Dutch population. Commencing in late September 1944, the famine reached its peak in February 1945 and continued until the Allied victory in early May. Because this half-year of Nazi-inflicted starvation occurred during one of the coldest winters on record, neither food nor fuel could be moved by barges, and the populace lived without electricity or heat. Mortality more than doubled, accounting for over 20,000 deaths (Burger et al. 1948; Stein and Lumey 2000; Stein et al. 1975a), and this number does not include the approximately 110,000 Jews deported to concentration camps, never to return (Trienekens 2000). During the German army occupation-induced famine, the caloric ration was reduced from ~1800 to 400 to 800 calories per day. Although children and to some extent pregnant and lactating women received extra rations during the early part of this disaster, they too suffered starvation (Roseboom et al. 2001a, 2006; Smith 1947b; Stein et al. 1972, 1975a, b; Susser and Stein 1994). Individuals exposed in utero during this tragedy have been traced in follow-up studies which provide important lessons on the effects of caloric restriction/malnutrition on subsequent disease prevalence in adulthood (De Rooij et al. 2007; De Rooij and Roseboom 2010; Lumey et al. 1993; Roseboom et al. 2001a, b; Slager et al. 1985; Stein et al. 1975a, 2007). Not only was the starvation period clearly defined, and food rations documented, but the population was ethnically homogenous (Burger et al. 1948; Lumey et al. 1993). Beginning in the 1970s, initial studies were conducted on men conscripted for military service at age 18. This was followed by studies in the early 1990s of the Dutch national psychiatric registry and in the late 1990s and early 2000 analyses in which individuals were traced from birth records of a given institution. These latter more contemporary studies of "Dutch Hunger Winter Families" are considered the most definitive (Lumey et al. 2011).

16.3.1 Maternal and Infant Characteristics

A beneficial unintended consequence of this tragedy is that long-term sequelae have been defined in terms of the starvation period while in utero, e.g., during early, mid-, or late gestation (Table 16.1). Among the cohort of antenatal food-deprived adult women, fertility was halved (Stein and Susser 1975a; Stein et al. 1975a), and

Table 16.1 Maternal and newborn sequelae of Dutch Hunger Winter

	Control[a]	Trimester of exposure			Reference
		1st	2nd	3rd	
Maternal characteristics					
Primiparous (%)	37	43	30	21*	Lussana et al. (2008) Painter et al. (2005a) Roseboom et al. (2001b, 2003)
Weight at end of pregnancy (kg)[b]	68.0 ±8.5	68.4 ±8.7	63.4* ±8.0	62.6* ±7.4	Lussana et al. (2008) Painter et al. (2005a) Roseboom et al. (2001b, 2003)
Weight gain during third trimester (kg)[b]	3.7	5.7	5.0	0*	de Rooij et al. (2007) Painter et al. (2005a) Roseboom et al. (2001b, 2003)
Newborn characteristics					
Birthweight (kg)[b]	3.4 ±0.5	3.4 ±0.4	3.2* ±0.4	3.1* ±0.4	de Rooij et al. (2007) Lumey et al. (1993) Lussana et al. (2008) Painter et al. (2005a) Roseboom et al. (2001b, (2003, 2006) Smith (1947b) Stein et al. (1975a, 1995)
Birth length (cm)	50.6 ±2.1	50.9 ±2.1	49.8 ±1.9	49.4 ±2.1	Lussana et al. (2008) Painter et al. (2005a) Roseboom et al. (2001b, 2003)
Head circumference (cm)	33.1 ±1.6	32.8 ±1.3	32.2* ±1.4	32.3* ±1.6	Lussana et al. (2008) Painter et al. (2005a) Roseboom et al. (2001b, 2003)
Ponderal index (kg/m^3)	26.4	26.1	26.0	25.8	Painter et al. (2005a) Roseboom et al. (2001b, 2003)
Stillbirth rate (%)	2.0	3.5*	2.1	2.0	Stein et al. (1975b)
Infant mortality (%)	1.2	2.6*	1.9*	2.4*	Stein et al. (1975b)
	4.0			8.0*	Trienekens (2000)

*$p < 0.05$

[a]Control values are the mean of values born before and conceived after the famine (Roseboom et al. 2001a)

[b]Mean values of reports of several cohorts of individuals

maternal weight gain during pregnancy was small and essentially absent during the last trimester (Lussana et al. 2008; Roseboom et al. 2003; Stein et al. 1995).

Infants' birthweights were significantly lower among those exposed during the second and third trimesters (the latter being the period of most rapid fetal growth), with regional variation (for instance, over 340 g less in Amsterdam and about 200 g less in Utrecht (Lussana et al. 2008; Roseboom et al. 2003, 2006; Smith 1947a, b) (Table 16.1). In addition to being lighter, shorter, and thinner (Stein et al. 1975a; Stein and Susser 1975c), infants had decreased head circumference; nonetheless, ponderal index (100 × weight in kg/length in m^3) did not differ significantly

(Roseboom et al. 2001b, 2003). Placental weight and placental index (ratio of placental to fetal weight × 100) was increased in pregnancies exposed during the first trimester, suggesting compensatory growth to optimize fetal nutrition. Stillbirth rates were highest in the cohort experiencing first trimester starvation, while infant mortality was increased for those exposed at any time during gestation (Stein and Susser 1975a, b; Stein et al. 1975a).

16.3.2 Metabolic Sequelae

Those individuals exposed to famine late in gestation demonstrated impaired glucose tolerance (De Rooij et al. 2006c), with elevated 2-h glucose and insulin values (Painter et al. 2005a; Ravelli et al. 1998; Roseboom et al. 2001b), insulin secretion (De Rooij et al. 2006a), and an increase in type 2 diabetes (Kyle and Pichard 2006; Painter et al. 2005a; Roseboom et al. 2001b, c, 2003) (Table 16.2). Infants exposed during the third trimester tended to be small throughout their lives. Those normal weight newborns who experienced starvation during the first trimester but enjoyed the benefit of plentiful nutrition following birth were twice as likely to consume a high-fat diet and become obese (Stein et al. 2007) with an atherogenic lipid profile (Lussana et al. 2008) as adults. In females exposed to famine early in gestation, both waist circumference and body mass index were increased significantly (Ravelli et al. 1999). The genesis of these and other metabolic changes suggests disturbed hypothalamic-pituitary homeostasis or other central mechanisms. Caution must be exercised in interpretation of these results, however, as the choice of control subjects and sampling variability may have influenced the findings (Stein et al. 2009b). An association also has been shown between quantitative fingerprint markers (fingertip ridge count contrast between digits 1 and 5), which is determined by both genetic and non-genetic factors (Kahn et al. 2008).

In terms of growth per se, one of the best-characterized epigenetically regulated gene loci is that for insulin-like growth factor 2 (IGF-2) (Smith et al. 2006). In the blood of periconceptional exposed survivors, DNA methylation of the *igf-2* gene was decreased significantly, compared to unexposed same sex siblings (Heijmans et al. 2008), as was that for the promoter region of *ins-igf* gene, while that for *lep* was increased (Tobi et al. 2009). Individuals exposed in mid-gestation also showed Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-gamma 2 gene (De Rooij et al. 2006b). This was the first evidence in humans that environmental conditions early in gestation can persistently alter epigenetic profiles.

Table 16.2 Metabolic and cardiovascular sequelae of Dutch Hunter Winter

	Control	Trimester of exposure			Reference
		1st	2nd	3rd	
Metabolic sequelae					
Plasma glucose 2 h (mmol/1)[a]	5.8	6.1	6.1	6.3*	Painter et al. (2005a) Ravelli et al. (1998) Roseboom et al. (2001b)
Plasma insulin 2 h (pmol/1)[a]	170	207*	190	200	Painter et al. (2005a)
Impaired glucose tolerance prevalence (%)[a]	15	16	14	21*	Roseboom et al. (2001b)
Body mass index (kg/m^2)[a]	28.9 ±4.9	28.4 ±4.7	28.2 ±4.4	28.5 ±4.5	Lussana et al. (2008) Ravelli et al. (1999) Roseboom et al, (2001b)
Cardiovascular sequelae					
LDL/HDL cholesterol[a]	2.5 ±0.9	2.6–3.3* ±1.1	2.6 ±1.1	2.7 ±0.9	Lussana et al. (2008) Painter et al. (2005a) Roseboom et al. (2000a), (2001b), (2003)
Factor VII (% of standard)[a]	131	117*	133	131	Painter et al. (2005a)
Coronary artery disease (%)	3.3	8.8*	0.9	2.5	Ravelli et al. (1999) Roseboom et al. (2003)
Hypertension (systolic blood pressure, mmHg)				↑ 0.8 mmHg per 1% decrease in protein/ CHO	Roseboom et al. (1999), (2001d)
Microalbuminuria			↑ 2×		Painter et al. (2005a)
Obstructive airway disease (%)	16.4	23.0	24.8*	15.0	Lopuhaa et al. (2000) Painter et al. (2005a)
General health					
Perceived poor health (%)	4.9	10.3*	3.7	6.4	Painter et al. (2005a) Roseboom et al. (2001b), (2003)

$p < 0.05$

[a]Mean values of reports of several cohorts of individuals

16.3.3 Cardiovascular Sequelae

In concert with the relative prosperity that followed the war, the cohort that experienced first trimester famine demonstrated a significantly greater prevalence of cardiovascular disease, with an earlier onset of coronary artery disease (Roseboom et al. 2000a, 2001a, 2006). Despite having normal birthweight, those exposed in early pregnancy demonstrated atherogenic lipid profiles, suggesting

altered lipid metabolism (Lussana et al. 2008; Roseboom et al. 2000a, 2006) (Table 16.2). Women, age 59, exposed at any time showed increased total and low-density lipoprotein (LDL) cholesterol and triglycerides (Lumey et al. 2009). The coagulation factor VII was decreased following exposure early in gestation (Roseboom et al. 2000b). Famine-associated differences in mortality may have contributed to these findings, as those who died may have had higher concentrations of factor VII than those who survived (Kyle and Pichard 2006). These alterations may reflect disturbed hepatocyte function, a decrease in their numbers, or other invidious changes.

Overall, while no increase was observed in the prevalence of hypertension in survivors of intrauterine famine (Roseboom et al. 1999), among individuals who had been small at birth, blood pressures tended to be higher later in life. That elevated blood pressure seen in offspring was shown to be related to lowered maternal intake of protein in relation to carbohydrate during the third trimester (0.8 mmHg increase per 1% decrease in protein/carbohydrate), suggesting a link to macronutrient ingestion (Roseboom et al. 2001d, 2006) (Table 16.2). Men, but not women, who experienced the famine in utero had a significantly greater prevalence of hypertension associated with enlarged placental surface area, perhaps as a compensatory mechanism to optimize nutrient transfer (van Abeelen et al. 2011). Both males and females subjected to deprivation at any period showed earlier onset of coronary artery disease (Painter et al. 2006c) and decreased common and internal carotid artery lumen diameter and compliance (Painter et al. 2007a) with decreased thickness of the intima and media (Painter et al. 2007b).

16.3.4 Related Sequelae

That cohort of infants exposed to maternal caloric restriction in mid-gestation, a time of organ development, developed a greater incidence of obstructive airway disease with bronchitis and other pulmonary diseases (Lopuhaa et al. 2000). They also gave evidence of renal disease by microalbuminuria with decreased creatinine clearance (Painter et al. 2005c; Roseboom et al. 2006). In women exposed during early pregnancy, breast cancer also was increased significantly (Elias et al. 2004b; Painter et al. 2006a; Roseboom et al. 2006). In terms of general health, at age 50, twice as many of individuals exposed during early gestation perceived themselves as being less healthy (10.3%), as compared to controls (4.9%) (Painter et al. 2005a; Roseboom et al. 2003) (Table 16.2). In response to a more complete health questionnaire survey at age 59, preconception exposure to famine was associated with lower measures of mental and physical well-being (Stein et al. 2009a), although up to the age 57, no increase in mortality has been shown in either men or women (Painter et al. 2005b). Also of relevance are the limited findings of individuals exposed to famine during childhood or adolescence (ages 2–20). In terms of metabolic disease, the mitogenic peptide insulin-like growth factor 1 (IGF-1) was increased in adulthood, as was its IGF-binding protein, with

decreased IGF-binding proteins 1 and 2 (Elias et al. 2004a). Women who suffered starvation during both prenatal development (Painter et al. 2008b) and childhood (age 2–12) (Elias et al. 2005) were significantly less fertile, and among these (age 2–6) the onset of menopause was advanced almost 2 years (Elias et al. 2003). Again, these findings support the idea that the hypothalamic-pituitary-ovarian axis is particularly sensitive to nutritional deprivation during childhood. Those females exposed to starvation during childhood (ages 2–9) experienced a significant increase in breast cancer in adulthood (Elias et al. 2004b). Despite being well fed as they grew into adulthood, in one study the offspring of survivors (F2 generation) also showed significantly lower birthweights (Painter et al. 2008a; Susser and Stein 1994).

16.3.5 Neuropsychological Sequelae

Several sub-studies of the Dutch Hunger Winter have focused on neurodevelopmental defects. Initially among the cohort of 18-year-old men subjected to antenatal famine, no evidence was detected of mental impairment at the time of compulsory military induction, and this was confirmed in another group that included men and women at age 59 $\pm$ 1 years (De Groot et al. 2011). In contrast, notable among survivors (particularly males) of those conceived during the most severe period of the famine was a doubling in prevalence of the central nervous system anomalies hydrocephalus, neural tube defects with anencephaly, and/or spina bifida (Brown and Susser 1997; Susser et al. 1996, 1998). Because perinatal mortality was doubled in this cohort, the prevalence of these disorders may have, in fact, been much greater (Susser et al. 1996). Using more narrow definitions of famine exposure, spina bifida was increased only in males, while that for anencephaly was increased greatly in females (Brown and Susser 1997). Females also demonstrated a significantly higher prevalence of cerebral palsy, spastic diplegia, and epilepsy (Brown and Susser 1997). An increased prevalence of cerebral palsy among those exposed during the second and third trimesters (Stein et al. 1975a; Susser et al. 1996) suggests gender-specific nutritional effects.

Another issue of major concern is that among men and women exposed to famine during the first trimester, schizophrenia (Brown and Susser 2008; Hulshoff Pol et al. 2000; Susser and Lin 1992; Susser and Stein 1994; Susser et al. 1996, 1998, 2008) and schizophrenia spectrum personality disorders (Hoek et al. 1996) were increased two- to threefold. These changes were associated with decreased intracranial volume and an increased number of brain anomalies, including focal hyperintensities predominantly in white matter, which may reflect myelin loss (Hulshoff Pol et al. 2000). These findings stress the critical importance of the early days and weeks of gestation in neurogenesis and brain development. A more recent study of men and women, age 56–59 who experienced first trimester nutritional deprivation, disclosed decreased cognitive function, motor learning, and selective attention debility that may be ascribed to accelerated cognitive aging

(De Rooij and Roseboom 2010). The peak incidence of schizophrenia, schizoid personality, and congenital neural defects occurred in the same birth cohort (Susser et al. 1998). Also of concern, the prevalence of antisocial personality disorders was increased significantly among men exposed during the first or second trimesters (Neugebauer et al. 1999), and the risk of drug addiction was doubled (Franzek et al. 2008). In addition, men (but not women), exposed during the second and third trimesters, showed a near doubling of other affective mood disorders (Brown et al. 2000), associated with a significant decrease in head circumference (De Rooij et al. 2010). Again, these results emphasize the importance of optimal nutrition during the early periods of neurogenesis and during brain development (Bota et al. 2003). Table 16.3 summarizes the major sequelae of the Dutch Hunger Winter by trimester of exposure and to a limited extent that nutritional deprivation during childhood.

Because the Hunger Winter occurred at a well-defined time and place, the scope and severity of the famine was described carefully, and the health effects of birth cohorts exposed at various times during gestation carefully documented; the "Dutch famine study" has become a classic public health epidemiologic investigation (Kyle and Pichard 2006; Lumey et al. 1993, 2011; Stein et al. 1975a, b; Susser et al. 1998). In a study of DNA methylation in 422 individuals at age 59 ± 1 years who were exposed to the famine during the antenatal period, the time period of 1–10 weeks, but not subsequent 10-week periods, was associated with increased methylation of

Table 16.3 Summary of long-term sequelae of Dutch Hunger Winter[a]

Trimester of exposure			
Early	Mid	Late	Childhood
Metabolic			
Obesity (♀ only)	Impaired glucose tolerance	Impaired glucose intolerance	↑ IGF-1 and IGFBP-3
Cardiovascular			
Atherogenic lipid profile (↑LDL/HDL cholesterol)			
Altered blood coagulability (↓ factor VII and fibrinogen)			
Coronary artery disease	Microalbuminuria	Hypertension	
Neuropsychiatric			
Congenital neural defects	Cerebral palsy	Cerebral palsy	
Schizophrenia			
Schizophrenia spectrum disorders	Affective mood disorder		
Substance dependence			
↓ Cognitive function			
Overall			
Perceived poor health			↓ Fertility ↑ Breast cancer

[a]Modified from Roseboom et al. (2006)

CpG dinucleotides (Tobi et al. 2015). Thus, this time of embryonic development during early gestation appears to be uniquely sensitive to nutritional influence.

16.4 Other Antenatal Maternal Starvation Studies

Studies from several other countries under circumstances very different from that in the Netherlands suggest the important role of antenatal nutrition and its deficiency in the pathogenesis of disease in the adult. One of the most severe and devastating famines in recorded history occurred from 1959 to 1961 in the Wuhu region of Anhui Province, central eastern China. Precipitated by social turmoil of the "Great Leap Forward" (Chen and Zhou 2007), by some estimates, 30–40 million people died (Smil 1999). Similar to studies from the Netherlands, adult offspring of starved mothers showed a marked increase in prevalence of type 2 diabetes with hyperglycemia (Li et al. 2010). In women, the prevalence of obesity also was increased (Huang et al. 2010), and alarmingly in both men and women, the risk of schizophrenia was doubled (St. Clair et al. 2005) (for review, see Yang 2012).

Another example comes from Nigeria, in which individuals exposed to famine in utero and/or during infancy during the war in Biafra (1967–1970) also demonstrated a significantly increased prevalence of type 2 diabetes and hypertension, with these sequelae appearing earlier in life and being more severe (Hult et al. 2010). A limitation of this report from West Africa is the lack of birthweight data and the inability to separate the effects of famine during fetal life from that of the newborn period. Although some have questioned the relation of antenatal food deprivation to subsequent mental disease in the offspring, given the markedly different ethnic and social circumstances, the similarity of findings as a consequence of the famines in China and Nigeria with that in The Netherlands has helped to reinforce the concept that metabolic and cardiovascular disorders and/or schizophrenia may develop as a consequence of nutritional deficiency during gestation, rather than that of culture, ethnicity, or other factors (Neugebauer 2005).

16.5 A Perspective on the Fetal Origins of Adult Health and Disease

Of relevance to sequelae of the Dutch Hunger Winter and other famines is a brief consideration of the concept of the developmental origins of adult health and disease. From the standpoint of physiology of the fetus and newborn infant, the concept of antenatal origins of disease in the adult is of special relevance. As noted, this is because during the course of gestation, a number of stresses to the mother (including food deprivation, compromised oxygenation, and exposure to environmental toxins) may "program" the embryonic and fetal brain, cardiovascular

system, and other organs (Rabadán-Diehl and Nathanielsz 2013; Hanson and Gluckman 2014). "Programming" constitutes a general process whereby a stimulus or insult at a critical period of development has lasting or lifelong consequences. As early as the 1930s, in Sweden and Great Britain, the role of the perinatal environment to mortality as an adult was suggested (Kermack et al. 1934). A mid-century report from Baltimore noted a familial pattern of doubling of coronary artery disease associated with sibling stillbirth and infant mortality rates (Rose 1964). Growth rate changes resulting from "congenital protein-calorie deficiency" (for instance, Platt 1966; Platt et al. 1964). Citing evidence from infancy to adolescence of factors leading to elevated serum lipids and blood pressure, with impaired glucose tolerance and obesity, in the early 1970s, based on data from the Framingham study of heart disease, two of its leading investigators concluded that atherosclerosis and coronary artery disease in adults are a "pediatric problem," stressing the importance of the pediatrician in preventive medicine (Kannel and Dawber 1972). From Norway, reports showed a strong correlation between deaths from arteriosclerotic heart disease in men aged 40–69 between 1964 and 1967 and infant mortality one-half a century earlier (Forsdahl 1973, 1977, 1978). From Finland, among men born in Helsinki during the years 1924–1933, those individuals with low birthweight and low ponderal index and with rapid catch-up growth rates and high body mass index experienced increased mortality from coronary artery disease (Eriksson et al. 1999).

Commencing in the mid-1980s, the late British epidemiologist David James Purslove Barker (1938–2013), of the University of Southampton, and colleagues noted the paradox that, while in general the rate of cardiovascular disease increases with prosperity, in an affluent nation, it is the poorest who suffer the highest prevalence of these disorders. In associating the elevated rates of ischemic heart disease in adults from England and Wales (from 1968 to 1978), with birthweights and infant mortality rates half a century earlier (from 1921 to 1925), they proposed the idea that cardiovascular disease in the adult can have its origins during antenatal life (Barker 1995a; Barker et al. 1989a, b, 1993; Barker and Martyn 1992; Barker and Osmond 1986a). Soon, the developmental origin concept was expanded to include type 2 diabetes (Hales et al. 1991), various markers of aging (Sayer et al. 1998), and other conditions in the adult (Barker 1995b, 1998, 2004a, b; Godfrey and Barker 2001) including breast cancer (Trichopoulos 1990). A proper interpretation of this relationship was based on several factors, including the association of neonatal deaths with low birthweight, the role of antenatal versus postnatal environment, the seemingly paradoxical increase in several diseases with affluence, and related factors that reflect "... variations in nutrition in early life, which are expressed pathologically on exposure to later dietary influences" (Barker and Osmond 1986b, p. 1081; Poston and Hanson 2014).

In a study of 3600 infants born in 1946 in the UK, birthweight was associated positively with cognitive ability at age 8, and subsequently up to age 26, as well as with the level of education achieved (Richards et al. 2001). In association with effects of maternal nutrition deprivation on the developing fetus per se, placental growth and development also may be altered, thus altering the hormonal milieu

with subsequent behavioral and hormonal consequences in the adult (Barker 1992, 1994, 2003; Barker et al. 1989a, 1995, 2002; Gluckman and Hanson 2006; Gluckman et al. 2008; Hanson and Gluckman 2014). These also include cortisol secretion later in life (Reynolds et al. 2007; Tu et al. 2007) and immune dysfunction (Götz et al. 2007; Merlot et al. 2008). Special hallmark features of such antenatal "programming" include critical periods of vulnerability, failure or unsatisfactory completion of specific developmental milestones, association with functional defects, the permanent nature of such sequelae, and its heritability. (A number of details of the developmental origins hypothesis have been reviewed elsewhere; Barker and Clark 1997; Barker and Osmond 1986a, b, 1987; Barker et al. 1989a, b, 1993; Bateson et al. 2004; Gluckman et al. 2008; Green and Hanson 2004; Hanson and Gluckman 2014; Law and Shiell 1996; Lucas et al. 1999; Martyn et al. 1996, 1998; Nijland et al. 2008; Osmond et al. 1993; Painter et al. 2006b.)

Several hypotheses have been proposed to account for the phenomena of genetic and epigenetic factors influencing fetal/adult growth, development, and long-term sequelae (Bateson et al. 2004). These include the "thrifty genotype" hypothesis to explain the origin of type 2 (non-insulin-dependent) diabetes among people as they become acculturated, adapting Western-style diets, so that calories could be stored more efficiently in times of plenty (Neel 1962), and the "thrifty phenotype" hypothesis which postulates that impairment of nutritional supply in early life results in permanent changes in tissue/organ structure and function to conserve glucose and prioritize development of the brain, heart, and other vital organs in infants who experience intrauterine growth restriction (Billack et al. 2012; Hales and Barker 1992, 2001; Ong and Dunger 2000; Prentice et al. 2005; Stöger 2008). A related hypothesis proposes that epigenetic alterations in gene expression can be heritable and may be reversible (Holness and Sugden 2006). Genomic imprinting is an epigenetically/environmentally driven phenomenon that determines the trajectory of fetal growth. Such imprinting profiles in the placenta from birth cohorts of individuals exposed to different environmental stimuli may contribute to the diagnosis and possible intervention plans for the affected new born (Lambertini 2014).

In laboratory animals, a host of studies have demonstrated a relation between intrauterine stress to the fetus, particularly that of maternal dietary imbalance, hypoxia, and/or emotional trauma, and subsequent disease in the adult (Rabadán-Diehl and Nathanielsz 2013; Gheorghe et al. 2010; Gluckman et al. 2008; Hanson and Gluckman 2005; Jansson and Powell 2007; McKinney and Bunney 1969; Robinson 1977). For instance, a considerable body of evidence is accruing on the role of antenatal protein restriction and disorders of the systemic renin-angiotensin system (Goyal et al. 2009), as well as that of the brain (Goyal et al. 2010), lung (Goyal et al. 2011a), and placenta (Goyal et al. 2011b), with sexually dimorphic programming of hypertension (Goyal and Longo 2012). In addition, the studies of Lubo Zhang and colleagues at Loma Linda University demonstrate the role of antenatal nicotine administration to pregnant rats in epigenetic responses. Significant alterations include those of altered myocardial function (Meyer and Zhang 2007) and susceptibility to ischemia (Lawrence et al. 2008), the expression pattern

of angiotensin I and II receptors in the brain (Mao et al. 2008), evidence of oxidative stress and vascular dysfunction (Xiao et al. 2011), as well as other significant changes in the adult offspring (Li et al. 2012). In developed countries, human studies suggest that epigenetic phenomena particularly are discernible when there is mismatch with antenatal inadequacy, followed by a relative lack of such stress during infant life and childhood (Gluckman and Hanson 2006; Hanson and Gluckman 2014). With their well-characterized roles in cell growth and development, in particular the hypothalamic-pituitary axis and glucocorticoids are important to fetal programming (Moisiadis and Matthews 2014a, b; Xiong and Zhang 2013). An investigator who has contributed greatly to understanding the fetal origins of adult disease particularly that of the heart is Lubo Zhang of Loma Linda University. In commenting upon the work, he summarized some of his contributions to the study of fetal stress and development programming of ischemic-sensitive phenotype in the heart.

Heart disease is the leading cause of death in the USA, with ischemic heart disease a major cause of morbidity and mortality, yet the molecular mechanisms remain largely elusive. In addition to other risk factors, large epidemiologic and animal studies have shown a clear association of adverse intrauterine environment with increased risk of ischemic heart disease in adulthood. Multiple animal models of fetal stress caused by gestational hypoxia, malnutrition, or fetal exposure to glucocorticoids, nicotine, and cocaine have demonstrated heightened heart vulnerability to ischemic injury in offspring (Bae and Zhang 2005; Bae et al. 2003, 2005; Lawrence et al. 2008, 2011; Li et al. 2003, 2004; Meyer et al. 2009a, b; Patterson and Zhang 2010; Patterson et al. 2010, 2012; Tong and Zhang 2012; Tong et al. 2011, 2013; Xiong et al. 2012; Xue and Zhang 2009; Xue et al. 2011; Zhang et al. 2009).

Hypoxia is a common gestation insult to the fetus. There is a profound clinical relevance in understanding the relationship between in utero oxygen insufficiency and the risk of ischemic heart disease later in life. We demonstrated in rats that maternal hypoxia (10.5% O_2) resulted in premature exit from the cell cycle in fetal cardiomyocytes (Bae et al. 2003; Tong et al. 2011, 2013). Further studies revealed that hypoxia via activation of endothelin-1 at the critical window of the heart development inhibited cardiomyocyte proliferation and decreases cardiomyocyte endowment in the developing heart (Paradis et al. 2015), which may negatively impact cardiac function later in life. Indeed, our studies demonstrated that intrauterine hypoxia led to a decreased cardiomyocyte endowment in adult rats and an increased vulnerability to ischemic-reperfusion damage (Li et al. 2003). The mechanisms linking fetal stress and programming of ischemic-sensitive phenotype in the heart remain poorly understood. Among other mechanisms, glucocorticoid plays a central role in response to fetal stress. Timing and level of antennal glucocorticoid exposure are key determinants of cardiovascular disease risk alter in life. Glucocorticoid acts mainly through the glucocorticoid receptor (GR), and the GR is highly expressed in fetal cardiomyocytes. Recently, we showed that dexamethasone acting on

glucocorticoid receptors inhibits proliferation and stimulates premature terminal differentiation of cardiomyocytes in the developing heart via increased DNA methylation in a gene-specific manner (Gay et al. 2015). Studies in rats have demonstrated that maternal hypoxia results in heightened cardiac vulnerability to ischemic and reperfusion injury in offspring and abolishes the protective effects by whole-body heat stress (Li et al. 2003, 2004; Patterson et al. 2010, 2012; Xue and Zhang 2009; Xue et al. 2011).

Several genes in the developing appear to be sensitive to chronic intrauterine hypoxic stress, including PKCε, eNOS, HSP70, β_2-andrenoceptors, angiotensin II receptors (ATRs), and glucocorticoid receptors, which may be of critical importance in development programming of ischemic-sensitive phenotype in the heart. PKCε is arguably one of the most important cardioprotective genes and consequently has been studied most extensively in the field of fetal programming of ischemic heart disease. Increasing evidence suggests that the epigenetics of gene expression patterns has a crucial role in developmental programming of adult disease. Epigenetic mechanisms are essential for development and differentiation and allow an organism to respond to the environment through changes in gene expression patterns. DNA methylation is a chief mechanism in epigenetic modification of gene expression patterns and occurs at cytosine of the dinucleotide sequence CpG. Methylation in promotor is generally associated with transcription repression of the associated genes. We demonstrated that intrauterine stress induces DNA methylation of CpG dinucleotides of transcription factor binding sites causing epigenetic repression of PKCε gene and the activity. In rats, both nicotine and cocaine mediated repressive effects on PKCε gene via increased DNA methylation of CpG dinucleotides of Spl (-346, -268) and EGR-1 (-1008) binding sites (Lawrence et al. 2008, 2011; Meyer et al. 2009a; Zhang et al. 2009). Similarly, prenatal hypoxia increased methylation of Spl (-346, -268) and EGR-1 (-1008) binding sites at the PKCε promoter in the fetal heart (Chen et al. 2013: Patterson et al. 2010). Both Sp1 and Egr-1 binding sites play an important role in regulating PKCε promoter activity (Meyer et al. 2009b; Zhang et al. 2009). Interestingly, in the embryonic rat, ventricular H9c2 cell line PKCε promoter was differentially regulated compared to the whole hearts with both having increased methylation of EGR-1 (-1008). Although H9c2 cells retain many properties consistent with freshly isolated cardiomyocytes, they lack the contractile properties and are capable of continuous growth. These findings may reflect the unique relationship of the direct effects of hypoxia on isolated cells versus whole organs and systemic responses. In animals, additional factors such as catecholamine and stress hormones may in conjunction with hypoxia produce a different methylation pattern of the PKCε gene. Interestingly, the nuclear levels and binding affinities of both Sp1 and Egr-1 were affected by hypoxia (Patterson et al. 2010). Consistent with these findings, the use of 5-aza-2-deoxycytidine reversed the hypoxia-induced methylation of Spl (-346, -268) and EGR-1 (-1008) and restored PKCε expression (Chen et al. 2013; Patterson et al. 2010). This pattern of increased promoter methylation persevered into adulthood (4, 5).

On the other hand, hypoxia is known to increase oxidative stress in cardiomyocytes, which plays a role in the development of heart disease. The mitochondria is believed to be the major source of hypoxia-derived reactive oxygen species (ROS) with complex III of electron transport chain being the most likely source. In fetal hearts, *N*-acetylcysteine and tempol (ROS scavengers) but not apocynin (inhibitor of NADPH oxidase) blocked the hypoxia-induced repression of PKCε expression and restored normal basal levels of methylation and Sp1 binding (Patterson et al. 2012). Consistent with these findings, studies have demonstrated that norepinephrine through NOX1-dependent and cocaine through NOX-independent mechanisms reduce PKCε gene expression and activity in fetal rat hearts. Of importance, administration of *N*-acetylcysteine during intrauterine hypoxic insult blocked myocardial vulnerability to ischemic-reperfusion injury in adult offspring by preserving normal postischemic LVDP and LVEDP in male rats subjected to prenatal hypoxia (Patterson et al. 2012). These findings point to redox-sensitive pathways as mediators of epigenetic repression of cardioprotective genes and subsequent increased cardiac vulnerability to I/R injury. They also suggest that the repressive intrauterine effects of hypoxia on cardioprotective genes may be ameliorated by increased antioxidant defense in utero.

We also demonstrated that antenatal hypoxia reduced the expression of GR in fetal and adult offspring heart leading to increased AT_2R density and subsequent cardiac vulnerability to I/R injury (Xue et al. 2011). Interestingly, 5-min pretreatment with the AT_2R inhibitor (PD123, 319) blocked prenatal hypoxia-mediated enhanced injury during I/R injury (Xue et al. 2011). These findings indicate hypoxia modifies GR expression leading to increased myocardial injury-enhanced AT_2R expression.

Of importance, there were significant sex differences in the effect of maternal hypoxia on cardiac vulnerability in adulthood. Maternal hypoxia increased the susceptibility in ischemia-reperfusion injury in male but not female rat offspring (Xue and Zhang 2009). Although the extent of gender-specific difference in gene expression patterns is not known in fetal hearts, PKCε is a suitable example of the gender disparities. Investigation has revealed reduced expression of PKCε protein in male rats compared to female rats. Maternal hypoxia caused significant decreases in PKCε mRNA and increased methylation of both SP1 binding sites (-346 and -268) for fetuses and adult offspring. The degree to which methylation was increased in female hearts was considerably lower than male hearts. Fetal hearts exposed to maternal cocaine responded in a similar sex-dependent manner (Zhang et al. 2009). Maternal cocaine exposure caused increased CpG methylation for SP1 binding sites -346 and -268 in male and -268 only in female offspring (Zhang et al. 2009). Likewise, maternal nicotine exposure caused increased susceptibility to ischemia-reperfusion injury in both male and female offspring, with poorer recovery in female offspring (Lawrence et al. 2008). PKCε protein was significantly reduced in both male and female hearts exposed to prenatal nicotine, which suggests nicotine induces a different pattern of regulation in fetal hearts (Lawrence et al. 2008). There is a general

tendency for female offspring to be more resistant to cardiovascular diseases induced by prenatal stressors (Xue and Zhang 2009; Zhang et al. 2009). Although the exact mechanism is not clear, we have found significantly higher expression of ERα and ERβ in female fetal hearts compared to males, suggesting a novel protective mechanism of ER by binding to the Egr-1 binding site in epigenetic regulation of PKCε gene expression patterns in the early developmental stage (Chen et al. 2013; Patterson et al. 2010).

Although a considerable body of phenomenological observations has been amassed, in a deep sense the fundamental cellular and molecular mechanisms of "programming" and its effects are largely unknown (Egli et al. 2008). In addition, antenatal and postnatal exposure to environmental toxins (such as ethanol in the genesis of fetal alcohol syndrome) and their timing in relation to birth, severity of stress, and duration are issues with profound implications for health and disease as an adult (Agin 2010; Landrigan et al. 2004). The scope of developmental toxicology is beyond the limits of this essay, however.

16.6 Critiques of the "Fetal Origin" Hypothesis

Despite the evidence noted above, one must acknowledge a number of caveats regarding the interpretation of these studies. For instance, one may ask which is worse for mortality risk, nutritional deprivation during fetal development, early childhood, or in adult life. One group suggests that the correlations found between infant mortality from 1895 to 1908 and adult mortality from various causes from 1969 to 1973 were generally much attenuated or abolished by controlling for indices of present-day socioeconomic circumstances. They further suggest that the conditions that led to higher infant mortality persisted throughout the adult life of the geographically located population (Ben-Shlomo and Davey Smith 1991). These authors also suggest that to determine a definitive basis for the DoHaD hypothesis requires epidemiologic studies from infancy throughout the full course of life (Ben-Shlomo and Davey Smith 1991). The DOHaD hypothesis also was tested in a meta-analysis of 15 longitudinal and four case-control epidemiologic studies which had supported that hypothesis. The authors rejected the hypothesis, based on inconsistencies both between and within the reports, inadequate correction for confounding factors, and nonspecific relationships (Elford et al. 1991). In a further critique of ten epidemiologic reports that associated circumstances early in life with cardiovascular disease in the adult, these authors stressed the invaluable role of epidemiologic studies in generating hypotheses, as opposed to testing them (Elford et al. 1992). In conclusion, they noted the failure of these reports to satisfy criteria for causality, inconsistencies between and within studies, and lack of specificity in formulation of hypothesis and the observed relationships and cautioned that confounding variables prevented firm conclusions (Elford et al. 1992). A commentary in *The Lancet* also raised a number of the same issues in regard to

interpretation of the data from many of the studies reported (Kramer and Joseph 1996).

A corollary consideration concerns the extent to which malnutrition in utero determines the occurrence of diabetes and coronary heart disease in adulthood. Some quote the results from the Leningrad (now St. Petersburg) siege study. In this cross-sectional study, 169 subjects were exposed to malnutrition in utero (intrauterine group) during the 1941–1944 siege, 192 subjects were born in Leningrad just before the siege and before rationing began (infant group), and 188 subjects were born concurrently with the first two groups, but outside the area of the siege (unexposed group). The authors concluded that intrauterine malnutrition was not associated in adulthood with glucose intolerance, dyslipidemia, hypertension, or cardiovascular disease. The authors did observe a correlation of intrauterine malnutrition with systolic blood pressure to body mass index, however, and evidence of endothelial dysfunction with elevated levels of von Willebrand factor (Stanner et al. 1997). A subsequent report has emphasized dissimilar aspects of the Leningrad experience with that in the Netherlands (Stanner and Yudkin 2001).

A further consideration comes from the reexamination of findings in the Oxford Nutrition Survey in World War II some 50 years later (Huxley et al. 2000). This study comprised two groups of pregnant women in the UK: the first were 120 - working-class women in the spring of 1942 that received no nutritional supplementation, while the second group included 253 women in 1944 that received supplementation during pregnancy (Huxley et al. 2000). Mothers were studied until after delivery, and offspring were followed to test the fetal origins of disease hypothesis. The authors found no evidence of an inverse association of low birthweight with subsequent elevation in blood pressure (Huxley et al. 2000). Two further reports of the analysis of this data from five decades earlier arrived at a similar conclusion, i.e., that neither birthweight nor maternal nutritional status has little relevance to later in life measurements of blood pressure (Huxley et al. 2002) or coronary heart disease (Huxley and Neil 2004). Others also have challenged the DOHaD hypothesis (Anonymous 1989; Lumey et al. 2011; Whincup et al. 1992).

As has been argued by many, a key consideration in this regard is that one's entire "life course" is critical in considering the vast scope of health and disease as an adult (Elder 1994; Floud et al. 1990; Halfon and Hochstein 2002; Lucas 1991; Lucas et al. 1999; Lucas and Morley 1994; Lynch and Davey Smith 2005). At the turn of the century, the *International Journal of Epidemiology* devoted a special issue to the "life course" approach to chronic disease in the adult. An introductory editorial outlined the conceptual models of life course epidemiology, distinguishing "critical" from "sensitive" periods of life, separating individual from intergenerational determinants of health, and related epidemiologic considerations. In closing, the authors presented the challenge of elucidating the mechanisms that account for social, geographical, and temporal patterns of disease distribution in our societies (Ben-Shlomo and Kuh 2002).

As noted, although a considerable body of phenomenological observations has been amassed, the fundamental cellular and molecular mechanisms of fetal

"programming" and its effects as a consequence of malnutrition or other environmental factors are largely unknown. Clearly, one must move beyond phenomenology to discover the signal transduction and gene regulation mechanisms that underlie epigenetic-mediated expression of proteins to account for the several diseases observed in later life. Some related challenges are listed below. What are the periconceptional pathways by which parents contribute more than genetic material to the offspring? What are the effects of parental exposures on the genomic integrity of gametes? What manner do exposures at specific stages of gamete development influence epigenetic marks in oocytes and sperm? How early in development this may begin? What are the means by which epigenetic marks survive zygotic reprogramming to be retained within the embryo? What are the mechanisms that account for the time lag between exposure during a critical period and the appearance of sequelae many years later? Quite obviously, studies in experimental animals will be of utmost importance to discover these mechanisms. Analysis of such reports, even though they are limited, is beyond the scope of this review however.

16.7 Malnutrition During Pregnancy as a Global Health Problem

Beyond considerations of fetal and newborn development, famine and starvation, of whatever magnitude or scope, are curses on humankind that must be eliminated at all cost. As described, the classic "experiment" on the invidious effects of antenatal famine/malnutrition, the Dutch "Hunger Winter," has provided sobering evidence of the role of timing and severity of such stress to the embryo/fetus in terms of subsequent disease in the adult. In particular, the "Hunger Winter" and its sequelae offer an appreciation of the effects of nutritional deprivation at specific gestational ages in affecting one's "life course" of health and disease. Epidemiologic studies from the famines in China and Nigeria support these observations. In addition, a report of increased prevalence of psychological and behavioral problems in offspring of women who experienced *hyperemesis gravidarum*, a relatively common condition of early pregnancy associated with nutritional deprivation, cannot be ignored (Mullin et al. 2012). Thus, it is at our peril that we minimize these reports, for we live in an age of uncertainty with global threats of major economic or political disruptions in supply of foodstuffs. These have a clear biological, psychological, and sociological consequences. Given the prevalence of nutritional deficiencies in pregnant women throughout the world, one cannot overestimate the importance of ensuring proper nutrition to attain the full genetic potential of their offspring.

Because optimal nutrition, or the lack thereof, relates so closely to developmental physiology, the global challenges presented regarding the role of nutrition and other environmental factors on childhood development are many (Morgane et al.

1993; Olness 2003). Famine and malnutrition are not only a burden on human health. By leaving in their wake deep-seated metabolic, cardiovascular, neural, and psychiatric disorders, they are an impediment to human progress. As cautioned by one group, "... for the millions of children around the world who begin their lives in adverse circumstances, we should be mindful of what is known about sensitive periods and act with alacrity to improve the lives of these children before neural circuits become well established and, thus, difficult to modify" (Fox et al. 2010, p. 36). For clinicians, public health workers, and the political establishment both in the USA and around the globe, a pressing challenge is to confront the growing inequalities among social groups with the widening gap between those of wealth and the underclass. To ensure optimal systemic, intellectual, and social development of the next and future generations, our responsibility is to guarantee that every pregnant mother is provided with proper nutrition for her growing child. Ensuring such optimal nutrition is a realizable achievement, and one hopes that it may be possible to define how prospective parents can select with care their lifestyle choices and adopt interventions to protect their children from adverse outcomes. This will not occur, however, by a market-driven economy, but rather by the determination of healers, the makers of public policy, and the political will of the leaders of nations.

16.8 Further Questions to Consider

Recent years have witnessed an almost exponential expression of papers on epigenetics, from epidemiologic studies and phenomenology to exploration of fundamental mechanisms including those on programming. Rather than a review article, this chapter attempts an historical survey of the discovery and evolution of the field of epigenetics. A critical aspect of this subject is the many vital questions that require investigation. Several examples follow:

- What are the mechanisms by which DNA methylation, histone modifications, noncoding RNAs, and other changes play a role in epigenetics regulated?
- What are the relations among the several epigenetic markers? For instance, what is the extent to which DNA methylation affects histone modifications or vice versa?
- To what extent is gene expression regulation by microRNAs, long noncoding RNAs, and other RNAs involved in regulation of other epigenetic mechanisms?
- What is the role of transcription factors in the regulation of these mechanisms?
- What is the role of these various epigenetic mechanisms in the regulation of polymerase II and transcription?
- To what extent is the regulation of these several mechanisms similar or different in various cell types?

Ultimately, the foundation to reduce morbidity and mortality in adulthood must recognize the contribution of both genetics, as well as epigenetic considerations in the disease process that results from acute and developmental origins.

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Chapter 17
Some Aspects of the Developing Brain and Nervous System

17.1 Overview

As knowledge of human development increases, the sobering reality is becoming evident that the foundations for much of our life as adults, including our state of mind and life, are established in our mother's womb prior to birth. Growth and development of the brain, the most complex organ not only in the body, but probably in the universe, is unique in many respects. As with other cells, those of the nervous system have identical genomic DNA sequences (the template of our heredity and instruction sets for gene expression); however, they develop into strikingly different and distinct phenotypes. Neurons, the cells responsible for signaling, conducting, and communication, convert a variety of stimuli into control of short- and long-term memory, consciousness, and behavior. By late gestation in the developing fetus, following a "brain growth spurt" neuron number is established with only modest postnatal neurogenesis other than in the dentate gyrus of the hippocampus and the periventricular and subventricular zones. Supporting astrocytes and glial cells follow a similar course, with a delay of several weeks. Maturation of oligodendrocytes with formation of myelin, follows a much later time course. In view of the complexity of orchestrated neurogenesis with axon and dendrite formation during this period of rapid growth, specialized cell type differentiation with their neurotransmitters, migration, the connectivity of literally billions of synapses, and selective cell death, the brain is exquisitely sensitive to factors that may alter and interfere with its normal pattern of growth and development.

In terms of cerebral development and gene expression, and their neurophysiologic correlates, several issues are of relevance. Beginning shortly following conception, and continuing until at least the third decade of life, development of the brain with its consequent function is an enormously complicated process, far beyond the limits of this review. Several fundamental observations may be in order, however. Brain development, with the main cell lineages neurons, astrocytes, and

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oligodendrocytes, is accomplished by spatial and temporal regulation of specific gene expression patterns for optimal growth, maturation, and cognitive maturation. These processes may reciprocally influence and reinforce one another and may be divided into prenatal and postnatal periods. During the 1970s, John Dobbing (1922–1999) and colleagues of the University of Manchester demonstrated in a number of mammals that when brain weight is plotted against age, it grows in a sigmoid trajectory, the transit period of rapid growth being the "brain growth spurt" (Dobbing 1974; Dobbing and Sands 1973, 1979). Timing of this growth spurt, as well as its development in general, varies—occurring prenatally in precocial species, those that can ambulate and are relatively independent at birth (guinea pig, monkey, sheep) or postnatally in altricial or non-precocial species (rat, rabbit, pig). For humans, following the first trimester during which period the gross brain shape is determined, this growth spurt with neurogenesis is intermediate or perinatal, occurring during both late gestation and early neonatal life (Altman and Das 1965; Bayer 1989; Dobbing 1968; Dobbing and Sands 1973, 1979; Rakic 1975, 2003, 2005).

Because the growth spurt encompasses an enormous number of anatomic, metabolic, and neurochemical events, each with their behavioral associations, this critical period of developmental plasticity varies dramatically with cell type and location. Thus, it is associated with enhanced vulnerability to nutritional restriction, hypoxia, or other stress, factor that must be taken into consideration when comparing or extrapolating from one species to another (Altman et al. 1970; Dobbing 1974; Dobbing and Smart 1974). As emphasized in recent studies, the concept of the brain "growth spurt" may, in fact, be misleading, as each brain region with their specific cellular units has a unique period of neurogenesis, neural migration, synaptogenesis, myelinization, and gliogenesis. These events, occurring ". . . prior to the major growth spurt cannot be ignored since this . . . cell division growth spurt, is a transient window of neurogenesis for the total macro- neuronal population, which is ultimately responsible for the development of the larger circuit formations and for the basic architectonic organization of the brain" (Morgane et al. 1993, p. 98). For instance, several lines of evidence demonstrate electrocortical activity (Aresin 1962), neurophysiologic function in the brain stem respiratory center (Windle et al. 1938), as well as spinal motor reflexes (Windle and Fitzgerald 1937), as early as 3 months' gestation. Complicating this scenario is the fact that the developmental profile may differ significantly in the premature newborn infant, as compared with that of the full-term infant (Kosmarskaya 1963), and a host of factors may alter other aspects of development (Windle 1967). Several reviews include detailed summaries of specific developmental events in the human brain (for instance, see Williams 1989).

At the time of birth, the human brain weighs ~350 g, that is about 10% of body weight, but it accounts for about one-quarter of the basal metabolic rate (Holliday 1971; Williams and Herrup 1988). In proportion to whole body mass and metabolism, the brain of the fetus is relatively large, comprising about 27% of adult brain weight, with its mass continuing to increase in the infant and child largely as a result of myelin deposition (Davison and Dobbing 1966; Dobbing and Sands 1973, 1979).

Because a number of physiologic adaptations tend to protect the developing brain, in the infant with nutritional deprivation or other causes of intrauterine growth restriction, the brain and head size are relatively preserved, with "brain sparing," in relation to body size and girth (Dobbing 1981). The human cerebral cortex constitutes six laminated layers of neurons with arrays of intersecting radial columns. During development, excitatory projection neurons that originate from the proliferative periventricular zone (PVZ) or subventricular zone of the embryonic cerebral vesicles migrate along elongated radial fibers to form the vertically oriented radial columns (Mountcastle 1997; Szentágothai 1978; Torii et al. 2009). Of fundamental importance in terms of development and providing scaffolding for neuronal migration and terminal location are cortical radial glial cells, a distinct cell class which displays unique features in each species studied (Rakic 2003). Such neural development and diversity is determined by several major factors which can vary considerably, including the number of stem or precursor cells from which the population is derived, the duration of the regional proliferative period, and the cell cycle duration (McConnell 1991; Sur and Rubenstein 2005; Williams 1989).

Cerebral cortical development with its many nuclei, tracts, and other structures must proceed in an exquisitely orchestrated progression of cell migration and differentiation, with the complement of neurons being relatively complete at the time of birth, while glia and astrocytes continue to develop at a more leisurely pace (Takizawa et al. 2001). By 2 years of age, brain volume has achieved 80–90% of its lifetime maximum (Pfefferbaum et al. 1994), with increasing myelination continuing into and beyond young adulthood (Sowell et al. 2004). In primates, the perinatal period sees the emergence of unique cellular patterns, cytoarchitecture, and neurochemical maturation. Throughout adolescence, this is followed by further neuronal growth and differentiation with synaptogenesis and circuit organization (Levitt 2003). As may be self-evident, synaptogenesis, with orchestrated development of the synapses per se, the pre- and postsynaptic neurons with their neuroligand and neurolexin connecting cell adhesion molecules, their vesicles of neurotransmitters, and other specialized features, is critical for neuronal function and cognition (Soler-Llavina et al. 2011; Südhof 2008). Importantly, glial cell number appears to be adjusted proportionally to match the neuronal population (Williams and Herrup 1988). During the time of rapid neuronal growth in the last trimester of pregnancy, the cerebral, so-called, resting-state networks (as determined by functional magnetic radiation imaging), although developing at different rates for various systems (visual, auditory, somatosensory, motor, and others), are mature near term, prior to experiencing or being a consequence of related cognitive functions (Doria et al. 2010). Despite the remarkable advances of contemporary neuroscience and dedicated study during the 1990s "Decade of the Brain" (Goldstein 1994) and beyond, we are only in the infancy of understanding nuances of cerebral development and function (Aguirre et al. 2010; Bota et al. 2003; Edlund and Jessell 1999; Guillemot et al. 2006; Jessell and Sanes 2000; Price et al. 2006; Rakic 2005).

17.2 Developmental Neurogenesis

Also at mid-century, Donald Olding Hebb (1904–1985) of McGill University postulated that "adaptability" or "plasticity" of the developing brain was accomplished by "strengthening synapses," in the absence of structural reorganization (Hebb 1949). In the early 1960s, Roger Wolcott Sperry (1913–1994) of the California Institute of Technology proposed a "chemoaffinity" theory in which:

> . . . the establishment and maintenance of synaptic associations were conceived to be regulated by highly specific cytochemical affinities that arise systematically among the different types of neurons involved via self-differentiation, induction through terminal contacts, and embryonic gradient effects. . .. A necessary conclusion from these results [is] that the cells and fibers of the brain and cord must carry some kind of individual identification tags, presumably cytochemical in nature, by which they are distinguished one from another almost, in many regions, to the level of the single neuron; and further, that the growing fibers are extremely particular when it comes to establishing synaptic connections, each axon linking only with certain neurons to which it becomes selectively attached by specific chemical affinities.
>
> (Sperry 1963, pp. 703–704)

Sperry's idea was that selected molecules would serve as labels or markers that direct migration and the formation of unique patterns of synaptic connections between and among neurons, to establish ". . . the developmental pattern of central nervous organization" (Sperry 1963, pp. 703–704). In 1981, Sperry was awarded the Nobel Prize in Physiology or Medicine for his perceptive studies concerning the functional specialization of the cerebral hemispheres.

During the era of Sperry's contributions, Joseph Altman of the Massachusetts Institute of Technology first proposed continued neurogenesis in the adult brain, with structured plasticity (Altman 1962). Later, axonal elongation and synaptic reorganization were demonstrated in response to injury (Raisman 1969), and soon, it was shown that experience alone could affect structural changes in pre- and postsynaptic neurons (Greenough et al. 1978). In support of Sperry's hypothesis of neural cytochemical affinities, studies during the past decade have established the role of Down syndrome cell adhesion molecule (DSCAM) and related proteins in determining specific neural connections (Matthews et al. 2007; Schmucker et al. 2000; Wojtowicz et al. 2007). A galaxy of DSCAM isoforms are essential for a robust system of orchestrating the complexity of self-avoidance and normal axonal and dendritic patterning (Hattori et al. 2009; Zipursky 2010). In support of Altman's hypothesis, in the adult brain of many species, stem cell (e.g., progenitor cell-derived) neurogenesis continues in the subventricular zone (Reynolds and Weiss 1992; Richards et al. 1992) and in the subgranular zone of the dentate gyrus of the hippocampus (Gage et al. 1995; Kriegstein and Alvarez-Buylla 2009; Kuwabara et al. 2009; Ming and Song 2011; Palmer et al. 1997; Suh et al. 2007; Zhao et al. 2008).

The extracellular matrix, its proteins, and integrin cell surface receptors also play a critical role in neurogenesis and axonal guidance (Garcion et al. 2001; Rønn et al. 1998). Assumptions of the extent to which these findings in laboratory animals

apply to humans need to be reassessed, however. Studies of human surgical and autopsy brain specimens, from birth to ninth decade of life, disclose that the migratory cell stems that originate in the SVZ, while present in newborn infants, disappear by the 18th month of life (Sanai et al. 2011). The authors also describe for the first time a large number of tangential migratory neurons, originating in the SVZ and destined for the olfactory bulb, that coalesce into the medial branch of a rostral migratory stream that reaches the ventromedial prefrontal cortex (Sanai et al. 2011). Many intricacies of neurogenesis in developing and adult brain, and the regulation by cell-intrinsic programs and cell external cues, have been reviewed by others (Cohen and Greenberg 2008; Edlund and Jessell 1999; Gage 2000, 2002; Kinter 2002; Qian et al. 2000; Rakic 2000, 2003).

As noted above, in the early to mid-1960s, several important contributions were made to the cellular aspects of brain development and neuronal myelinization and some aspects of their relation to maternal nutritional status (Davison and Dobbing 1966; Dobbing 1974; McIntosh et al. 1979). In addition, a monumental series of experimental studies contributed to understanding the importance and basis of critical periods in neural development. At this time David Hunter Hubel and Torsten Nils Wiesel, at Harvard Medical School, described the organization of neural circuits in the primary visual cortex where information from the retinas is processed. They identified an aspect of neural organization crucial for combining information from the two eyes to reconstruct accurately the three-dimensional world. In these studies, they detailed the manner in which visual nerve axons from the lateral geniculate nucleus of the thalamus, the brain's relay center for visual information received from the retina, fan out into a broadband to terminate in the visual cortex in alternating eye-specific zones referred to as cortical orientation and ocular dominance columns.

Hubel and Wiesel also performed a series of experiments in which they demonstrated that following birth, suturing the eyelids closed over the eyes of kittens for 2–3 months, severely impaired development and maturation of neurons of the visual cortex and cells in the lateral geniculate body, altering the structure of the cortical ocular dominance columns (Wiesel and Hubel 1963a). As a consequence, even though the eyes were uncovered within a few weeks, the animals were functionally blind. In contrast, suturing the lids of one eye shut resulted in cells that would have fired in response to the closed eye instead responded to the open eye, thereby resulting in amblyopia. Shutting the eye of an adult cat did nothing, demonstrating that visual cortex cells were programmed during a critical developmental window during the first weeks to month of life. This research demonstrated clearly that in the sensory development of the brain, early visual experience plays a critical role in the origin of visual cognition and that the relative level and synchrony of activity exerts a powerful influence on the development of brain circuitry. The concept that "neurons that fire together wire together" subsequently was supported by several lines of evidence. These and other studies led to the idea that synchronous presynaptic and postsynaptic activity could couple release and uptake of synaptotrophins, thereby modulating synaptic connections (Hubel and Wiesel 1965; Snider and Lichtman 1996; Wiesel and Hubel 1963b). For their

discoveries concerning neurogenesis and the visual system, Hubel and Wiesel shared with Roger Sperry the 1981 Nobel Prize in Physiology and Medicine (Hubel 1982; Wiesel 1982).

A 1964 3-day symposium in Paris, *Regional development of the brain in early life*, was organized jointly by the Council for International Organizations of Medical Sciences and the *Délégation à la Recherche Scientifique et Technique*. This multidisciplinary gathering included a number of prominent neuroanatomists, histologists, neurochemists, physiologists, electrophysiologists, and clinicians. In addition to sharing their latest findings on brain development, their goal was to compare methodologies and approaches and to develop meaningful collaborations among scientists from different disciplines (Minkowski 1967). Contributions included several dozen aspects of neurogenesis, myelinization, synaptic development, the role of nerve growth factors, enzyme and neurotransmitter activities, and electrophysiologic correlates (Minkowski 1967).

In 1970, Marcus Jacobson (1930–2001) of Johns Hopkins University synthesized contemporary ideas from organ systems, cellular, and subcellular studies on the development of the nervous system in vertebrates as well as invertebrates, stressing the organization of various aspects of neuronal function (Jacobson 1970). Less than a decade later, he updated this synthesis with recent discoveries placed within the context of the historical evolution of concepts and understanding (Jacobson 1978). More recent studies have confirmed that normal development of the visual cortex depends critically upon appropriate neural information being transmitted from the retina (Rakic 1976, 1988; Rakic and Riley 1983), and rather than being preprogrammed, this development is very much experience dependent (Jandó et al. 2012).

For neurons and other brain cells, among the most sophisticated in the body, despite the differentiation signal having been experienced only once during the earliest development, cell identities are maintained for a lifetime (Ringrose and Paro 2004). Much of this developmental gene switching on and off in the transition from a single fertilized cell to fully formed organism follows elegantly orchestrated and regulated epigenetic mechanisms (Bale et al. 2010; Borrelli et al. 2008; Jiang et al. 2008). For the major brain cells, deviation in the normal pattern of gene expression from neural progenitor cell to mature neuron or glia may lead to altered phenotype, and such altered gene expression may be evidenced by neuropsychiatric disorders. (This occurs as well in laboratory animals, which display numerous lethal embryonic null mutants.) As can be appreciated, because of the complexity and specificity of neural regulatory circuits, classical analysis of individual genes or genome-wide analysis in the whole brain, or its major parts, is inappropriate.

Gene regulation per se in development of the brain is beyond the scope of this chapter; however, a consideration of some basics may be relevant. During the past several decades, studies in the mouse, with many genes and cell lineages similar to the human, have revealed a number of fundamental molecular mechanisms with the proneural and other genes required underlying brain development. These include aspects of cell differentiation with neural induction from precursor cells in the neural plate; regionalization of the neural tube along the dorsoventral and

anteroposterior axes; neurogenesis with generation of neurons, astrocytes, and oligodendrocytes from multipotent progenitor stem cells located in the SVZ of the embryonic neural tube; the regulation of selective neuronal survival and apoptosis; the regulation of neural cell migration with patterning and guidance to site of function; the acquisition of differentiated features; the formation of synapses among appropriate neurons with development of circuitry; and others. The cerebral cortex is composed of two major neuronal populations; projection or pyramidal neurons are glutamatergic and excitatory utilizing *N*-methyl-D-aspartate (NMDA) receptors, while inhibitory interneurons utilize the neurotransmitter gamma-aminobutyric acid (GABA) and its receptors (Guillemot et al. 2006; Jessell and Sanes 2000; Price et al. 2006; Smith and Greenfield 2003). Recent evidence suggests that GABA also plays a critical role in the development of neural progenitor cells (Yuan 2008). In the brain, as in all organs, interactions among gene, gene products, and small molecules are responsible for the regulation of all cellular processes, including those of cell survival, proliferation, and differentiation. These interactions are organized into complex lattice structures and/or networks that modulate intracellular signaling, metabolism, and gene regulation. In terms of regulatory mechanisms, it is clear that these involve complex gene regulation by a number of transcription factors.

17.3 Cognitive Development

The relation of development of the brain to cognitive development is, quite obviously, a vital area of consideration. Because of its complexity, extensive literature, and somewhat peripheral relation to fetal-neonatal physiology per se, however, it cannot be considered at length (see Nelson et al. 2006). Pioneer work on development of the nervous system in vertebrates commenced with the monumental studies of George E. Coghill of the University of Kansas and the Wistar Institute of Anatomy and Biology, Philadelphia. Working chiefly with the larva of the salamander *Ambystoma*, over several decades, Coghill formulated a "universal law" that the overall pattern of development of the central nervous system and associated behavior (integrative action) dominates the partial or individual sequential patterns (reflex or analytical action). That is, the organism is not the sum of its localized reflexes, but rather "a totally integrated matrix." As with his contemporary Joseph Needham, Coghill rejected metaphysical vitalism, appreciating that embryology, as a science, arose from the domain of morphology. However, in contrast to Needham's biochemical perspective, Coghill held that it was from a behavioral vantage point that physiological implications of complete integration could be understood (Coghill 1929). Coghill recognized the possibilities of fresh discoveries and insights, with concepts such as:

> . . . totipotence, pleuripotence, organizers, gradients, all of which have meaning only as the organism is regarded . . . as a dynamic pattern in time . . . the neuron-embryologic study of behavior shows that events within a behavioral system can be understood scientifically only as their relation is known to subsequent as well as antecedent phases of the cycle.
>
> (Coghill 1933, p. 137)

Coghill concluded this essay with his credo:

> My own working hypothesis holds that the relation of cause and effect, in a purely scientific discipline, is a space-time relation within a unitary system, that the living organism is such a system, and that I can not fully perceive any phase of this relation or part of this system without perceiving the system as a whole. Upon this hypothesis my understanding of an even scientifically or experimentally requires knowledge of the future as well as of the past of the system in which that event occurs. If this be philosophy, I would call it a philosophy, not of *being*, but of *becoming*; not of *life*, but of *living*—which is itself my supreme experiment.
>
> (Coghill 1933, p. 138)

In his 1928 lectures at University College London, Coghill summarized these concepts, presenting the background for his passion to perform studies in parallel on the development of behavior in combination with morphologic studies on nervous system development. As noted earlier, it was Donald Barron's knowledge and appreciation of Coghill's studies in the salamander that prompted him, upon meeting Joseph Barcroft in 1934 and learning that he had purchased a large number of pregnant ewes, to ask whether he proposed to study the development of the nervous system in the mammalian fetus. Nonplussed by this question, but intrigued, Barcroft asked Barron to ". . . tell him [all] about it." This was a seminal event in the genesis of the disciplines of fetal physiology that illustrates its cross-disciplinary nature (Coghill 1929). As an aside, in 1937 Coghill with Wolfram Karl Legner (1902–1981) published an English translation of one section, "Embryonic Motility and Sensitivity" of Preyer's *Specielle Physiologie*. . . (Preyer 1937). Preyer's *The mind of the child*. . . also was translated into English (Preyer 1901).

A mentor of Coghill was Charles Judson Herrick (1868–1960), of the University of Chicago. Influenced greatly by his elder brother Clarence Luther Herrick (1858–1904), who died early in his career, "CJ," as he was known by friends, dedicated himself to integrating several disciplines in understanding the nervous system. With detailed histologic analysis, combined with functional studies of the Tiger salamander and primitive mammals, Herrick established the brain as a "working mechanism," with the nervous system organized for responses of the body as a whole (Herrick 1924, 1926, 1929). A "behaviorist," near the conclusion of his life, he expressed his views on humanity's responsibilities in regard to conduct and contribution to civilization, concepts that are relevant half a century later. In the conclusion to his introduction, Herrick affirmed:

> I did not devote sixty years to intensive study of the comparative anatomy of the nervous system merely to collect dead facts or add to the score of "accumulative knowledge". I wanted to find out what these animals do with the organs they have and what they do it for, with the expectation that this knowledge would help us to unravel the intricate texture of the human nervous system and show us how to use it more efficiently. The unflagging toil of hundreds of qualified experts in diverse fields of science has abundantly fulfilled this

> expectation, but we have disastrously failed to apply the knowledge to anything even resembling successful treatment of the disordered behavior that now prevails in our troubled world. We can do better if we really want to and are willing to pay the price.
>
> (Herrick 1956, pp. 7–8)

In addition to other honors, Herrick was a member of the National Academy of Sciences (Bartelmez 1973). Founding of the American School of Psychology has been credited to the Herrick brothers in collaboration with Coghill (Roofe 1971). Perhaps the most satisfactory theoretical treatment of the concept of neural-based hierarchic order was given by Paul A. Weiss, whose experimental studies of the semiaquatic salamander of the genus *Triturus* (newt) provided Coghill's concepts with a solid empirical basis.

The mid-twentieth century witnessed the waning of behaviorism and the gradual ascendance of cognitive psychology. Two important contributions to a widened understanding of development of the nervous system and cognitive psychology were the 1948 Hixon Symposium *Cerebral mechanisms in behavior* at the California Institute of Technology and the Dartmouth Conference on Learning Theory held 2 years later. The Hixon Symposium chaired by Lloyd Alexander Jeffress (1900–1986) of the University of Texas, Austin, and Henry Walker Brosin (1904–1999) of the University of Chicago (and later at Pittsburgh) included a dozen and a half of the most outstanding cognitive scientists in the world. Also attending were individuals from emerging fields of cybernetics and information processing such as the mathematician John von Neumann (1903–1957) of the Institute of Advanced Study, Princeton University, who was involved with, among other things, development of the first electronic computer, ENIAC, and the future two-time Nobel Laureate, chemist Linus Carl Pauling (1901–1994) of the California Institute of Technology. Another participant was Warren Sturgis McCulloch (1898–1969) of the University of Illinois, Chicago, and later the Massachusetts Institute of Technology. A neurophysiologist, McCulloch was interested in parallels between the nervous system and logical machines and in his papers such as "A logical calculus of the ideas immanent in nervous activity" (McCulloch and Pitts 1943) contributed to neural network theory, the theories of automata, and cybernetics and provided a foundation for certain theories of the mind. For the most part the participants challenged the inadequacies of behaviorism, sparking a new direction of psychological thought and thinking in regard to development of consciousness and the mind (Jeffress 1951). In a discussion of "The problem of serial order in behavior," Weiss observed:

> . . . while the physiologist and psychologist deal with the ready-made machine of the nervous system . . . the embryologist must explain just how such an immensely intricate, yet orderly, thing can develop, . . . the relative autonomy of structural patterns of activity, and the hierarchical principle of organization . . . the nervous system is not one big monotonic pool whose elements can be freely recombined in any number of groups, thereby giving an infinite variety of nervous responses . . .. The working of the central nervous system is a hierarchic affair in which functions at the higher levels do not deal directly with the ultimate structural units, such as neurons or motor units, but operate by activating lower patterns that have their own relatively autonomous structural unity .... The principle point is that the rhythm is not something generated through an input rhythm, but is itself a primary

> rhythm which may be released and even speeded up or retarded by the input, but is not derived from the input. So we have experimental evidence that autonomy of pattern, rhythmic automatism, and hierarchical organization are primary attributes of even the simplest nervous systems, and I think that this unifies our views of the nervous system.
>
> (Weiss 1951, pp. 140–142)

A member of the National Academy of Sciences (1947), in 1979 Weiss was awarded the National Medal of Science (Overton 1997). At the Dartmouth conference/seminar, the participants considered the current status, and problems with, the theories that occupied dominant positions in the field of learning theory of five distinguished cognitive psychologists. Sigmund Koch (1917–1996) of Duke University identified many inadequacies in the hypothetico-deductive behavioralist system. Other participants noted weaknesses of other theories of learning, particularly as they failed to meet the necessary and sufficient criteria for a theory, as required by the then prevailing philosophy of science, logical positivism (Estes 1954).

Another pioneer in the field of cognitive development was Jean William Fritz Piaget (1896–1980), of Geneva's International Bureau of Education and later with the International Centre of Genetic Epistemology which he founded (1955) and directed. From his studies in infants and children, Piaget postulated that individuals pass through stages of development regarding several operational stages or models (biological, sociological, sensorimotor/adaptive, logical and figurative thought) which allow them to think in new, more complex manners (Piaget 1936, 1953, 1937, 1954). Although many of Piaget's theoretical claims have been discredited, phenomena he discovered such as object permanence during infancy and the developing complexity of conversations in children continue to attract interest (Walkerdine 1990). Another controversy in cognitive development's early years was that of "nature versus nurture" or nativism versus empiricism. As now recognized, this is a false dichotomy in light of the tsunami of research supporting the role of epigenetics and other factors in gene activity and the development of neurons and other brain cells (see Davies 2001).

A related area of cognitive development is that of ante- and postnatal influences on cognition, learning, and function. The psychologist and director of the Institute for the Study of Child Development at the Robert Wood Johnson Medical School, University of Medicine and Dentistry at New Jersey, Michael Lewis has focused on several aspects of intellectual development and the challenges of their understanding. His edited 1986 volume *Learning disabilities and prenatal risk* includes major sections on prenatal factors (drugs, pollutants, smoking, drinking, and undernutrition) and perinatal factors (maternal medications, obstetrical trauma, and infections) on brain development and compromised cognition and learning (Lewis 1986).

17.4 Cerebral Blood Flow in the Fetus and Newborn

As one of the, if not the most, important organs in the body, and as an illustration of the regulation of blood flow during development, it may be appropriate to consider briefly that to the brain and the extent to which the regulatory mechanisms differ from that of the adult. Cerebral blood flow (CBF) is the complex, multi-feedback, integrated response to numerous regulatory influences including transmural pressure gradients, shear stress, and perivascular neuronal activity as well as chemical, endocrine, and metabolic factors originating in the brain and circulating blood (Bevan and Bevan 1981, 1994; Busija and Heistad 1984). The "holy grail" of cerebrovascular biology and essence of homeostasis is the hierarchy of regulatory mechanisms that couple CBF to tissue metabolism. In contrast to the idea that the fetus and newborn are simply small adults, it is critical to remember that not only do stimuli reaching immature cerebral arteries differ from those of the adult, but so too the responses of these vessels to those stimuli differ dramatically (Bevan et al. 1980; Duckles and Banner 1984; Goyal and Longo 2012; Goyal et al. 2009, 2012; Longo and Goyal 2013; Longo et al. 1996a, b, 2000; Pearce et al. 1991, 1999, 2003).

As for the adult, the maintenance of well-regulated cerebral vascular tone and blood flow is essential to the developing organism. For the fetus and newborn infant, in part, this is because the brain is uniquely susceptible to a broad variety of injuries and insults, the majority of which culminate in diverse patterns of encephalopathy. A major pathogenic factor in these disorders is dysregulation of cerebral blood flow with intracerebral hemorrhage. Hemorrhage into the germinal matrix and periventricular region, which occurs in about 2–5 per 1000 live births, is associated with the development of spastic motor deficits associated with cerebral palsy, convulsive disorders, and other neurological diseases (Doyle et al. 2010; Groenendaal et al. 2010; Munck et al. 2010; Sheth 1998) and/or with cognitive attention deficiencies (Aarnoudse-Moens et al. 2009; Del Toro et al. 1991; Johnson et al. 2010; Msall 2010). Among very preterm and very low birthweight (<32 weeks' gestation; <1500 g) and extremely preterm and extremely low birthweight (<28 weeks' gestation; <1000 g) infants, the prevalence of brain damage is particularly high (Ferriero 2004; Stoll et al. 2010; Vannucci and Vannucci 2004; Volpe et al. 2011). The consequent pathology can result in severe neurological sequelae with lifelong personal, social, and economic consequences. Because of their major clinical relevance, cerebrovascular responses to ischemia, hypoxia, hypercapnia, and other pathophysiological stresses have attracted considerable investigative effort. Nonetheless, many fundamental issues regarding CBF regulation in response to increased neuronal activity and other physiological stimuli remain uncertain, which complicates understanding of how this regulation changes with development.

During the past several decades, studies have revealed important aspects in the fundamental signaling transduction mechanisms that regulate cerebrovascular contractility of smooth muscle cells (SMC) in the course of maturational development

(Goyal et al. 2009, 2010a, b; Longo and Goyal 2013; Longo et al. 1996a, b, 2000; Pearce et al. 1999, 2003). Some of these important mechanistic differences include unique features of the relation of function to structure (Goyal et al. 2012), the role of endothelium-mediated prostacyclin, and eicosanoid, nitric oxide, and cyclic nucleotide relaxation mechanisms (Leffler et al. 1999, 2001, 2005). In terms of contractile mechanisms, the relatively immature organism is characterized by the unique role of calcium (Ca^{2+})-dependent receptor-second messenger coupling with plasma membrane L-type Ca^{2+} channels showing a virtual dependence of the immature organism on extracellular Ca^{2+} (as opposed to intracellular Ca^{2+} stores in adult) for Ca^{2+}-dependent thick (myosin) filament regulation (Blood et al. 2002; Long et al. 1999; Nauli et al. 2000). In addition, plasma membrane K^+ channels (in particular the big conductance calcium-sensitive potassium (BK) channel) play a major role in regulating Ca^{2+} entry into the fetal cerebral SMC (Long et al. 2000a, b). The BK channel in the fetus shows greater conductance and is more sensitive to intracellular Ca^{2+} concentration (i.e., has a lower Ca^{2+} set point) (Lin et al. 2003). The BK channel in fetal cerebral arteries also is most sensitive to protein kinase A (PKA) phosphorylation, in contrast to the adult which is more sensitive to protein kinase G (PKG) phosphorylation (Lin et al. 2005, 2006). Additionally, Ca^{2+} stores in fetal SMC sarcoplasmic reticulum demonstrate much less releasable Ca^{2+} than adult, either en masse or as "sparks," thus accounting, in part, for the developing cerebrovasculature being more dependent on extracellular Ca^{2+} for contraction (Long et al. 1999, 2000b). Also in the developing fetus, the relative density of $alpha_1$-adrenergic receptor subtypes (A, B, D) is much less than that of the adult. These also differ strikingly in their responses, the B and D subtypes being more responsible for trophic responses (Goyal et al. 2010b). Additionally, many elements of the non-Ca^{2+}-dependent pathway of protein kinase C (PKC) to specific enzymes such as the mitogen-activated protein kinase (MAPK) cascade, extracellular regulated kinases (ERK1/2), and the downstream effectors Rho A, Rho kinase (ROCK), myosin light $chain_{20}$ (MLC_{20}), and others differ dramatically in the fetus, compared to adult (Goyal et al. 2009, 2010a; Longo et al. 2000). For instance, compared to adult cerebrovascular SMCs, which show little activity of Rho kinase-mediated mechanisms, fetal arteries show great dependence on ROCK. In turn, developing SMC lack the adult sensitivity to CPI-17 inhibition of MLCP (Goyal et al. 2009). Of particular significance, based on these and other studies, compared to the more mature vessels, those of the developing organism display much greater Ca^{2+} sensitivity for contraction. Thus, in terms of overall regulation one may postulate a "ying and yang" phenomenon, wherein a combination of elevated Ca^{2+} sensitivity and reliance on extracellular Ca^{2+} characterize early development, whereas the mature adult cerebrovasculature is dependent on intracellular Ca^{2+} stores. Also compared to adult SMCs, those of the developing fetus and newborn demonstrate manifold differences in gene regulation of critical signal transduction pathways (Goyal and Longo 2012).

Taken together, these studies demonstrate profound differences in cerebral artery relaxation/contraction mechanisms as a function of developmental age and emphasize the need to understand the biochemical and molecular basis of these

changes. Further complicating cerebrovasculature maturation is the marked heterogeneity observed among vessels from different species, arteries of different size, and among similarly sized arteries from different vascular beds. Nutritional history and health status also have an important bearing, not only on vascular characteristics per se but also on the rate at which these characteristics change with postnatal age. The emerging picture is one of the highly dynamic vascular phenotypes that change in concert with its environment (Longo and Goyal 2013).

As the cerebrovascular smooth muscle cells and vessels are smaller with less connective tissue (Goyal et al. 2012), commonly the age-related differences in cerebrovascular contractile mechanisms have been attributed to vascular structural and functional immaturity (Bevan and Su 1973; Toda 1991). This is the case, particularly as it relates to the relative inability of vessels to constrict with enough force to prevent propagation of transient arterial blood pressure increases to the microcirculation, i.e., the "hypo-contractile" hypothesis. Alternatively, increased likelihood of cerebral artery rupture may result from a greater abundance and/or potency of relaxant mechanisms in less mature vessels, the "hyper-relaxation" hypothesis (Longo et al. 1996b; Nauli et al. 2000; Pearce et al. 1991; Pryds 1991). On one hand, reduced tone and contractility of cerebral arteries may be appropriate for the relatively low perfusion pressures typical of fetal life in utero. On the other hand, we know little of how this may be a consequence of important differences from the adult in relation to the fine structure and vascular reactivity, as well as differences in patterns of stimuli received. Unfortunately, little is known about the basis of this vulnerability in terms of cerebrovascular structure and function.

In perspective, major differences in cerebrovascular relaxation/contraction mechanisms in the fetus and newborn, as opposed to the adult, are the degree to which specific elements and signal transduction pathways and their interactions differ as a function of developmental age. Clearly, important differences in the manner in which these mechanisms function distinguish the developing cerebral vasculature from that of the adult. Thus, one might be tempted to ask: are these developmental changes merely an epiphenomenon or are they of fundamental biologic significance? Considered from one point of view, cerebral artery relaxation and contraction mechanisms in the fetal and newborn organism work amazingly well. Nonetheless, the significant differences in mechanistic coupling and interaction may be a key factor in the vulnerability of the cerebral vasculature to dysregulation in response to hydrostatic pressure surges, hypoxia, and other stress (Longo and Goyal 2013).

That elements of the cerebral artery agonist-mediated, presynaptic and postsynaptic, relaxation, and contraction mechanisms should be regulated independently and show differing responses during the course of maturation should come as no surprise. This only furthers the view that homeostatic responses differ in the immature as opposed to the more mature animal. These include the structural transition from immature "synthetic" to the more mature "contractile" smooth muscle cell phenotype, accompanied by major structural changes of essentially every element. For developing cerebral arteries, the character of many of the

responses is similar to those of the adult, with the trend that the magnitudes of changes are smaller for contractility and larger for changes in receptor density. Maturation of many of the smooth muscle cell mechanisms also illustrates the concept of "developmental plasticity." Overall, multiple mechanisms are recruited in response to normal development, both to promote relaxation (hyper-relaxation hypothesis) and to attenuate contraction (hypo-contractile hypothesis). Within this framework, the multiple independent mechanisms are heterologous and unique for each signal transduction pathway. A caveat of most of these studies is that a limited number of age groups were examined, e.g., preterm fetus, term fetus, newborn, and adult. Thus, the true developmental profiles of variable changes with maturation are either unknown or poorly described. Nonetheless, it is the late fetal and newborn period, that phase upon which most studies have focused, that has high relevance to dysregulation and disease.

In light of the above, one might ask: what do the present findings mean for future research? A number of major challenges lie ahead. Biocomplexity poses perhaps the greatest challenge, to understand the manner in which cell and tissue functions emerge from the interactions within the manifold array of cellular, biochemical, and molecular networks. During recent years, linear models of signal transduction have provided useful information on the role of various receptors, second messengers, enzymes, and other cascade elements in terms of development of cerebrovascular function and dynamics. Nonetheless, as we progress, a more encompassing network model incorporating interactions among a host of molecules will be required to understand the emergent properties of activity and function of the diverse cerebrovascular cells. Thus, in our effort to unravel the underlying basis of biocomplexity, we must move beyond the phenomenology of signaling pathways, to consider their interactions, feedback mechanisms, and temporal regulation in health as well as disease (Ingber 1997, 2003a, b). In terms of development, an important lesson is the plasticity of signal transduction mechanisms in a given cell type and/or vessel. This suggests that continued exploration of these regulatory mechanisms is likely to be rewarded with unexpected, important new findings and insights. Although the general principles of signaling are becoming clarified, a challenge is to elucidate the details of the regulatory mechanisms of information flow, signal amplification, feedback regulation, and crosstalk among different pathways.

In terms of cerebral blood flow dysregulation in the fetus and newborn infant, many factors have profound implications as the basis of hypo- and hyperreactivity, vessel rupture, intraventricular hemorrhage, and long-term neurological sequelae. In addition and of consequence, these studies have implications for the developmental "programming" during fetal and neonatal life of cerebrovascular, cardiovascular, and other diseases in the adult. Thus, one must ask, what do these findings mean in terms of the clinical care of premature and other newborn infants and other patients? Hopefully, this synthesis will have vital implications for diagnosis and potential therapy of those infants with dysregulation of cerebral blood flow, as well as relevance to the broad field of preventive medicine and public health. Recent advances are promising in that they point the direction for moving beyond descriptive phenomenology to investigation of fundamental cellular and molecular

regulatory mechanisms and understanding in a deeper sense. Of unprecedented opportunity and great promise, the rapidly growing diversity and power of new investigative tools and technology offer the opportunity for further insights into the role of development in regulation of the cerebrovasculature. Hopefully, a new generation of studies of gene regulation, protein-protein interactions, and related mechanisms will yield key information that will add to, and stimulate further, rapid advancement in this field of critical importance. The translational application of this knowledge to the prevention and amelioration of cerebrovascular complications in the fetus and newborn infant constitutes one of our greatest challenges for the future (Longo and Goyal 2013).

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Chapter 18
Related Developments in Fetal and Neonatal Endocrinology

The second half of the twentieth century saw a virtual explosion of research in fetal and neonatal physiology. In the UK, to a great extent, this was a result of the work of the Dawes group at the Nuffield Institute and also to investigators at Cambridge University. In Australia, this was a consequence of development of the focal groups in Melbourne, Adelaide, and Sydney and, in Canada, developments at London and Toronto in Ontario. Without question, Dawes and the Nuffield Institute "school" must be credited with the diaspora of gifted investigators who migrated to academic institutions in these and other countries. Advances proceeded in essentially every aspect of developmental physiology and biochemistry. With the education of talented pediatricians and obstetrician gynecologists committed to improve the health and well-being of their patients, increasingly "translational" and clinical research was included. Thus, it is perhaps appropriate to consider some highlights of these contributions, such as those in reproductive endocrinology, growth and metabolism, nutrition, neurodevelopment, cardiovascular, pulmonary and renal function, immunology, and so forth. In each of these areas, discerning clinicians and basic scientists have collaborated to apply theory to the evaluation and management of major diseases that result in morbidity and mortality. As a caveat, it must be appreciated that the synopses that follow are not in any sense exhaustive or complete. The goal here is to give a taste of some of the exciting developments during this period, in the application to discoveries in basic physiology/endocrinology and their contributions to and implications for perinatal and neonatal medicine. (With the citations provided, the interested reader can pursue these topics further.)

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18.1 The Beginnings of Reproductive Endocrinology and Medicine

The early twentieth century saw the emergence of endocrinology and reproductive medicine. Ernest Henry Starling (1866–1927) had coined the term "hormone" and with William Maddock Bayliss (later Sir William; 1860–1924) developed the theory of endocrine control of internal secretions (Bayliss and Starling 1904; Starling 1905). Experiments quickly established the vital role of the pituitary in orchestrating the functions of other glands and tissues (Crowe 1910; Cushing 1910; Houssay and Biasotti 1930), including the role of the adenohypophysis in the regulation of growth (Evans and Long 1921). Other studies demonstrated the ovarian dependence of the menstrual cycle that experimental hypophysectomy profoundly affected the reproductive system and the oxytocic action of posterior pituitary hormone (Dale 1909). Additional contributions to reproductive endocrinology at this time included the first definite description of the cyclic changes in the endometrium with the menstrual cycle and the attribution of pregnancy-induced changes in the mammary glands to hormones derived from the developing fetus and placenta.

In terms of biologic and physiologic development, one may consider two complementary threads, the differentiation of structure and the integration of function. Rather than being separate abstractions, these are aspects of a unitary process. Early in the century, the various embryological contributions and other discoveries were placed in perspective by Francis H. A. Marshall of the Universities of Edinburgh and Cambridge in his *The Physiology of Reproduction* (Marshall 1922). In this encyclopedic synthesis, Marshall reviewed existing knowledge in reproduction in its broadest sense, including reference to over 1000 works. In the preface, he presented the caveat, "It may be objected that, for a book on physiology, too much space is devoted to the morphological side of the subject. This has been done purposely, since it seemed impossible to deal adequately with the physiological significance of the various sexual processes without describing the anatomical changes which these processes involve" (Marshall 1922, p. 2). Focused chiefly on reproductive biology, Marshall presented insights to endocrinology such as that the existence and respective roles of follicular and luteal hormones were essential to ovulation (long before either was isolated). With consideration of embryology, placental development, and related topics, importantly these volumes contributed to the science that allowed the genesis of fetal and neonatal physiology. Quickly appreciated as a masterpiece, a second edition of Marshall's *magnum opus* was called for, which appeared in 1922 following the "Great War" and included chapters on the new field of biochemistry of the ovary and the testicle (Marshall 1922). In the introduction to his 1936 Croonian Lecture "Sexual Periodicity and the Causes Which Determine It" Marshall observed:

> . . . the great majority of animals, both vertebrate and invertebrate, not to mention plants, have a more or less definite season of the year at which they breed . . . There is no month of the year at which some species does not have its breeding season, and yet for the particular

> species in question the season is most regular . . .. In view of the general correlation between the seasonal and the sexual cycles it must be assumed that these stand in the relation of cause to effect.
>
> (Marshall 1936, p. 423)

Marshall's postulate led to a number of fundamental questions regarding mechanisms of animal breeding and reproductive physiology (Marshall and Hammond 1946). As Sir Alan Sterling Parkes (1900–1990) noted in his obituary of Marshall:

> Scientists are of many kinds, but inspiration flows most fruitfully from those who are able, by some gift withheld from lesser men, to divine the richness of uncharted country and sense the vital landmarks. Thus do they avoid the barren places and the morasses of unimportant detail which engulf so many. To these, discovery is an art rather than a science, a *matter* of instinct rather than of intellectual machinery. Such was Marshall.
>
> (Parkes 1950, p. 248)

In reproductive endocrinology, the late 1920s and the 1930s were a time of ferment. Selmar Aschheim (1878–1965) and Bernhard Zondek (1891–1966) had announced their test for diagnosing pregnancy (Aschheim and Zondek 1928) and also had isolated pituitary gonadotrophic hormone (Zondek and Aschheim 1928). Steroid hormones also were under active investigation. Edgar Allen (1892–1943) and Edward Adelbert Doisy (1893–1986) isolated estrogen (Allen and Doisy 1923), and George W. Corner and Willard Myron Allen (1904–1993) discovered progesterone (Corner and Allen 1929), Guy Frederick Marrian (1904–1981) isolated both pregnanediol and estriol (Marrian 1929, 1930), and Adolf Frederick Johann Butenandt (1903–1995) crystallized both progesterone (Butenandt 1934) and androsterone (Butenandt 1931). Butenandt and Doisy would win Nobel Prizes for their fundamental contributions (1939 and 1943, respectively). With these and other advances, a third edition of Marshall's *Physiology of Reproduction* was planned. However, because of World War II, the greatly enlarged (three volumes) multiauthored edition, edited by Sir Alan, did not appear until 1952 (Parkes 1952–1966; also see Parkes 1950; Polge 2006).

18.2 Fetal-Neonatal Endocrinology

A critical issue in developmental physiology in regard to the regulation of growth of the fetus and its placenta is that of the role of hormones in this growth and specialized tissue and organ function and the extent to which these hormones derive from the mother, the placenta, and/or the fetus per se and the extent to which they are interactive. In mid-century, Alfred Jost (1916–1991) of Paris demonstrated, in embryonic rabbits, that decapitation had little or no effect on growth of their trunk or limbs (Jost 1947). Thus, somewhat surprisingly, somatic growth and development including the cardiovascular, respiratory, and other systems were demonstrated to proceed independently of pituitary growth hormones or input from the central nervous system. In an effort to understand these regulatory mechanisms, in rabbits and rats, Jost performed a number of studies on the effects of removal of

specific organs including the hypothalamus, thyroid, parathyroid glands, and testis on fetal growth and the development of other organ systems (Jost 1961, 1966, 1968, 1969; Macnaughton 1969).

In large part, the concept of the placenta and fetus, in concert with the mother, consists of a "maternal-placental-fetal unit" for hormonal synthesis and metabolism is derived from the work of Egon Diczfalusy and his collaborators. In the case of steroid biosynthesis, by aromatization the placenta converts fetal 16α-hydroxy-dehydroepiandrosterone sulfate into estriol. This then is secreted into the maternal circulation and is the main estrogen metabolite excreted in the mother's urine. The fetus also can initiate the pathways for conversion of cholesterol to pregnenolone and on to dehydroepiandrosterone sulfate. Thus, the placenta, in concert with several fetal organs, plays a unique role in the regulation of steroid hormone production in pregnancy (Diczfalusy 1964). Shortly following his graduation from medical school in Hungary (1949), Diczfalusy moved to Stockholm, Sweden, where his investigations centered upon the metabolism of steroids in the fetus and placenta, their interconversion, and functional roles (Diczfalusy 1962, 1970, 1974; Mathur et al. 1970). He rose to become professor and head of the Reproductive Endocrinology Research Unit at the Karolinska Institute. Unique in the study of fetal physiology, these historic studies were conducted in the fetuses of women having elective termination of pregnancy. Although raising ethical considerations regarding human experimentation and fetal research (see below), one must remember that these studies were performed under strict guidelines of the Medical Research Council of Sweden. With the work of Jost and others, these studies marked the beginning of the discipline of endocrinology of the "maternal-placental-fetal unit" (Klopper and Diczfalusy 1969).

A related contribution of vital importance to the reproductive sciences was the discovery of the prostaglandins. In 1934, Ulf Svante Hansson von Euler (1905–1983) first reported that the lipid fraction of human seminal fluid, which he called prostaglandin, possessed potent contractile activity of smooth muscle (Euler 1934). Three decades later, Sune Karl Bergström (1916–2004) with Bengt Ingemar Samuelsson and colleagues determined the chemical structure of several of the specific prostaglandin molecules (Bergström et al. 1962, 1964; Bergström and Sjövall 1960).

Critical to the elucidation of many aspects of the pharmacology and metabolism of eicosanoids are the contributions of Sir John Vane, who in 1953 earned his doctoral degree in pharmacology with Dawes at the Nuffield Institute. A graduate of the University of Birmingham, Vane majored in chemistry at a time in which the stress was on chemical synthesis rather than experimentation. During a career advisory session, his professor of chemistry asked him about his plans following graduation. Vane, who at this time was rather disillusioned about the field, responded "Anything but chemistry" (Moncada 2006, p. 403). For his doctoral studies in pharmacology at Oxford, he worked with Professor Joshua Burn, the head of that department, and with Dawes with whom he published two manuscripts in the *Journal of Physiology* (Dawes and Vane 1956; Dawes et al. 1953). Following a postdoctoral fellowship at Yale, he joined the Institute of Basic Medical Sciences

located in the Royal College of Surgeons complex across from Lincoln's Inn Fields. Here, during the late 1950s and the 1960s, he developed the blood-bathed superfusion bioassay that has proven so valuable (Vane 1964). In this sensitive bioassay, the properties of minute amounts of biological chemicals can be analyzed, allowing determination of the manner in which vasoactive substances are handled in the circulation (Moncada 2006; Vane 1964, 1969). One of Vane's key discoveries was that angiotensin I is converted to angiotensin II on passage through the pulmonary circulation, rather than forming in the plasma as had been assumed (Bakhle et al. 1969). Not long thereafter, it was established that angiotensin-converting enzyme (ACE) is localized at the luminal surface of pulmonary endothelial cells (Ryan et al. 1975). (With about one-half of the vascular endothelial cells residing in the lung, it is perhaps not surprising that that organ serves metabolic functions.) In the mid-1960s, a Brazilian pharmacologist, Sergio Ferreira, joined Vane as a postdoctoral fellow. With him he carried a vial of the dried extract of the venom of an Amazon viper (*Bothrops jararaca*) to study its properties, which he had shown potentiates the actions of bradykinin. Following other studies, Ferreira, Vane, and colleagues determined that the venom contained an inhibitor of the angiotensin-converting enzyme (Aiken and Vane 1970; Ferreira 2000; Greene et al. 1972). This work led to the discovery of serpasil, the first ACE inhibitor used in the treatment of hypertension. By the early 1970s, the "Vane Bioassay Cascade" was used by many investigators. Subsequently, Vane also elucidated the mechanism by which nonsteroidal anti-inflammatory agents such as aspirin (acetylsalicylic acid) inhibited prostaglandins synthesis (Ferreira and Vane 1979; Vane 1971, 2002).

In 1973, Vane moved to the Wellcome Foundation to become director of research and development. In studies of anaphylaxis in guinea pigs, in addition to the release of prostaglandin E_2 and $F_{2\alpha}$, with collaborators he discovered a third compound they named PGX. This was quite unstable, had potent vasodilatory activity, and inhibited platelet aggregation (Bunting et al. 1976; Moncada et al. 1976). This they soon named prostacyclin (PGI 2), a major product of arachidonic acid metabolism (Moncada and Vane 1979). The physiologic properties and ubiquitous nature of prostaglandins displayed balance, such as between the constrictor activity of thromboxane A_2 formation by platelets and prostacyclin production by the vascular wall (Bunting et al. 1983).

In 1986, Sir John moved to the William Harvey Research Institute, which he established at St. Bartholomew's Hospital Medical College. There, he focused on elucidating selective inhibitors of cyclooxygenase 2 (COX-2) and the interplay between nitric oxide and endothelin in the regulation of vascular function (Moncada 2006; Vane et al. 1994). Discovery of the important role of these compounds in the regulation of a number of biological functions, including health and disease, opened the whole field of prostaglandin research with its great impact in reproductive physiology (Vane 1982; Vane and Bergström 1979). These contributions were followed by award of the 1982 Nobel Prize in Physiology or Medicine to Bergström, Samuelsson, and Vane.

In his essay "My life and times . . .," Sir John reviewed many aspects of these studies. He concluded:

> My life and times with enzymes and mediators have been a fascinating detective story; finding previously undiscovered pathways and interactions that have led to important new concepts and drugs. I want to thank all those colleagues, PhD students, technicians and postdocs who have contributed to the excitement of my research. . ..
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> (Vane 2001, p. 797)

Of Sir John Vane, Sir Salvador Moncada of the University College London has written:

> John was an ingenious, hands-on pharmacologist, able to generate meaningful hypotheses almost effortlessly. He was a gifted speaker and writer, a motivator and a teacher to several generations of pharmacologists. He had a great understanding of biological processes and a keen eye for the behavior of the tissues in his beloved bioassay. One of his favourite phrases to students reporting back to him with results that they did not understand was 'the tissues never lie'—it is the interpretation that can fail.
>
> (Moncada 2006, p. 409)

Gautam Chaudhuri, a doctoral student of Vane in the 1970s and now at the University of California Los Angeles, has written:

> Many of us who were mentored by Sir John were trained as classical pharmacologists. While Professor Dawes was pursuing fetal physiology, Sir John focused on vasoactive substances. This was not surprising as mentors and mentees do seem to part as time progresses. Professor Vane was a brilliant scientist and went on to train numerous individuals who have and continue to occupy high positions in academia. In addition, many individuals who worked in his lab as visiting scientists also have received wide recognition.
>
> While pursuing my Ph.D., I was lucky to come in contact with illustrious people who also considered Sir John a mentor and friend. These included Professor Sir Salvador Moncada, currently of the University College, London. Sir John's students and collaborators have done exceedingly well: Professor Moncada was elected as a Fellow of the Royal Society of London and is also a Foreign Associate of the National Academy of Sciences, USA. Sergio Ferreira was also elected as a Foreign Associate of the National Academy of Sciences, USA. Another student of his, Roderick Flower, who completed his PhD under his mentorship, was elected to the Royal Society. This all emphasizes the role of Geoffrey Dawes in training a unique individual who won the Nobel Prize, and also trained other illustrious scientists.
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> (Letter from GC to LDL, 20 July 2012)

From the 1960s to the present, the field of developmental endocrinology has virtually exploded, with increased understanding of the role of glucocorticoids, the insulin-like growth factors 1 and 2 (IGF-1, IGF-2), and other hormones and mitogenic peptides in the regulation of growth and development. One who contributed greatly to this field was Geoffrey Donald Thorburn (1930–1996), a graduate in medicine from the University of Sydney (1956). In the mid-1970s, Thorburn spent 5 years at the Nuffield Institute, before returning to Australia to head the Department of Physiology at Monash University. Particularly important contributions were those relating to the hormonal regulation of parturition (Bassett and Thorburn 1969; Bassett et al. 1969; Thorburn and Challis 1979), including the major role of

prostanoid production by the placenta in this regard (Rice and Thorburn 1989; Thorburn 1991). Many aspects of Thorburn's investigative studies have been reviewed (Jenkin et al. 2009). A long-time colleague of Thorburn, who continued to pursue an understanding of the hormonal regulation of parturition and other aspects of developmental endocrinology, was John R. G. Challis, most recently with the Michael Smith Foundation for Health Research, Vancouver, British Columbia, and who generously prepared the Foreword for the first edition of this volume (Challis and Brooks 1989; Challis et al. 2000; Thorburn and Challis 1979).

At Cambridge University, Robert Semple Comline (1920–1998) with his colleague Marian Silver (1928–1994) continued in the Barcroft-Needham-Hammond-McCance-Widdowson tradition of pursuing several facets of fetal-neonatal growth and development and endocrinology. Particular contributions included that of function of the developing adrenal gland in fetal homeostasis (Blaschko et al. 1967; Comline et al. 1965; Comline and Silver 1961, 1966). In addition to the lamb (Comline and Silver 1972; Fowden et al. 1989), the Cambridge group studied comparative metabolism in several developing species including the cow (Balfour and Comline 1962; Blaschko et al. 1967; Comline and Silver 1976; Comline et al. 1974), horse (Fowden et al. 1980, 1984), and pig (Comline et al. 1979; Silver et al. 1986, 1988).

18.3 Developmental Neuroendocrinology

Notable in regard to neuroendocrine aspects of brain development was the work of Geoffrey Wingfield Harris (1913–1971). After working at Cambridge under F.H.A. Marshall, Harris qualified in medicine at St. Mary's Hospital, London (1939); he then returned to Cambridge University. Still later, he held the FitzMary Chair of Physiology at the University of London (1952). Commencing in 1962, he served as Dr. Lee's professor of anatomy at Oxford University. With a singular passion, Harris recognized and pursued the relation of the hypothalamus to the pituitary gland in regulation of the gonadotrophins. He was the first to appreciate the role of the hypophyseal-pituitary portal circulation as the highway by which endocrine messages produced by the hypothalamus reached the anterior pituitary gonadotrophs. Harris termed this material "luteinizing hormone-releasing factor," later called gonadotrophin-releasing hormone (GnRH). His studies laid the foundation for reinvention of the hypothalamus as an active partner with the anterior pituitary in vital aspects of endocrinology, especially the reproductive sciences. This concept that the brain regulates the neuroendocrine system by a remarkably orchestrated pattern of synthesis and secretion of a family of peptide hormones has been described by numerous workers in the field (De Groot and Harris 1952; Donovan 1972; Harris 1948, 1952, 1955, 1961, 1964a, b, 1970, 1972; Harris and Campbell 1966; Harris and Donovan 1966; Harris and Jacobsohn 1952a, b; Harris and Levine 1965; Naftolin et al. 1971a, 2007; Raisman 1997; Reichlin 1964; Vogt 1972).

Frederick Naftolin, director emeritus of the Reproductive Biology Research and codirector of the Interdisciplinary Program in Menopause Medicine at New York University, worked with Harris from 1968 to 1970. He recalls:

> While [Geoffrey] Dawes was making his observations on the cardio-respiratory side, there was ferment in South Parks Road about the sexually-determined development of the regulation of gonadotrophins. Years before, Geoffrey Harris and collaborators had refined the work of [Carroll Athey] Pfeiffer (Pfeiffer 1936) and others to identify the role of the hypothalamus, rather than the pituitary, in gender-specific gonadotrophin regulation. (Harris 1964a; Harris and Levine 1965). We worked to further this body of evidence. This was the time when objective, sensitive, precise and practical hormonal measurements were becoming available. Since I had worked on an assay for human LH [luteinizing hormone] as an endocrinology fellow with Charles Alvin Paulsen (1924–2008), my D.Phil thesis project was to develop and apply an immunoassay for rat LH.
>
> The laboratory worked mainly on rats, though we occasionally studied rabbits and humans. Rats are born during the period of sexual differentiation of the regulation of gonadotrophin regulation. The developmental window is open from a couple of days before parturition until the end of the first two weeks of life (MacLusky and Naftolin 1981). During this . . . "critical period", the hypothalamic circuitry for gonadal hormonal suppression (estrogen) of GnRH (negative feedback), followed by disinhibition of GnRH release by the preovulatory surge of circulating estradiol (positive feedback), or for blocking the surge, is laid down in the rat hypothalamus. Females have both negative- and positive-feedback regulation of gonadotrophin secretion, while males have only negative feedback regulation. Females exhibit positive feedback and this is the default circuitry of the hypothalamus. This developmental sexual dichotomy is the outcome of a further elaboration of the developmental program in males that blocks the ability of the hypothalamus to disinhibit GnRH secretion, as in positive feedback. This "sexual differentiation of the brain" is caused by exposure to testosterone from the testis during this perinatal period (Harris and Naftolin 1970; MacLusky and Naftolin 1981). The timing of the "critical period" for brain sexual differentiation is species-dependent and usually between E15 to P15. Conveniently, rats are born at E21-22, so there is a window of opportunity to study experimentally the mechanism of the testes' effect on their brain sexual differentiation in rats that extends from E~17 to P~14 (MacLusky and Naftolin 1981). Studies administering sex steroids rather than minced testis showed that testosterone or its metabolites will masculinize the rat's regulation of gonadotrophins as an adult irrespective of the genetic sex (Jaggard and Bradbury 1961). But, while testosterone may be secreted from the testis, the molecular mechanism of its action was not known. This was especially true since evidence had also been reported indicating that natural and synthetic estrogens and partially aromatized androgens also could masculinize the genetic female rat's control of gonadotrophins (MacLusky and Naftolin 1981). So, at the end of the 1960s the active agent at the level of the brain's cells remained unresolved (Naftolin 2012).
>
> The occurrence of the critical period at the time of parturition and thereafter in rodents is a boon to experimental testing and treatment; the work had progressed to the point of requiring blood hormone measurements to measure the results of manipulating the system. Charles S. Corker and I were the two graduate students tasked with this job. Corker and his supervisor, Donald Exley solved the measure of estradiol in the circulation by the novel use of sex hormone binding globulin (SHBG) to bind pictogram amounts of estradiol in the plasma to measure the competition of endogenous estradiol for radio-labelled estradiol (Corker et al. 1970). I utilized the cross-reaction of rat luteinizing hormone (LH) with anti-human chorionic gonadotrophin to develop a radioimmunoassay to measure rat LH. (Naftolin 1970). The combination allowed the first concurrent measures of blood

estradiol and LH, and showed conclusively that the estrogen surge preceded the LH peak in humans and rodents (Brown-Grant et al. 1970; Corker et al. 1969; Harris and Naftolin 1970). Under the direction of Keith Brown-Grant we then turned our attention to brain sexual differentiation of gonadotrophin secretion.

The pioneering work by Pfeiffer showed that the organization of the central axis that silences positive feedback in rats arises from the testis, and this effect occurs during the perinatal period in genetic males and females. The presence of the ovary is of no moment in this aspect of development (Pfeiffer 1936). Parenthetically, Pfeiffer thought that this effect was at the level of the adenohypophysis. He later confided to me that this bias was the result of the enormous influence at the time of Harvey Cushing and his preoccupation with the pituitary as the main controlling influence over the regulation of endocrine function (Pfeiffer, personal communication). However, Harris and Jacobsohn showed definitively that the organization of positive feedback and its expurgation by the testis is independent of the pituitary; the location of sexual differentiation is in the hypothalamus (Harris and Jacobsohn 1950). That this regulation by the hypothalamus was due to the intactness of the pituitary-portal system was the subject of a an acrimonious and public debate between Harris and the Queen's physician, Sir Solly Zuckerman (1904–1993) (Thomson and Zuckerman 1955). Harris was ultimately proven correct and [in Zuckerman's review of Harris' monograph *Neural control of the pituitary gland* (Harris 1955)] received a qualified admission (Zuckerman 1956). Harris had this framed and hung in his office. Nonetheless, Zuckerman continued to bitterly deride Harris' work, even after his death in 1971, which could have played a role in Harris not receiving the Nobel Prize. In 1977, the Prize was awarded to Andrew Viktor Schally and Roger Charles Louis Guillemin [which they shared with Rosalyn Sussman Yalow (1921–2011)] for the discovery of the chemical makeup of the hypothalamic releasing hormones. [Prior to receiving the Nobel Prize, Guillemin had been awarded the National Medal of Science (1976) by President Gerald Rudolph Ford (1913–2006; President 1974–1977)]. Fortunately, Professor Harris lived to relish his victory over Zuckerman, and this was the subject of conversations over our evening sherry in his office. Sadly, Zuckerman continued to incorrectly disclaim Harris' having proven that the portal blood mainly flows from the hypothalamus to the pituitary, and not the other way around, even after Harris' death. This scurrilous behavior caused me to walk out during a lecture he gave at the Oregon Primate Center.

As the GnRH neurons that secrete into the portal vessels are located in the hypothalamus and are targeted by hypothalamic neurons, brain sexual differentiation occurs in this area of the brain (Harris and Naftolin 1970; MacLusky and Naftolin 1981). Working with Keith Brown-Grant and Allan U. Munck, another visitor to the Department, we tested and found wanting the idea that testosterone was organizing the male rat brain via formation of ring A-reduced products (Brown-Grant et al. 1971). This opened the way for the ultimate testing and proof that male rats converted the testosterone from the fetal-newborn testis to estrogen that organized the hypothalamic circuitry for monotonic control of the gonadotrophins. This formulation depended on the proof that the brain could convert androgen to estrogen, and was accomplished working with Kenneth John Ryan (1926–2002) [at the University of California, San Diego] shortly after I completed my training at Oxford. (Naftolin et al. 1971a, 1975). We studied dissected hypothalamic tissue from mid-trimester female and male fetuses. Promptly after delivery of fetuses that had been terminated by saline infusion into the amniotic sac it was determined that there were no signs of life and the brain was removed and bloc-dissected to furnish the anterior and posterior hypothalamus. I included the temporal lobe as a putative negative control. The method of proving the presence of aromatization in the tissue was the same as the Ryan lab had been using to study placental aromatization of androgens. This is a method based on incubation of the homogenized tissue with radio-labelled androgen (^{14}C-androstenedione)

and performing a phenolic extraction to isolate the estrogens. The extract then is treated to form phenolic conjugates, acetates in this case, which are then extracted and recrystallized to constant specific activity. The products are compared with authentic estrogen acetates to prove that newly formed estrogen has been made by the microsomes in the brain homogenates (Naftolin et al. 1971b, 1972a). The proof of the presence of aromatase in neurons was substantiated by the discovery of aromatase in synaptosomes for rat brain. While not yet explained, this finding is challenging as it implies additional effects of aromatase in the brain other than as would result solely from the production of estrogen. We have commented on the interesting possibilities and hope someday to discover the true meaning of this interesting finding (Naftolin et al. 1996).

The discovery of aromatization by brain cells was a byproduct of studies begun in Oxford on brain sexual differentiation that led to the "Aromatization Hypothesis". The demonstration of this novel biology led to important changes in the understanding of brain metabolism and action. There remain many unexplained ramifications of this work (Naftolin et al. 2007; Panzica et al. 2012). Regardless, the discovery that set them off would not have been possible without the support of many seasoned scientists for a naïve young academic (Naftolin 2012).

Other Oxford by-products of [Harris'] interest in understanding the fetal-neonatal development of control of the gonadotrophins included observation of the pulsatile release of LH in humans (Naftolin et al. 1971a, 1972b), and evidence that male rams are seasonal breeders with low LH in the off-season. This work also included the first observation of the pulsatile nature of LH secretion (Katongole et al. 1971). Since Professor Harris was the person who in 1936 had postulated that the hypothalamus secreted factors into the pituitary-portal vessels that arrived at the adenohypophyseal cells and released the pituitary trophins (Harris and Naftolin 1970), our work at the Department of Human Anatomy included testing purified sheep luteinizing hormone releasing factor (LRF) on human subjects and show that it induced LH secretion (Naftolin et al. 1971a). Harris was elated with these studies, but could not proceed with the sequencing of the molecule because his biochemist collaborator, the Imperial Chemical Industries' ace chemist Harry Gregory, was occupied with . . . bradykinin [which had been discovered earlier (Rocha E Silva et al. 1949)]. Later, after Professor Harris' premature death the actual sequence of thyrotrophin releasing factor and LRF were determined and the peptides could be re-named releasing hormones. The determination of the chemical structure of releasing hormones contributed to the 1977 Nobel Prize [being] awarded to Guillemin and Schally. It was very sad that Harris could not have shared the medal that he so richly deserved.

The time spent in Oxford branded not only my professional identity; it indelibly laid an experimentalist's circuitry in my brain. Inspired by Harris, we followed the discovery that formation of estrogen by the brain furnished the mechanism of testosterone's organization of the male rodent brain's gonadotrophin release pattern (Naftolin et al. 1972b; MacLusky and Naftolin 1981), with exposition of the effect of estrogen on developing hypothalamic neurons and circuitry (Nilsen et al. 2000), and the cellular mechanisms by which the hypothalamus undergoes synaptic plasticity and the midcycle release of LH to trigger ovulation (Naftolin et al. 2007).

The work on fetal brain neuroendocrine development and function that Professor Harris, Keith Brown-Grant, and others accomplished in Oxford during [the] same period that Dawes was exploring fetal-neonatal cardio-pulmonary development and function was little known to the other side. However, it can now be seen as an important companion chapter in the Oxford contribution to developmental neuroendocrinology.

(Naftolin 2012; Letter from FN to LDL, 28 December 2009)

18.4 Hormonal Regulation of the Timing of Birth

A clear and overwhelming contribution to research during the past half century is understanding that the timing of birth is a complex, multifactorial process of interrelated biologic mechanisms. The idea that the human fetus plays a role in the initiation of parturition had long been implied by the clinical observation of prolonged pregnancy in cases of anencephaly of the developing fetus. For instance, in 1933 from the Women's and Maternity Hospitals, University of Liverpool, Percy Malpas (1901–1980) reported that in 9 of 44 cases (20%) of anencephaly, pregnancy was unduly prolonged, as it was also in 3 cases each of hydrocephalus and spina bifida. In several of the anencephalic infants, the pituitary gland and the adrenal glands were absent (Malpas 1933). Others reported similar findings (Comerford 1965; Rea 1898). In animals, several lines of evidence also support this concept. For instance, greatly prolonged gestation occurs in Guernsey (Kennedy et al. 1957) and Holstein-Friesian (Holm et al. 1961; Jasper 1950) cattle, in which the fetus demonstrated central nervous system anomalies including aplasia of the anterior pituitary gland and in some cases adrenal insufficiency. About the same time as these reports in cattle, facial congenital malformation was recognized in 1–8% of newborn sheep whose mothers had pastured in certain alpine meadows in Southwestern Idaho. This anomaly was restricted to the head and varied from classical cyclops with a single eye to deformity of the upper jaw. A veterinarian with the US Department of Agriculture Research Service in Utah, Wayne Binns (1911–1994) and colleagues observed that the most severe of these cases were associated with prolonged gestation. The local ranchers did not believe the condition to be hereditary, but rather it resulted from some environmental toxin (Binns et al. 1959) ingested by the ewe early in gestation (Binns et al. 1960). Soon it was discovered that in those sheep which had grazed on the plant *Veratrum californicum* on days 10 to 15 of gestation, the period of gestation was markedly prolonged in a manner similar to that described in cattle. In addition, the fetus demonstrated cyclops with multiple intracerebral abnormalities including absence of the hypothalamus and pituitary gland as well as hypoplasia of the adrenal glands (Binns et al. 1964).

It was not until the studies of Graham Collingwood Liggins (later Sir Graham; 1926–2010) of Auckland, New Zealand, and colleagues, however, that the essential role of the fetal hypothalamic-pituitary-adrenal axis in the initiation of parturition was established. An obstetrician gynecologist at the National Women's Hospital with a passion for research, Liggins was influenced by a colleague Albert William Liley (later Sir William Liley; 1929–1983) who had revolutionized the management of obstetrical patients with the rhesus hemolytic disease *erythroblastosis fetalis* by intrauterine transfusion of the anemic fetus (Liley 1963). Tackling the problem of premature labor "Mont" (for his childhood fascination with Monty the Mouse) Liggins devised a plan to elucidate the mechanism of the timing of parturition on animals. The hope was that this understanding might lead to the prevention of preterm birth. Believing this involved the fetal pituitary gland, using

lambs from a nearby agricultural research station, he taught himself to perform fetal hypophysectomy (Watts 2010). Then on a sabbatical with Peter Carleton Kennedy (1923–2006) at the University of California Davis, Liggins electrocoagulated the pituitary gland of 17 fetal lambs during the last third of gestation (normal term ~147 days). With ablation of 70% or more of the hypophysis, somatic development was retarded, including that of epiphyseal centers, with hypoplasia of adrenal cortex, thyroid gland, and interstitial cells of testis, and other changes, as compared to controls. These findings demonstrated the critical role of the pituitary gland during antenatal, as well as postnatal development (Liggins and Kennedy 1968; Liggins et al. 1967). In 2000, this latter paper was featured as a classic paper in the American Journal of Obstetrics and Gynecology. Although these fetuses subjected to hypophysectomy were alive at delivery, they demonstrated extensive endocrine and metabolic disturbances (Liggins and Kennedy 1968). Along the way, Liggins also was first to establish the role of glucocorticoids in maturation of the lung (Liggins 1969a, b).

In a further study of fetuses of 24 pregnant ewes, Liggins, Kennedy, and Louis Wilkins Holm (1938–2008) performed pituitary ablation by electrocoagulation between 93 and 142 days of gestation. Following destruction of 70% of the pituitary gland or destruction of the fetal hypothalamus with section of the pituitary stalk, pregnancy was prolonged from 158 to 187 days' gestation (Liggins et al. 1967). In a subsequent report, Liggins also demonstrated the luteolytic role of prostaglandin F2α in sheep parturition (Liggins and Grieves 1971). As a vital contribution, these studies established the essential role of the fetal pituitary and adrenal glands, and associated hormonal changes, for the initiation of parturition (Liggins et al. 1973). As a caveat, while Liggins and his colleagues were careful to point out differences between the physiologic and endocrinologic characteristics of the pregnant ewe and the human, they noted the potential for this experimental model to explore in humans mechanisms of the initiation of labor and, hopefully, gain understanding of the premature onset of labor.

Critical to understanding the hormonal changes associated with parturition, and the hormonal events of the fetus with those of the mother, were contributions of John R.G. Challis and Robert Brian Heap and colleagues in the early 1970s. With the use of a newly developed radioimmunoassay for total estrogen, Challis, still a doctoral student at Cambridge University, demonstrated in sheep an abrupt and dramatic 10- to 20-fold rise in this hormone just before the onset of parturition (Challis 1971). Later, this was shown to be associated with increased output of prostaglandin F2a from intrauterine tissues (Challis et al. 1972). Subsequently, at Harvard University, working in the laboratory of Kenneth Ryan, Challis correlated the prepartum changes of estrogen in the rabbit with a concomitant fall in progesterone in both plasma (Challis et al. 1973) and myometrium (Challis et al. 1974a). In primates, however, it was difficult to demonstrate peripheral plasma changes of progesterone before birth (Challis et al. 1974a, b), and attention then turned to hormone levels in the amniotic fluid as a reflection of intrauterine steroid changes (Mitchell et al. 1976) and to the importance of paracrine processes in the regulation of labor. In rabbits, they also showed that the progesterone fall was associated with

prostaglandin $F_{2\alpha}$-induced luteolysis (Challis et al. 1974c) and that ovarian granulosa cells produced the prostaglandin (Erickson et al. 1977). Challis' latter work was developed at the Nuffield Institute, in collaboration with Geoffrey Thorburn and Jeffrey Robinson.

Of vital importance to fetal development is a well-orchestrated maturation and interaction of the hypothalamic-pituitary-adrenal axis. Some aspects of this have been reviewed above. In addition to corticotropin-releasing hormone (CRH), arginine vasopressin (AVP) was discovered to be an adrenocorticotropic hormone (ACTH) secretagogue. Although corticotrope stimulation releases ACTH relatively early in development, the sensitivity of adrenal cortical cells to ACTH appears relatively late in gestation. Complicating an understanding of this regulation is the fact that during the last few weeks of gestation, CRH is expressed in large amounts by the placenta (Gitau et al. 2004; Majzoub and Karalis 1999; Mastorakos and Ilias 2003). Proopiomelanocortin (POMC), the primary precursor of several anterior pituitary peptides, is enzymatically cleaved to yield $ACTH^{1\text{-}39}$ (Bell et al. 2005). Because in some early studies of ACTH biology the specificity of $ACTH^{1\text{-}39}$ antibodies used were relatively poor, confusion arose as to changes and role of ACTH in adrenocortical function (see Rose et al. 2011). Clearly, evidence indicates that the bioactive $ACTH^{1\text{-}39}$ concentration increases near term (but not in proportion to the large amount of circulating placental-derived CRH (Castro et al. 1992; Lockwood et al. 1996)), and this roughly correlates with a plasma surge in cortisol (Castro et al. 1992). In the near-term fetal sheep, the anterior pituitary responsiveness to AVP also increases strikingly (Fora et al. 1996; Perez et al. 1997). These studies of ACTH responses to AVP by fetal anterior pituitary cell in vitro contrast with an in vivo study were done by Ede Marie Apostolakis (1943–2010) that indicates selective antagonist of the AVP V1 receptor does not affect ACTH secretion in the fetus during the last 2 weeks of pregnancy (Apostolakis et al. 1991). Thus, fetal cortisol in circulation is not simply regulated by a negative feedback control of modulators of ACTH secretion.

The work by Liggins advanced the notion that a prepartum rise in fetal corticosteroids could provide a signal, as part of the maturation of the ovine fetal hypothalamic-pituitary-adrenal axis, for initiation of labor in sheep. Mean ACTH and cortisol concentrations increase in the circulation of fetal sheep as pregnancy nears term, most dramatically in the 72 h preceding birth (Bassett and Thorburn 1969; Liggins et al. 1977b). This reflects an increase in pulsatile frequency and amplitude of cortisol secretion (Apostolakis et al. 1992; Yellon and Apostolakis 1994). However, the rise in fetal cortisol may not entirely be driven by an enhanced pulsatile secretion of ACTH. Rather enhanced responsiveness to ACTH by the adrenal appears to promote the systemic rise in fetal cortisol. In this regard, another group that has contributed greatly to an understanding development of fetal adrenal cortex cortisol biosynthesis, a reflection of organ maturation and a possible role in the initiation of parturition, is that of Elvie Marelyn Wintour and her colleagues at the Howard Florey Institute for Experimental Physiology and Medicine at the University of Melbourne (MacIsaac et al. 1989; Tangalakis et al. 1990; Wintour 1984; Wintour et al. 1985, 1986). During the last few weeks of gestation, the ovine

fetus demonstrates a progressive increase in corticotropin-releasing hormone (CRH) mRNA in the paraventricular nucleus of the hypothalamus. This is coupled with an increase in POMC concentration in the pituitary with enhanced POMC processing during late gestation, resulting in enhanced ACTH secretion (for review, see Challis et al. 2001). Beyond the hypothalamic-pituitary portion of the axis, critical maturational changes at the level of the adrenal gland also occur in concert with increases in ACTH (Braems et al. 1998). During the latter part of gestation, there is an augmentation of adrenal responsiveness to ACTH, mediated not only by increase ACTH receptor expression but also by increased ACTH receptor binding and adenylate cyclase response to ACTH (Durand et al. 1980; Saez et al. 1984; Tangalakis et al. 1990). The rise in ACTH and cortisol in fetal circulation as pregnancy nears term suggests both a reduced anterior pituitary responsiveness to the negative feedback actions of cortisol and increased adrenal responsiveness to ACTH.

Charles Andrew Ducsay at Loma Linda University and Dean Allen Myers at the University of Oklahoma have shown that in late gestation, enhanced ACTH signaling contributes to the ontogenic rise of key steroidogenic enzymes such as the cytochrome P450, family II, subfamily A, polypeptide 1 (CYP11A1), and cytochrome P450 (CYP17) (Myers et al. 2005). These investigators also showed that as term approaches, maturational changes in the hypothalamic-pituitary-adrenal axis are responsible for the observed increase in fetal plasma cortisol (Harvey et al. 1993; Magyar et al. 1981), in part this being regulated by activation of endothelial nitric oxide synthase (Ducsay and Myers 2011). Of particular note, acclimatization to high altitude long-term hypoxia is associated with activation of the HPA axis, increased expression of adrenal endothelial cell nitric oxide synthase (Monau et al. 2009), with the NO inhibiting ACTH-induced cortisol secretion (Monau et al. 2010). The superimposed stress of more severe hypoxia may overcome the NO inhibition of steroidogenesis via induction of protein phosphatases and other factors (Ducsay and Meyers 2011), and cortisol excretion is enhanced (Myers and Ducsay 2012).

Collectively, Liggins and colleagues made monumental contributions with several major reviews about the role of the fetal hypothalamic-pituitary-adrenal axis in the initiation of labor in sheep (Liggins 1969a, 1988; Liggins et al. 1977a). Effects of ACTH or cortisol infusion into the sheep fetus to induce premature labor did not occur following an infusion of mineralocorticoid. Although preterm birth was specific for treatment, the effect to induce labor was dose dependent. Moreover, evidence suggested that the sustained rise in cortisol was independent of pulsatile ACTH secretion and cortisol treatment may feed-forward to promote basal secretion of ACTH (Apostolakis et al. 1994). Such effects of cortisol may involve neural control of ACTH release. Based on data indicating high concentrations of corticotropin-releasing hormone in the median eminence of the hypothalamus and that innervation of the latter arises in the parvocellular portion of the paraventricular nucleus, Thomas Joseph McDonald and Peter William Nathanielsz of Cornell University, Ithaca, NY, tested the hypothesis that elimination of this innervation would result in a significant delay in the onset of parturition. At 120 ± 2

days gestation, by stereotaxis, they placed electrolytic lesions in the paraventricular nuclei. They also placed small glucocorticoid implants adjacent to these nuclei to block further the physiologic ACTH response to hypoxemia and hypertension (McDonald and Nathanielsz 1991). In the lambs so lesioned, labor failed to be initiated by 157 dpc, at which time the pregnancy was terminated for tissue collection. Additionally, lesioned fetuses failed to demonstrate an ACTH increase in response to severe hypotension (50% decrease in blood pressure) and failed to demonstrate the normal near-term increase in circulating ACTH and cortisol (McDonald and Nathanielsz 1991). Because the hippocampus has been shown to modify function of the hypothalamic-pituitary-adrenal axis, in a further study, McDonald and colleagues stereotaxically transected the dorsal columns of the hippocampal fornix to test this hypothesis. With no effect on fetal hormonal levels or gestational length, the authors concluded that the lack of hippocampal maturity creates a lack of negative feedback that leads to increased ACTH in fetal circulation (McDonald et al. 2006).

Despite the extent of our knowledge of the events leading to the timing of parturition in sheep and other ungulates, these mechanisms and the relative roles of endocrine, paracrine, and other factors vary widely among species and may not involve signals from the fetal adrenal. This concern is relevant for the sheep as well, since cortisol infusion to sustain physiological prepartum peak concentrations in fetal circulation for more than 9 days did not induce preterm parturition. Lack of evidence in other species to support a role for fetal adrenal gland in the process of parturition leaves open the question of the signal arise that initiates the onset of labor in sheep. It is sobering to appreciate that we still are far from understanding these fundamental mechanisms of hypothalamic-pituitary-adrenal axis and associated regulation in humans or other primates.

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Chapter 19
Further Developments in Fetal and Neonatal Physiology

> *. . ..It is not in the nature of things for any one man to make a sudden violent discovery; science goes step by step, and every man depends on the work of his predecessors. When you hear of a sudden unexpected discovery—a bolt from the blue, as it were—you can always be sure that it has grown up by the influence of one man on another, and it is this mutual influence which makes the enormous possibility of scientific advance. Scientists are not dependent on the ideas of a single man, but on the combined wisdom of thousands of men, all thinking of the same problem, and each doing his little bit to add to the great structure of knowledge which is gradually being erected.*
>
> Lord Rutherford (in Cohen 1960, p. 112)

19.1 Pulmonary Physiology and Respiratory Distress Syndrome

An example of the manner in which discoveries in fundamental physiology and biochemistry have combined with clinical investigation to impact care of the newborn infant, perhaps no more striking instance, is that of an understanding of the lung and the changes that occur at birth. Survival at birth depends on the optimal development and maturation of the lung in utero. Disorders of lung growth, maturation, and regulation of respiration continue to be among the most important problems with which the neonatologist has to deal. As noted above, in the USA, as well as throughout the world, premature birth occurs in 7–12% of pregnancies, a major complication of which is pulmonary immaturity with resultant respiratory distress syndrome (RDS). Affecting more than 10% of infants born prematurely, RDS is characterized by tachypnea, cyanosis, grunting, and intercostal and subcostal chest wall retractions. The potentially devastating consequences, with both short- and long-term complications, include alveolar rupture, development of pneumothorax, pulmonary interstitial emphysema, and other conditions. In the USA alone during the 1950s, this condition claimed the lives of more than 10,000 infants a year. A monumental discovery was that inadequate pulmonary surfactant

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was associated with altered pulmonary function and respiratory disease of the newborn. In this instance, the genesis of a clinical problem was elucidated in the laboratory by collaboration of basic scientists and clinicians, driven by curiosity and working to saving the lives of prematurely born infants.

It was Richard Eric Pattle (1918–1980), a physicist turned physical chemist, working at the Chemical Defence Experimental Establishment, Porton Down, Salisbury Wiltshire, in the south of England, who in studying the behavior and prolonged stability of bubbles in pulmonary edema fluid from rabbits, which had been subjected to war gases such as phosgene, suggested the presence of an "anti-pulmonary edema factor." Pattle reported that bubble films were resistant to antifoam, and their stability "... is due to an insoluble surface layer on the bubbles... that must... have formed the original lining layer of the fine air spaces." In contrast to rather transitory bubbles from serum or other bodily fluids, these bubbles remained stable for an hour or more. He noted that the surface tension of the lung bubbles is therefore "zero" and that in the small alveoli with "sharply curved, and probably moist" walls, if surface tension were that of ordinary liquids, "... enough suction would be exerted to fill the alveoli with a transudate from the capillaries. Means for keeping the surface tension low must therefore be part of the design of the lung." Pattle continued that it "... is thus evident that the alveoli are lined with an insoluble protein layer which can abolish the tension of the alveolar surface." This unique substance also was present in the lungs from the mature fetus as well as the adult. Pattle concluded that his findings suggest that this substance is "... secreted in the depths of the lung" (Pattle 1955, pp. 1125–1126). In a further note, Pattle demonstrated what has been referred to by some as "Pattle's Peculiar Protein" (Hughes 2001, p. 4), in the lungs of birds, reptiles, and amphibians (Pattle and Hopkinson 1963). In his major review of this field of research, including that in the main classes of vertebrates, he detailed a number of problems to be explored, such as the cells of origin, the biochemistry of the surfactant protein, and the mechanism(s) of synthesis. Pattle concluded, "The finding that the lung lining substance appears only late in foetal life ... suggests that the absence of the lining substance may sometimes be one of the difficulties with which a premature has to contend; such a defect may possibly play a part in causing some cases of atelectasis neonatorum ... These matters need experimental investigation" (Pattle 1958, pp. 239–240). Pattle prepared several other reviews of his work (Pattle 1965, 1966).

The pulmonary alveoli that were lined with an aqueous layer had been postulated in the previous year by Charles Clifford Macklin (1883–1959) of the University of Western Ontario (Macklin 1954). Only later was it discovered that several decades earlier, Kurt von Neergaard (1887–1947) of the University of Zurich published "New interpretations of basic concepts of respiratory mechanics. The retractile force of the lung, dependent on the surface tension in the alveoli" (von Neergaard 1929). In this report, von Neergaard explored the role of surface tension as a critical force in pulmonary expansion and contraction, demonstrating that these could not be explained solely by elasticity of tissue elements. von Neergaard summarized his major findings as follows.

The so-called lung elasticity—one of the fundamental concepts of the mechanism of breathing—to now has been attributed . . . to the elasticity of the elastic fibers. Contrary to these previously held ideas we find:

1. The total recoil of the lung consists of two components, the greater effect from surface tension, the lesser important from true tissue elasticity. Quantitatively the most important portion of the lung recoil is based on that due to the surface tension on the terminal membrane boundary between alveolar epithelium and the alveolar air.
2. The importance of surface tension in lung recoil has been investigated experimentally by measuring so-called elasticity [or pressure-volume] curves of animal and human lungs before and after elimination of surface tension. Eliminating the surface tension can be achieved by complete filling of the lung with a physiological gum arabic-electrolyte solution in a differential vacuum method. By this filling, the terminal membrane between alveolar epithelium and alveolar air is eliminated. . ..
3. The measurements show that, depending on the state of expansion, the recoil due to the surface tension is on average two or three times greater than that of the actual tissue elasticity. . .. From the difference between the total recoil and the partial component of true tissue elasticity, the curve of the apparent elastic effects of surface tension of the alveoli decreases in comparison to other physiologic solutions by becoming enriched with surface-active substances. . ..
4. Apart from the experimental aspect, a theoretical explanation is proposed, which is based on the laws of surface tension and anatomy. In different ways, values for the effect of surface tension have been calculated, which come very close to the experimentally determined values. The recoil due to the surface tension is a function of the radii of curvature of the alveoli. These conform in the expiratory phase to flat spherical sectors with a relatively large radius, i.e., a relatively small surface recoil. With maximal inspiration, the alveoli approach their equivalent hemisphere-like shape with the smallest possible radius, but the effect of surface tension is great. The analysis of the curves, taking into account the theory, suggests that in situations of lower states of expansion [or stretch/elongation], the reduction in size of the alveoli brings with it a reduction in volume of the small alveolar ducts. These test results support the concept . . . regarding the supportive and stabilizing function of the elastic fibers.
   With the recognition of the importance of the physico-chemical force, the surface tension, one of the basic concepts of respiratory mechanics, one of the forces necessary for life, has been transferred from the realm of morphologic viewpoint into the area of functional classification [or evaluation].
5. The possibility of short term changes of the recoil forces of the lung exists. Firstly, through nervous pathways the curvature of the alveoli and thus the effect of the surface tension can be modified by the sphincter like smooth muscle structure at the base of the alveoli. Secondly, the recoil force can be increased or decreased by fluctuations in the H^+-ion concentration (CO_2 tension) in the alveoli.
6. The consequences of the concept of so-called elasticity of the lung in the newborn are discussed. Here too there is a significant role of surface tension in the alveoli, an essential factor.

(von Neergaard 1929)

von Neergaard also noted that for the newborn infant, "... a lower surface tension would be useful for the respiratory mechanism, because without it pulmonary recoil might become so great as to interfere with adequate expansion," and "surface tension as a force counteracting the first breath of the newly born should be investigated further" (von Neergaard 1929, pp. 393–394). [Note. Only recently did I learn that an alternative translation has been presented elsewhere (von Neergaard 1975, pp. 289–290)].

After pointing out several issues with Neergaard's report, John A. Clements has written:

> Neergaard's paper will always remain a classic in the literature of pulmonary surface tension. It was the first of its kind, and its inventiveness and scholarship are legendary. It is likely that we shall never know exactly what ideas and events led to Neergaard's association of surface tension with lung elasticity, but it is clear that he understood the nature of surface tension and capillary phenomena before he did his definitive work. . . . His prior papers show that he was well educated in physics, and familiar with concepts of equilibrium, mechanical forces, and mathematical description of physical systems. He understood the origin of surface tension in intermolecular attractive forces and in the asymmetry of the force field on molecules in the limiting layer. He was aware that surface tension causes "all the phenomena of capillary chemistry." It appears that he was well prepared for his work on alveolar surface tension. In fact he was building on knowledge that had been accumulating for at least three centuries. ... Neergaard's paper was a *tour de force*, a beautiful creation apparently sprung fully formed like Athena from Zeus' brow, a startling marriage of the most sophisticated pulmonary physiology of his time with the classical theory of capillarity. Unhappily, it was a case of pearls before swine, for this wonderful paper was largely ignored during the next 25 years. . . . Perhaps von Neergaard did not promote the ideas sufficiently.
>
> (Clements 1996, pp. 210–212)

In his insightful historical review of research on pulmonary surfactant, Julius H. Comroe, Jr., refers to von Neergaard's work as "premature science," in the sense that at that time there was no established paradigm or physiologic role to which this discovery contributed (Comroe 1977a). Although von Neergaard attempted to isolate a surface-active material, he was not successful (Clements and Avery 1998).

Concurrently with Pattle, and independently, in attempting to understand the basis of seemingly conflicting data from other investigators, John A. Clements and colleagues at the US Army Chemical Warfare Laboratories, Edgewood Arsenal in Maryland, were exploring some aspects of pulmonary responses to nerve gas poisoning. Among other responsibilities, Clements was placed in charge of overseeing some research on pulmonary mechanics being conducted under the aegis of a US Army contract in Boston. This experience stimulated further his interest in pulmonary surface tension. Based on the assumption that this fluid had a fixed surface tension as that of plasma (40–50 dynes cm^{-1}), one investigator had calculated the alveolar surface area to be only one-tenth that estimated from morphometric analysis (Radford 1954). Questioning this result, Clements modified a Langmuir-Wilhelmy balance for his studies and developed the methodology to quantify surface tension of pulmonary extract in several species (rat, cat, and dog). In contrast to the assumption that surface tension remained constant, Clements showed that upon compression, pulmonary surfactant allowed surface tension to decrease to near zero (~10 dynes cm^{-1}) (Clements 1957; Clements et al. 1961). As was clear, this was the "anti-atelectasis factor" that allowed alveoli to remain open at end expiration and presented a fresh paradigm for understanding alveolar stability. Clements and colleagues proposed:

> The uniformity of the result obtained in these several methods and preparations argues strongly for the presence of a peculiar material on the alveolar surface. Its minimum coefficient of compressibility is between 0.01 and 0.02 cm/d. This value places it in the

> category of 'liquid' films. . .. The film can apparently be reversibly compressed to 50% of its initial area. After further compression, the film apparently ruptures on re-expansion. This suggests a solidification or gelation, or folding on marked compression which is irreversible.
>
> (Brown et al. 1959, p. 720)

In several reports, Clements has described the development of this line of investigation (Clements et al. 1958; Clements 1962) and corrected several errors suggested by others (Clements 1996). As an aside, Clements' seminal paper of 1957, in which he announced the discovery of pulmonary surfactant, was rejected by the journal *Science* and finally was published in what he described as a non-peer-reviewed *déclassé* rag. Despite the journal in which the paper appeared, it became a citation classic (Clements 1997, p. 5). In 1974, Clements was elected to the National Academy of Sciences.

A decade previously, Peter Gruenwald, a Viennese pathologist first working at the Margaret Hague Maternity Hospital in Jersey City, and later at the Mt. Sinai Hospital in Baltimore, had observed that in infants who were stillborn, the alveoli were distended with fluid. In contrast, those infants who died soon after birth showed abnormal patterns of aeration with some groups of aerated alveoli alternating with areas of atelectasis. In measuring the pressure required to inflate these lungs, Gruenwald determined that with air this required pressure was twice that with saline. Experimenting with a surface-active compound to decrease the pressure required to inflate the lungs with air, he concluded that surface tension plays a major role in the initiation of breathing and expressed doubt that hyaline membranes were responsible for resorption atelectasis (Gruenwald 1947). Clements has written:

> Gruenwald was a prophet crying in the wilderness. He had little contact with respiratory physiologists until 10 years later (he and I became collaborators for a short time, after the discovery of lung surfactant) . . . and his message, like Neergaard's, was lost to mainstream thought. In fairness it should be added that his observations did not explain why some infants experienced respiratory distress and others did not.
>
> (Clements 1996, p. 217)

Other reports noted the "liver-like" state of the airless newborn lungs, with the presence of a "vernix membrane" (Miller and Hamilton 1949) or "hyaline membrane" (Blystad et al. 1951; Tran-Dinh-De and Anderson 1953) lining the alveoli. The fibrinoid content of these membranes, suggesting their origin from blood plasma that had leaked into the alveoli, was soon established (Gitlin and Craig 1956).

In the mid-1950s, the pediatrician/neonatologist Mary Ellen Avery (1927–2011), at the Johns Hopkins Hospital, developed a keen interest in the respiratory problems of the newborn as she observed too many premature infants with RDS struggle for a gasp of air, many too die. Later working with the physiologist and authority on pulmonary mechanics Jeremiah (Jere) Mead (1920–2009) at the Harvard Medical School and School of Public Health in Boston (Mead 1961; Mead et al. 1957), she recognized the importance of Clements' findings and visited him to learn how to use the surface balance. She has written:

> Around Christmas-time 1957, I drove to Edgewood and visited Clements. I saw the surface balance, returned to Boston, and decided to explore the reason why lungs of infants who died of hyaline membrane disease never had foam in their airways. It is obvious that the reason they lacked foam was that they did not have surfactants with the capacity to reduce surface tension when surface area was reduced. It was straightforward to demonstrate that deficiency in surfactants on a surface film balance modeled after that of Clements.
>
> (Avery 2011, p. 1082)

Avery and Mead then applied findings from the work of Clements and Pattle to the treatment of infants with respiratory distress and hyaline membrane disease. Regarding the nature of lung fluid extracts from markedly premature, as compared to mature, newborn infants, Avery and Mead concluded:

> The results show that without exception the surface behavior of lung extracts of the nine infants with hyaline membrane disease was different from that of infants dying from other causes and the same as that of infants smaller than 1200 gm. This suggests that the disease is associated with the absence or delayed appearance of some substance which in the normal subject renders the internal surface capable of attaining a low surface tension when lung volume is decreased.
>
> (Avery and Mead 1959, p. 521)

They then related their results to the pathogenesis of the disease:

> Thereafter [following the first inspiration], during expiration, the alveolar surface of the normal lung would have diminished tension [<10 dynes cm^{-1}]. . ., thus reducing the tendency of the air spaces to collapse. On the other hand, in a lung lacking this lining material, surface tension would tend to remain high [>20 dynes cm^{-1}] during expiration; the air spaces would be unstable, and some would collapse.
>
> (Avery and Mead 1959, pp. 521–522)

Avery and Mead summarized their findings:

> Recent observations suggest that a low surface tension in the lining of the lung may permit stability of the alveoli at end-expiration. Lacking such a material, the lung would be predisposed to collapse. Measurements of the surface tension of lung extracts confirm the presence of a very surface-active substance in lungs of infants over 1100–1200 gm, and in children and adults. In lung extracts of very small premature infants and infants dying with hyaline membrane disease the surface tension is higher than expected, suggesting that the surface active material is deficient. The possible role of this deficiency in the pathogenesis of hyaline membrane disease is discussed.
>
> (Avery and Mead 1959, p. 523)

Avery has noted the financial circumstances of grant support (or lack thereof) for these now classic studies:

> It is perhaps pertinent to note that the studies done in 1957–59 were as a special fellow of The National Institute of Neurological Diseases and Blindness, but without any separate grant support. The facilities and supplies were provided by the Department of Physiology, Harvard School of Public Health, but since all the equipment was assembled from laboratory leftovers, the cost was minimal. I made the first trough from a wooden slide box lined with paraffin; only after the original observations did we splurge on a teflon trough, made by a plastics manufacturer in Cambridge.
>
> The essential attribute in the environment was the encouragement to pursue new ideas, and a receptivity even to their initial halting presentation. . .
>
> (Avery 1977, p. 3)

As noted by Nicholas Macy Nelson, Emeritus Professor of Pediatrics at Pennsylvania State University and who had worked in Boston with Clement Smith:

> Present-day Fellows who have had their 'breakthrough' research underappreciated can take heart from the pediatric *Alte Herren* [old men] of 1959 that Mel's paper on the high surface tensions she found in [premature infant] lungs was of insufficient interest [to be accepted] for presentation at the spring pediatric meetings.
>
> (Nelson 2008, p. e4)

This work of Avery and Mead was to provide a scientific rationale for the treatment of respiratory distress syndrome in the newborn with continuous positive airway pressure (CPAP; see below). Later, upon returning to the Johns Hopkins University in Baltimore, in a study comparing two 5-year periods (1944–1948 and 1954–1958), in which the first group had experienced higher ambient oxygen concentrations important in the pathogenesis of retrolental fibroplasia, Mary Ellen Avery and Ella Hutzler Oppenheimer (1897–1981) described the pathologic findings in the lungs in live-born, premature infants who had died of hyaline membrane disease. Of interest in this regard, they found no significant difference between the two groups (Avery and Oppenheimer 1960). Avery was the recipient of many awards and honors. Among others, these include the National Medal of Science from President George Herbert Walker Bush (1991), election to the National Academy of Sciences (1994), President of the American Association for the Advancement of Science (2003), and receipt of the American Pediatric Society's highest award, the John Howland Medal (2005) (Hostetter 2005). Avery has presented a historical review of hyaline membrane disease, including categorizing the critical observations, epidemiology, diagnosis, surfactant and its physiologic role and biosynthesis, approaches to therapy, and early studies on prevention with antenatal glucocorticoids (Farrell and Avery 1975), and has recounted some personal aspects of her vital contributions (Avery 1975, 1995, 2000, 2011; Avery et al. 1986).

At the time of Mary Ellen Avery's receipt of the John Howland Medal, Lewis R. First of the University of Vermont sang a song from the Broadway show "Mame," with the lyrics rewritten:

> You helped the tiniest infants survive—Mel
> You made neonatology thrive—Mel
> You've mentored thousands of trainees
> And given us advice that's always right.
> You've smartened all of our brainees
> And helped us set our goals and hit new heights!
> Tonight we all just want to say wow—Mel
> You've earned the award we fondly call How—Mel
> Your many contributions
> Have helped children who are sick and who are well.
> We owe so much to what you've done
> From you we've learned at least a ton
> To us you're truly number one—here's to Mel!
>
> (Hostetter 2005, p. 1315)

Observing the previous era of surfactant research as rather "monastic," John A. Clements has written:

> With Avery and Mead's publication . . . the subject entered its secular phase. . . . The flood gates were open, and everyone could see the potential applications to clinical problems. Surfactant research became respectable, grant money started to flow into it, and an exponential increase in publications began. Over the next 35 years the field diversified and became fair game for biochemists, anatomists, cell biologists, molecular biologists, and therapeutic adventurers, myself included. Nowadays, surfactant substitution treatment saves the lives of thousands of infants with respiratory distress syndrome every year and substantially reduces the cost per survivor, and it may well be applicable to other lung diseases. While the necessary knowledge was being painfully extracted from a reluctant Mother Nature, a rich harvest of information was garnered about lung biology, hormonal control of development, and several related subjects. Some observers of the history of surface tension in the lungs may bemoan the loss of our monastic *Weltanschauung* [conception of the world]. Not I.
>
> (Clements 1996, pp. 225–226)

Following these discoveries, a number of questions became evident regarding this substance that could lower the surface tension in alveoli with changes in the respiratory cycle (Comroe 1977a, b, c, d). Julius Comroe summarized these succinctly:

> What is surfactant? Where does it come from, where does it go and what regulates its formation and movements? Can it or a similar substance be prepared synthetically and used as replacement therapy? Can the physician, instead of nature, make an immature lung become mature? Can the physician not only maintain the life of a newly born baby until the lung matures but also ensure normal postnatal function of all organs?
>
> (Comroe 1977c, p. 497)

On the basis of roentgenographic evidence, hydrostatic pressure changes, and latex filling of the vessels, earlier, Charles C. Macklin had suggested that inspiration and expiration are associated with distension and contraction, respectively, of the pulmonary arteries and veins, with them serving as an "accessory heart." He referred to this as the "lungbeat" that mirrored the heartbeat (Macklin 1946, p. 229). Later, Macklin postulated that alveolar type II cell (pneumocyte) was the source of the lining layer of the alveoli, which he believed was mucoid and maintained ". . . constancy of the surface tension of the alveolar wall . . . a matter of paramount importance" (Macklin 1954).

At this point, a critical challenge for clinicians was that of identifying before birth those infants who would be born prematurely without adequate pulmonary surfactant. By biochemical analysis, in 1961, three groups discovered that the surface-active compound contained a large component of phospholipids as well as protein. Clements, having joined the faculty at the University of California, San Francisco, with his colleagues established that phospholipid dipalmitoyl phosphatidylcholine (lecithin) was the most abundant component and a critical surface-active compound (Klaus et al. 1961). With the use of infrared absorption spectroscopy, Pattle and a colleague also demonstrated that lecithin was a component (Pattle and Thomas 1961). The same year, Sue Buckingham (1923–1969) of Johns Hopkins recognized in sheep the phospholipid nature of this material

(Buckingham 1961). In the developing mouse, Buckingham with Avery then demonstrated the temporal correspondence between the appearance of osmiophilic lamellar inclusions in alveolar type II cells and the capacity to lower surface tension (Buckingham and Avery 1962). Later studies would establish the role of these osmiophilic lamellar bodies as the origin of the phospholipids of pulmonary surfactant. For instance, by subcellular fractionation, Clements and coworkers demonstrated that the mitochondrial fraction of alveolar cells provided the richest source for this activity (Klaus et al. 1962; also see Clements 1997; Clements and Avery 1998), presumably because it included type II cell lamellar bodies.

A decade later, by analyzing fluid from the newborn lung as a function of gestational age, Louis Gluck (1924–1997) with his colleagues at the University of California San Diego established that lecithin increases dramatically at ~35 weeks of gestation. By measuring in amniotic fluid the concentration ratio of lecithin (L) to sphingomyelin (S) (the latter of which remains constant or decreases slightly, e.g., the L/S ratio), Gluck provided clinicians with a diagnostic tool, the L/S ratio, to gauge the maturity of the developing fetus and, in instances of premature labor, to alert attending physicians to the need for special care (Gluck et al. 1971; Gluck and Kulovich 1973). The following year, Clements with coworkers described a relatively simple qualitative "shake test" that could be performed at the bedside to establish the degree to which adequate disaturated lecithin was present in the fetal pulmonary and amniotic fluid and therefore be predictive for respiratory distress syndrome (Clements et al. 1972). Clements group also identified a surfactant-specific protein (King et al. 1973), which subsequently led to the discovery of several proteins (surfactant proteins A–D) associated with the lipids of pulmonary surfactant (Wright and Clements 1987). A number of other aspects of the biochemistry and physiology of pulmonary surfactant have been reviewed (Halliday 2008; Tierney 1989; van Golde et al. 1988), as well as that of respiratory distress syndrome (Reynolds 1975; Stahlman 1984; Tooley 1977).

Following the discovery and introduction of the animal-derived Tokyo-Akita (TA) surfactant for replacement of deficient surfactant in the lungs of RDS premature newborns (Fujiwara et al. 1980), the field of exogenous surfactant therapy of such infants has been revolutionized (Collaborative European Multicenter Study Group 1992). Thurman Allen Merritt with Gluck group in San Diego and Mikko Hallman of the University of Helsinki, Finland, pursued studies on the benefits of surfactant therapy for infants with RDS. For instance, in five very low birthweight (<1000 g) RDS infants, a bolus of human surfactant isolated from amniotic fluid at the time of cesarean section produced a dramatic improvement of symptoms and blood gas values which lasted 8–15 h (one infant later died) (Hallman et al. 1983). This group also showed that 22 infants of similar RDS status demonstrated dramatic improvement with a lower rate of complications, compared to the controls which received only intermittent ventilation (Hallman et al. 1985). In an expanded clinical trial of preemies (24–29 weeks gestation with RDS, a L/S ratio <2, and absence of phosphatidylglycerol), 31 surfactant-treated infants showed significantly less requirement for respiratory support and fewer cases of bronchopulmonary dysplasia, pulmonary interstitial emphysema, or pneumothorax. The number of deaths in

this group was less than one-third that of controls (Merritt et al. 1986). These dramatic results were confirmed in an even larger study of surfactant-treated RDS preemies; however, "rescue treatment" given almost 4 h after birth showed no significant improvement (Merritt et al. 1991).

As is well remembered, the disastrous epidemics of poliomyelitis in the first half of the twentieth century, and particularly by mid-century, struck fear in the hearts of all, regardless of ethnic group or social status. These almost annual plagues had a profound impact on society and medicine (Daniel and Robbins 1997; Paul 1971). To help compensate for the polio-associated central nervous system bulbar respiratory failure and weakened respiratory muscles, the use of the Drinker style negative pressure "iron lung" helped to preserve many whose lives otherwise would have been lost (Drinker and McKhann 1929). In this era, a major problem in the management of infants was ventilation in those with respiratory distress syndrome. Modification of the "iron lung" and other inventions played a key role in saving the lives of infants with this disease. Although it is unknown who first used assisted ventilation for infants, the Scottish-born American inventor Alexander Graham Bell (1847–1922) designed and built a whole body, negative pressure respirator for use with newborns. Presented at a Montreal meeting of the American Association for the Advancement of Science, his contribution was "... met with little enthusiasm" (Stern 1970, p. 24). In the late 1950s and early 1960s, several groups refined this modality, using negative pressure for infants with respiratory failure (Stahlman et al. 1965). Leo Stern of the Montreal Children's Hospital, McGill University, described in some detail the experience of that group and the criteria for such therapy (Stern 1970; Stern et al. 1970). Other early workers employed intermittent positive pressure ventilation for this purpose (Adamson et al. 1968; Heese et al. 1963; Owen-Thomas et al. 1968; Swyer 1970; Thomas et al. 1965). Complicating this picture, however, were reports of severe "respirator lung" disease (now known as bronchopulmonary dysplasia) with a high mortality rate that followed these endeavors (Hawker et al. 1967; Northway et al. 1967). A 1969 Paris conference of experts weighed the role of assisted ventilation in the treatment of these infants and considered the extent to which the possible complications outweighed the potential benefits (Symposium... 1970; Philip 2003).

Despite these setbacks, long-term positive pressure ventilation proved its value (Donald and Lord 1953; Donald et al. 1958; Smythe and Bull 1959). For instance, on the basis of observation of 150 infants with respiratory distress syndrome over 4 years, Robert Usher of the Royal Victoria Hospital, Montreal, reviewed many features of the disease. In addition to assisted ventilation with oxygen, he stressed the need for intravenous glucose and sodium bicarbonate to correct hypoglycemia, acidosis, and electrolyte imbalance with hyperkalemia (Usher 1959, 1961). Of interest, the editor of the journal in which one of his original papers was published amended a series of seven caveats concerning the validity of Usher's recommendations (Usher 1961).

About this time, Maria Delivoria-Papadopoulos and Paul Robert Swyer at the Hospital for Sick Children and the University of Toronto reported on a rather extreme case for that time, of an 1800 g infant of 34 weeks gestation, who, with

assisted pulmonary ventilation, survived cardiorespiratory arrest and RDS with no neurological sequelae at 6 months of age (Delivoria-Papadopoulos and Swyer 1964). Shortly thereafter, George Albert Gregory and the San Francisco group reported their striking success with the use of continuous positive airway pressure (CPAP), rather than intermittent pressure, in the management of premature infants with respiratory distress syndrome (Gregory et al. 1971). The dramatically improved oxygenation demonstrated by these workers no doubt was a consequence of ventilating incompletely collapsed air spaces, as well as reducing regional vascular resistance and the right-to-left shunt. Of the 20 infants studied, 16 survived (80%) including 10 that weighed <1500 g at birth. This is compared with a survival rate of 20% or less in the controls (Gregory et al. 1971). Soon, other reports verified the use of CPAP in increasing survival of infants with RDS (for instance, see Krouskop et al. 1975). Because oxygenation is a function of mean airway pressure, the use of positive end-expiratory pressure can result in requirements for high peak inflation pressure and fractional concentration of inspired O_2 (Boros et al. 1977; Delivoria-Papadopoulos 2003). Gregory has given a historical account of the development of CPAP (Gregory 2004).

During this period, several other problems concerned fetal-neonatal pulmonary physiology. One was the urgent need to quantify certain aspects of pulmonary function in the newborn infant. This was advanced by the development of a body plethysmograph with a pneumatic cuff seal for such studies by Kenneth William Cross of St. Mary's Hospital Medical School, London (Cross 1949; Cross and Warner 1951). Another problem was that of understanding the factors that regulated fluid secretion in the lung of the fetus, and its reabsorption in the newborn following birth, as conflicting findings had been reported in terms of fluid dynamics and mechanisms. Following a report that the periarterial tissue and lymphatic vessels of the lungs of newborn rabbits were quite distended with eosinophilic staining liquid (believed to be rich in protein; Aherne and Dawkins 1964), the pediatrician-physiologist Leonard Birnie Strang (1925–1997) of the University College Hospital (UCH), London, tackled this problem. For a number of years, he was at the Postgraduate Medical School, Hammersmith Hospital, London. Later, he worked at the Nuffield Institute with Dawes and then with Clement Smith in Boston. In the lamb and newborn infant, Strang performed a series of studies to establish some basics such as the mechanisms of liquid uptake from the lungs, showing a striking increase in lymph flow during the first hours following the onset of respiration (Strang 1967). He also performed a series of studies on growth and development of the fetal lung; pulmonary permeabilities of ions and nonelectrolytes; fluid secretion rates, composition, and mechanisms; fluid reabsorption following birth; the role of thyroid hormones and hydrocortisone in the reabsorptive process; and so forth. In a monograph (Strang 1977b) and several reviews (Strang 1976, 1977a, 1989, 1991), Strang summarized his work. These studies were crucial to understanding some basic features of the lung during the perinatal period. In 1990, Strang was awarded the James Spence Medal for "... excellence in research, in teaching, and in clinical care," by the British Paediatric Association (now the Royal College of Paediatrics and Child Health) (Lloyd 1990; Oliver 1997). (Other neonatologists and

physiologists who previously had received that distinction included Robert A. McCance, 1961; Geoffrey S. Dawes, 1969; Kenneth W. Cross, 1979; Elsie May Widdowson, 1981; and Sir Peter Tizard, 1986.)

Regarding Strang and the Postgraduate Medical School at the Hammersmith Hospital, John Burnard West, of the University of California San Diego, has written:

> We were together in the Postgraduate Medical School at Hammersmith Hospital ... for several years starting in the late 1950s. Another Australian, Kemp Fowler, had just built the first mass spectrometer specifically designed for medical research and we were busy trying to get as much information as we could from expired gas. The mass spectrometer gave a rapid readout of PO2, PCO2, and PN2. I remember that Leonard worked on a method of measuring blood gases by equilibrating a small bubble of air with blood, and subsequently measuring the PO2 and PCO2 in the air bubble using the mass spectrometer. This, in effect, was an extension of the method introduced by Richard Lord "Dick" Riley [1911–2001] at [Johns] Hopkins for measuring blood gases. [see Permutt 2002] I don't think much came of Leonard's work in this area, or at least I cannot see a publication related to it on PubMed. However, blood gas electrodes began to be used shortly afterwards so maybe that put an end to that line of research.
>
> I knew Leonard and his first wife fairly well and used to visit them in Barnes from my flat in Hammersmith. We spent some evenings playing chess together. I also visited Leonard when he was working in the Nuffield Institute with Geoffrey Dawes, and I remember attending an experiment where they were looking at pulmonary vascular resistance in the newborn lamb.
>
> I spent a year with Hermann Rahn (1912–1990) in Buffalo in 1961–1962 and Leonard was in Boston at the same time. I visited him on one occasion in their home to the west of Boston. An incident here made an impression on me. Leonard had a health problem (it may have been a renal stone) and the first thing he did was take a flight back to London. I sometimes cite this as an example of the fact that a good test of where somebody regards his home is where he goes if he gets sick.
>
> As I am sure you know, Leonard was a great Francophile. I think he had spent vacations in France as a medical student. After he retired from UCH he went to live in *Volx* in *Haute Provence*. My wife and I visited him and his second wife there on a couple of occasions. Leonard tried hard to integrate himself into the local community although I am not sure how successful that was. *Volx* was very parochial and few foreigners lived there. I know that Leonard spent some of his time translating [the French novelist and critic Marcel] Proust [(1871–1922)].
>
> Leonard was one of the most charming, generous and good-natured persons that I ever knew. He coped with his disability very well although I think when I visited him in *Volx* he found getting around rather tiring. He was a wonderful person and many people at UCH remember him very happily.
>
> (Letter from JBW to LDL, 24 August 2010)

As noted, pulmonary surfactant was shown to contain a lipid component, disaturated phosphatidylcholine, and a complement of several proteins, surfactant proteins (SP)-A, (SP-A), SP-B, SP-C, and SP-D, the synthesis of both components of which is stimulated by cortisol (Liggins 1994; Rooney 1985). In the main, these proteins are produced by so-called type II pneumocytes or alveolar cells. As an aside, several of these proteins (SP-A and SP-D) also may play a critical role in stimulating immune responses, thus modulating host defense in the lungs and protecting against infections to which the newborn is vulnerable (Wright 2006).

Following the discovery of surfactant, the race was to develop and patent the "Holy Grail" of synthetic substitutes, artificial surfactant, to be used on infants with respiratory distress syndrome, and to discover the methods to administer these compounds in the optimal manner. (For review, see Comroe 1977c; Robillard et al. 1964; Scarpelli 1995.) Nonetheless, despite all that has been learned concerning the composition and function of pulmonary surfactant, little was known of the fundamental cellular and molecular mechanisms that regulate prenatal type II cell maturation, surfactant protein and lipid synthesis, and related mechanisms and phenomena (Perez-Gil and Weaver 2010). As a measure of success, with advances in the understanding of pulmonary physiology of the newborn infant, in the USA, the number of infants who die from RDS has dropped exponentially from that of the 1950s to the present.

With advances in cellular and molecular biology, during the past several decades, a new era of surfactant biology has emerged (Whitsett 2014). Detection of disaturated palmitoyl phosphatidylcholine stimulated the search to understand at a deeper level surfactant biochemistry and physiology. The finding that the biophysical behaviors of lipid-rich surfactant extracts differ from those of synthetic phosphatidylcholine-lipid mixtures suggested that other compounds contribute to the unique surface tension characteristics of pulmonary surfactant. This led to the discovery of SP-B and SP-C, critical proteins in surfactant function (Notter et al. 1987; Whitsett et al. 1986a, b), and its ability to reverse atelectasis in the preterm rabbit (Rider et al. 1993). In conjunction with the advances in clinical application of surfactant replacement therapy of the 1980s were the purification, amino acid sequencing, and preparation of antibodies and molecular probes to clone the cDNAs and genes that encode surfactant proteins A to D to reveal their functions (Ariki et al. 2012; McCormack and Whitsett 2002; Shulenin et al. 2004; Whitsett and Weaver 2002; Whitsett et al. 2010). These advances helped to bring understanding of fundamental issues regarding development of the lung, its maturation, surfactant protein gene mutations, surfactant protein structure and function, and more specific diagnosis and treatment for pulmonary diseases (Whitsett 2014).

19.2 Corticosteroids and Maturation of the Fetal Lung

Along this line, as is widely recognized, the cellular and molecular biology of growth and development of the lung is extremely complex (Copland and Post 2004). As noted earlier, it was in fetal sheep in the early 1960s that the virtuoso "Mont" Liggins and his coworkers at the University of Auckland demonstrated the ability of adrenal glucocorticoid hormones to induce the enzymes required for surfactant synthesis and thus maturation of the lungs of the premature infant (Liggins 1969, 2000; Mescher et al. 1975). In five of the cattle with prolonged gestation due to pituitary and adrenal anomaly referred to above, delivered by hysterotomy, these authors administered hydrocortisone for up to four days

following delivery in an attempt to optimize their respiration (Holm et al. 1961). Liggins later recorded:

> I remember one morning . . . there was a lamb lying in a cage with its mother. A lamb that had been infused as a fetus with cortisol. And to my surprise this lamb was still breathing, not very healthy breathing, but it was alive and breathing. It had no right to be. It was so premature that its lungs should have been just like liver, and quite uninflatable. And this struck me as surprising.
>
> (Watts 2010, p. 1140)

The value of steroids soon was confirmed by Avery and her group (DeLemos et al. 1970). Liggins and the pediatrician Ross Nisbet Howie of the National Women's Hospital, Auckland, later reported a clinical trial of 213 mothers in spontaneous premature labor, or who otherwise were to be delivered before 37 weeks, who were given betamethasone. In 77% of the 117 mothers so treated, delay in delivery of at least 24 h was achieved. In these infants, the incidence of both respiratory distress syndrome and neonatal mortality was markedly decreased (9% versus 26%; $P < 0.01$; 3% versus 15%; $P < 0.01$, respectively). None of the infants whose mothers received steroid died of hyaline membrane disease or intraventricular cerebral hemorrhage (Liggins and Howie 1972). Liggins later recorded that the manuscript for this paper initially was submitted to the journal *Lancet* but "... was rejected on grounds of lack of general interest" (Liggins 1982, p. 305). In the fetus, Liggins and colleagues also demonstrated that variables such as lung volume and levels of saturated phosphatidylcholine, the major surface-active component of pulmonary surfactant, were correlated more closely with cortisol levels than with gestational age (Kitterman et al. 1981). Others demonstrated the value of glucocorticoids in lung maturation in the fetal baboon (Kotas and Kling 1979).

Mary Ellen Avery has made several important points in regard to the challenges of antenatal steroid use. First, she pointed out that it was Florence Moog (1915–1987), an anatomist at Washington University, St. Louis, who, in studies of the appearance with maturation of phosphatase in the duodenum of the suckling mouse, demonstrated the role of glucocorticoids in mediating this response (Moog 1953). Thereby, Moog's studies initiated the idea of the role of hormones on organ maturation. These observations stimulated Sue Buckingham to test this idea, demonstrating in the fetal rabbit the role of corticosteroids in maturation of pulmonary phosphatase (Buckingham et al. 1968). Unfortunately her untimely death prevented her from completing more definitive studies along this line of investigation.

Avery also has commented on the lack of enthusiasm and, in fact, overt hostility on the part of professional colleagues, reviewers of manuscripts, and others, regarding the antenatal administration of glucocorticoids to pregnant women in the event of preterm labor (Avery 1984a, b). For instance, she quotes one such hostile reaction:

> . . .the article under discussion deals simply with long-term follow-up rather than efficacy in preventing the problems of prematurity. (In fact, their data show no significant differences between treated and placebo groups with regard to weight or gestational age at delivery). In

> the collaborative study on steroid administration, there was no significant difference in effect of steroids, except in a very small subgroup of white, non Hispanic patients. Dr. Avery's final sentence 'The failure to do so (treat with glucocorticoids) is costly in terms of morbidity and, I believe, constitutes poor practice,' is clearly a biased opinion and inappropriate … Their use is still experimental and should be limited to those institutions where protocols can be strictly adhered to … After a follow-up program of only 3 years, and a questionable risk/benefit ratio, Dr. Avery's dare to use steroids for this indication or practice poor medicine has no place in either modern perinatology or *The Journal*.
>
> (Avery 1995, p. 134)

Avery recalled the tongue-in-cheek wisdom of the German philosopher Arthur Schopenhauer (1788–1860) regarding the response to new ideas:

> The first response is that it is ignored. The second is that it evokes hostility in the form of many attempts to disprove it. Finally, it merges in with the collective wisdom so that the response is "haven't we known this all along?"
>
> (Avery 1995, p. 133)

Several years following the introduction of corticosteroids, a large prospective double-blind study demonstrated the role of antenatal steroids in the prevention of respiratory distress syndrome in those infants who were markedly premature (incidence of 21 versus 59% in non-treated controls, with both severity less and death rate lower, $P < 0.05$ for each; Papageorgiou et al. 1979). With these studies, a new era of fetal-neonatal medicine was launched. Nonetheless, it was a decade until a 1994 NIH-sponsored "consensus" advocated antenatal steroid treatment in women in spontaneous premature labor (NIH 1994), and the use of this modality became the "standard practice." A late twentieth-century meta-analysis of randomized trials of antenatal corticosteroid therapy from 1972 to 1994 provided irrefutable evidence of the efficiency and virtue of such therapy (Crowley 1995). To accelerate fetal pulmonary maturation and the production of surfactant, a 2000 NIH Consensus Conference *Antenatal Corticosteroids Revisited and Repeat Courses* recommended further that a single course of antenatal steroid therapy be given for pregnant women between 24 and 34 weeks gestation who are at risk of delivering within 1 week. They concluded that the then current benefit-risk data were insufficient to support the routine use of repeat or rescue courses of antenatal corticosteroids (NIH 2000). A decade later, the Gainesville group demonstrated the role of pulmonary mineralocorticoid receptors in mediating the composition of fluid in the fetal lungs (Keller-Wood et al. 2011). Liggins has reviewed the major organ systems, including the thyroid gland, lungs, liver, and adrenal, in which a maturational response to glucocorticoids has been demonstrated (Liggins 1994). He also wrote a thoughtful account of these many contributions (Liggins 2000).

The widespread use of antenatal glucocorticoid therapy to enhance fetal lung maturation also has stimulated investigation of the extent to which this alters the function of the hypothalamic-pituitary-adrenal axis. Several studies, in fact, suggest that this is the case in terms of attenuated adrenal cortical response to stimulation (Davis et al. 2004, 2006; Ng et al. 1997). Clearly, many factors are involved in hypothalamic-pituitary-adrenal axis regulation, and these must be taken into account in terms of antenatal programming of adult health and disease (Rose et al. 2011).

A related major issue in this regard is that of the regulation of the growth and development of the lung. Beyond the maturing morphology (Burri 1984) is that of signaling networks (Ornitz and Yin 2012), growth factors (Alphonse and Thébaud 2011; Meller and Bhandari 2011), mesenchyme (Ahlfeld and Conway 2012), and even the mechanical movements per se in stimulating cell proliferation (Skinner 1989). As can be appreciated, this topic requires an extended chapter of its own.

In response to my questions regarding several aspects of the development of surfactant and the use of corticosteroids and lung development in general, Alan Hall Jobe (1944–) who has contributed greatly to this area wrote:

> My introduction to perinatal research was during my neonatology fellowship in 1975 at University of California San Diego in the lab of Louis Gluck. He had developed the lecithin/sphingomyelin (L/S) ratio (Gluck et al. 1971) and, with Mikko Hallman, had described the changes in phosphatidylinositol and phosphatidylglycerol in amniotic fluid to further refine testing for fetal lung maturation (Hallman et al. 1976). Following discussions with Hallman, I began experiments to understand the "mass action" of surfactant and its components. The initial studies used radiolabeled precursors of phospholipids to measure incorporation (synthesis) into the lung tissue and labeling of lamellar bodies in type II cells prior to secretion into the airspaces of newborn and adult rabbits (Jobe 1977; Jobe et al. 1978). That information, together with measurements of pool sizes of the major surface active substance in surfactant—saturated phosphatidylcholine—allowed me to estimate the slow secretion and subsequent clearance and recycling of surfactant components, metabolic characteristics that proved ideal for surfactant treatment (Jacobs et al. 1982). These metabolic studies in rabbits were then expanded at Harbor-UCLA, with the help of my long-time colleague Machiko Ikegami, to term newborn and premature lambs to allow us better to understand the metabolism and function of surfactant in a large animal models for translation to infants (Jobe et al. 1980, 1983). The metabolic studies of the phospholipids were expanded to include the metabolism and function of the surfactant proteins (Ikegami and Jobe 1998; Jobe et al. 1981).
>
> In Japan, Tetsuro Fujiwara had first described the clinical response of infants to surfactant treatment in 1980 using a surfactant prepared from cow lung (Fujiwara et al. 1980). The investigations that allowed for this initial successful trial primarily came from the reports of surfactant treatment effects from Sweden by Bengt Robertson, a pediatric pathologist, and Goren Enhorning, an obstetrician, who worked together with surfactant treatment models with preterm rabbits (Enhorning and Robertson 1972; Enhorning et al. 1973). At UCLA, Forrest Adams worked with Fujiwara and Ikegami to demonstrate striking surfactant treatment responses in preterm lambs, and that experience resulted in Fujiwara's clinical report in 1980 (Adamson et al. 1968). Dr. Ikegami joined me at Harbor-UCLA and we performed extensive studies of surfactant treatment responses, distribution, and the effects of surfactant treatments on endogenous surfactant metabolism using both preterm sheep and rabbit models. A ventilation system for preterm rabbits was an adaptation of that used by Bengt Robertson, and we collaborated with him on studies with animal source and synthetic surfactant (Robertson et al. 1985; Ueda et al. 1994a). Using these models we demonstrated the phenomenon of surfactant inactivation by proteinaceous and inflammatory material that entered the airspaces with lung injury (Ikegami et al. 1984). We also demonstrated how the preterm lung used the surfactant given for treatment as substrate to reprocess and improve surfactant function (Ueda et al. 1994a, b). These concepts proved to be central to understanding how surfactant treatments work in the preterm lung (Jobe 1993).
>
> We also became interested in the interactions between surfactant and lung maturation, and with the support of the pediatric endocrinologist Delbert Fisher, we characterized the synergetic maturational effects of glucocorticoids and thyroid axis hormones on surfactant

function in preterm rabbit and sheep models (Ikegami et al. 1987, 1991). The initial work was done at Harbor-UCLA, but in 1998 I initiated a now 25 year collaboration with John Newnham in Perth, Western Australia to explore lung maturation using intra-amniotic injections rather than fetal catheterizations to expose fetuses to test drugs (Jobe and Ikegami 1993). We found that antenatal corticosteroids improved lung function by rapid effects on lung structure before surfactant pools increased (Ikegami et al. 2010; Willet et al. 1997). The antenatal corticosteroids changed the dose-response curves for multiple assessments of lung function for both endogenous and treatment doses of surfactant (Seidner et al. 1988). The corticosteroid treatments also decreased the inhibition of surfactant function in the injured preterm lung (Ueda et al. 1995). Synergistic effects of the combined exposures to antenatal corticosteroids and postnatal surfactant also could be demonstrated in humans (Jobe and Ikegami 1993). This US-Perth collaboration has characterized extensively the multi-organ system effects of antenatal corticosteroids in fetal sheep models.

Although we surveyed many hormones for lung maturational effects *in vivo*, none other than corticosteroids were practical clinically. However, clinical reports had suggested that chorioamnionitis was associated with less respiratory distress syndrome (RDS) and Mikko Hallman and his collaborations made the observation that intra-amniotic IL-1 induced lung maturation in fetal rabbits in 1997 (Bry et al. 1997). With his help, we confirmed that observation in fetal sheep and utilized our research program with Dr. Newnham in Perth to explore how fetal exposure to inflammation caused lung maturation and modulated the fetal immune system and virtually every organ system in the fetus. Of note, our colleague Matt Kemp demonstrated striking inflammatory effects in the fetal skin (Kemp et al. 2011). The fetal response differed for exposures to lipopolysaccharide (LPS), interleukin-1 (IL-1), live Ureaplasma, or live *Candida albumins*, demonstrating the range of fetal responses to different inflammatory challenges (Collins et al. 2010; Kramer et al. 2009; Payne et al. 2014). LPS-induced inflammation caused a larger and more consistent lung maturational response than did antenatal corticosteroids, but both stimuli given together further promoted lung maturation (Kuypers et al. 2012). These observations in sheep help explain the relatively low rate of severe RDS even in infants with birth weights <1 kg. Most of the multi-systemic effects (fetal inflammatory responses) from intra-amniotic pro-inflammatory mediators such as LPS are transduced by the initial increases of IL-1 in the fetal lung and gastrointestinal tract (Kramer et al. 2009). Fetal exposure to intra-amniotic LPS or live Ureaplasma can tolerize the fetus to a second pro-inflammatory exposure—effects that may result in long-term programing of the immune system (Kallapur et al. 2007, 2011). Our studies of fetal inflammatory responses from chorioamnionitis in sheep are not directly translatable to the preterm human because of the different placentation in primates. Therefore, at the Primate Center at UC Davis we have initiated studies of intra-amniotic LPS, IL-1, and live Ureaplasma in *Rhesus macaques* to explore the link between chorioamnionitis and preterm labor. These studies focus on the inflammatory cells—primarily neutrophils—that are recruited to the decidua and produce pro-inflammatory products associated with preterm labor (Kallapur et al. 2013; Presicce et al. 2015).

A byproduct of our studies with maternal corticosteroids in the sheep are a number of observations that strongly suggest that the currently preferred corticosteroid for clinical use—a 50/50 mixture of betamethasone-phosphate (Beta-P) and betamethasone-acetate (Beta-Ac) is not the optimal therapy for fetal maturation (Jobe et al. 1998, 2009). Fetal intramuscular treatment with the Beta-P and Beta-Ac mixture causes some lung maturation and no fetal growth restriction. In contrast, maternal treatment causes fetal growth restriction and more lung maturation, but at much lower blood levels of free Beta in the fetus than the fetal treatment. The Beta-Ac component causes blood levels of Beta that are 10-fold lower in the mother and almost undetectable blood levels of Beta in the fetus, yet lung maturation is equivalent to maternal treatment with the Beta-P and Beta-Ac mixture (Jobe et al. 2009). We hypothesize that the maternal corticosteroid treatments signal via the

a b

Fig. 19.1 (**a**) Geoffrey S. Dawes CBE, March 1981. (**b**) Opening the Sunley Research Centre, Charing Cross, London (1985)

placenta to cause the pleotropic effects on the fetus that do not require direct fetal exposure to the glucocorticoid. Ongoing experiments will evaluate that hypothesis. My goal is to develop an inexpensive and safe antenatal treatment for low resource environments.

I also have been interested for many years in how to decrease the injury of the preterm lung from mechanical ventilation. Our studies have used a model to exteriorize the fetal sheep head and chest while maintaining placental perfusion (Kallapur et al. 2007). We then expose the fetal lung to an injury such as stretch (tidal volume) and then return the fetus to the uterus or deliver and ventilate the preterm newborn. Our goal is to learn how injury to the preterm lung is initiated and progresses toward bronchopulmonary dysplasia. We know that, as with chorioamnionitis, the initial inflammation is IL-1 mediated and antenatal corticosteroids, surfactant treatment, and positive end expiratory pressure can mitigate the injury (Berry et al. 2011).

RDS is the disease initially associated with neonatology, and its successful treatment is a tribute to the advances in perinatal medicine. The mortality from RDS was about 2.5 per 1000 live births in the late 1960's with no effective therapies available (Fig. 19.1). While antenatal corticosteroids and surfactant treatments decreased mortality after 1990, the major decreases in mortality occurred because of better general care practices. RDS is no longer a lethal disease unless the infant is extremely premature or there are complicating abnormalities.

(Letter from AHJ to LDL, 6 March 2015)

19.3 A Tribute to "Mont" Liggins

At a 1988 symposium in Rotorua, New Zealand, Advances in fetal physiology…, to mark "Mont" Liggins' retirement from teaching and clinical responsibilities, four dozen associates and fetal-newborn physiologists from around the globe gathered to

review their latest work and to honor the "Master of New Zealand Midwifery." As noted by the organizers:

> Few individuals have had such impact on the development of a field of science as Mont Liggins has had on the development of fetal physiology over the past two decades. Mont has had a significant influence on many aspects of fetal physiology. He pioneered the experimental use of the fetal sheep for studies of fetal physiology. His discoveries of the role of glucocorticoids in the maturation of the lung and on the initiation of parturition determined the path of fetal physiology for many scientists. He contributed to the rediscovery of fetal breathing movements and has studied the significance of these and hormonal factors in lung maturation. Other interests have included the control of fetal growth and fetal prostaglandin physiology. His studies of the pregnant diving seal show the diversity of his interests. His personal qualities have endeared him to colleagues throughout the world.
>
> (Gluckman et al. 1989, p. v)

For the evening's banquet, David J. Mellor of New Zealand's Massey University prepared "A Tale of Mont (or a Montage of Events)":

> In the '50s and '60s physiologists well knew
> That oxygen became short as the fetus **grew**,
> And therefore proposed that to escape this dearth
> Mechanisms **unknown** made the mother give birth!
> But then we all **learnt** from Barron at Yale
> That such **hypoxia's** a myth, supply doesn't fail!
>
> While some workers thought the fetus was passive,
> Most thought it a parasite, its burden so massive
> Its demands would increase 'till burden so massive
> Its demands would increase 'till the **dam** was compelled,
> By dint of long straining, to see it expelled!
> And thus it was by most fetologists figured
> That the process of birth was by the mother triggered!
> There now enters one who is known to us all,
> He's casually dressed and not **very** small,
> He hails from New Zealand, the islands 'down-under,'
> And he sets about blowing these theories asunder,
> For he saw that the fetus with a hole in the head
> Would stay in the uterus until it was dead!
>
> Of course it was Mont who cleared the confusion
> By causing early birth with a **fetal** infusion;
> He showed us how the adrenals get bigger
> To help produce cortisol, the **fetal** trigger
> Which breaks down the barriers that prevent admission
> To the outside world at parturition.
>
> But not only that, for Mont was to show
> How cortisol's needed to make the lungs grow,
> And with other hormones acting they also mature,
> So the young once born can easily procure
> The oxygen it needs in its first vital breath,
> Without which new life soon leads to death!
> This 'Tale of Mont' having progressed thus far
> Now sees him working in Antarctica;
> But the scene's not pretty, our tone must descend,

For there's Mont with his arm up a seal's rear end;
The danger's not small and nor is the smell,
But a progesterone test will do just as well!
Few would dispute that Mont is a winner,
Certainly no one attending this dinner,
Most pause to listen when he rises to speak,
He's a mountain among us, a veritable peak,
He's no mere knoll, he's one of our big 'uns,
The tallest among us—we **salute** Mont Liggins!

David Mellor, July 1988 (Mellor 1989, p. vii)

In his introduction to this symposium, Geoffrey Dawes reviewed "Mont" Liggins' many contributions to science and to life. These included not only studies on the role of the fetal hypothalamic-pituitary-adrenal axis in the timing of parturition but also the role of glucocorticoids in the development of the lung and the value of antenatal glucocorticoid therapy in lung maturation in women with impending premature labor. In regards to Liggins' studies, Dawes observed, ". . . the impact of this discovery on perinatal research was great. Here was evidence of endocrine participation in the physiological preparation for birth. The impact has not yet subsided." He cautioned, however, "The details of the cellular mechanisms are still controversial, and yet further attention has been directed to lung structural development, as a determinant of viability" (Dawes 1989, p. 5). Dawes concluded by remarking upon Liggins' studies in the Weddell seal and not only its cardiovascular responses to both water immersion and free diving (Dawes 1989; Zapol et al. 1979) but the implications for the fetus in utero (Hill et al. 1987). Dawes concluded his tribute to Liggins, a consummate fetal physiologist and fellow fly fisherman, with a quote from Izaak Walton (1593–1683) that there ". . . is another reason of the preference accorded to it, since there is more merit and therefore more pleasure, in excelling in what is difficult" (Dawes 1989, p. 10).

As an aside, from the Sovereign of the United Kingdom in 1980, Liggins received the Polar Medal for his studies in Antarctica. That same year, the Royal Society of New Zealand awarded Liggins the Hector Medal [in honor of Sir James Hector (1834–1907)] for his outstanding scientific research. A decade later (1991), the Queen knighted Liggins, and in 2002 upon her visit to New Zealand, Queen Elizabeth II officially opened the perinatal research unit, named in his honor, the "Liggins Institute," at the University of Auckland.

One who spent several sabbaticals working with Liggins, Robert Kenwood Creasy, formally of the University of California, San Francisco, and the University of Texas, Houston, has written:

> Not much question that he and Dawes opened up the science of fetal physiology. Although initially interested in oral contraception, and assisting with fetal hemolytic disease studies, he soon began his career-defining pursuit, namely the etiology of parturition. He decided to follow a recent hypothesis that the onset of labor was initiated by the fetus, and not the mother as most held, and developed a series of innovative techniques in a chronic sheep model. Mont's quest, initially very much on his own, and with VERY meager support or direction from others, developed the ability to discern the role of the fetal pituitary through a rather ingenious method of transecting the pituitary stalk in a living fetus. He then went on with others, such as Geoffrey Thorburn of Australia to elucidate the mechanism of the onset of parturition in the sheep. Unfortunately, despite his efforts that mechanism of parturition

in humans was still not known at the time of his passing away. However, he was able with his intellect to show that the fetus plays a key role in humans as well.

With these techniques he was able to induce premature delivery by giving ACTH or cortisol to the fetus. He then recognized the importance of the unexpected, namely that the prematurely delivered fetus has partly aerated lungs at a time when they should have been solid. From the above observations to a rigidly controlled clinical trial, he established that the use of antenatal steroid administration to mothers about to deliver prematurely would dramatically lower respiratory distress syndrome and demise in the human neonates. This result has had a major clinical impact throughout the world and was arguably the most important single advance in obstetrical care in the last half century.

He then continued his parturitional studies further elucidating various processes involved, frequently collaborating with the world's leading fetal physiology investigators. He also continued to be involved with studies of the fetal lung. Indeed his counsel and wisdom were continually sought after by visiting investigators throughout the years. He always remained interested in helping those interested in a research career and or clinical medicine. He also played a significant role for many years in investigating the diving mechanisms in pregnant Antarctic seals and their fetuses. These studies were productive and resulted in many novel concepts.

Lastly, having worked with Mont in multiple settings in New Zealand, Antarctica, and the US, and with a wide variety of people, it was remarkable to see him drop everything he was working on and concentrate on helping others, including my two small children when they would come bouncing into the lab on a Saturday morning. He gave major advice to many on their various projects, including my own quest to develop a model of fetal growth restriction in the sheep that would mimic much of that seen in humans.

A true friend and mentor to a very large number of scientists, and certainly one of the Fathers of Fetal Physiology.

(Letter from RKC to LDL 26 May 2012)

As must be clear, this example of the manner in which basic physiology and biochemistry have interacted with clinical medicine to relieve suffering and save lives is one of the most noble examples of the possibilities of translational medicine that one can imagine.

19.4 Blood and Hematology

In early gestation, erythrocytes are produced in the embryonic yolk sac, liver, and perhaps tissues other than the bone marrow. The erythrocytes formed are of larger size, nucleated, and contain more hemoglobin than those of later gestation. During the course of gestation, these fetal erythrocytes demonstrate a number of unique characteristics (Keleman et al. 1979). In the late nineteenth century, on the basis of alkali denaturation rates in adults and newborn infants, Ernst Körber (ca. 1832–ca. 1896) of the University of Dorpat first demonstrated that fetal hemoglobin differed from that of the adult (Körber 1866), and this was substantiated several decades later (Krüger 1888). As is well established, the hemoglobin molecule consists of four heme groups (an organic moiety, protoporphyrin, and central iron atom) bound to the protein globin. The primary structure of hemoglobin is genetically determined by the globin chain amino acid sequence. During the mid-1930s and

thereafter, several studies established the nature of the hemoglobin of the fetus (Hgb F), as opposed to that of the adult (Hgb A) (Brinkman and Jonxis 1935, 1936; Huehns and Beaven 1971; Jonxis 1949; Schroeder 1980). The resistance of fetal hemoglobin to acid elution distinguishes these cells from those of the adult (Kleihauer et al. 1957), a characteristic used to detect fetal erythrocytes in the maternal circulation. In a series of studies on the development of embryonic hemoglobin, Ernst Reinhard Huehns of the University College London with colleagues examined the blood of human embryos of gestational age <90 days by starch-gel electrophoresis. By measurements of electrophoretic mobility, absorption spectrum, and alkaline denaturation, these workers demonstrated new hemoglobins, Gower 1 and 2, consisting of epsilon chains ($zeta_2$ $epsilon_2$ and $alpha_2epsilon_2$, respectively). These represent the earliest globin chain synthesis in the embryo, preceding that of gamma chains and later the beta chains (Huehns et al. 1964a). These workers also illustrated the dramatic changes in non-alpha chains during the course of gestation (Huehns et al. 1964b). In the 1980s and 1990s, the genetic basis for these changes was elucidated with demonstration of the alpha-like and beta-like genes on chromosomes 16 and 11, respectively, and nuances in the combinations of alpha, beta, gamma, and delta peptide chains were discovered (Perrine et al. 1988; Thompson et al. 1991). Recent years have witnessed fresh insights into the complexities of the developmental molecular mechanisms of the beta-globin switch, by which gamma globin expression is silenced. These involve glucocorticoid-mediated changes (Zitnik et al. 1995) and interactions of the transcription factors Krüppel-like factor 1 (KLF1), B-cell lymphoma/leukemia 11A (BCL11A), GATA binding protein 1 (GATA1), and others (Sankaran et al. 2011; Zhou et al. 2010).

X-ray crystallographic studies demonstrate that oxyhemoglobin (R form) and deoxygenated (T form) hemoglobin differ in conformation, oxyhemoglobin being the most compact (Perutz 1964). For the interaction of globin chains with organic phosphates, these conformational changes are critical (Perutz 1970). As noted earlier, a crucial factor in determining O_2 uptake in the lung or placenta and its delivery to tissues is that of hemoglobin O_2 affinity. In general, each mole of hemoglobin can combine with four moles of oxygen (Scherrer and Bachofen 1972). By heme-heme interaction, as oxygen binds to a given heme group, it more readily binds to other heme groups. This mechanism gives the oxyhemoglobin saturation curve its sigmoid shape, as first demonstrated by Christian Bohr (1855–1911) of the University of Copenhagen (Bohr 1905). An equation that describes the oxyhemoglobin saturation (or dissociation) curve was formulated by the 1922 Nobel laureate Archibald Vivian Hill (1886–1977) of the University College London (Hill 1910). (For review of this field, see Astrup and Severinghaus 1986.)

In the adult, the *alpha*/*beta* chain dimers bind allosteric cofactors such as organic phosphates, chloride ions, and protons which decrease the hemoglobin affinity. In the erythrocyte, that organic phosphate in greatest concentration is 2,3-diphosphoglycerate (2,3-DPG) (Benesch and Benesch 1967; Chanutin and Curnish 1967; also called 2,3-bisphosphoglycerate). By cross-linking the *beta*

chains with stabilization of the quaternary structure of deoxyhemoglobin, 2,3-DPG decreases hemoglobin O_2 affinity. In fetal erythrocytes with *alpha*/*gamma* chain dimers, such binding is minimized, resulting in increased O_2 affinity (Bauer et al. 1968, 1969; Delivoria-Papadopoulos et al. 1971a; De Verdier and Garby 1969; Salhany et al. 1971). During the latter third of gestation, the fetal erythrocyte concentration of 2,3-DPG (called by some during the late 1960s and 1970s, the "Direct Path to Grants") rises to adult levels at term (~5 mM L^{-1}). During the first year of life, this increase continues, in concert with a decrease in hemoglobin F, before decreasing again to adult levels (Delivoria-Papadopoulos et al. 1971b; De Verdier and Garby 1969). (For review, see Delivoria-Papadopoulos and McGowan 2011.)

It was in the beginning of the twentieth century that Karl Landsteiner (1868–1943) discovered several isoagglutinins in human blood that were capable of agglutinating erythrocytes from another individual (Landsteiner 1900). Shortly thereafter, Jan Janský (1873–1921) discovered that blood could be classified into four major groups, O, A, B, and AB (Janský 1906/1907). Then, with the onset of World War II, almost simultaneously both Philip Levine (1900–1987) and Rufus E. Stetson (1886–1967) (Levine and Stetson 1939) and Landsteiner (Landsteiner and Wiener 1940; Wiener and Peters 1940) discovered the Rh antigen. Later, with a development that has contributed enormously to fetal medicine, Levine and coworkers first described Rh incompatibility between a mother and her newborn infant (Levine et al. 1941).

Prior to this time, occasional transfusions of anemic newborns with erythroblastosis fetalis were performed as a life-saving measure, but the results were variable. With the discovery of the Rh factor came an understanding that with Rh antigen on the infant's erythrocytes (inherited from the father but lacking in the mother), the mother develops an antibody to the Rh antigen which crosses the placenta to destroy fetal red blood cells. Soon Louis K. Diamond (1902–1999) and colleagues, of Boston's Children's Medical Center and Lying-In Hospital, developed the technique for treating such infants with exchange transfusion. Their initial report was on 391 cases over the previous 4-year period (Diamond et al. 1951).

In a historic perspective, Diamond recorded:

> Thus, it was imperative to remove not only the infant's antibody-coated Rh-positive red cells but the plasma containing free anti-Rh gammaglobulin as well. A needle puncture of the longitudinal sinus was too dangerous for this purpose; multiple peripheral veins and arteries were too delicate for routine and lengthy procedures, but there was the umbilical vein, invitingly large and patent. At first, we tried to enter it with large steel needles but these could not be maintained in situ for long. Rubber catheters became a problem as well as a risk, being too narrow and clot-promoting when blood was withdrawn. Fortuitously, we learned about a plastic, nonwettable, nonirritating polyethylene catheter being used by . . . our pediatric neurosurgeon. He had found that the plastic could be chemically sterilized and reused, was nonirritating to the tissues, and retarded clotting . . .. Preliminary in vitro and animal implantation experiments were reassuring, and initial trials of umbilical vein catheterization and blood exchange were successful.
>
> The value of exchange transfusion as a therapeutic measure can be appreciated by the statistics; in the 1950s, with about 4 million births per year in the U.S., 1/200 infants would have had EF [erythroblastosis fetalis] due to maternal Rh sensitization, a conservative estimate of 20,000 infants/year. Before exchange transfusion became routine, mortality was

> close to 50%, including deaths from kernicterus and its late complications. The new treatment reduced this mortality to 10% or less, so that at least 8000 newborn infants a year were saved in the U.S. alone.
>
> (Diamond 1976, pp. 2–3)

At this time, it became evident that spectrophotometry of red blood cell hemolysis products in amniotic fluid could be useful in the diagnosis and prognosis of Rh disease (Bevis 1952, 1956; Liley 1961). Albert William Liley (later Sir William) of the University of Auckland originated the idea of intrauterine fetal blood transfusion in the treatment of severe Rh disease (Liley 1963, 1964). Soon, the validity of this approach was confirmed by others (Freda and Adamsons 1964). Beyond the treatment of *erythroblastosis fetalis* per se, Liley's studies demonstrated that the pregnant uterus could be invaded without placing in jeopardy the life of the fetus or the mother (Liley 1971a). In addition, Liley initiated the concept of treating the fetus as a patient, initiating a "paradigm shift" in clinical obstetrics. Liley later described this contribution:

> The idea of fetal transfusion originated from two aspects of amniocentesis and one of these was a mishap. First, because of the diagnostic precision of amniocentesis, it was possible to define very clearly a group of babies affected so severely and so early by hemolytic disease that were beyond the aid of conventional therapy. It was very frustrating to have to put a diagnosis on a baby which was virtually a sentence of death and then sit back and watch the baby die. Second, the mishap was due to the fact that occasionally at amniocentesis in very severely affected babies—especially with a large anterior placenta to obscure landmarks—I accidentally needled the distended fetal abdomen. Instead of getting deep yellow, cloudy, amniotic fluid, I got brilliant, golden, clear fluid which was obviously ascitic fluid; this windfall was easily confirmed by injection of contrast medium. Now this had not been intended, and initially it was rather disconcerting, but it did not appear to disturb the fetus—who was a write-off anyway. However, it occurred to me that if we could needle the fetal peritoneum without even trying then perhaps we could do it deliberately and put it to some good use.
>
> . . . the possibility of using this route for transfusion was attractive and the only question then was whether the fetus could take up sufficient cells rapidly enough to relieve anemia. We were preparing to conduct some experiments in the occasional neonate who required laparotomy (e.g., diaphragmatic hernias) to check uptake rates from the peritoneal cavity but abandoned this preliminary project when a fortuitous visitor passed through Auckland on her way home to the United Kingdom. This was a young English lady, aged 22, a geneticist who had been working in Nigeria on her favorite topic of sickle cell disease. With her she had some beautiful blood slides from neonates and infants, homozygous for HbS, who had been given normal cells intraperitoneally. There were floods of normal cells in their peripheral blood, and this was good enough evidence for us that cells could be taken up from the peritoneum in massive quantity and at a relatively rapid rate.
>
> We therefore went directly to the fetus. Both our staff and our isoimmunized patients were quite familiar with needling pregnant uteri, the patients were no strangers to disappointment and under no delusion regarding fetal prospects without treatment and, although a number of my senior colleagues did not really expect that this business would get off the ground, at least they thought it was reasonable and worth a try—which was a great assurance and encouragement.
>
> (Liley 1971b, p. 303)

In another publication, Liley asked, is there a final solution to the problem? After considering several alternatives, he concluded that we are engaged in a war that we cannot win and from which we cannot disengage. Thus, he suggested that it seems

both premature and arrogant to speak of the conquest of hemolytic disease (Green 1985; Liley 1977). As noted, some of the historical aspects of exchange transfusion have been reviewed (Diamond 1976, 1983). Within a decade, direct transfusion into the umbilical vessels was being performed (Berkowitz et al. 1986a, b). The importance of Liley's work lies in marking the beginning of fetal medicine, for within several years, cells from amniotic fluid samples would be used for chromosomal analysis to determine the absence or presence of genetic disorders, and numerous other conditions could be diagnosed and treated.

Prevention of Rh sensitization in the fetus and newborn then resulted from the work of two groups of investigators who independently conceived and developed the idea. Vincent J. Freda (1927–2003) and John G. Gorman of Columbia University with William Pollack of the Ortho Research Foundation started with the known concept that passive immunity, under proper conditions, can block active immunization (Freda and Gorman 1962). These workers first developed the specialized hyperimmune anti-Rh gamma-globulin (RhoGAM) preparation for providing passive immunity which is now used worldwide (Mittendorf and Williams 1991). In Rh-negative male volunteers, they then tested their hypothesis by intravenous administration of Rh-positive erythrocytes followed by injections of the Rh antibody (Freda et al. 1964; Gorman et al. 1964). The authors concluded:

> To sum up, we are cautiously optimistic that prevention of erythroblastosis is possible. We feel that the directions that must be followed are now clear. However, there remains a vast amount of applied research to be done before preventive therapy of hemolytic disease of the newborn can become a practical reality. This must be vigorously pursued.
>
> (Gorman et al. 1964, p. 549)

Subsequently, these investigators instituted a trial of passive immunization in Rh-negative mothers at risk and found a similar protective effect (Freda et al. 1965, 1966). Concurrently with the New York group, Ronald Finn (1930–2004), Cyril Astley Clarke [later Sir Cyril (1907–2000)], and their associates of Liverpool, England, demonstrated the protective effect of Rh antiserum in blocking sensitization (Clarke and Finn 1977; Finn et al. 1961). With other groups throughout the world, they established the clinical effectiveness of this prophylaxis. Paradoxically, Dr. Freda's original application for a grant to support this monumental research was rejected as unfeasible. Both Freda and Finn, with their colleagues, have written accounts of their achievements (Finn et al. 1977; Freda et al. 1977). In 1980, Freda, Gorman, and Pollack with Sir Cyril Clarke and Finn shared the Albert Lasker Clinical Medical Research Award.

19.5 Hyperbilirubinemia and Kernicterus in the Fetus and Newborn

In association with Rh disease and other hemoglobinopathies, a critical problem, particularly among premature newborns, was hyperbilirubinemia with the development of kernicterus which may be toxic to brain cells. Kernicterus (German *kern*,

kernel nucleus; Greek *okterus*, jaundice) is staining of and damage to brain centers, particularly the basal ganglia, by unconjugated, indirect bilirubin, e.g., that is not bound to plasma albumin. In the newborn infant, this may occur as a consequence of polycythemia with lysis of erythrocytes or the congenital defect in bilirubin metabolism, hereditary hyperbilirubinemia, and Crigler-Najjar syndrome type I. Kernicterus also may be a consequence of Rh incompatibility between mother and fetus, with hemolysis of fetal erythrocytes and release of unconjugated bilirubin into the circulation. Christian Georg Schmorl (1861–1932), pathologist at the University of Dresden, coined the term *kernicterus* (Schmorl 1904); however, the condition itself had been described by several previous workers (see Hansen 2000; Sourkes 1997).

In the "pre-intensive care" era (e.g., prior to ~1965), newborns, who in general were more than 28 weeks gestation and weighed more than 1250 g, often developed hyperbilirubinemia with kernicterus, not always as a consequence of hemolytic disease. Initially, these infants were believed not to develop this pathology unless the serum bilirubin level exceeded 18–20 mg dL^{-1}, and the only effective therapy was exchange transfusion (Lucey 1960). From this time to ~1980, the era of "early intensive care," emerging technologies and sub-specialization with the development of neonatology, was associated with survival of infants <28 weeks gestation who weighed <1000 g. With smaller and smaller preemies surviving, these years saw many in which kernicterus developed at relatively low bilirubin levels (10^{+} mg dL^{-1}).

An important contribution during this era was the serendipitous observation that the introduction of a regimen of administering antibiotics to premature infants during the first 5 days of life could result in a higher death rate with an increased incidence of kernicterus (Andersen et al. 1956). With the mid-century development and introduction of new antibiotics, several workers recommended that their administration to premature newborns would lower mortality rates. While believing this to be a reasonable idea, to help provide statistical evidence for this regimen, William Aaron Silverman (1917–2004) and colleagues at the Columbia University commenced a clinical trial to compare the results of the proposed new therapy (oxytetracycline) with that of an "established regimen" (penicillin plus sulfisoxazole). To demonstrate a 25% reduction in mortality, they calculated that 100 infants in each treatment group would be required. Concurrently, for another trial, bilirubin levels in the infant's blood were being measured (Andersen et al. 1956). Silverman has recorded that part way through the trial, his colleagues informed him that most of the kernicterus was occurring in the penicillin/sulfisoxazole group:

> I was astounded by this announcement and quickly "peeked" at the outcome in 192 infants who had been enrolled thus far. The findings were so shocking that the trial was stopped. The fact that the formal trial had "saved" half of the infants from exposure to the unsuspected hazards of the accepted treatments was undeniable, but this experience made it clear that the fixed-sample-size design must be modified to provide protection against unexpected effects in clinical studies.
>
> (Silverman 1979, p. 3)

Regarding the statistical method, Silverman observed:

> Naively, I thought that suspicion and antipathy toward the use of statistics in medicine (by no means rare at the time) would surely change. The power and inherent safety of scientific method, embodied in the small-sample techniques that had been developed by R.A. Fisher, had been so well documented in agricultural research and were so well accepted in preclinical studies, I was certain that medicine's irrational resistance could not last for long. After a quarter of a century of observing innumerable examples of our failure to learn from past disasters, I can see that my early optimism was completely misguided.
>
> (Silverman 1979, p. 3)

Subsequent studies demonstrated that sulfisoxazole displaced bilirubin from its albumin-binding site, thus permitting the unbound bilirubin to diffuse into the brain (Odell 1959). As is now appreciated, there is no precise bilirubin threshold, as many factors including degree of prematurity, hypoxia, acidosis, hypothermia, hypoglycemia, hypoproteinemia, sepsis, and certain pharmaceutical agents, either in isolation or in combination, can augment susceptibility to brain damage (Ackerman 1970; Ackerman et al. 1970; Gartner et al. 1970; Hansen 1993; Keenan et al. 1972; Lucey 1972a).

Also during this time, the therapeutic value of phototherapy, which had been reported from Britain (Cremer et al. 1958), was recognized (Ives 1992; Lucey 1960, 1969, 1974, 1977; Lucey et al. 1968), along with more aggressive exchange transfusion. Ever the pediatric *provocateur*, the "father" of "Lucey lights" has raised a number of questions and considered controversial issues regarding this therapy (Lucey 1972b, 1974). From the 1980s onward, the era of "intense intensive care," with discontinuation in NICUs of the antiseptic benzyl alcohol, the incidence of hyperbilirubinemia encephalopathy in premature newborns virtually disappeared (Watchko and Oski 1992). The American Academy of Pediatrics has published guidelines on the management of hyperbilirubinemia (Am. Acad Pediatr. 2004). Nonetheless, despite being largely of historical interest, many questions remain about the basic pathophysiology and neuropathology of this devastating disorder (Newman and Maisels 1992). For his many contributions, in 2010, Lucey was recipient of the John Howland Award of the American Pediatric Society (Lucey 2010).

19.6 Immunology

A remarkable aspect of reproduction is that a pregnant woman with a functional immune system can succeed in maintaining a semiallogenic fetus to term without immunologic rejection. Mammalian pregnancy requires protection against immunologic rejection of the developing fetus bearing discordant paternal antigens. Fetal immunologic competence has been reported as early as 13 weeks gestation, with synthesis of complement and its components (Kohler 1973; Stabile et al. 1988), although near-term values are only about one-half those of the adult (Adinolfi

1977). Immune evasion in this developmental context entails silenced expression of chemoattractant proteins (chemokines), thereby preventing harmful immune cells from breaching the maternal-fetal interface. Thus, a critical question is by what mechanism does the mother not reject the normally developing embryo/fetus? During the 1950s and 1960s, several groups began to explore the ontogeny of fetal immune tolerance. Initially, this work was stimulated by the idea that because the maternal organism does not reject the fetal allograph, it must exist in an immunologic-deficient state. Over several decades, this was replaced by a more contemporary view of a genetically programmed sequence of immunologic maturation that proceeds in an orderly manner (Hayward 1978; Miller 1966). For instance, it had been demonstrated that at 110–117 dpc, the sheep fetus rejected maternal skin grafts (Schinckel and Ferguson 1953). In addition, on the basis of experiments in mice and chickens, Rupert Everett Billingham (1921–2002) and coworkers at the University of London advanced the concept that "actively acquired immunologic tolerance" in the mouse develops in response to fetal exposure of foreign antigens (Billingham et al. 1953). Rather than a placental "barrier," several studies demonstrated the facility with which gamma globulins cross the placenta from the mother to the fetus (Bangham 1961; Brambell 1961). Subsequently, Arthur Matthew Silverstein and colleagues at the Johns Hopkins University reported immunologic rejection in the fetal lamb as early as 77 dpc (Silverstein et al. 1964). Even removal of the fetal thymus, which was believed to release cellular or hormonal immunologic factors, failed to suppress antibody formation (Silverstein and Kraner 1965). Silverstein also demonstrated considerable temporal differences in the development of the immune system among several species, e.g., that immunologic competence to various antigens does not develop simultaneously, but rather at different times during gestation (Silverstein 1964, 2009).

A study that provides insights into the mechanism of maternal-fetal tolerance suggests that previous ideas have been overly simplistic. Specifically, one group has proposed that a subset of $CD4^+$ helper T cells, so-called peripheral (extrathymic) T cells, with activation of the transcription factor FOXp3 act through sophisticated molecular mechanisms to enforce maternal-fetal tolerance (Samstein et al. 2012). In addition, in mice, it was demonstrated that fetal wastage triggered by *Listeria monocytogenes* infection is driven by placental recruitment of CXCL-9-producing inflammatory neutrophils and macrophages that promote infiltration of fetal-specific T cells into the decidua. Maternal CD8+ T cells with fetal specificity upregulated expression of the chemokine receptor CXCR3 and, together with neutrophils and macrophages, were essential for *L. monocytogenes*-induced fetal reabsorption. Conversely, decidual accumulation of maternal T cells with fetal specificity, and fetal wastage, was extinguished by CXCR3 receptor blockade and in CXCR3-deficient mice (Chaturvedi et al. 2015). Importantly, these results suggest that functionally overriding chemokine silencing at the maternal-fetal interface promotes the pathogenesis of antenatal infection. This also supports the concept that the therapeutic reinforcement of this pathway may represent a universal approach to mitigate immune-mediated pregnancy complications.

By what other mechanisms does the fetus develop its immunologic tolerance? A number of reports have demonstrated transfer of proteins, cells, and other foreign antigens across the placenta from the mother to the fetus and vice versa. For instance, beginning at about 16 weeks, the placenta actively transports the small IgG immunoglobulins from the maternal to the fetal circulation. In fact, fetal immunoglobulins are almost totally maternal IgG that is transferred by a syncytiotrophoblast receptor-mediated process. The bulk of IgG transport occurs during the last month of pregnancy (Simister 2003), with infants not attaining adult levels until 3 years of age. In contrast, the maternal IgA, IgE, and IgM antibodies do not cross to the fetus in appreciable amounts; thus, the fetus and newborn infant have only low levels of these immunoglobulins (Kane and Acquah 2009; Palmeira et al. 2012). Maternal cells also may cross the placenta and engraft into human fetal tissues, resulting in "maternal microchimerism" (Adams and Nelson 2004). The mechanisms by which the fetal immune system recognizes and responds to such antigens are unclear. A novel concept of fetal tolerance is that maternal cells (perhaps pluripotent stem cells that have crossed the placental barrier and not been eliminated by fetal natural killer cells) "instruct" activated T lymphocytes (regulator T cells) of the fetus to become tolerant of self-antigens and reactive against those that are foreign, thereby suppressing fetal immune responses (Hayward 1978; Mold et al. 2008). In the human fetus, lymphocytes that can recognize antigens appear as early as 12 weeks gestation. Multiple factors influence responsiveness of these cells, enhancing their capacity to make IgM antibody, including regulation of T lymphocytes, deficiency of number or function of macrophages, and altered number or function of B lymphocytes (Hayward 1978). For both the fetus and newborn, the complex set of immunologic demands in response to potentially harmful inflammatory immune responses, and protection against infection both in utero and following birth, present challenges of enormous scope (Levy 2007; Solomon 1971). Particularly sobering is the realization that we know so little regarding tolerogenic versus immunogenic forms of microchimerism, fetal-maternal immune mechanisms, and the mechanisms of immunologic responses in the newborn (Burlingham 2009).

19.7 Chronic Catheterization of the Fetus

In the mid-1960s, a technical advance was made that would present a "sea change" to the study of fetal physiology and related functions. Since the time of Paul Zweifel and the late nineteenth-century investigators, an issue in the study of the ruminant or other large mammalian fetus in utero was the necessity for the fetus, with the ewe, to be anesthetized for performance of the required studies. This resulted in a biologic Heisenberg uncertainty principle (after Werner Heisenberg), e.g., that in performing a given measurement, one changed the value of that variable being measured. For fetal physiology, these measurements under anesthesia were "acute," rather than reflecting the true undisturbed steady-state conditions of intrauterine

existence. In 1965, the Yale group, led by Donald H. Barron and Giacomo Meschia, reported on their use of plastic catheters, chronically implanted in the fetal vasculature and other sites. With this technique, one could study blood gas values and other variables for several weeks following recovery from the anesthesia and surgery required for their implantation. Importantly, Meschia and colleagues demonstrated for the first time that, contrary to the accepted belief (as had been "demonstrated" by Barcroft) that fetal arterial PO_2 and [HbO_2] decreased significantly near term, this was not the case, but rather these values remained constant (Meschia et al. 1965). This breakthrough, not appreciated by many for a decade or more, revolutionized the study of the developing fetus in a truly physiologic state. This innovation by Meschia, Barron, and colleagues is a worthy example of the manner in which a methodologic advance can contribute to a conceptual "paradigm shift" in the course of science. Meschia has written on some aspects of this contribution (Meschia 2006). Following his retirement from Yale, Barron accepted the Julius Wayne Reitz (1908–1993) Chair of Reproductive Biology at the University of Florida, Gainesville, where he continued studies on uterine and fetal blood flow and oxygen consumption. Barron liked to remind students that "science begins with a question while technology begins with a task." He also stressed that a carefully conceived hypothesis could be tested effectively in many ways. Pondering alternative approaches to the design of an experiment, he taught, "... was not only valuable intellectually, but often revealed more elegant, robust, and efficient methods of answering important, complex questions. Alternatives need not be obstacles" (Bartol 2000, p. 6). In regard to placing problems in their proper perspective, Barron noted, "I concluded a long time ago that I had plenty of information, but little knowledge concerning it." As a pedagogue he observed, "Good teaching consists in asking a question the student feels compelled to answer for himself" (Johnson and Friedrich 1985). Regarding his rejection of plans for retirement, he observed, "... I feel like a boy who gets paid to go fishing", and "as long as it's fun and I can still play the game ... I'll find some aspect to study" (Anonymous 1981, pp. 15–16).

From the University of British Columbia, the 16th Baron of Corcomroe, Anthony Manning Perks, recalled memories of Dr. "B" at Gainesville:

> My post in Zoology also offered me freedom to go down to work with ... Sidney Cassin, at the University of Florida, where I had been the first Postdoctoral Fellow (they say that when I die, they will stuff me, and put me in a glass case, next to the first Graduate Student!). It was through Sid that I first met Dr. Donald Barron, Sir Joseph Barcroft's closest co-worker, to whom he dedicated his pioneer book. Dr. Barron was retired from Yale, and held in great honour, in the Ob/Gyn Department in Florida, down on the 3rd Floor. Sid introduced me to Barron as having worked with Geoffrey. There was a brief hesitation, but slowly our relationship blossomed, especially when Barron realized that I had started my career in Cambridge, in Charles Darwin's old college, Christ's Church. It soon became clear how much Barron missed Cambridge, and regretted leaving it, we often shared memories of the many eccentric professors (like ourselves!), who we had both known. From that point on, Barron and I became close friends. On one occasion, Geoffrey visited Florida, and Sidney and I took him down to talk to Barron; Geoffrey listened attentively to Barron, who was still fascinated by the placenta (although now without a lab). It was clear that there was great respect between the two—and yet a feeling of distance. It was only later I was told (so far as

I know, accurately) that when the Nuffield Institute first opened, Barron, with his huge accomplishments in fetal physiology, had applied for the Directorship. However, Oxford chose its own, and gave the job to Geoffrey, a bright, but less experienced young man. It was then that I realized the reason for Barron's reticence when we first met! I was a Dawesman! So happy I overcame this.

Later, there were other experiences involving Dr. Barron. When I was writing a review, I asked him whether he could give me the original reference to William Harvey's "*De Generatione*. . .." Barron thought for a minute, then said, "I think I can help you". Next day, he came shuffling slowly along the long corridor, with a book in his hand. "This is what you want. I'm sorry. This is the Frankfurt printing; I could not afford the earlier one". It was the original book!!! From the 16 hundreds! Barron's books came into my life a second time. One day I went down from Sid's lab in Florida, after surgery, to get a cup of coffee. As I passed the library, I saw some students pawing through a pile of books, free, and left sitting in the hot sun, in the courtyard. It was after Barron had, sadly, died. I went over to look, and saw one of the students throw down an old, almost derelict, brown book. I picked it up, and realized that these "discards" were Barron's old books. The book thrown down was not only the first book on Fetal Physiology, Barcroft's "Researches on Fetal Life", but it was the first book, the proof copy, occasionally annotated by Barcroft himself, and printed on irregular browned sheets of wartime paper. I grabbed it, together with Barron's own 1977 reprint, and a pile of books of medical history, such as the life of Galen. I still have them all, and treasure them.

(Letter from AMP to LDL, 23 April 2010)

Later Perks wrote:

In addition, your recent letter started me thinking more about Dr. Barron. I gave a number of memories in my previous letter, but more came streaming back over the weekend. . .. Their only merit is that I knew Barron well during his late retirement years, in Florida, when he seemed to be a little on his own.

Many mornings, on my visits to Gainesville, as I drove into the Medical School, along 13th Avenue, I would overtake Barron on his large, heavy bicycle. He would be stopped at the side of the road, dressed in his usual white pith-helmet, looking for all the world like a British colonel in the Indian Army of Victorian times. His bicycle would be leaning on a fence, while he himself was engrossed in writing on a notepad. Apparently, whenever a new idea occurred to him he stopped wherever he was, even in the countryside, and put it down, before he lost it. Once in his office, he would put on a long, white lab-coat, just as if he still had a lab—I think because he really missed having one. I do the same, not to emulate him, but for the same reason! He would always wear the same lab coat to seminars, the only one to do so, and sit towards the back, near a beautiful painting of himself in his gorgeous Cambridge Sc.D. robes . . ..

I would often go to his office, and talk to him about his current interests, often historical ones: I shared these interests, but the busy clinicians had neither the time nor the interest for such things. He was much on his own, and shy in social settings. He had taken a great interest in such people as Sidney Ringer [(1835–1910)], and I always encouraged him to publish what he had found—but he never did, and much was lost. Once he said that he had submitted a paper to an historical journal, but when it was turned down, never did again. I wonder whether the referees ever realized that he had known many of these people, famous in the history of medicine. What a loss! He would often talk of his summer visits to the "Krakenhaus" where Sigmund Freud [(1856–1939)] had worked. He would disappear into the library until evening, and then meet his wife (who he always referred to as "Mrs. B") on a certain bench, in a certain park, at a certain time: they seemed to have a very ordered life, but a very close one.

(Letter from AMP to LDL, 26 April 2010)

19.8 Cardiovascular Physiology

Shortly following the introduction of chronic catheterization of the ovine fetus, Abraham Morris Rudolph and Michael A. Heymann, at the University of California, San Francisco, introduced the use of microspheres, labeled with various radioactive nuclides, to quantitate cardiac output and the regional distribution of blood flow to the brain, heart, and other organs in the fetus (Rudolph and Heymann 1967). Again, because microspheres did not alter the course of blood flow, and there was no significant recirculation, this methodological development changed the course of cardiovascular research at the organ level, not only the fetus but also that for the newborn infant as well as adult (Heymann et al. 1977; Rudolph 1985). In further contributions with this methodology, Rudolph and colleagues reported on many aspects of fetal cardiac function and blood flow in the umbilical vessels, the superior and inferior vena cavae, and the vascular shunts including the *ductus arteriosus* (Hoffman 2002; Rudolph 1996; Rudolph and Heymann 1970), as well as the responses to hypoxia and other stress (Itskovitz et al. 1982 1987, 1991; Rudolph 1984). In an essay, "The fetus at Everest or Death Valley," Rudolph correctly dismissed the concept that the fetus is at "Mt Everest in utero." Yet, despite the fact that the fetal basal temperature exceeds that of the mother by only 0.5 °C (in the sheep; Lotgering et al. 1983), he compared it to being at the Death Valley (Rudolph 1989). The justification for this latter metaphor is unclear when the in utero existence in a rather watery *milieu* is hardly that of a scorchingly hot, semiarid desert. Clinically and importantly, Heymann, Rudolph, and colleagues used the prostaglandin synthesis inhibitor indomethacin to close the *ductus arteriosus* in premature infants (Heymann et al. 1976) and prostacyclin E_1 to maintain potency of the *ductus* in infants with pulmonary atresia (Clyman et al. 1978; Heymann and Rudolph 1977).

As noted, since the beginnings of fetal physiology, a critical issue has been the extent to which functions in the exteriorized fetus may be regarded as truly physiologic, as compared to that of the relatively undisturbed state in utero. The earliest such comparison of the chronically catheterized in utero fetus with that of acute fetal exteriorization under local or general anesthesia ex utero demonstrated significantly increased vascular resistance with a 37% decrease in umbilical blood flow and a small, but significant, increase in umbilical venous O_2 tension (Heymann and Rudolph 1967), presumably associated with prolonged transit time in the placental exchange area. Later, Dawes would argue for the need for, and validity of, both approaches (Dawes 1984, p. 262). In a subsequent report, Rudolph considered the continued need for studies of organ-based cardiophysiologic systems in a world in which cellular and molecular biology constitutes the major emphasis. Here, he argued for the need for continued exploration of the mechanisms of cardiovascular responses to hypoxia, the role of mechanical loading on myocardial growth, and the effect of congenital heart disease with altered circulating patterns on cardiovascular growth and development (Rudolph 1996).

At the University of California, Los Angeles, Nicholas S. Assali was another leader in investigation of fetal and maternal physiology. One of the developers of the electromagnetic flowmeter, Assali pioneered techniques for the measurement of uterine blood flow and utero-placental-fetal metabolism (Assali et al. 1960; Ladner et al. 1970) and studied the development of autonomic and neurohumoral regulation of fetal and neonatal cardiovascular function (Assali et al. 1969, 1970, 1977, 1978; Nuwayhid et al. 1975; Woods et al. 1977). With the use of their flowmeter, this group also presented quantitative data showing the decrease in *ductus arteriosus* blood flow to almost zero following ventilating the fetal lungs with oxygen, the flow returning to normal when the inspired gas was switched to nitrogen. In these studies this group also quantified in the fetus the relatively low vascular resistance and high cardiac output, presumably to compensate for its relative low O_2 content (Assali and Brinkmann 1972; Assali et al. 1970). In general, the studies of this group were in anesthetized ewes with the fetus either marsupialized to the uterine and abdominal walls or in utero. In comparing these experimental animals to those that were chronically instrumented and unanesthetized, the chief physiologic differences observed were that in the later maternal arterial blood pressure was lower as was the arterial PO_2, presumably because the acute group were artificially ventilated. Assali maintained that for the fetus, the physiologic cardiovascular responses were similar (Assali et al. 1974). As an aside, one study reported that as a consequence of chronic instrumentation, fetal body weight and thymus weights were decreased by 15 and 27%, respectively, while the liver to body weight ratio was increased to 24% (Clark et al. 1990).

In other studies of fetal cardiovascular function, Raymond Gilbert, at Loma Linda University, demonstrated that the relatively high cardiac output observed in the fetus (several times that of adult) is achieved by two mechanisms. One, both right and left ventricles eject blood into the aorta. Two, both ventricles are working at near maximum capacity; that is, they are at the top of their respective cardiac function curves (Gilbert 1980). Because the ventricles are quite sensitive to afterload, e.g., arterial blood pressure (Gilbert 1982), when the fetal cardiovascular system is stressed (as with hypoxia of a few hours duration), cardiac output either remains unchanged or decreases (Gilbert 1998). With longer-term hypoxia of several months, fetal cardiac output is decreased by ~25% because of reduced function of both ventricles (Kamitomo et al. 1992, 1994). With acute hypoxia, fetal cardiac output is redistributed to the brain, heart, and adrenal glands at the expense of the rest of the body (Peeters et al. 1979), and with long-term hypoxia, this creates the potential for asymmetric growth restriction (Kamitomo et al. 1993). The reduced myocardial function is brought about by a decreased myofibrillar response to intracellular calcium concentration, which probably is mediated by reduced myofibrillar Mg^2-activated ATPase (Browne et al. 1997a, b). In addition, the coronary vasculature of the long-term hypoxic fetus exhibits reduced contractile responses to extracellular K+ and to intracellular calcium concentrations. The reduced coronary vascular contractile response to intracellular calcium is mediated completely by changes in calcium sensitivity through the Rho kinase pathway (Maruko et al. 2009).

19.9 Related Fields of Research

Many organ systems are not considered in this review. Thus, quite obviously, there are many topics in fetal-newborn physiology the history of which are worth study. For instance, blood flow to the gravid uterus is a major consideration in terms of fetal homeostasis and well-being. Several recent reviews place this field in perspective (Paradis and Zhang 2013; Pastore et al. 2012; Sen et al. 2013; Thornburg and Louey 2013; Xiao et al. 2010; Zhu et al. 2013). A related area of fetal physiology of great relevance is that of amniotic fluid metabolism, rates of turnover, and regulation (Brace et al. 2004; Jellyman et al. 2009; Robertson et al. 2009). Yet another is the circadian periodicity of the developing fetus and the fact that for its circadian rhythmicity, the fetus is dependent upon maternal melatonin which crosses the placenta (Yellon and Longo 1987, 1988).

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Chapter 20
Additional Clinical Aspects of Developmental Physiology and Clinical Care

The woman about to become a mother, or with her newborn infant upon her bosom, should be the object of trembling care and sympathy wherever she bears her tender burden or stretches her aching limbs . . . God forbid that any member of the profession to which she trusts her life, doubly precious at that eventful period, should hazard it negligently, unadvisedly, or selfishly.

(Oliver Wendell Holmes 1842/1843, p. 503)

20.1 Neonatal Intensive Care in Preterm Birth

As noted, a major public health issue is that of preterm birth, which occurs in 10–12% of pregnancies in the USA, an incidence which appears to resist improvement despite advances in the practice of maternal-fetal medicine. Importantly, this complication of pregnancy accounts for about 75% of perinatal mortality and often is associated with several serious long-term developmental and health issues for the infant who survives. As noted earlier, preterm birth is defined as that <37 weeks gestation, while very preterm birth is that <33 weeks gestation and extremely <28 weeks gestation (Reece and Hobbins 2007). Appropriate for gestational age, preterm infants must be distinguished from small for gestational age infants, defined as ≤ 10 percentile (Zhang et al. 2010), although it is recognized that small infants are not necessarily growth restricted, and those with growth restriction are not necessarily small (Bhide 2011; Zhang et al. 2010). In part, a consequence of maternal and/or fetal infection (Abramowicz and Kass 1966; Romero et al. 1988), preterm birth has many causes, and a more clear understanding of the mechanisms of this scourge is essential to its amelioration. Also as noted earlier, several aspects of the basis for antenatal steroid therapy for pulmonary development have been established (Ballard and Ballard 1995; Ballard et al. 1980). Nonetheless, a considerable body of evidence supports the value of this modality in lessening infant morbidity and mortality (Bonnano and Wapner 2009), and a number of considerations remain to be resolved such as the ideal drug, dosage regimen, indications for retreatment, and so forth.

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During the first half of the twentieth century, premature and other newborn infants were under the care of nursery nurses, midwives, and obstetricians. There were few incubators, no advanced technology, and no rooming-in. Although pediatrics was a well-defined specialty, emphasis was on the care of older children, and few had experience with the care of newborn infants, much less those that are premature (Cone 1979). Beginning in the late 1950s and during the 1960s, a milestone of extreme importance was the organization of neonatal intensive care units (NICUs) at major medical centers. Quite obviously, the salvage of premature infants is intertwined with that of the development of NICUs and their enlightened utilization. In turn, this critical advance depended upon new insights and understanding of the basic physiological concepts being discovered in respect to the lung, the heart, temperature regulation, and other considerations. Of vital importance, the establishment of NICUs with increased sophistication, and with widespread access based on the existence of regionalization, has allowed the development of a national network that is saving the lives of many premature or otherwise ill newborns who in former times would have died soon after birth. Thus, a brief review of some aspects of these symbiotic developments may be appropriate.

Several authors have presented a historical perspective on the origin and development of neonatal care and the subspecialty of neonatology (Cone 1983, 1985; Desmond 1998; Dunn 1998; Philip 2005). Over the centuries, a number of works have included aspects of pediatrics and care of the newborn (Radbill 1971), and only a brief survey will be presented here. An eighteenth-century milestone in this regard, for its innovation and attempt to correct erroneous concepts, was the 1748 letter to one of the governors of the recently established Foundling Hospital, London. In this, William Cadogan (1711–1797) of Bristol laid down rules on the nursing, feeding, and clothing of infants, particularly those that were puny (Cadogan 1748). This essay was of great importance, as in eighteenth-century England, infant welfare was much neglected through the ignorance of mothers and midwives as well as physicians, and "foundlings," abandoned infants with their excessive mortality, were a common feature of life. Established by Royal Charter in 1739 and formally opened two years later, the Foundling Hospital was dedicated to the education and support of these deserted infants and young children. Cadogan wrote his essay with the hope that the Hospital would "... be a Means not only of preventing the Murder of many, but of saving more by introducing a more reasonable and more natural Method of Nursing" (Cadogan 1748, p. 3). Called by some "the Father of Child Care" (Rendle-Short and Rendle-Short 1966), Cadogan's pamphlet of only 34 pages had a much wider influence than its size might suggest. He presented a scathing view of caretakers, who because of traditional prejudices "... are capitally mistaken in their management of children," their care having "... no real Foundation in nature" (p. 4). In establishing his case, Cadogan referred to the London Bills of Mortality in which one may observe that "... almost Half the Number of those, who fill up that black list, die under five Years of Age" (p. 6). As examples of mismanagement, he noted that an infant's head would be bandaged tightly (to improve its shape and to aid in closure of the anterior *fontanelle*) and that infants would be stuffed into tight flannel and its limbs swathed with unyielding

wrappers (to prevent bowing of the legs and spinal deformities, which probably occurred as a result of rickets). Counter to these practices, Cadogan recommended loose clothing and freedom of movement, as well as exposing the infants to fresh air so that they would not become a "... Hot-bed Plant" (p. 9). Also of importance, Cadogan advocated breast-feeding infants by the mother rather than a wet nurse and delaying until several months of age feeding with simple and light solid food rather than "overfeeding" them early with "heavy" foods (Rendle-Short 1960). Six years following the appearance of this work and his move to London, Cadogan was elected a physician of the Foundling Hospital (Munk 1861).

In the early 1800s, recognition of the special needs of premature infants stimulated Johann Georg von Ruehl (1769–1846) of St. Petersburg, Russia, to invent an incubator for newborns (Cone 1981). Others also credit Etienne Stéphane Tarnier (1828–1897), one of the great *accoucheurs* and chief of obstetrics at *L'Hôpital Maternité*, Paris, with pioneering care of the premature infant. In about 1880, Tarnier introduced the use of an incubator he modeled upon that used for chicks. By 1893 the unit at the *Maternité* was expanded into a *Pavillion des Enfants Debiles* [Pavillion of Weakling Infants] with a dozen incubators (Henry 1898; Pinard 1909; Toubas and Nelson 2002). Following establishment of *crèches* [day nurseries] for the care of infants of the increasing numbers of women who worked in factories, in 1876 the *Sociéte de Allaitement Maternalle* [Society for the promotion of breast-feeding by mothers] was founded to provide homes for expectant mothers during the last weeks of pregnancy, prior to their entering a maternity hospital for confinement. To encourage breast-feeding, for up to a year, financial and other assistance was given to mothers who nursed their infants. Importantly, Pierre Constant Budin (1846–1907), a student of Etienne Stephane Tarnier who became chief obstetrician at the *Hôpital de la Charité* (1882) and at *L'Hôpital Maternité* (1895), recognized that a major factor in infant mortality was the lack of proper care and feeding of those born prematurely. Budin initiated what became known as the *puériculture* [child care] movement, with the development of centers for *Consultation de Nourrissons* [newborn infant consultation] (Budin 1900). Mothers who delivered in maternity hospitals were encouraged to return at regular intervals so that their infant could be weighed and examined, and often the mother received an honorarium based on the baby's progress and fitness (McCleary 1933).

In the provinces, with less access to specialized maternity hospitals, the French infant welfare movement took a different form. Here centers for *Goutte de Lait* [drops of milk] were dedicated to supporting breast-feeding; providing clean, sterilized milk for those bottle-fed; and weighing the infants to follow their development and well-being. To emphasize that cow's milk constituted a poor substitute for mother's milk, their motto *Faute de mieux* [for lack of the best] was affixed to the baskets containing bottled cow's milk for those mothers who did not breast-feed. In general, the mothers would return weekly to have their infants weighed and examined, and every effort was made to promote the child's well-being. A British senior medical officer in the UK Ministry of Health, George Frederick McCleary (1872–1962), has given a detailed history of the early years of the infant welfare movement in France, Great Britain, and America (McCleary 1933). Budin

promoted the idea ("Budin's rule") that the bottle-fed newborn should be given milk daily equal to no more than one-tenth its body weight (Budin 1900; Toubas 1992) and did much to promote the education of mothers in child care. In his essay "Incubator-Baby Sideshows," William Silverman (1917–2004) described the manner in which, beginning in the late-nineteenth century, incubators with premature infants were featured at national and international exhibitions (Silverman 1979, 1980a). In 1891, a leader in this crusade, Alexandre Lion of Nice, established an infant charity in Paris, and several other cities, in which preemies were displayed in a *couveuse* [hatchery or incubator] of his design. A British magazine article of the time featured in some detail photographs and description of Lion's *Oeuvre Maternalle des Couveuses D'enfants* [The baby infant charity] in Paris (Smith 1896).

In turn, Budin's protégé Martin Arthur Couney (ca. 1860–1950) in 1896 (the same year as Lion and perhaps under Lion's direction) exhibited their latest incubator with living premature babies, at the *Berliner Gewerbe Ausstellung* [World Trade Exposition in Berlin]. This exhibit, the *Kinderbrotonstalt* [child hatchery] of six preemies, each in an individual incubator, drew considerable attention, its popularity being a forerunner of many similar demonstrations to follow. These included the 1897 Victorian Era Exhibition in London (at which, because British infants were not allowed to be shown, premature infants were brought from France); the 1898 Trans-Mississippi Exhibition in Omaha, NB; the 1900 Pan-American Exhibition in Buffalo, NY; the 1904 Louisiana Purchase Exhibition in St. Louis, MO; the 1915 Panama Pacific International Exhibition in San Francisco; the 1930 Chicago World's Fair; and others (Baker 2008; Raju 2006; Silverman 1979; Winks et al. 1997).

In America, it was Joseph Bolivar De Lee (1869–1942) of the Chicago Lying-In Hospital, and a leader in the emergence of obstetrics as an academic discipline (De Lee 1913), who in 1900 established an incubator station at the Lying-In. This included an ambulance service with an incubator in which to transport babies from where they were delivered at home to the hospital. Another important figure in this regard was Julius Hayes Hess (1876–1955), a pediatrician at the Michael Reese Hospital, the University of Illinois, and the Cook County Hospital. In 1914, Hess opened a unit for premature infants at the Sarah Morris Hospital (part of the Michael Reese Hospital), enlarging it several years later. Hess also fostered the idea of regionalization of healthcare, with the premature unit serving the greater community. A prolific author, among other works, Hess wrote *Premature and Congenitally Diseased Infants* (Hess 1922), which helped to increase the attention of obstetricians, pediatricians, and others of the importance and value of these infants and the possibility of saving their lives. In considering the special problems of these newborns, Hess emphasized the need for proper hospital-based facilities and an understanding of the infant's physiologic needs vis-à-vis maintenance of temperature, hydration, and feeding and avoidance of infection. In the Preface he wrote:

> As part of the great movement toward conserving and developing the individual to his highest point of health efficiency, as an important factor in national health, and as an effort directed toward the source of a considerable morbidity, the care of premature infants and the conservation of their flickering lives has a prominent place.
>
> (Hess 1922, p. vi)

Hess then acknowledged his "... indebtedness to Dr. Martin Couney ... for his many helpful suggestions in the preparation of the material for this book" (Hess 1922, p. vi). Working with Hess' group, Couney had a large display of "incubator babies" at Chicago's 1933–1934 Century of Progress Exhibition as well as at the New York World Fair of 1939–1940 (Silverman 1979). In 1941, Hess updated his volume, with an emphasis on the nursing care of "preemies" (Hess and Lundeen 1941), and later summarized his three decades of experience in their care (Hess 1953). Many of these early developments have been reviewed (Cone 1983; Dunham 1957).

In Birmingham, England, in 1929 a small maternity home, the Sorrento Maternity Hospital, was opened for the care of pregnant women with special problems. Two years later (1931) a young physician with obstetrical experience, Victoria Mary Crosse (1900–1972), was appointed to establish the "premature baby ward," a special unit to care for those infants born throughout the city. Soon, this included a motor service to transport such "preemies" from outlying areas of the city. In 1945, Crosse, by this time Birmingham's chief obstetric officer and senior medical officer, published a volume *The Premature Baby*. In this work, she stressed the strict requirements with attention to detail and unremitting oversight for midwives, nurses, and physicians working together to minimize morbidity and mortality in this group of fragile infants (Crosse 1945). In his Foreword to this volume, the genesis of which he stimulated, Sir Leonard Parsons emphasized Crosse's nonpareil success in saving the lives of these infants (Crosse 1945, p. v). The following year in the introduction to his Blair-Bell memorial lecture delivered to the Royal College of Obstetricians and Gynaecologists, "Antenatal Paediatrics," Parsons quoted the lines of the English Poet Laureate John Edward Masefield (1878–1967):

> In the dark womb where I began
> My mother's life made me a man;
> Through all the months of human birth
> Her beauty fed my common earth;
> I cannot see, nor breathe, nor stir
> But through the death of some of her
>
> (Masefield 1927, p. 71)

Sir Leonard followed with the prophetic words, "The paediatricians of the future must be concerned with the well-being of the child from the moment of its conception and sometimes even before that event ... When obstetricians, paediatricians and social workers combine together ... the future will indeed be radiant with promise" (Parsons 1946, pp. 2 and 15).

One who was influenced greatly by these "incubator babies" displays was Arnold Lucius Gesell (1880–1961), a child psychologist and pediatrician at Yale University. A pioneer in the study of infant development and "the beginnings of the human mind," Gesell filmed the "fetal infants" and their reactions at the New York

World's Fair, obtaining material for his monograph *The Embryology of Behavior* (Gesell 1945), *The First Five Years of Life* (Gesell 1940), and other works (Radbill 1972). Gesell also obtained considerable information on newborn development from the "experiment" of "rooming-in." This was introduced in 1944 at the Yale New Haven Hospital by Edith Banfield Jackson (1895–1977), who soon after delivery placed newborns on the gurney with their mothers for "bonding" (Cooke 1954; Jackson 1991).

Although criticized by many as overt commercialism and exploitation, there can be no doubt that with their incubators, hand feeding, and care, Couney and others saved the lives of a small army of premature infants, who otherwise would have died. Their work drew public and professional attention to the special needs of these special newborns (Gartner and Gartner 1992). Nonetheless, as Silverman has cautioned, "It would be fatuous to attach deep significance to this odd chapter in medical history...." He pointed out that this latter enterprise was, to a great extent, the result of the activities of a single person, Couney, and had relatively little impact per se in the development of the care of premature and immature infants at either a national or international level. Tellingly, Silverman compared some aspects of contemporary NICU theatrics to those of the "incubator baby" sideshows, finding in both a "... disturbing detachment from reality" and a "narrowly focused response to [a] ... complex problem." He closed one of his essays with the comment *Plus ça change, plus c'est la même chose* [the more things change, the more they are the same] (Silverman 1979, p. 140).

In 1914, August Ritter von Reuss (1879–1954) of the University of Vienna published the first comprehensive volume on diseases of newborns (von Reuss 1914; English translation 1920). In his 1939 Presidential Address to the American Pediatric Society, Clifford Grosselle Grulee (1880–1962), of Evanston, IL, reviewed a number of important problems presented by the newborn infant. In acknowledging the need for pediatrics to assume a more active role in this regard, he noted, "In previous times the problems of the newborn child have been the province of the obstetrician, a field in which he has taken comparatively little interest and to which he had contributed little .... As pediatricians we have but scratched the surface" (Grulee 1939, pp. 2–7). It thus was in the mid-twentieth century that interest in, and primary responsibility for, care of the newborn infant shifted from the obstetrician to pediatrician. In 1952, Arthur Hawley Parmelee (1943–2006) of the University of California Los Angeles, published his *Management of the Newborn* (Parmelee 1952), one of the first such works in the modern era. With development of neonatal intensive care units, as known at present, the terms "neonatology" and "neonatologist" had their origin (Schaffer 1960, p. 1). In contemplating the challenges presented by the rapidly burgeoning field of neonatal medicine, Alexander James Schaffer (1902–1981) of Baltimore admitted, "We ... [are] confronted with the apparently insurmountable obstacle of our own limitation of knowledge" (Schaffer 1960, p. v).

In the present era, it is perhaps difficult to appreciate the primitive state of care of the newborn, particularly the premature infant, as late as the mid-twentieth century. This deficit included little or no appreciation of the healthcare needs of such infants

and a degree of fatalism regarding their outcome, the lack of appropriately trained subspecialists in neonatology, a lack of understanding of the pathophysiology of the many physiologic abnormalities and clinical conditions these infants face, as well as an absence of equipment and instruments designed specifically for their management. Based on relatively rapid advances in understanding pulmonary and other aspects of physiology, within a brief period, NICUs blossomed at several academic medical centers in the USA, including Yale; Harvard; Vanderbilt; the University of California, San Francisco; Stanford; the University of Colorado; and others (Cooke 1954). A pioneer in this regard, in the emerging discipline of neonatology and the development of neonatal training programs, was Clement A. Smith of the Boston Lying-In Hospital. For over three decades, "Clem" Smith worked in concert with an outstanding group of Harvard fellow physician-scientists, Duncan Earl Reid (1905–1973), chair of obstetrics; Stewart Hilton Clifford (1900–1997), chair of pediatrics; Claude Alvin Villee, Jr. (1917–2003), in biochemistry; and Kurt Benirschke (1924–2016) and Shirley Driscoll in pathology, to convert the essentially custodial care of preemies, into the aggressive management that developed "neonatal intensive care" units (Nelson 2008). This research and patient management involved further developments for the premature infant of incubators (also called Isolettes), microtechniques in blood gas analysis, respirator support, transcutaneous oxygen monitoring, umbilical vessel catheters, early administration of fluids (Hansen and Smith 1953; Nicolopoulos and Smith 1961), total parental nutrition (Wilmore and Dudrick 1968), and related techniques. All in all, these advances constituted a campaign to create an extrauterine "womb" in which to keep these newly born fetuses alive.

Of note, a number of these technological advances, such as electrical pressure transducers, rapid and sensitive recorders, the plethysmograph and pneumotachograph, rapid gas analyzers, cardiopulmonary monitors, and sophisticated and quantitative theories of gas exchange, respiratory mechanics, and cardiovascular mechanisms, occurred as a consequence of the US space program. Instruments designed to monitor vital signs of astronauts were adapted for use in medical centers. In the development and inclusion of neonatal intensive care units as key components of nurseries, Smith and his colleagues played a major role. In 1946, following the publication of Smith's *Physiology of the Newborn Infant* (Smith 1945), Sir Joseph Barcroft sent him a photograph and wrote:

> My dear Smith ... as regards the photograph I am proud to send it to you. Concerning its effect, you remember that the essence of missionary enterprises such as yours is to expel the people one by one—in the case of (my) disciples I think it was 70 of them—from the centre at which they were all over the world. I am sure the effect of the photograph in your Laboratory will be very similar! These photographs are always rather stilted affairs.
>
> (Avery 1976, p. 857)

Quite obviously Smith rigorously followed Sir Joseph's counsel, training over four dozen fellows who became leaders in the discipline and missionaries to the world. In a highly cited essay, "The Valley of the Shadow of Birth," Smith used the metaphor of crossing a deep valley with its turbulent river for childbirth and its hazards (Smith 1951). The paper is of historical interest, as it also reviews

contemporary ideas and issues concerning prematurity, respiratory distress syndrome, and retinopathy of the newborn in the premodern era. In 1975, Smith was the first recipient of the Virginia Apgar Award of the American Academy of Pediatrics (Nelson 2008), and the following year, he received the John Howland Award of the Pediatric Research Society (Avery 1976; Smith 1976).

Louis Gluck has given an account of the development of NICUs and the concept of "rooming-in" at the Yale New Haven Hospital (Gluck 1970, 1992). Others have recorded aspects of the competing ideas regarding construction of a special nursery for critically ill premature infants (Butterfield 1992; Little 1992; Sinclair et al. 1981; Sunshine 1992), and Paul Swyer outlined physiological principles on which to base such intensive care (Swyer 1975). Within a relatively few years, model programs were developed at many centers for the care of high-risk mothers and their infants (Sunshine and Quilligan 1974). In the 1960s, although initially respirators were used only when other modalities of therapy had proven ineffective, by the early 1970s, with their benefits 1.5 kg (~3 pounds) infants were surviving almost routinely. An appropriate therapy of these fragile beings did not develop without some serious problems in relation to iatrogenic sequelae, as a consequence of the preemies being delivered and intensive care given to keep them alive at ever earlier and earlier gestational ages (Gellis 1976; Lubchenco 1976; Moore 1976).

A neonatal care center of note was that developed in the early 1960s by the pediatrician Mildred Thornton Stahlman (1922–), at the Vanderbilt University Medical Center, in Nashville, TN. Here, she first used an infant-sized Drinker iron-lung, tank-type, negative-pressure respirator to save a baby with RDS (Cotton 1996). In scientific investigations, Stahlman has emphasized the intimate interrelationship of basic laboratory studies with clinical research in improving patient care (Stahlman 1984, 1989, 2005). In her 1985 Child Health Day Address at the NICHD, Stahlman gave a historical perspective of great relevance today. She has written:

> The technology needed to study the physiology and biochemistry of both normal and sick prematures and sick term infants adequately was simply not available. Nothing was the right size, from respirators to endotracheal tubes, from blood sample size to such simple things as cardiac electrodes. The long-honored "hands-off" policy in sick prematures had not created a demand, either entrepreneurial or commercial. On this backdrop of ignorance of normal physiology and biochemistry, every new bit of scientific information was exciting, each modifying, in its time, the ability to manage derangements in newborn physiology which impacted on outcome.
>
> The first so-called NICU's were all begun by people who were already trained in research techniques, usually with animal models or in adults. These pioneers, unwilling to accept the status quo, designed, usually with NIH funding, nursery settings where scientific measurements could be carried out, even on very sick and very premature infants. Their nurseries were usually backed up by an animal laboratory or a basic biochemical laboratory, where clinical problems recognized in the nursery could be modeled, or biochemical systems examined in vitro. These data were taken back to the bedside for clinical trial, and, if successful, became part of a therapeutic regimen.
>
> (Letter from MTS to LDL, 23 December 2009)

Stahlman then reviewed specific examples of management of asphyxia of the newborn, the transitional circulation, respiratory distress syndrome, hyperbilirubinemia, and parental alimentation. She concluded:

> What can we learn from this recital of past changes in patient care resulting from research? First: research implies understanding. In every clinical trial, the physician-scientist asks the parent or the patient to join with him in taking a chance, however small, in testing a new idea, a new mode of therapy, a new approach to management. This may be better than the old way of doing things, but may, not only not be better, but actually be worse, not just in the short term, but also in the long-term effects, apparent only years later. The development of committees for the protection of human subjects addresses this problem, but in reality, they only assess informed consent. . .
>
> Second: advances in science move slowly, in fits and starts, rather than smoothly uphill. Each piece of new information builds on all the previous ideas and data and trials, provided by others. We build on the past, and no new information, however trivial it seems at the time is wasted, if it is true.
>
> Third: The translation of new ideas into clinical care, even those proven superior by careful trials, is a slow process. We are all creatures of habit, and old ways die hard. Colleagues have to be convinced, editors have to be convinced, nurses have to be convinced, hospital administrators have to be convinced. It is like the turning of a super-tanker at sea—the order is given and the rudder is turned long before the wide arc of direction/change is accomplished. So it is with clinical research.
>
> Fourth: . . . so many new changes have improved outcome in the NICU, public expectation has been raised unrealistically for salvage of the unsalvageable, saving of the hopeless. The public must be kept aware of current limitations and of the continuous need for research support. We, on the other hand, must never accept these current limitations as permanent. They are only a temporary resting place, a place for seeing into the future. Tennyson wrote "yet all experience is an arch wherethrough gleams that untravelled land, whose margin fades forever and forever as I move."[1] So let it be with us.
>
> Fifth: The role of the physician-scientist in this relationship is crucial, and must be nurtured and preserved. We are told that he is a disappearing breed, with increasing pressures of hard to get research money, and private practice incentives, both driving him toward other career goals. It takes many post-graduate years to be a competent neonatologist and at the same time, a competitive scientist. Recent trends in funding of research training have made it increasingly difficult to achieve this status. Finally the NIH has recognized this as a national crisis, and steps are being taken to assure that, if motivation can be restored, training funds for career physician-scientists will be available.
>
> (Letter from MTS to LDL, 23 December 2009)

Several years earlier, Stahlman had observed:

> Throughout the history of newborn intensive care, research has played a major role. Many early intensive care units were established by research funds, and research has been the engine that has powered success in all its aspects. The failures have arisen largely from the acceptance of a new mode of management or a new technology before adequate and definitive controlled clinical trials have demonstrated its efficacy or potential harm. Management practices that were designed for or only applicable in specific situations have crept into generalized use, and, once in the mind-set, removal is virtually impossible.

[1] "Ulysses" a Poem by Alfred Tennyson (1809–1892; 1st Baron Tennyson)

> The importance of basic research in the understanding of fetal growth and development, the transition from intrauterine to extrauterine life, and the problems facing the premature or sick newborn cannot be overestimated. Basic research arises from an observation that cannot be explained by known facts and that challenges the observer to ask why, not how. This generates a cascade of hypotheses to be tested, each based on the addition of information derived from answering the prior question. The satisfactory answering of a question may depend on the level of the development of a given technology at a particular point in time, but technology has never posed a question or generated an original idea. The few people who stubbornly ask why are our national treasures. The translation of their basic principles into medical practice is the last and least original step in the production of better medical management that takes full advantage of the most advanced technology. The relative place of this final step in the scientific advancement of medical knowledge should never be forgotten.
>
> (Stahlman 1989, p. 1791)

As Stahlman has reflected, "In the long run it is the intellect, the integrity, and the conscience of the physician-scientist that must fulfill the tenet [of Hippocrates (460–375 BCE)] *primum non nocere* [first do no harm]" (Stahlman 1992, p. 20). As with the institution of intensive care units for adults, the concept of NICUs rapidly spreads throughout the developed world (for instance, for the UK; see Christie and Tansey 2001).

Another champion for the vital necessity of neonatal intensive care for the premature infant was William Silverman (1959, 1970). In a 1989 review of "Neonatal Pediatrics at the Century Mark," Silverman divided neonatal intensive care into the first 70 years, a "pastoral era," and the last 30 years, as a more intense "mechanistic era" extending to the present. Following a review of the vicissitudes during these years, he cautioned:

> After basic mechanisms concerning isolated phenomena have been worked out and rigorously battle-tested in preclinical studies, there is an understandable temptation to translate the hard-won information into practical action as soon as possible. But the lesson of history in neonatal medicine is very clear: at every turn, in the search for effective treatments, there are traps to snare the impatient innovator. It has been said that nature is a tyrant queen: make a mistake and she cuts off your head.
>
> In medicine we are always tempted by the optimistic vision of a dramatic therapeutic success. Our daily experience with misery and death makes us desperate to help, but, paradoxically, we need to be preoccupied with the *negative* aspects of innovation. The reason for this guarded attitude goes back to the fact that our observations about complex events in the natural world are never complete. There are, in fact, no criteria that would even allow us to judge completeness. Nonetheless, doctors, like engineers (and unlike scientists), are obliged to act on the basis of incomplete information about material phenomena. Action in the applied sciences is always an informed compromise. Compared with the small pond of knowledge in medicine, our ignorance is Atlantic.
>
> (Silverman 1989, p. 165)

A decade later, Nicholas M. Nelson categorized the first half century (1900–1950) as an "Era of Compassionate Observation" during which physicians practiced minimal intervention (Nelson 2000). This was followed by an "Era of Passionate Intervention" (ca. 1950–1980) during which a "... cornucopia of technology" helped to erect the edifice of contemporary neonatology. This, in turn, has

been succeeded by the "Era of Reflective Consolidation" (ca. 1980–2000) that presently exists (Nelson 2000, pp. 731–733). As Nelson emphasized, the advances that helped to create the newest era had their roots in sociology, technology, biology, and physiology.

As noted, in conjunction with conceptual advances in understanding physiology per se, of vital importance were those of medical technology. For the management of respiratory problems, Forrest Morton Bird (1921–2015) created and marketed the first low-cost, medical ventilator on a large scale, the *Bird Universal Medical Respirator* (1955, 1958; *Bird Mark 7*). This invention contributed greatly to management of individuals with respiratory failure and related conditions. Bird referred to this as the "Model T Ford" of respirators because it was easy to maintain and repair (http://www.latimes.com/local/obituaries/la-me-forrest-bird-20150803-story.html). For premature and other newborn infants, in 1970 the *BabyBird* respirator was introduced. In 1992, the *VIP Bird®Infant Pediatric System* was introduced. Recipient of many honors, Bird was inducted into the National Inventors Hall of Fame (1995), and he was awarded the US Presidential Citizens Medal (2008) and the National Medal of Technology and Innovation (2009).

Other vital technological advances to lessen morbidity and mortality in the prematurely born infant included microanalysis for blood gases, electrolytes, bilirubin, and so forth and specially designed equipment such as plastic small-bore endotracheal tubes, umbilical vessel catheters, electronic monitoring of newborn vital signs and oxygenation status, transcutaneous oxygen monitor, small infusion pumps, equipment to provide parental nutrition, and other advanced devices. In conjunction with these developments, advances in care of the newborn includes the maintenance of thermal stability and nutrition, antibiotics to combat infection, and other measures (Dunn 2007; Philip 2005), including institution of the "Usher regime" of intravenous bicarbonate infusion to correct metabolic acidosis, with glucose and insulin (Usher 1959, 1963).

In the USA and other developed countries, the 1970s saw an additional important factor in the success of NICUs in saving the lives of critically ill infants and decreasing their morbidity. This was the appreciation of the necessity of regionalization of healthcare, with such facilities being located in tertiary medical centers (Butterfield 1992; Lewis 1977; Lucey 1973; McCormick et al. 1985). A Committee on Perinatal Health of the National Foundation—March of Dimes—and its report "Toward Improving the Outcome of Pregnancy" also played a role in this regard (Committee on Perinatal Health 1976). In view of the "staggering cost" and "scarcity of personnel" for comprehensive care to mothers and infants at high risk (Committee on Perinatal Health 1976), the guidelines for regionalization, avoiding duplication of effort, became a standard for which to strive. This concept was stimulated, in part, by a demonstration in Quebec, Canada (Carrier et al. 1972; Usher 1970). Here, establishment of a province-wide program that mandated transfer of high-risk newborns to regional centers quickly reduced morbidity and mortality. Soon, this concept was inaugurated throughout Canada. Importantly, Usher and others recognized that more was needed than simply providing intensive care for the newborns per se. To further improve survival, they advocated

establishment of "perinatal centers," in which high-risk mothers would deliver their infants, which then could receive optimal professional intensive care (Segal 1972; Swyer 1970; Usher 1970). An additional argument for regionalization of care was that it was only in such tertiary centers with a number of infants that randomized controlled clinical trials could establish the therapeutic value of various modalities of prevention and therapy (Lucey 1973; Nesbitt 1974), e.g., "evidence-based" medicine. Jerold Frances Lucey of the University of Vermont, and editor of *Pediatrics*, with colleagues also advanced the concept of calculating birthweight to gestational age ratios to compare statistics from different tertiary care centers (Philip et al. 1981). In addition to major advances in technology and therapy, attention to sociological and psychological considerations has been important in care of these fragile and usually premature human beings at the beginning of life. In her *Newborn Medicine and Society*, Murdina MacFarquhar Desmond of Baylor College of Medicine, Houston, TX, has chronicled the history of care of the newborn infant with the evolution of neonatal intensive care in its social context (Desmond 1998).

Peter McNaughton Dunn has presented several accounts of the development of perinatal medicine and neonatal intensive care in the UK (Dunn 1978, 1983, 1998, 2003, 2007). In addition, he has written several dozen essays that feature the "great physicians" in *Perinatal Lessons from the Past* and which have appeared in the *Archives of Disease in Childhood*. Of special relevance, Dunn has pointed out the serious challenges that faced those who, in the early years (1950s and 1960s), devoted themselves to newborn care. These included the relative neglect of intensive perinatal care by pediatricians and obstetricians, the lack on the part of many in authority of the importance of the problem, inadequate staff, the absence of training programs in neonatology, the lack of appropriate equipment, little experience with the clinical problems with which one dealt, and the multiple errors made in dealing with these complicated issues (Dunn 1998, 2007). Dunn also has documented the struggles and development during these years of special care baby units, as appreciation for the need of specialized neonatal care followed by the 1948 enactment of the National Health Service. Dunn has documented from that time forward many aspects of the advances in newborn care, particularly those that are premature. About this time, an editorial in *The Lancet*, while in part addressing the issue of retrolental fibroplasia, noted:

> Most of our nurseries for the newborn are still without the means of monitoring environmental and arterial oxygen, even though the necessary equipment has been available for at least a decade; and resident paediatric staffing of all but the largest maternity units is virtually non-existent. The facts are sombre. Each year in Britain more than 17,000 babies die in the perinatal period, a number equivalent to deaths from all causes over the next 28 years of life. Almost as many deaths take place during the first week of life as during the whole of the remainder of childhood. Analysis suggests that this mortality might be reduced by at least a third and possibly by half if modern knowledge and resources in perinatal care were made generally available throughout the country. . . . Surely the time has come to recognise on both humane and economic grounds that the cost of continued perinatal neglect is far too great.
>
> (Anonymous 1974, pp. 437–438)

As observed earlier, advances in neonatal care included continuous positive airway pressure to improve ventilation, attention to thermal stability, monitoring acid-base status, preventing retinopathy from excessive O_2, hypoglycemia, the use of parental nutrition, and other modalities. Importantly, Dunn also has offered a number of personal recollections of his role in advancing the frontiers including helping to found and foster the British Association of Perinatal Medicine, its contributions to life, and incorporation into a multidisciplinary body with profound influence for good (Dunn 2003, 2007). In addition, he has recorded efforts of the joint British Paediatric Association-Royal College of Obstetricians and Gynaecologists Liaison Committee under his chairmanship (1978), to prepare a comprehensive series of recommendations to improve care during the perinatal period (Dunn 1978, 2007). During the 1970s these special care baby units evolved into full-fledged NICUs and have played a vital role in increasing fetal and neonatal survival and well-being (Dunn 2007).

A Wellcome Trust "Witnesses to Twentieth Century Medicine" conference, chaired by Robert (later Sir Robert) Boyd of the University of Manchester, also has reviewed many aspects of development of neonatal intensive care in the UK and their dependence upon, and close association with, advances in fetal and neonatal physiology (Christie and Tansey 2001). As noted, the 1974 *Lancet* editorial "The Price of Perinatal Neglect" drew attention to the extreme need for properly trained neonatologists and appropriate care of the newborn. After mentioning many of the iatrogenic-induced diseases including retrolental fibroplasia with blindness (excessive oxygen), hypothermia, hypoglycemia, kernicterus (excessive vitamin K), "gray baby" syndrome (chloramphenicol), and others, the editorial writer emphasized the fact that in Britain, although first-day mortality among low-birth-weight newborns decreased in the previous decade, an excessive number continue to die. "Analysis suggests that this mortality might be reduced by at least a third and possibly by half if modern knowledge and resources in perinatal care were made generally available throughout the country. Moreover, mortality is only a fraction of total perinatal morbidity" (Anonymous 1974, p. 437).

Several reviews have explored various aspects of the history and immense contributions of neonatology (Cone 1983, 1985; Yaffe 1992), including the evolution of neonatal intensive care in terms of the "hands-off" years prior to 1950 (Robertson 2003a; Silverman 1989), the "heroic" years from 1950 to 1970 (Robertson 2003b), and the "experienced" years, 1970 to 2000 (Robertson 2003c). Guidelines for resuscitation of the newborn have been presented by the American Heart Association (Kattwinkel et al. 2010).

20.2 Retinopathy of Prematurity

As noted, management of the premature infant has presented numerous challenges. A report of O_2 requirements in early life documented the relative lack of data and disagreement regarding the available data on the optimal arterial blood O_2 levels for

the newborn. As it became appreciated that the skin color of premature neonates was an unreliable indicator of their state of oxygenation, a related question concerned the extent to which these infants suffered from "subcyanotic anoxia" (Smith and Kaplan 1942). In the misguided belief that following birth the infant should quickly achieve arterial blood gas values similar to those of the adult, following its first use in the late 1920s and early 1930s oxygen became widely overused (Raju 1999). Placing the situation in perspective, William Silverman observed that O_2 therapy had become an "albatross" for the neonatologist (Silverman 2004). Also as Silverman noted, one of the factors leading to hyperoxygenation was the fact that, in the early years, incubators were a kind of pressure cooker, with tight-fitting gaskets and novel O_2 intake float valves, almost insuring maintenance of elevated O_2 levels (Silverman 2004).

A bittersweet sequela in the treatment of infants with respiratory disease syndrome with elevated oxygen levels (for many of whose lives were saved) was the development of what originally was referred to as retrolental fibroplasia (RLF) with blindness. The "first epidemic," of what later would be called retinopathy of prematurity (ROP), was reported by the Boston ophthalmologist-pathologist, Theodore Lasater Terry (1899–1946), who termed it *amblyopia ex anopsia* [dull vision that results from disuse] (Terry 1942). Terry believed this to be a consequence of persistence of the hyaloid artery with fibroblastic overgrowth of persistent *tunica vasculosa lentis* [vascular coat of the lens] (Terry 1943). In his 1944 report of 160 cases from Boston, Chicago, and other cities, Terry introduced the term retrolental fibroplasia, pointing out that this condition develops in about 10% of premature infants weighing less than 3 pounds (Chandler 1947; Terry 1944, 1945). Numerous reports soon appeared from other centers (Hepner et al. 1950; Hipsley 1952; Kinsey 1950, 1951; Kinsey and Zacharias 1949). At Johns Hopkins Hospital, Baltimore, ophthalmologist husband and wife team William Councilman Owens (1917–2006) and Ella Uhler Owens (1914–1999) demonstrated that RLF developed postnatality in 12% of infants weighing less than 1360 g (3 lbs.) and that it did not involve the hyaloid vascular system (Owens and Owens 1949a). Although they believed the condition to be a consequence of imbalance of vitamins A and E and that it could be prevented by treatment with the antioxidant d-1 alpha tocopherol acetate (a form of vitamin E) commencing in the first week following birth (Owens and Owens 1949b), this proved not to be the case. In a subsequent report, Owens and Owens placed the RLF prevalence higher, at 15% in infants who weighed less than 1360 g, and recorded that in these infants the first ophthalmologic changes occur at 4 weeks of age, with the retrolental membrane fully formed by 4 months of life (Owens and Owens 1950). By mid-century RLF had become the most common cause of blindness in children (Tasman et al. 2006).

The role of oxygen in the genesis of this disorder first was suggested by a comparison of the incidence of RLF in the USA where O_2 therapy was used freely, with that in the UK where it was used more sparingly. In conjunction with hyperoxia, Kate Isabel Campbell (1899–1986) of Melbourne associated RLF with the development of edema in loose connective tissue in the premature infants. In a survey of three Melbourne nurseries, she reported that of 123 infants in a "high-

oxygen" group, 23 developed RLF (18.7%). In contrast, among infants in the "moderate-oxygen therapy" group (for which the families were charged for each tank of oxygen used), only 4 of 58 (6.9%) developed this disorder. Campbell concluded that in cases of cyanosis, O_2 only should be given in amounts to "... keep the infant's colour satisfactory" (Campbell 1951, p. 49). In 1966, the University of Melbourne conferred upon Campbell the degree doctor of laws *honoris causa* [with public esteem and honor].

Following a 1947 visit to the USA, during which she learned of the increasing prevalence of RLF, the Birmingham, England, neonatologist Victoria Mary Crosse reported on the low prevalence of this disorder in her experience (14 cases in over 6000 deliveries of preemies from 1945 to 1950) and that 12 of the 14 cases had been given continuous O_2 for periods varying from 2 to 5 weeks (Crosse 1951). About this same time, from experimental studies in newborn kittens exposed to 60–70% O_2, the first ophthalmic pathologist in the UK, Norman Henry Ashton (1913–2000), and colleagues of the University of London demonstrated RLF to be a consequence of hyperoxia-induced vasoconstriction (vaso-obliteration), followed upon return to atmospheric air by "a reopening of the vessels." Many vessels remained permanently obstructed by collapse or blood clot, however, so that the normal architecture was not restored. "The reformed network was grossly abnormal, haemorrhages occurred, retinal re-vascularization recommenced from the disc, blood vessels grew into the vitreous, and retinal detachment developed.... These phenomena are regarded as significant in the genesis of retrolental fibroplasia in man" (Ashton et al. 1953, p. 520). Over the next decade, Ashton would explore several aspects of the role of oxygen in the development of the retinal vasculature (Ashton 1966, 1970a, b; Ashton and Blach 1961; Ashton and Pedler 1962). As head of the Institute of Ophthalmology, Ashton became a world authority in many aspects of this field. A recipient of many honors, he was a fellow of the Royal Society and in 1976 was made commander of the Order of the British Empire (Luthert and Langley 2005).

Also at this time, in rats and mice (but not opossums) subjected to 60–80% oxygen for several days, Arnall Patz (1920–2010), of the District of Columbia General Hospital, Washington, DC, and subsequently of the Wilmer Eye Institute, Johns Hopkins University, Baltimore, also demonstrated the role of oxygen as being a causative factor in the genesis of RLF (Patz et al. 1952, 1953; Tasman et al. 2006). Later, he defined this association further (Patz 1955; Patz and Eastham 1957a, b). For his many contributions to life, and to understanding the pathogenesis of retinopathy of the newborn, in 2004 Patz was awarded the Presidential Medal of Freedom.

Another early report that demonstrated the virtues of limiting ambient O_2 levels was that of Joseph Dancis and colleagues at the New York University-Bellevue Medical Center. Reporting on 148 surviving infants of birthweights less than 2000 g over a two and one-half-year period, these workers demonstrated that not one case of cicatricial retrolental fibroplasia was observed among those infants who had received less than 40% O_2, administered for brief periods as possible and only when clinically required (Guy et al. 1956).

Because so much controversy surrounded the early reports of RLF, the policies regulating the use of oxygen changed periodically, a reflection of the limited knowledge regarding O_2 metabolism and toxicity (Obladen 2009; Tin and Gupta 2007; Vento 2014). Under the aegis of the National Institute of Neurological Diseases and Blindness, 18 major centers in the eastern USA agreed to cooperate on a prospective randomized control clinical trial which involved about 750 premature infants (grouped by weight 1000 g or less, 1001–1250 g, and 1251–1500 g). The study compared the use of "routine" (high) ($FIO_2 > 50$) oxygen for 28 days with the "curtailed" ($FIO_2 < 50\%$) oxygen (the latter given only for cyanosis or respiratory difficulty). In these two oxygenation groups, the observed RLF incidence were 22.6% and 6.6% ($p < 0.01$), respectively (Kinsey 1956; Kinsey and Hemphill 1955). Thus, the role of hyperoxia was confirmed in the human newborn, and O_2 use was recommended to be limited to less than 40% for as short a period of time as possible. Nonetheless, this study left many questions unanswered, including the genesis of retinopathy of prematurity in this infant cohort. As Silverman has critiqued, unfortunately debate ended and the mantra "under 40% is safe" was accepted as gospel; and if an infant developed RLF, it was held as proof that the 40% FIO_2 limit had been exceeded (Silverman 2004, p. 395). Two decades later, in an attempt to define further the level of arterial O_2 tension and exposure duration responsible for development of retrolental fibroplasia, under the aegis of the National Society for the Prevention of Blindness, many of the same investigators conducted a further five university center studies of 589 low-birth-weight infants (Kinsey et al. 1977). Because of failure to maintain rigorous methods, this report continued the confusion. Also, in a letter to the editor, two "disenchanted investigators" soundly criticized the study, not only pointing out methodologic deficiencies but stressing the need for a large properly collaborative controlled trial to address the issues involved (*Les Chermignonards Désenchantés* 1977). In reaction to this anonymous critique, the original authors wrote a blistering response (Kinsey et al. 1978). Aside from demonstrating again an association of RLF with O_2 levels among the low-birth-weight (<1200 g) infants, the study emphasized that intermittent and infrequent blood gas measurements may not reflect true arterial O_2 values (Kinsey et al. 1977; see Lucey 1977). In commenting on this "second epidemic" of RLF (Phelps 1981), Jerold Lucey cautioned "... no criteria exist which guarantee the safe use of oxygen in very low birth weight infants ... It is time to admit our ignorance and to begin new studies which might help us in finally preventing this disease" (Lucey 1982, p. 497).

As a "parable for modern man," Silverman documented aspects of this tragedy including the design of clinical studies such as those quoted. Silverman, and others, emphasized the requirement for controlled clinical trials to establish optimal therapies for medical conditions about which little is known (Silverman 1980b, 1987). A report of the "early treatment for retinopathy of prematurity cooperative group," in which almost 7000 infants from 26 centers in the USA were screened, concluded that among those preemies weighing <1251 g or less, the incidence of ROP, its time of onset, rate of progression, and timing of prethreshold disease have changed little during the previous two decades (Good et al. 2005). A subsequent retrospective

study, based on the National Inpatient Sample maintained by the Ageing for Healthcare Research and Quality of 34 million live births from 1997 through 2005, reported on ROP incidence of 15.6% in premature infants (many of which had associated intraventricular hemorrhage) whose length of hospital stay was more than 28 days (Lad et al. 2009).

The paradox remains in treating critically ill newborns that, although lower levels of O_2 reduce the risk of retrolental fibroplasia, levels that are too low increased deaths from respiratory distress (Stenson 2011), and some believe that it may increase the chance of survival with cerebral palsy or other neurologic disorder. Several reports consider the dilemma of optimal oxygenation while avoiding the hazard of hyperoxia with superoxide generation with consequent damage (Fabian et al. 2008; Saugstad 2008; Saugstad and Aune 2011; Saugstad et al. 2011, 2012). In *A Guided Step into the Unknown*, Silverman reviewed many aspects of clinical trials and human experimentation (Silverman 1985). As observed by others, during the past half century or more, "oxygen must have been given to more infants than any other medical product . . . yet we still know very little about how much . . . [they] actually need, or how much it is wise to give. . . . Fifty years of observational study have gotten us nowhere" (Tin 2002, p. 615; see also Tin and Gupta 2007; Tin et al. 2001). More recently, details of the pathophysiologic mechanisms of early vascular disease have been suggested, with a two-phase hypothesis distinguishing between physiological retinal vascular development and vasoproliferation, as well as aspects of management (Hartnett and Penn 2012), with target ranges for arterial [HbO_2], in preterm infants (Dawson et al. 2010; Tarnow-Mordi et al. 2010; Vento 2014). The most recent European guidelines (Sweet et al. 2013) recommend maintain [HbO_2] at 90–95% in very preterm infants up to those 36 weeks of age. Nonetheless, many questions remain unanswered.

20.3 Transcutaneous O_2 Measurements

As noted, the moments of transition from fetus to newborn poses an extraordinary challenge for the neonate, especially for one that is premature. Indeed, a number of such infants require vigorous resuscitation to achieve postnatal stability. While in the delivery room and thereafter, positive pressure ventilation and oxygenation are the most relevant interventions. Nonetheless, a challenge for neonatologists is that of the infant O_2 needs during resuscitation. While hyperoxia is associated with the generation of oxidative stress and reactive oxygen species, hypoxia also can be associated with these as well as a number of serious short- and long-term sequelae. As may be appreciated from the above account of the role of oxygen in retinopathy of prematurity, of critical importance is the necessity of monitoring O_2 levels in arterial blood and tissues. Supplemental O_2 is of course vital to neonatal resuscitation and many aspects of intensive care. A two-edged sword, in the premature infant O_2 excess with toxicity, is associated with severe disorders in addition to ROP including bronchopulmonary dysplasia (Merritt et al. 2009), neurologic disabilities

(Marlow et al. 2005; Wolke et al. 2008), and many other conditions (Sola 2008). To complicate the picture, controversy continues to surround several aspects of the issue of monitoring O_2 levels (Klein et al. 2010; Merritt and Mazela 2010).

Following the invention of O_2 and CO_2 electrodes, and their fabrication for application in anesthesia and intensive care medicine, these were adapted to measure continuously and in a noninvasive manner blood gas values through the skin. Originally developed to measure the metabolic rate of skin, and based on earlier studies by others, in the late 1960s Dietrich Werner Lübbers (1917–2005) of the Department of Applied Physiology, University of Marburg, commenced developing methods to measure capillary [HbO_2] levels (Lübbers 1966, 1981, 1987) and transcutaneous PO_2 and PCO_2 levels ($tcPO_2$ and $tcPCO_2$, respectively; Lübbers et al. 1973, 1979). Lübbers has reviewed the theory and development of these methods (Lübbers 1979, 1981, 1987). Soon, he was joined by the husband and wife team Albert and Renate Huch, who, stimulated by the intermittent fetal scalp blood sampling studies of Erich Saling of Berlin (see below), sought to develop a method to measure continuously O_2 levels in the presenting fetal scalp during late labor and delivery. They also sought to correlate these measurements with electronic FHR rates (Huch and Huch 1979, 1985; Huch et al. 1981; Lübbers et al. 1973). Because of its complexity, a number of problems plagued this transcutaneous methodology including maintenance of electrode contact during the course of labor, calibration of the electrode, differing reflection coefficient of skin versus that of hemoglobin, the nonhomogeneous distribution of capillaries and hemoglobin in the skin area studied, and the need to heat the skin to 43° to 45 °C or produce hyperemia by other means to arterialize the capillary bed. In a collaboration with the pediatrician Gösta Rooth (1918–2008) at the University of Lund (and later at the University of Uppsala), Sweden, using a PO_2 electrode on an adult, they obtained transcutaneous PO_2 measurements that mirrored closely (with a delay of ~30 s) that of arterial blood (Huch et al. 1972, 1973a). Subsequent studies in newborn infants confirmed these findings and demonstrated that while this methodology was impractical for the fetus during the course of labor and delivery, it had great potential for the newborn (Huch and Huch 1979, 1985; Huch et al. 1973b, 1981).

Also in an effort to monitor continuously arterial blood gas values by transcutaneous microelectrodes, anesthesiologist and authority in blood gas analysis John Wendell Severinghaus of the Cardiovascular Research Institute, University of California, San Francisco, and colleagues worked to develop these for both $tcPO_2$ (Severinghaus et al. 1978a) and $tcPCO_2$ (Severinghaus 1977; Severinghaus et al. 1979). In several reports he described their fabrication, calibration, selection of skin site, preheating, estimation of skin diffusion resistance, and computation of arterial PO_2 and PCO_2 from the $tcPO_2$ and $tcPCO_2$ values (Severinghaus 1982, 1983; Severinghaus et al. 1978b). In newborn infants, his group established the surprisingly close relationship of $tcPO_2$ to arterial PO_2 (Peabody et al. 1978) and showed that the heating power used by these electrodes usefully monitors mean arterial pressure in the compromised newborn (Peabody et al. 1979). A 1978 international symposium, held in Marburg, West Germany, under the aegis of the National Foundation March of Dimes, presented almost 90 reports reviewing various aspects

of the techniques and results of transcutaneous O_2 and CO_2 monitoring in obstetrics and pediatrics (Huch et al. 1979).

As technology has improved and the cost of equipment decreased, over the past several decades, transcutaneous measurements of arterial PO_2 and PCO_2 have become routine. In addition, pulse oximetric estimation of arterial oxyhemoglobin saturation (reported as SpO_2), developed in the 1980s and 1990s, has significantly reduced the need for transcutaneous measurements. Nonetheless, despite several clinical trials, the dilemma of maintaining optimal oxygenation while avoiding the *Scylla* of hyperoxia and *Charybdis* of hypoxia remains a challenge in neonatal care (Rosychuk et al. 2012; Saugstad and Aune 2011; Saugstad et al. 2011; Vento 2011).

20.4 Thermoregulation

Because of several factors such as its relatively large skin surface area to body mass, its limited ability to generate heat through muscular contraction (shivering thermogenesis), and its relatively poor thermal insulation from the environment, the newborn infant is vulnerable to body heat loss and hypothermia. Thus, following birth, temperature of the mammalian newborn falls. Beginning in the late-nineteenth and early-twentieth century, the importance of regulation of newborn temperature came to be appreciated. Understanding the physical/chemical processes by which an infant, particularly the premature, regulates its body temperature as a homeotherm (or endotherm, e.g., maintains constant body temperature), as opposed to a poikilotherm (or ectotherm, e.g., having body temperature equal that of ambient temperature), was aided by the development of incubators. A 1930 study from Boston had suggested that the premature infant could not maintain body temperature equal to that in utero because of incomplete neural development. It also suggested that subnormal temperatures were a "characteristic" of prematurity and that attempts to maintain its body temperature at 37 °C (98.6 °F) may be detrimental to optimal growth, even leading to death (Blackfan and Yaglou 1933). Not until the mid-1950s, however, did a series of randomized, controlled, clinical trials by William Silverman, and colleagues at Columbia University, establish the vital importance of thermal stability for survival of preterm infants (Silverman 1959; Silverman et al. 1958). In turn, this work was based, in part, on that of Richard Lawrence Day (1905–1989), originally at Columbia University and later at Cornell (Day et al. 1943), and others (Mann and Elliott 1957; Mordhorst 1932), demonstrating that premature infants, most of whom were one week of age or older, increased their metabolic rate on exposure to cold air. These studies also demonstrated that newborn survival was significantly greater in an atmosphere of 80–90% relative humidity, as compared to that at 30–60% (incubator air temperature was 84 °F, 28.3 °C in both groups). Of note, core body temperature of infants maintained at the higher value of relative humidity was significantly greater, leading the authors to postulate a "normothermic hypothesis" that survival was optimized in an environment that maintained their temperature at the elevated

temperature (Silverman and Blanc 1957). Silverman also reported lower mortality at a higher ambient temperature (89 °F, 31.7 °C; Silverman 1959). Silverman later recognized that the lower mortality at high humidity was due to less evaporative heat loss and, in very early preemies, small increases in body temperature contributed greatly to survival (Silverman 1994). Following the early studies, factors regulating infant heat production versus heat loss were elucidated (Sinclair 1970). Findings suggest that to maintain normal physiologic functions, infants required a "neutral thermal environment" or "thermal neutral zone" dependent upon gestational age and birthweight. Shortly thereafter, incubators with temperature servo control were introduced.

In regard to heat production in the developing organism, studies at Oxford's Radcliffe Infirmary demonstrated in newborn kittens and rabbits a striking rise in O_2 consumption following infusion of norepinephrine, a phenomenon that was essentially absent by 20 days of age (Scopes and Tizard 1963). Soon, based on the large increase in heat production by the newborn rabbit upon exposure to cold, David Hull (later Sir David) and his Oxford colleagues identified brown adipose tissue as a specialized heat-producing organ for the newborn infant (Aherne and Hull 1964, 1966; Dawkins and Hull 1964; Hull 1966). Brown adipose tissue in the newborn, much of it located around arteries in the neck, in the mediastinum, and in the abdominal cavity, had been recognized earlier, as being similar to intrascapular and auxiliary fat, as well as that of the "hibernating gland" located in the mediastinum and neck of hibernating mammals (Carlier and Evans 1903; Rasmussen 1923). This tissue also was known to have a rich blood supply and a wealth of mitochondria and to be of vital importance in the regulation of body temperature (Brück 1961; Johansson 1959; Dawkins and Hull 1964). As a site of non-shivering thermogenesis, brown adipose tissue content is greatest in the premature infant, decreasing with age (Aherne and Hull 1966; Dawkins and Scopes 1965; Hull 1966). This tissue is now recognized to be a primary site of energy expenditure through sympathetic-initiated thermogenesis, mediated, in part, by epigenetic-mediated conversion of circulating thyroxine (T4) to the more active triiodothyronine (T3) with upregulation of mitochondrial uncoupling protein (Cannon and Nedergaard 2004). Thus, brown fat mitochondria generate heat without producing energy in the form of ATP. For newborns of differing gestational age and size, a range of temperatures that constitute the "neutral thermal environment" has been found to minimize energy expenditure (Hey 1975). Arlin Brice Blood and Gordon Gilbert Power of Loma Linda University have reviewed in extenso thermoregulation in the fetus and newborn infant, its regulation by O_2 availability, hormones, and other factors (Power and Blood 2011).

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20.5 Some Aspects of the Development of Maternal-Fetal Medicine

From a clinical standpoint, an obvious necessity during pregnancy is the assurance of fetal well-being culminating in the delivery of a healthy infant. Thus, periodic assessment of the developing fetus is key to reducing the risk of antepartum fetal death. During the first decade of the twentieth century, the field of maternal-fetal medicine commenced initially as a social movement of maternal and child health. A veritable public crusade flowered for the improved care of pregnant women and their children, an advance that had its roots in concern for the astonishingly high death rates during the first few years of life. Pediatricians, obstetricians, nurses, public health workers, and women's groups strove to establish well-baby clinics, assure clean milk supplies, and eliminate the waves of epidemic infections that accounted for a huge loss of life. Soon it was realized, however, that without properly managed pregnancy, the benefits of pure milk were limited. Concurrently the works of John William Ballantyne (1861–1923) at the University of Edinburgh (Ballantyne 1902–1904) and John Whitridge Williams (1866–1931) at Johns Hopkins University (Williams 1903) helped to focus attention on several issues, including the enormous numbers of mothers who died of pregnancy-related disorders. Another focus of attention was on the diseases of young children that had their origin during fetal life and the realization that, to a certain extent, the fetus could be treated by correcting the underlying pathologic condition in the pregnant mother. These ideas contributed to the origins of prenatal care (Longo 1988).

In 1909 the Association for the Study and Prevention of Infant Mortality was organized to promote studies in maternal and child care (Williams 1910). Soon thereafter and as a consequence of a 1912 White House Conference on Standards of Child Welfare, the Children's Bureau of the Department of Labor was established. Its mission was for the purpose of "... investigating and reporting on all matters pertaining to the welfare of children and child life among all classes of people" (Longo 1988, p. 7). Under the leadership of Julia Clifford Lathrop (1858–1932), the Bureau conducted numerous early studies relating to maternal and child health. At that time in the USA, leaders in promoting more scientific obstetrics and pediatrics included obstetricians John Whitridge Williams and Joseph B. DeLee and pediatricians such as Abraham Jacobi (1830–1919) (Jacobi 1887) and Luther Emmett Holt (1855–1924) (Holt 1894). In the UK, these included obstetricians such as John Martin Munro Kerr (1868–1960), William Blair-Bell (1871–1936), and Sir John Harold Peel (1904–2005) and pediatricians such as Sir Leonard Parsons (Parsons and Barling 1933).

Until mid-twentieth century, the only way of interrogating the developing fetus in utero was by assessing its movements or stethoscopic auscultation of the heart. As noted in an editorial of the time, entitled "Foetal Medicine—Who Is to Practise It?":

> Up to now the formidable inaccessibility of the human foetus has meant that foetal medicine (apart perhaps from foetal electrocardiography) has virtually not existed. In an

> age when Man has been able to measure most things from an atom to a galaxy, it is thus paradoxical that to measure his own size during the most critical and precarious period of his life, he still has to depend upon the extreme fallibility of the palpating hand.
>
> (Anonymous 1966, p. 453)

After pointing out that to this time, because of the obstetrician's prime interest in the pregnant mother, and that of the pediatrician in the newborn, "... the foetus has been nobody's baby," the editor noted further:

> With the advent of the techniques of amnioscopy and foetal blood sampling ... and of amniocentesis and foetal transfusion ... we witness the end of the long period of foetal inaccessibility and, we hopefully believe, the start of the science of foetal medicine.
>
> (Anonymous 1966, p. 453)

The editorial concluded, "... is a new kind of doctor needed, at least at the academic level, who, by combining the interests and skills of both the obstetrician and the paediatrician, can act as the foetus's doctor?" (Anonymous 1966, p. 453).

As noted, two visionaries in the development of what today we call maternal-fetal medicine were John William Ballantyne of Edinburgh and John Whitridge Williams of Johns Hopkins University. Ballantyne introduced the idea of a pro-maternity hospital to provide inpatient antenatal care with preventive medicine for obstetrical patients (Ballantyne 1901, 1923). He also ardently advocated the study of the pathology of the fetus and newborn infant and published a two-volume *arbeit* on the subject (Ballantyne 1902–1904). In addition to his contributions in the development of academic departments of obstetrics and gynecology in America and his authoritative textbook of obstetrics (Williams 1903), Williams is noted for his role in the development and popularization of prenatal care and the child heath movement (Longo 1981; Williams 1910, 1915). It has been suggested that because of unprecedented advances in and emphasis upon gynecologic surgery, during the first half of the twentieth century, the visions of these two leaders were eclipsed (Dunn 2007).

In terms of the management of labor and delivery, contributions that helped to set the stage for what was to follow were the mid-century graphical analysis of the course of labor (Friedman 1955) and scoring of cervical dilatation for the elective induction of labor (Bishop 1964). Again, beyond witnessing just a new era in clinical medicine, the second half of the twentieth century may be regarded as a sweeping renaissance in concepts, technology, and care. As a consequence of advances in basic as well as clinical sciences, increasing knowledge with "translation" to the bedside contributed to a number of aspects of health and the diagnosis and treatment of disease. Among numerous diagnostic and therapeutic advances, treatment of the fetus in utero as a patient became a practical reality, overcoming the view, held until then, that the intrauterine sanctuary was inviolate (Rooth and Saugstad 1985; Saling 2006; Saling and Arabin 1988). With the ever-increasing complexity and challenge of providing optimum care for two patients, obstetrics has given birth to the field of maternal-fetal medicine, a subspecialty further dividing within itself. As noted, during the early- to mid-1930s, Nicholson Eastman

and colleagues had demonstrated acidosis with elevated lactate levels in the umbilical cord blood of depressed neonates (Eastman 1932; Eastman and McLane 1931).

Also in the 1950s, Virginia Apgar developed her scoring system in an attempt to quantify the well-being of the newborn infant (Apgar 1953). With L. Stanley James and coworkers, she quantified the degree of neonatal depression by correlating umbilical cord blood pH with the Apgar score at one and 5 min (James et al. 1958). As noted, also during this era, advances in hematology contributed to the virtual elimination of death from Rh disease (Bevis 1952; Diamond et al. 1951; Finn et al. 1961; Freda and Gorman 1962; Liley 1961, 1963, 1964).

Thus, at mid-century, considerable emphasis in clinical obstetrics was beginning to be placed on fetal assessment. Beyond clinical evaluation of "high"-risk pregnancy, quasi-objective determinations included those of fetal size, height of the uterine fundus, auscultation of fetal heart rate, and monitoring fetal movements. During the next decade, more objective measures of evaluating fetal well-being became widely accepted. These included electronic monitoring of fetal heart rate (EFM) (which allowed the continuous assessment of one aspect of the fetal state during the course of labor; see below) and measurements of hormones such as estriol, human chorionic gonadotrophin, and somatomammotropin and sampling of amniotic fluid (amniocentesis) for measures of hemolytic pigments, pulmonary surfactant activity, cells for cytogenetic diagnosis and chromosomal analysis (Fuchs et al. 1956/57; Fuchs and Riis 1956; Tabor et al. 1986), and other constituents (Harman 2009; Parer 1991). Increased facility in amniocentesis resulted from advances in ultrasonic imaging.

In an attempt to determine its state of well-being, an additional important contribution of fetal physiology to clinical practice in this era was that of sampling blood directly from the fetus. In 1960, Erich Saling of the *Städt Frauenklinik und Hebammenlehranstalt* [City Clinic for Women and Institution for Midwife Instruction], Berlin, developed the idea of obtaining a small aliquot of fetal blood from the scalp or other presenting part during the course of labor. This approach overcame centuries of ethical and emotional barriers. In his report, "New Procedures for Examining the Fetus during Labor: Introduction, Technique, and Basics," Saling described his pioneering approach to assess the fetal condition by obtaining fetal scalp blood. He championed the concept of combining pH with abnormal fetal heart rate pattern (Saling 1962). Subsequently, direct blood sampling of the fetal umbilical cord for diagnosis and treatment was introduced, and the new field of maternal-fetal medicine blossomed (Huntingford 1964; Morris and Beard 1965; Nicolaides 1986; Saling and Schneider 1967). Saling has described the development of his ideas along this line, much of this work originally being associated with an attempt to diagnose and treat severe Rh incompatibility (Saling 1959, 1961b). With the development of micro measurement techniques for blood sample analysis, Saling published his report of obtaining fetal scalp blood during labor (Saling 1961a, 1962, 1966, 1981, 1985). Regarding its clinical utility, Saling maintained that in cases of abnormal heart rate patterns fetal scalp blood analysis enables one to establish the presence or absence of fetal hypoxemia or acidosis. In addition, such analysis may

help one to determine whether tocolysis (inhibition of uterine contractions) or rapid operative delivery is required (Saling 1985).

The introduction of this approach was not without criticism, and with current advanced methodology, the sampling of scalp blood has become uncommon. Saling reported that his initial grant application to support these studies was rejected. The reviewers believed fetal blood sampling to be "... ethically inadmissible to break the taboo of the unborn infant" and that the presence of the *caput succedaneum* would eliminate the validity of the blood sample measurements (Saling 1985, p. 109). Another whose voice was raised against this approach was that of Nicholas Assai. He argued:

> In recent years, we have witnessed an avalanche of papers written by various investigators all exalting the value of fetal blood pH determination and proclaiming it as the key to the mystery of intrauterine life. A rush has been set off to obtain blood samples from the fetal caput, buttocks, feet, or any other accessible anatomical part with utter disregard for such accuracy prerequisites as anaerobic collection, knowledge of sample origin—whether arterial or venous, stagnant or freely circulating, uncontaminated, and so forth. I am sure that this is a temporary overenthusiasm on the part of clinical investigators, and that the pH fever will subside as soon as we learn the true meaning of fetal blood pH and its relation to the over-all picture of fetal acid-base balance and fetal-maternal interrelationship.
>
> (Assali 1967, p. 325)

Following Saling's introduction of the fetal scalp sampling technique, it remained for another two decades before blood samples were taken directly from the umbilical vessels with the fetus in utero, e.g., percutaneous umbilical blood sampling (Daffos et al. 1983).

This period also witnessed methods of culturing fetal cells from the amniotic fluid (Jacobson and Barter 1967) and examining the chromosomes for infant gender (Shettles 1956) or congenital abnormality. Discovery of the fetal-specific alpha-fetoprotein (Bergstrand and Czar 1956; Gitlin and Boesman 1966), its elevation in cases of neural tube defects (Brock and Sutcliffe 1972) and lower than normal levels in fetuses with trisomy 21 (Merkatz et al. 1984), served as a marker for prenatal screening. With the advent of electronic fetal heart rate (FHR) monitoring (see below and Hon and Quilligan 1968), associations could be made between FHR patterns and neonatal outcome. With introduction of the oxytocin challenge test (Ray et al. 1972), the FHR response to uterine contractions could be assessed. With the observation of an accelerated heart rate during spontaneous uterine contractions (Lee et al. 1975; Trierweiler et al. 1976), this then served as a basis for the fetal activity determination test (Lee et al. 1976), later renamed the non-stress test.

By allowing, for the first time, visualization of many aspects of fetal and placental growth and development, the introduction of ultrasonography of the pregnant woman revolutionized obstetrics (Donald 1969, 1974; Holmes and Howry 1963). Among other considerations, this permitted assessment of the fetal "biophysical profile," e.g., measurements of fetal breathing movements, gross body movements, muscular tone, reactive heart rate, and amniotic fluid volume. To provide a dynamic assessment of well-being, each of these variables could receive a score of zero or two (Manning 1999; Manning et al. 1980; Platt et al. 1983).

Continued technological advances have allowed detection of various indices of fetal growth (brain, biparietal of head, chest, abdominal dimensions, and other descriptors) and a host of congenital anomalies. As a critical component of a healthy pregnancy, and the long-term health and well-being of the offspring, assessing fetal growth and defining its restriction has become an important aspect of contemporary maternal-fetal medicine. Among many considerations:

> A population reference is often established on the basis of a large sample size (ideally representing the underlying population), with a study population that includes both low-risk and high-risk pregnancies and both normal and abnormal perinatal outcomes. On the other hand, a standard usually is based on low-risk pregnancies with a normal outcome. When the "population reference" and the "standard" are applied to an individual fetus or infant, interpretation of the findings differs. Use of a population reference will yield a relative fetal size in relation to the total population; a standard will assess a fetal size in comparison to normally grown fetuses. Thus, a standard may have more clinical utility than a population reference.
>
> (Zhang et al. 2010, p. 522)

As suggested in an editorial that accompanied this report, one should limit the term "small for gestational age" to the fetus and newborn infant whose weight is <10th percentile for population-based gestational age and limit the designation of "fetal growth restriction" (FGR) to those fetuses and infants whose growth is believed to be less than optimal, "... recognizing that all SGA infants are not all FGR, and that FGR infants are not all SGA, SGA would be based on growth percentiles, and FGR would be based on evidence of pathologic growth" (Iams 2010, p. 513). The assessment of the health and vitality by not only 2D, 3D, and 4D ultrasonography and echocardiography but by sampling of fetal blood and tissues, chorionic villus sampling, amniotic fluid biomarkers, and other measures, as well as fetal therapy per se, has introduced a "brave new world" into the care and management of the pregnant woman and her developing infant. Consideration of all of these modalities is far beyond the limits of this essay. A striking aspect of these advancements was the rapidity with which they occurred; with the emergence of maternal-fetal medicine and high-risk perinatology, clinical obstetrics underwent a virtual revolution. In view of the seemingly innumerable aspects and complexities of high-risk diagnosis and care demanded in the present day, a number of papers and volumes may help to guide the perplexed (Bianchi et al. 2000; Creasy et al. 2004; Hansen and Sladek 1989; Harman 2009; Malcus 2004; Nageotte and Gilstrap 2009; Queenan et al. 2010; Reece and Hobbins 1999, 2007; Salvadori 1981). In terms of prenatal genetic diagnosis, two technologies have emerged that hold great promise. Following in vitro fertilization and prior to transfer to the uterus, preimplantation genetic diagnosis by removal of single cells from each blastocyst (day 3) can be assessed for single gene disorders as well as chromosome abnormalities (Munné 2003). In addition, cell-free fragments of fetal DNA in the circulating blood of the pregnant mother (Lo et al. 1997) can reveal molecular genetic disorders as specific markers become available to differentiate DNA of the fetus from that of the mother (Daniels et al. 2004; Kitzman et al. 2012; Lo 2005). In fact, recent reports demonstrate that the entire genetic sequence of the fetus may be

determined from a maternal plasma sample (Fan et al. 2012) which allows diagnosis of inherited and de novo genetic diseases. On one hand, such diagnostic ability will alert physicians to commence treatment immediately following delivery and even while the fetus is in utero (Bianchi 2012). On the other hand, major practical and ethical questions concern the manner in which prospective parents and physicians will use this genetic information.

Perhaps surprisingly, despite innumerable advances and the increase in knowledge, is that in so many areas our ignorance is profound. For instance, what is the physiologic basis for the premature onset of cervix ripening and labor, and why, despite our best efforts, is the prevalence increasing? To what extent does brain damage-neuronal injury occur during prenatal life, and what can we do to prevent/minimize this injury that can result in catastrophe? How can we improve and optimize the incorporation of advances in physiology and biomedical science into clinical practice? These are but a few of the issues we must contemplate. In her 1992 "50-year overview. . ." of the development of perinatal medicine, Mary Ellen Avery considered a number of advances that have contributed to significant decreases in fetal and neonatal mortality and morbidity during the previous half century. Among the lessons learned in this recital, she stressed that, ". . . we must build our interventions on the relevant basic science. Where that does not exist, we must make every effort with animal models and tissue culture methodology to unearth understanding of the appropriate regulatory mechanisms that may need to be stimulated." Avery concluded that if we dedicate ourselves to it, the capacity exists to reduce infant mortality an additional 50% (Avery 1992, pp. 49–50).

20.6 Some Aspects of Newborn and Child Care

Another of the mid- to late-twentieth century leaders in calling attention to the care of infants and children is Billy Franklin Andrews, for several decades professor and chairman of the Department of Pediatrics, the University of Louisville, Kentucky (Andrews 1970). Andrews stressed the concept of newborn infants being "therapeutic orphans" (Andrews 2004a, p. 671) and introduced the idea of the "six finger exercise" in considering the care of the newly born infant (cardiorespiratory, hematologic, nutrition and metabolism, infection, congenital anomalies), with the tied off sixth finger being *pediatric iatrogenica*, and the palm of the hand central nervous system (Andrews 1968, 2004a, b). Among colleagues, Andrews became known as "*Doctor Iatrogenesis*" (Andrews 2004a, p. 673). He also developed the "Billy Box" in which the infant could be kept warm and oxygenated for various studies (letter from BFA to LDL 3 August 2015).

An additional contribution by Andrews of note is "The Children's Bill of Rights." Written in 1968 (Andrews 1988), and quoted in many venues including the Congressional Record (1993), the United Nations (1989), and others, as well as being translated into several languages, the thesis of the "Children's Bill of Rights" bears testimony to all aspects of improved care for mothers and children (Andrews

1995). Andrews is the recipient of many honors and awards both in American and abroad.

The Children's Bill of Rights

We hold these truths to be self-evident:
That each newborn infant is the most perfect
and helpless of all of the creation;
That each newborn is as individual as the stars
of our universe;
That each newborn has the inalienable right to
be born wanted, loved and protected; and while
growing to maturity within and without the womb
that every measure possible, as is known, be undertaken
to afford the very best environment, nutrition and
opportunity for growth and development;
That proper shelter, nutrition, clothes, education and
health measures be provided each child to assure that
each, with maturity, can assume the full responsibilities
of adulthood and citizenship;
That the personhood of each child be fully appreciated
and that each be informed of all matters including
health as they grow in intellect and in capability;
and that they learn to be involved, as maturity
allows, and to participate in all decisions concerning
their well-being;
That when and if correction is deemed necessary
it will be applied with the greatest of respect
and care and without mental or physical abuse;
That we shall as a society make every effort to
establish for the children of today a firmer
footing that we have ourselves enjoyed in all ways;
That we have witnessed from the very mistakes
of nature much that has greatly benefited all of
mankind and that infants and children with birth
defects shall be our responsibility to rear to the
fullest potential possible that they, too, shall
share the rights to Life, Liberty, and the Pursuit
of Happiness which is their birthright;
And that we fully realize that the level of civilization
attained by any society will be determined by the
attention it has paid to the welfare of its infants
and children.
Therefore, in full awareness of these truths, we vow
upon our honor and all we hold to be sacred
to do our very best to bring about a better
world for those who succeed us in order to
repay our predecessors for our own gift of life.

Billy F. Andrews, M.D. 19 May 1968

Another who contributed to care of the infant is the late Kathryn Elaine Barnard (1938–2015), professor of nursing and founder and director of the Center on Infant Mental Health and Development at the University of Washington, Seattle. With expertise in the social and emotional development of infants and young children, she developed innovative techniques to improve parenting. These included exploring the connection between early stimulation of the senses with social, emotional, and behavioral growth. In the early 1970s, Barnard urged a change in the treatment of premature newborns who often were placed in an incubator for protection. To that end, she contributed to development of the isolette which rocks and soothes the infant. Studies demonstrated that gentle rocking led to faster weight gain and improved motor and sensory functions (see Barnard 1982, 1983). Barnard spent much of her career defining the newborn's "ecological niche" and developing programs to improve the infant's mental health, creating methods of sensory stimulation, as well as educating nurses, physicians, and parents in infant care in hospital and home. Physical contact with the infant, and gentle touching, she taught, was crucial to helping the infant toward understanding the world and making themselves understood (Barnard 1998; Barnard and Blackburn 1985; Barnard and Morisset 1995, Barnard et al. 1993). Barnard also was the recipient of numerous awards and honors.

An additional academician who contributed to assessment of the newborn neurologic state is Thomas Berry Brazelton of the Children's Hospital in Boston and Harvard Medical School. The Brazelton Neonatal Behavioral Assessment Scale is used throughout the world, which augments the pediatrician's awareness of, and attention to, the effect of young children's behavior, activity states, and emotional expressions on the ways in which their parents react to and thereby affect them. The Brazelton Scale evaluates not only the physical and neurological responses of the newborn but also their emotional well-being and individual differences (Brazelton 1973; also see Sameroff 1978).

20.7 Pathology of the Fetus and Newborn

During the latter nineteenth and early twentieth centuries, the field of pathology developed into a rather sophisticated science. It was not until near mid-nineteenth century, however, that the pathology of the fetus or newborn received dedicated attention. Edith Louise Potter (1901–1993), of the University of Chicago, changed that. Although seemingly rather removed from physiology, the pathologic contributions of Potter and her associates helped to advance the understanding of physiology. Initially, because an administrator in the Chicago Department of Health questioned the relation of the relatively high infant death rate to the general sanitation and health of the city, he promoted the idea of autopsies to ascertain cause of death. As a pathologist at the Chicago Lying-In Hospital, Potter gained enormous experience in this regard, performing over 10,000 infant necropsies in slightly over three decades. With the chairman of the Department of Obstetrics and

Gynecology, Fred Lyman Adair (1878–1972), in their 1940 report *Fetal and Neonatal Death*, in which they analyzed 526 fetuses and infants dying from 1931 to 1938, the leading causes, accounting for almost one-third of the total deaths, were anoxemia and intracranial hemorrhage (Potter and Adair 1940). Potter also published a monograph on Rh and its relation to congenital hemolytic disease and intragroup transfusion reactions (Potter 1947) and a major synthesis *Pathology of the fetus and newborn* (Potter 1952). In preparing the volume for pediatricians, obstetricians, as well as pathologists, she observed:

> The description of the body of a dead infant is of no value as an isolated piece of information, but if it is integrated with the various aspects of heredity, conception, development, intrauterine and extrauterine environment and behavior it becomes part of an important chronicle. Only by correlating all the facts of one case with all those of many cases can we hope to elicit the etiologic factors responsible for clinical and pathologic observations. . .. In addition to the ultimate aim of the pathologist, of immediate practical importance is the demonstration to the attending physician of the pathologic changes found in any fetus or infant who fails to survive and the correlation of these findings with the symptoms observed during life. When symptoms can be recognized as associated with specific pathologic processes a great stride has been made toward their prevention and cure.
> (Potter 1952, p. ix)

In 1946, Potter also reported on the findings of 20 infants with bilateral renal agenesis (Potter 1946b) and abnormal facies (Potter 1946a). These infants also had pulmonary hypoplasia, and later it was shown that this Potter's syndrome was a consequence of severe oligohydramnios (Thomas and Smith 1974). Several years before her death, Potter reminisced on her career and numerous contributions to perinatology (Potter 1989). From the Queen's University and Royal Victoria Hospital, Belfast, Ireland, pathologist John Edgar Morison (1912–2007) contributed *Foetal and Neonatal Pathology* (Morison 1952). In this work, Morison considered three aspects of developmental pathology: disturbances of prenatal life, adaptation to extrauterine existence, and infections in fetal and neonatal life (Morison 1952). As noted earlier, an additional individual who made considerable contributions to pathology of the fetus and newborn, many of which had implications for physiology, such as differentiating between those infants born preterm and those small for gestational age, was Peter Gruenwald (Gruenwald 1974). The contributions to pathology of the placenta have been noted above (Benirschke and Driscoll 1967; Fox 1997).

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Chapter 21
Governmental Support of Research in Fetal and Newborn Physiology

21.1 The Medical Research Council of Great Britain

> First I believe that this nation should commit itself to achieving the goal, before this decade is out, of landing a man on the moon and returning him safely to earth. No single space project in this period will be more exciting, or more impressive to mankind, or more important for long-range exploration of space; and none will be so difficult or expensive to accomplish.
>
> (Kennedy 1961)

> Scientists alone can establish the objectives of their research, but society, in extending support to science, must take account of its own needs.
>
> (Kennedy 1963)

To support the investigators who labor to advance knowledge in a given field of scientific research, a critical element is that of the financial resources for such endeavors. In the earliest years of experimental investigation, the occasional King and other patron gave off their largesse to support the William Harveys and his ilk. Contributing to the advancement of science in a significant manner, such support was limited and irregular (Frank 1980). In Great Britain, it was in the mid- to late-nineteenth century that the predecessors of the Medical Research Council (MRC) developed. At this time, tuberculosis was one of the nation's chief health problems. In 1901, a "Royal Commission Appointed to Inquire into the Relations of Human and Animal Tuberculosis" was established under the chairmanship of Sir Michael Foster, professor of physiology at the University of Cambridge, to determine whether the disease was the same across species and the extent to which they could infect one another. In a large part, and with recognition of the German and French governmental-sponsorship research to advances in medicine and healthcare, this study helped to crystallize in the minds of many leaders, political as well as academic, the concept that the promotion of medical research is a responsibility of the state. In 1891, "The British Institute of Preventative Medicine" had been established in London. In 1903, this title was changed to "The Lister Institute of Preventative Medicine." The National Insurance Act of 1911 stipulated that

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research not be limited to tuberculosis, and 2 years later (1913) a Medical Research Committee was established. As outlined by Thomson:

> The duties of the Committee will be to formulate the general plan of research and enquiry at the outset, and for each year to make arrangements for carrying it out, and to supervise its conduct so far as may be necessary, and in particular to secure adequate coordination of the various parts of the scheme. It will also deal with the collection and publication of information and of the results of statistical and other enquiries so far as suitable or necessary. For this purpose it will determine, subject to the assent of the Minister responsible for National Health Insurance, the expenditure of the money available each year; the total of the sums available … being about £57 000 per annum. Before the Minister responsible for National Health Insurance gives his final assent to the scheme of the Medical Research Committee for any year, he will receive criticisms and suggestions in regard to it from the Advisory Council for Research, which is being appointed for this purpose.
>
> (Thomson 1973, p. 22)

Within a few months, the Committee, under the chairmanship of John Fletcher Moulton (Lord Moulton; 1844–1921), had developed a "Scheme of Research" for "… the extension of medical knowledge with the view of increasing our powers of preserving health and preventing or combating disease…" (Thomson 1973, p. 28). From its beginning, the Medical Research Committee sought to support both basic and clinical research. In terms of the latter, one must understand that prior to World War I, the schools of medicine, particularly those in London, were private institutions, and there was little encouragement for research of a clinical nature (Booth 1989, 2003).

A key element in progress, and a foundation upon which medical research in Britain was to thrive, followed implementation of the proposal of Lord Chancellor Haldane, the "Haldane Principle," e.g., that scientific decisions regarding research proposals were to be made independent of governmental intervention (Thomson 1973, p. 41). At the end of the "Great War," the Committee acknowledged "… the urgent importance of using the resources of the Medical Research Fund to forward the science of experimental medicine and its study at the bedside in close conjunction with all of the resources of the modern laboratory" (MRC 1919, p. 28). Because of the lack of structured university-hospital alliance, and few physician-scientists to lead out in research in experimental medicine, it would be a decade or more before these ideals would be realized. In large part, it was the combined influence of preclinical scientists working with their clinically oriented allies, who advanced state funding for medical research. With the appointment of a Medical Advisory Committee in 1929, the funding of centers of clinical research increased to support the flourishing of clinical investigation. As chair of the "Royal Commission on University Education in London," Lord Haldane previously had made a vital contribution. This was the report (1913) noted earlier that recommended the initiation of changes that would lead to the establishment of contemporary academic departments of medicine and other clinical subjects, in which there was an appropriate emphasis on research (Royal Commission on University Education 1913).

A year following passage of a Ministry of Health Act (1919), the Medical Research Committee was reconstituted into the independent Medical Research Council, with a Royal Charter and a modest budget for "purposes of research," and an institute was established at Hampstead in north London for clinical research. During the two decades 1914 to 1933, the MRC was chaired by Sir Walter Morley Fletcher, a physician-physiologist and Fellow of the Royal Society. Fletcher stressed the importance of both the basic sciences and clinical research to the advancement of medicine and played a key role in furthering medical research in the UK. Fletcher's successor as MRC secretary, Edward Mellanby (later Sir Edward; 1884–1955), a pioneer in the study of rickets, also was a staunch supporter of clinical investigation and urged furtherance of studies on the nutritional basis of health and disease. Despite the considerable evidence from experimental studies, contrary views by some members of the UK Ministry of Health resulted in less than full commitment to the pursuit of nutritional studies (Petty 1989).

During the 1930s, the MRC provided funds for specific projects, as well as working to establish full-time academic positions at a number of medical schools throughout the UK (Booth 2003). Another field to which the MRC devoted considerable support was that of experimental radiology. Although to a great extent the focus of this support was on the biological effects of radiation, it included studies in diagnostic radiology such as that of Alfred E. Barclay (Cantor 1989). In the immediate post-World War II era, the MRC supported two vital studies that changed the progress of clinical research. An important concept of the MRC "Scheme of Research" was to engage in "all statistical investigations useful either as preliminary to research or confirmatory of its results." These included "enquiries relating to diet, occupation, habits of life and other matters bearing upon the incidence of disease" and to "collect and deal with all types of vital statistics," including those with an epidemiological bearing (Thomson 1973, p. 114). As is widely appreciated, contemporary statistics is the science of learning from data and of measuring, controlling, and communicating uncertainty. Thereby, statistics provides the critical navigation essential for supporting the advances in science and of society. A MRC grantee of note who developed the field of statistics was Karl Pearson (1857–1936) of the London School of Hygiene and Tropical Medicine, University College, London. Pearson's group included the noted biostatistician George Udny Yule (1871–1951), in concert with Sir Francis Galton (1822–1911) (Hill 1937).

Another key figure in the development of statistics for the biological sciences was Ronald Aylmer Fisher (later Sir Ronald; 1890–1962), who spent a decade and a half at the Rothamsted Experimental Station, located in Hertfordshire. Here, Fisher introduced the concept of random allocation of compared treatments to improve crop yields in agricultural research. Such analysis permitted chance to operate freely, thus allowing calculation of reasonable estimates of effect-size in a single growing season (Fisher 1926). This format of randomized controlled trial overcame the limitations of traditional methods of data collection, which could require many years. An example of the value of the randomized controlled clinical trial was that by Austin Bradford Hill (later Sir Bradford; 1897–1991) which established the

efficacy of streptomycin in the treatment of pulmonary tuberculosis (Streptomycin in Tuberculosis Trials Committee 1948). Another important study was the application of epidemiology to a clinical problem, e.g., the demonstration by Bradford Hill and William Richard Shaboe Doll (later Sir Richard; 1912–2005) on the relation of smoking cigarettes to lung cancer (Doll and Hill 1950). Although Fisher's, Hill's, and the others' approaches revolutionized research in many fields of science, several decades elapsed before this methodology was adopted into clinical medicine. In a prescient essay on the value of biostatistics in clinical and preventive medicine, Sir Austin Bradford Hill observed:

> . . . the physician's first duty is to his patient—to do all in his power to save the patient's life and restore him, as rapidly as possible, to health. That fundamental and ethical duty must never be overlooked—though with the introduction of better, brighter and ever more toxic drugs, and with the wide prevalence of surgical procedures . . . the onlooker may perhaps with good reason sometimes ask the clinician 'are you sure you know where that duty lies?' It seems to me sometimes to be unethical *not* to experiment, not to carry out a controlled clinical trial.
>
> (Hill 1963, p. 30)

Together these mathematically minded biometricians, whose chief interest at the time was the study of heredity and eugenics, revolutionized experimental science. With the use of mathematical methods, they invented the concepts of testing the null hypothesis, degrees of freedom, regression analysis, the correlation coefficient, analysis of variance, the chi-square test of statistical significance, and others that helped to develop the discipline we know as biostatistics (Higgs 2000). Also important for clinical and basic science investigators, the creation of these methodologies has proven time and again the superior power of the experimental over the observational approach to the evaluation and interpretation of data and/or the results of a given therapy.

In 1916, the Medical Research Commission, at the urging of Osler and several others, had recruited Thomas Lewis (later Sir Thomas; 1881–1945) as their first full-time investigator. A pioneer in cardiology and electromyography, Lewis later headed a MRC-supported Department of Clinical Research and Experimental Medicine at the University College Hospital, London. Of importance to biomedical research, Lewis was one of the architects of clinical research in all of Great Britain. By the early 1930s he was one of the most influential British academicians in this field (Lewis 1933), founding the Medical Research Society (1930). In his 1935 Thomas H. Huxley address, ". . . Clinical Science within the University," given at the University of Birmingham, Sir Thomas quoted Sir James Paget (1814–1899) in stressing the importance of clinical research:

> I feel sure that clinical science has as good a claim to the name and rights and self-subsistence of a science as any other department of biology; and that in it are the safest and best means of increasing the knowledge of diseases and their treatment. . .. Receiving thankfully all the help that physiology or chemistry or any other sciences more advanced than our own can give us, and pursuing all our studies with the precision and circumspection that we may best learn from them, let us still hold that, within our range of study, that alone is true which is proved clinically, and that which is clinically proved needs no other evidence.
>
> (Lewis 1935, p. 631; Booth 2003)

Nonetheless, some have held that during its early years, the MRC record of support for research in medicine was rather uneven (see Hamilton 2004; Timmermann 2008). Much of its funding was based on "word of mouth" recommendation for those in favor, rather than formal peer review (Boyd and Boyd 2010). Some outstanding early members of the council, and investigators supported by the MRC, included Sir Charles Scott Sherrington (1857–1952), Sir Frederick Gowland Hopkins (1861–1947), Sir Henry Hallett Dale (1875–1968), First Baron Edgar Douglas Adrian (1889–1977), and Baron Howard Walter Florey (1898–1968), each of whom was a Nobel Laureate (Thomson 1973–1975). Sir Hans Adolf Krebs (1900–1981) in his address "The Making of a Scientist" emphasized the critical role of the Medical Research Council in supporting first-rate investigators in Britain, a number of whom became Nobel Laureates (Krebs 1967).

Several historians have considered in detail aspects of the MRC and its support for biomedical research, both basic and clinical (MacNalty 1948), as well as educational (Poynter 1966). Specific contributions include establishment of the Royal Postgraduate Medical School (incorporated 1931, opened 1935) at the Hammersmith Hospital, London, which, in addition to its role as a teaching institution, had as its goal "... the pursuit of research and advance of medical knowledge" (Booth 1985, p. 1771; Newman 1966). Another MRC contribution of note was the establishment in 1947 of the "Unit for Research on the Molecular Structure of Biological Systems," currently the "MRC Laboratory of Molecular Biology" at Cambridge University. Following passage of the National Health Services Act in 1948, clinical research expanded greatly. The Northwick Park Institute for Clinical Research near Harrow (founded in 1994) was in part funded by the MRC. (See Austoker and Bryder 1989; Booth 1985, 1986, 2003; Newman 1966; Thomson 1973–1975; Timmermann 2008.)

21.2 The Medical Research Councils of Canada and Australia

In Canada, the Medical Research Council, modeled after that in the UK, had its beginnings in 1916. As chairman of the MRC Medical Research Committee, in 1938, Sir Frederick Grant Banting (1891–1941; who with Charles Herbert Best (1899–1978) codiscovered insulin) and Chester Bryant Stewart (1910–1999) traveled throughout Canada, meeting with over 300 medical researchers from Halifax, Nova Scotia, to Vancouver, British Columbia, to assess the state of Canadian biomedical science. The goal of this pilgrimage was to establish, more firmly, medical research throughout the provinces. To their dismay, however, in institutions other than McGill University and the University of Toronto, Banting and Stewart discovered a dearth of facilities, funding, or other infrastructure required for successful laboratory or clinical research. The Banting and Stewart report was of importance in establishing a knowledge base, upon which recommendations for increased and more systematic support could be sustained. In 1939, with the

interruption of World War II however, the schemes for improved organization had to be placed on hold. In returning to this issue following the war, in 1958, the Canadian Privy Council appointed a special committee under the chairmanship of the prominent physician-scientist Ray Fletcher Farquharson (1887–1965), to revive increased extramural support for medical research. At the heart of the Farquharson Commission, discussions were the issues of how to nurture young Canadian medical scientists and to support the work of established investigators. To what extent could the Dominion government provide for medical science? How would government support fit into the landscape of research funding, alongside other government ministries, and that of private philanthropy and the work of voluntary organizations?

In light of the continued state of discontinuity in resources and funding, in 1960 under the leadership of Farquharson, the independent Canadian Medical Research Council was founded, which would fund fundamental research in the basic biomedical sciences, as well as translational and clinical research. Farquharson noted "most important of all is the great expansion of medical research which follows in the wake of every advance. Each new discovery leads to further discovery; each advance in treatment throws new light on the fundamental nature of the affected disorder, demanding further investigation. Every new treatment whether successful or not is potentially dangerous, creating new problems" (Medical Research Council of Canada 2000, p. 15). In 2000, the Canadian MRC was transformed into the Institutes of Health Research, as the governmental agency responsible for all health research. With establishment of 13 "virtual" institutes throughout the provinces, in part, its mission was to increase funding for more research on targeted priority areas, build research capacity in underdeveloped areas such as population health and health services research, train a new generation of health researchers, and focus on knowledge translation, so that the results of research would be transformed into policies, practices, procedures, products, and services. Under the leadership of several outstanding scientists, the Canadian Institutes of Health Research has continued to grow, and the establishment of major centers of fetal and neonatal physiology, perinatology, and neonatology with world-class scientists attests to that commitment (http://www.cihr.ca).

In Australia, upon recommendation of a Royal Commission, the Federal Health Council was established in 1926, and a decade later, in 1937, the National Health and Medical Research Council was founded. In part, because of Australia's expansive enterprise in sheep farming, the MRC made major investments in veterinary medicine research, much of which concerned reproduction and the biology of the fetus and newborn.

21.3 The US National Institutes of Health

During the nineteenth century, epidemics of Asiatic cholera and yellow fever swept across the USA. It was not until the latter part of the century, however, that the government recognized the need for a national laboratory to study these and other

infectious diseases such as smallpox and tuberculosis. Thus, federally funded research originated in 1887, with formation of the Laboratory of Hygiene in the Marine Hospital Service on Staten Island, New York. Joseph James Kinyoun (1860–1919), a microbiologist, was the sole investigator. With advancements in understanding the role of microbes in human disease, the original mission was to eliminate infectious diseases. Congressional legislation expanded the laboratory in 1902, with divisions of chemistry and zoology. By 1912, when the Hygiene Laboratory had reached the maturity of a quarter of a century, it had moved to new facilities at 25th and E Streets in Washington, DC. Its name had been changed to the Public Health Service Laboratory, and its research programs expanded to include diseases other than those that were communicable. This led to creation of related federal agencies such as the US Food and Drug Administration (1906) and the Centers for Disease Control and Prevention (1946) (originally the Communicable Disease Center).

Without doubt, the transformation in the role of the federal government in economic and social aspects of national affairs that occurred during the depression era of the 1930s constituted a broad underlying aspect of the role of government in the major advances in public health and medical research that followed. For example, this transition in national concern set the stage for the legislation with great consequence for mothers and their children. In the Social Security Act of 1935, with the support of physicians and his wife Anna Eleanor (1884–1962), President Franklin Delano Roosevelt (1882–1945) included Title V that enabled the federal government to fund state programs for maternal and child heath units and services for crippled children. Two years later in 1937, not only was the Public Health Service Research Program 50 years old, but it had been reorganized into the National Institutes of Health (NIH). This imaginative and far-reaching legislation occurred largely as a result of Louisiana Senator Joseph Eugene Ransdell (1858–1954), whose bill to create the Institute to focus on the fundamental problems of the diseases of man was passed by Congress in 1930 and signed by President Herbert Clark Hoover (1874–1964). During these early years, the chief emphasis of the NIH were studies on infectious disease and cancer. By mid-decade, the National Institutes of Health was experiencing a shortage of space, in particular requiring quarters for housing research animals. About this time, a wealthy entrepreneur and philanthropist, Luke Ingalls Wilson (1872–1937), had offered the US State Department his magnificent estate, "Treetops" adjoining the Rockville Pike in Bethesda, MD, as a site for an institute to promote peace. This being of little interest to the State Department, then Surgeon General Thomas Parran (1892–1968) urged President Roosevelt to accept this windfall for the NIH and to allocate funds to construct the original three campus buildings. Lewis Ryers Thompson (1883–1954), who had served as assistant surgeon general, was appointed the first director of this newly reorganized NIH. In 1937, construction commenced on the Bethesda campus. With passage of the National Cancer Act by Congress that year, the National Cancer Institute was created, and paradoxically Wilson died of cancer soon thereafter. With establishment of this new Institute, the NIH became the National Institutes of Health. Medical research advanced not only at Bethesda,

but grants-in-aid were provided to scientists at nonfederal institutions. (For the History of the NIH to 1937, see Harden 1986.)

During the early 1940s, with the trauma and horrors of World War II, national attention and energies were diverted to a greater cause, the preservation of a free society. The evils of war were not without limited good effect, however. In 1944, Title III of the Public Health Service Act (Public Law 410) provided the US surgeon general to foster, conduct, support, and cooperate in wide-ranging research related to health and disease. Thus, the award of grants for nonfederal research and research training was extended beyond cancer to other health problems. An additional advance that eventuated from the war effort was establishment of the Office of Scientific Research and Development to coordinate and contract university-based research. This collaboration of the federal government with the nation's institutions of higher learning demonstrated the contributions to national problems such an alliance could achieve. With success of the Manhattan Project, the invention of radar, the commercial production of penicillin, and many other achievements, the contributions of science and technology generated high expectations for the solution of problems of national scope.

In late 1944, prior to the end of World War II, President Roosevelt contacted Vannevar Bush, a PhD in electrical engineering who served as his science advisor and director of the wartime OSRD, for his ideas on what the government might do to further research by public and private institutions. In response, Bush distilled the lessons of the wartime mobilization, outlining his visionary policies for the federal support of peace-time health and other research in his *Science—The Endless Frontier* (Bush 1945). This marked the beginning of modern-day governmental science policy. Bush foresaw both the vital need for government's support of fundamental biomedical research and the key role that the nation's universities and colleges could play in advancing knowledge in the health sciences by letting minds explore. In this analysis Bush proposed "Five Fundamentals" or basic principles that he believed must guide governmental support of civilian research, to achieve progress toward national goals in science, health, defense, and the economy. These included emphasis on basic research, stability of funding for five or more years for the support of long-term programs, oversight administration by citizens committed to promotion of the work of a given agency, and independence of the grantee institutions themselves with internal administrative control of policies and administration, and the scope of research performed must remain responsible and accountable to the president and to Congress. In following these principles, the several agencies have made a lasting contribution and legacy. Bush played an advisory role in the organization of scientific talent and resources into national laboratories that continued far beyond the war years. He worked to develop grant funding mechanisms to support university scientists and their research, without the heavy hand of governmental interference. Rather than conceptualize the science per se, Bush helped to create the organizations that supported and advanced science.

With termination of the wartime OSRD, university biomedical contracts were transferred to the NIH, while those in the physical sciences were placed under the

aegis of the Office of Naval Research (ONR). In 1950, with passage of the National Science Foundation (NSF) Act (Public Law 81–507), those OSRD programs of NIH and ONR not relating to health were transferred to the newly founded NSF. The following year (1951), the physicist and former OSRD director of field operations, Alan Tower Waterman (1892–1967), was appointed first director of NSF. In this role, he devoted his energies to ensure that the foundation became one of the world's finest government-supported organizations devoted to promotion of excellence in scientific research and education. Upon retiring from that position in 1963, Waterman was awarded the National Medal of Freedom.

It was in the late 1940s that national leaders conceived the concept of a highly focused effort to solve diseases of major impact on the nation's health. Following passage of the Mental Health Act of 1946, authorization was given for the National Institute of Dental Research and the National Heart Institute (1948). Passage of an "Omnibus Act" in 1950 empowered the US Surgeon General Leonard Andrew Scheele (1948 to 1956; 1907–1993) to establish several other disease-identified institutes including those for Neurological Disease and Blindness, Experimental Biology and Medicine (later renamed Arthritis and Metabolic Diseases), and Microbiological Institute (predecessor to Allergy and Infectious Diseases). With its broadened mandate, the NIH could address the basic and clinical aspects of health and disease on a wide front. Scheele later served as president of the World Health Organization.

In 1948 construction on the major building of the NIH Clinical Center commenced, and by 1953 it was completed to house a unique, world-class program of intramural basic, translational, and clinical research developed both to understand and to treat, beyond infectious disease and cancer, a number of diseases that plague society. Chiseled on the portals of the Clinical Center are the words of Jack Masur (1908–1969), designer and first director of that center from 1948–1951 to 1956–1969:

> Hospitals with long traditions of excellence have demonstrated abundantly that Research enhances the vitality of Teaching. Teaching lifts the standards of Service, and Service opens new avenues of Investigation.
>
> (see Longo 1988, p. 6)

Critical to the development of biomedical science, growth of the NIH budget was enthusiastically supported by the US Congress to ameliorate disease and improve the health of Americans. A key player in this growth with its exponential increase in influence of the NIH was James Augustine Shannon (1904–1994), director from 1955 until his mandatory retirement in 1968. An MD and PhD in physiology, who during World War II led a research group, and subsequently served as director of medical research and corporate vice-president of a major pharmaceutical company, Shannon had considerable insight into the potential and strength of biomedical research, evidenced, in part, by the number of gifted scientists and physician-scientists whom he attracted to the NIH. He also had a great gift to communicate this promise to presidents and leaders in Congress, who enacted the legislation required to support this expanded enterprise (Kennedy

1998). Under Shannon's leadership, America saw a "Golden Age" of biomedical research. In addition to growth in the intramural research program, Shannon oversaw an almost exponential increase in support of extramural research grant programs and the establishment of problem-focused study sections to develop and sustain quality and relevance of research proposals through rigorous peer review (Hannaway 2008). Working with the US secretary of the Department of Health, Education, and Welfare, Marion Bayard Folsom (1893–1976), and Congress, the 1956 Health Research Facilities Construction Act (PL 84-835) expanded construction of medical research facilities at a number of university medical centers to expand research capacity in the life sciences. Importantly, Shannon with the support of Secretary Folsom also cultivated its training and mentoring programs for the education of scientists and physician-scientists, including the Medical Scientist Training Program for MD-PhDs and Research Career Development Awards for young investigators. These awards transformed academic medicine to a profession that strove for supreme excellence in basic and clinical research, teaching, and patient care. Shannon later characterized these contributions as products of a "republic of science" (Shannon 1967, p. 100).

In his 1967 Alan Gregg (1890–1957) Memorial Lecture delivered to the Association of American Medical Colleges, Shannon recounted the role of the NIH in advancement of biomedical research and the health of Americans. He noted the need to maintain research on the cutting edge of science, the requirement to balance research and education, issues relating to the problems of academic centers in adapting to becoming major centers of research and their institutional responsibilities, and the need to maintain an optimal environment for the advancement of medical knowledge, to devising mechanisms for continued long-term support, and ensure that medical education reflects the progress in science and understanding (Shannon 1967). For his many contributions, including those of research on antimalarial drugs during World War II, Shannon was awarded the Presidential Medal of Merit (1948). He also received the Public Welfare Medal of the National Academy of Sciences (1962) and the National Medal of Science (1974). He is recognized by having the NIH Administrative Center (Building 1) named in his honor. Thomas J. Kennedy, Jr, has written of Shannon,

> Virtually none of today's active scientists have had any experience with the environment that prevailed in the world of biomedical research in the pre-Shannon era. In fact, there is hardly a person now alive who remembers.... But the institution to whose structure, function, growth, and development he added such a powerful impetus has in the four or so decades since he became its director changed the face of biological and medical science, medical practice, and human health almost beyond recognition.... Virtually every individual who ever worked closely with Jim Shannon remembered him as a heroic figure and almost unanimously rated their associations with him as the most enriching and memorable of their careers.
>
> (Kennedy 1998, pp. 373–374)

Much has been written about the concatenation of events that transpired during Shannon's tenure at the NIH. In part, because leaders in the executive branch of the US government displayed little enthusiasm for this nation's commitment to the

improvement of healthcare-directed research, two leaders in the legislative branch seized the day. Senator Joseph Lister Hill (1894–1984) (D-Alabama), chair of the Labor and Public Works Committee as well as its Health Subcommittee, and later of the Appropriations Subcommittee with jurisdiction of education, labor, and health, became known as the "... statesman for health" (Schaefer 1968). His colleague Congressman John Edward Fogarty (1913–1967) (D-Rhode Island) also became chair of the subcommittee for Labor, Health, and Education of the House Appropriations Committee. Together with Hill they became effective champions of medical research, and during their tenure the NIH budget and its overall research program blossomed (Schaefer 1968). Fogarty is honored by the NIH "John E. Fogarty International Center for Advanced Study in the Health Sciences." Hill, who in addition to many other pieces of legislation cosponsored the 1956 bill to transfer the National Library of Medicine to the NIH, is commemorated in the NIH Lister Hill National Center for Biomedical Communications at the National Library of Medicine. Regarding Fogarty and Hill, Shannon has written "The nation owes much to their courage, devotion, and skill" (Shannon 1967, p. 102).

Another key player in advancing an agenda for biomedical research, and advocate in the "health syndicate," was the politically connected, New York philanthropist and health activist Mary Woodard Lasker (1900–1994), who mobilized considerable support for biomedical research. In 1942, she, with her husband and advertising executive Albert Davis Lasker (1880–1952), established the Albert and Mary Lasker Foundation to raise awareness of major diseases and their need for greater funding for research. Mrs. Lasker effectively lobbied presidents and congressional leaders for funding for major diseases. Initially concentrating on cancer, mental health, and birth control, later, she expanded their agenda to include cardiovascular disease, hypertension, and arthritis. Together, the Laskers also played an important role in reorganizing the American Cancer Society to enlarge its public fundraising, publicity, and lobbying efforts. Mary Lasker received both the Presidential Medal of Freedom, the Nation's highest civilian honor (1969), and the Congressional Gold Medal (1989). The Lasker Awards of the Albert and Mary Lasker Foundation are a tribute to their legacy.

With an expanding social agenda, by 1961 a Center for Research in Child Health had been established in the Division of General Medical Sciences of the NIH. In addition, that year a task force had reported to President John Fitzgerald Kennedy (1917–1963) that research into the physical, intellectual, and emotional growth of children was severely handicapped by not having a centralized organizational structure. The group called for a new institute to launch a concentrated attack against disorders of development. Because at that time, by law, each institute had as its mission to increase understanding and to develop treatments for specific diseases, new laws had to be written and enacted. Thus on 17 October 1962, near the seventy-fifth birthday of the (by then designated) National Institutes of Health, Public Law 87-838 was passed, which authorized creation of the National Institute of Child Health and Human Development (NICHD), with a mandate to conduct research and training relating to maternal health, child health, and human development, and the National Institute of General Medical Sciences. A few months later,

the National Institute of Child Health and Human Development was physically established (Longo 1988). It is almost beyond comprehension to encompass and appreciate the good which has eventuated as a consequence of the founding and funding of the NICHD. In 2008, this was renamed the Eunice Kennedy Shriver National Institute of Child Health and Human Development to honor President Kennedy's older sister Eunice Kennedy (1921–2009), who had played a critical role in urging her brother, at that time who was not enthusiastic about the idea, that the improvements in health that would result from establishment of such an Institute would far exceed the initial costs required to bring them about (http://www.nichd.nih.gov/news/releases/eks_030308.cfm).

The year following founding the NICHD, President Kennedy became particularly mindful of the importance of research on the fetus and newborn. For it was at that time, 7 August 1963, that his son Patrick Bouvier was born by cesarean section at ~34 weeks' gestation (2.1 kg). With "hyaline membrane disease" (respiratory distress syndrome; see below), the infant was transferred to the Boston Children's Hospital Medical Center for treatment in a high-pressure oxygen chamber. Although initially the infant appeared to improve, despite heroic efforts he died 2 days following delivery. A related development of clinical relevance was the 1965 legislation to establish maternal and infant care programs. With establishment of children and youth clinics, comprehensive health services were provided to a high percentage of all children. In concert with societal mandate for improved clinical care, the NICHD increased its funding for training and education in research. Of importance to the field of fetal and neonatal physiology for the education of obstetrical gynecology physician-scientists, this included support for the Reproductive Scientist Development Program (Longo et al. 1999; Longo and Jaffe 2008) and, for their counterparts in pediatrics, the Pediatric Scientist Development Program (Hostetter 2002).

An additional vital milestone was that in 1968 of the National Library of Medicine (NLM) becoming a component of the NIH. With its online databases being accessible to scientists throughout the world, the NLM developed into the world's foremost biomedical communications resource. As has been observed by others, the decades since the end of World War II have seen the NIH expand into the premier biomedical research facility in the USA and the world. It is a noble and distinguished legacy of the manner in which governmental support of both fundamental and translational/clinical research has contributed to the betterment of humankind. As was observed by one individual in his testimony before a US Congressional Committee, "If we did not have the NIH we would have to invent it . . . [it] is one of the truly remarkable social inventions of the ages" (Bock 1980).

Several reports have reviewed the NIH contributions to science and the challenges in its maintenance of preeminence in biomedical research (Association of American Medical Colleges 1983; Harden 1986; Stetten and Carrigan 1984; Turner 1967). In commenting upon the NIH and its contributions to life, the essayist and former dean of the Yale and New York University Schools of Medicine, Lewis Thomas (1913–1993), observed:

> . . . it lifts the heart to look closely at one institution created by the United States Government which has been achieving, since its onset, one spectacular, stunning success after another. The National Institutes of Health . . . is one of this nation's great treasures. As social inventions for human betterment go, this one is standing proof that, at least once in a while, government possesses the capacity to do something unique, imaginative, useful, and altogether right.
>
> (Thomas 1984, p. xvii)

Of importance, each of these funding institutions, British, Canadian, Australian, American, and others, constitutes a vital organism in which the whole is greater than the sum of its parts. Each has a corporate existence that involves contributions by a large number of individuals, has a momentum that transcends individuals themselves, and is a corporate body which anyone would be honored to serve, but which no one can claim as its own. As demonstrated by considerable evidence, it is through national scientific organizations such as the MRC, the NIH, and others, and their support for individual grants for biomedical research, large program and center grants, training grants, predoctoral and postdoctoral scholarships, awards for sabbaticals, and other mechanisms, that progress in the scientific basis of medicine and health has been assured.

The National Institutes of Health (NIH) is the world's leading biomedical and behavioral research organization and spends about three-quarters of its nearly $30.1 billion budget on extramural grant research funding to support research in universities, medical schools, and research institutions (PMID27249058; NIH Budget History, NIH Extramural and Intramural Funding: FY 2014 Enacted 2014 [cited 2015], http://report.nih.gov/NIHDatabook/Charts/Default.aspx?showm=Y&chartId=283&catId=1).

Over the years since its creation, the NIH has undergone major transformations which reflect a combination of growth of the US scientific community, adaptations to advances in technology, new initiatives from discoveries that at its birth were foundational aspirations, and maturation of efforts to benefit an increasingly global perspective based upon public needs or interests. Innovations and new knowledge produced from investment in basic and clinical science research have proven of tremendous value to enhance the quality and longevity of life, for treatment of diseases, and economic growth. Evidence is clear that in addition to dramatic reduction in disease and suffering, new products, jobs, businesses, and even whole new industries have been created (National Academy of Sciences, National Academy of Engineering, and Institute of Medicine 2007). In each generation, the vicissitudes of public and political interests compete for government support (Longo and Power 1971). More recently, the NIH has responded through efforts of the deputy director for extramural research (Dr. Michael Lauer), as principal scientific leader and advisor to the NIH director on the NIH extramural research program, with the "Open Mike" forum (https://nexus.od.nih.gov/all/2017/05/02/nih-grant-support-index/). Recent discussions of funding concerns in the scientific community focus on the increasingly hypercompetitive system (Alberts et al. 2014) which threatens the future of biomedical research and the productivity of hundreds of thousands of scientists who epitomize the hope for discoveries of tomorrow's

cures to current and unanticipated maladies. This threat comes from multiple convergent factors, not just reduced overall real dollars that result in fewer grants being funded, but efforts to address pressures on the peer-review system which has undergone dramatic change over the years (Lindner et al. 2016; Eblen et al. 2016). Reviewers are challenged by the limits of expertise given the diversity and number of grants to evaluate within each study section, as well as the vertical integration and depth of specificity related to tissue, cells, and molecular pathways. The NICHD is looking to follow guidelines that support the best science based upon priorities to drive funding decisions (https://www.nichd.nih.gov/about/overview/directors_corner/prev_updates/Pages/082916-best-science.aspx, https://www.nichd.nih.gov/grants-funding/policies-strategies/strategies/Pages/FY2017-FAQs.aspx). The term "best" has multiple meanings, including applications that address pressing scientific or emerging public health needs and are highly innovative with high risk/high reward and that fill critical nonemergency gaps or other high program priority implications. The goal is to have more flexibility to fund beyond a payline score that may be considered somewhat of an artificial cutoff. Whether this approach can be applied across researcher-initiated or training grant application or whether certain grant programs for program projects or innovative exploratory research will continue is not known.

The dangers at hand are the decline of biomedical infrastructure resources by loss of seasoned investigators who train the next generation of critical thinking problem solvers and the discouragement of prospective trainees. A detailed report by FASEB, representing 30 scientific societies with over 125,000 members, contains extensive recommendations about how to sustain discovery in a strained biomedical research system (http://faseb.org/Portals/2/PDFs/opa/2015/10.23.15%20Sustaining%20Discovery%20for%20print%2031Aug15.pdf). In addition, NIH Director Francis Collins (appointed 2009) has announced plans to strengthen and stabilize research support to maximize the impact of public spending (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/new-nih-approach-grant-funding-aimed-optimizing-stewardship-taxpayer-dollars). Priorities include support for meritorious applications from early stage investigators, as well as early established mid-career investigators were mentioned. The utility developing a Grant Support Index (https://nexus.od.nih.gov/all/2017/01/26/research-commitment-index-a-new-tool-for-describing-grant-support/), with the idea that, over time and in close consultation with the extramural research community, expectation for total support provided to any one investigator may be reset without inflicting unintended harms or compromising scientific progress, productivity, or the stability of the intra- or extramural research ecosystem. The NIH, as stewards for investment in biomedical sciences by the American people, must protect the future of the biomedical research enterprise, regardless of budget situation. This can only come from balanced efforts to promote initiatives that are most important and impactful for the common good. These efforts are more challenging when support for biomedical research and training is perceived to dwindle as earmarks or big science projects appear to diminish the net availability of resources.

Of particular relevance for National Institute for Child Health and Development (NICHD), Dr. Diane Bianchi became the new director in November of 2016. A continued commitment to the NICHD Advisory Council's recommendations will promote collaborations with other NIH institutes and centers. Other efforts will support the new Environmental Influences on Child Health Outcomes (ECHO) program initiative, focused on understanding how air pollution, chemicals, and societal factors, including stress, sleep, and diet, might affect the health and development of children, as well as the Newborn Sequencing in Genomic Medicine and Public Health program with the National Human Genome Research Institute to understand newborn genomics. These efforts are essential to advance understanding and the remediation of several critical issues that afflict mothers and newborns, in part the result or work by the Pregnancy and Perinatology Branch of the NICHD which was established in 2005.

Two looming issues of high clinical relevance are the need for focus on maternal mortality and spontaneous preterm birth. Maternal mortality results from severe bleeding, mostly after childbirth (postpartum hemorrhage), and sepsis, unsafe termination of pregnancy hypertension, (preeclampsia), inadequate prenatal care, and diseases that complicate pregnancy (malaria, anemia, and HIV) (PMID25103301; http://kff.org/global-health-policy/fact-sheet/the-u-s-government-and-global-maternal-and-child-health/ <proper ref>) (WHO and UNICEF, *Countdown to 2015 Report* 2012 <proper ref>). For spontaneous preterm birth, 10–16% of all pregnancies occur before 37 weeks of gestation, an incidence in the USA that transcends across the demographics of race and origin (ethnicity). A detailed analysis relative to the USA is available in the 2016 Premature Report Card from the March of Dimes (http://www.marchofdimes.org/mission/prematurity-reportcard.aspx). Approaches to diagnose and treat spontaneous preterm birth are needed since related complications are the leading cause of death among children under 5 years of age according to the 2016 National Vital Statistics Reports (https://www.cdc.gov/nchs/data/nvsr/nvsr65/nvsr65_03_tables.pdf;, Table I-2 and 3>). Complications due to premature births along with low birthweight account for more than a quarter (35%) of newborn deaths, while the remainder are related to delivery difficulties, sepsis, congenital abnormalities, pneumonia, and other causes (Black et al. 2010; Wardlaw et al. 2014? UNICEF, *Committing to Child Survival: A Promise Renewed—Progress Report 2015*, 2015). Based upon data from the US National Center for Health Statistics, the average preterm birth rate of over 11% compares with 12% (range 5–18%) among lower-income countries in the survey of 184 countries worldwide (2016 World Health Organization report, http://www.who.int/mediacentre/factsheets/fs363/en/). Many maternal, but not paternal, factors have been associated with an increased risk of spontaneous preterm birth, including smoking, alcohol consumption, low body mass index, advanced age, and a short interval between pregnancies (Muglia and Katz 2010). Heritability from maternal and kinship relationships may contribute to 15–40% of preterm births (McPherson and Manuck 2016). The failure to address problems of maternal health and those that lead to preterm birth, whether spontaneous or from genetic, premature rupture of fetal membranes or inflammation-related causes, is a compounding risk due to the inevitable future

increase in incidence and cost to society when surviving premature born babies attain reproductive maturity and become pregnant.

The question at hand is who will carry the mantle to investigate important questions and research potential solutions. Obstetrics and gynecology departments receive the smallest amount of National Institutes of Health research funding and have significantly lower application success rates compared to pediatric, internal medicine, and surgery departments (Okeigwe et al. 2017). Other grant programs to enhance the competitive success of those in the field of perinatal medicine are discussed later, among them are Reproductive Scientist Development Program and the privately funded American Association of Obstetricians and Gynecologists Foundation (AAOGF) scholars (Chap. 28). Thus the training of aspiring Ob/Gyn physician-scientists not only allows the acquisition of skills to achieve independent research careers through appropriately mentored programs but also ensures that the health and well-being of women and their newborns are a research priority. Certainly in uncertain times ways must be found to persevere.

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Chapter 22
Bioethical Issues in Research on the Fetus and Newborn Infant

> *I decline to accept the end of man. It is easy enough to say that man is immortal simply because he will endure . . . I believe that man will not merely endure: he will prevail . . . The poet's voice need not merely be the record of man, it can be one of the props, the pillars to help him endure and prevail.*
>
> (William Faulkner 1950)

22.1 An Awakening of Responsibility

As noted earlier in reference to both performance of studies on fetal endocrinology and steroid metabolism, and in reference to other "experiments" for "pure science" and the conduct of double-blinded clinical trials in attempting to improve survival of very premature infants, a number of questions have arisen in regard to the ethics of experimentation. In addition to the issue of the culture wars of abortion, debates about the fetus, whether it be their bodies, rights, personhood, or perception of pain, involve arguments of scientific authority, political, religious, and social values, as well as other considerations (Dubow 2011; Morgan 2009; Reagan 2010). In addition, with the lifesaving technologies of maintaining viability of premature infants at ever earlier ages, and the recognition that a high percentage of such newborns face a life of neurologic disability, the debates can become intense. A number of gifted individuals have addressed these issues, and there are no easy answers. In his banquet address upon receipt of the 1949 Nobel Prize for Literature, in the early years of the Cold War era William Faulkner addressed the tragedy of that day, that of an almost universal fear for life and survival and our universal need for compassion and perseverance. In part, this involves our gaining an understanding of our origins.

Head of the Department of Experimental Medicine at Cambridge, and who, in addition to his legacy in nutrition and metabolic balance, contributed in a significant way to thinking on biomedical ethics, was Robert A. McCance. In his 1950 Presidential Address to the Section of Experimental Medicine and Therapeutics of the Royal Society of Medicine, McCance explored a number of ethical aspects of

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clinical investigation, as well as those of the basic sciences. He noted, "we should, I think, for present purposes, regard anything done to a patient, which is not generally accepted as being for his direct therapeutic benefit or as contributing to the diagnosis of his disease, as constituting an experiment, and falling, therefore, within the scope of the term experimental medicine" (McCance 1951, p. 191). Several years later, the Cambridge embryologist-endocrinologist Colin Russell Austin (1914–2004) organized a conference on *The mammalian fetus in vitro*. Here, about a dozen investigators addressed various aspects of maintaining the fetus ex utero, both for scientific study and preservation of life. In addition to consideration of marsupials and other species, these workers considered supporting the human fetus as early as mid-gestation by extracorporeal oxygenation with artificial placentae or other modalities. In addition to technical specifics, the participants considered social, ethical, and legal issues (Austin 1973). In his summary essay "The Road Ahead" (McCance 1973), McCance reviewed some of his thoughts from previous work in the field (McCance 1951, 1959), presenting guidelines and benchmarks for investigation of the human infant by both clinical investigators and basic scientists. In addition, he pointed out the problem for the investigator/clinician of performing experiments of "omission" (withholding treatment from a control) and those of "commission" (such as performing procedures for which there is no obvious need). Further, in asking "what... has gone wrong," he pointed out the controversy with physician-investigators, patients (or their parents), the law, and the "church" at odds in confrontation, the problem compounded by sensationalism by the mass media. McCance concluded that "tolerance and co-operation seem to be the best hope for clinical research... Discord will get us nowhere ... let us make progress together" (McCance 1973, pp. 364–366).

Ethical, legal, and moral aspects of clinical investigation have been considered during much of the twentieth century, if not before (see anthology of Ladimer and Newman 1963). However, it was at mid-century following the horrors of World War II and the Nuremberg Trials that ethical issues in regard to optimal care of patients and what might be regarded as "experimentation" came into focus (for instance, see Freund 1965) and were vilified by some (Pappworth 1967). In considering "Some Moral Dimensions of Medicine," the philosopher Samuel Enoch Stumpf (1918–1998) at Vanderbilt University in Nashville, TN, observed:

> Modern medicine has provoked some serious moral questions, not through malignant perversity, but because of the enormous momentum medical science has gained in the past few decades...
>
> There is the nagging question, What are the permissible limits and the proper conditions for experimentation on human beings? ...
>
> These decisions cannot be postponed indefinitely. It is crucial at this historic juncture that the enormity of the problem of discovering clear moral insights to delineate some acceptable boundaries and limits to the use of human beings in research, should not produce either an impatience or moral cynicism.
>
> (Stumpf 1966, pp. 460–468)

22.2 The Emergence of Bioethics

During the early 1970s, the field of "bioethics" (a term coined at this time; Potter 1970) came into being. Initially, its goal was to consider rather all-encompassing interpretation of global issues of long-range environmental concerns that is a "Bridge to the Future" of applying human values to biological knowledge (Potter 1971). Shortly thereafter, bioethics was redefined to address more specific dilemmas in clinical care (Hellegers 1977, 1978; Reich 1995). Of vital relevance to fetal and neonatal biology and clinical care was the founding in 1971 at Georgetown University of the "Joseph and Rose Kennedy Institute for the Study of Human Reproduction and Bioethics" (known as the Kennedy Institute) by that family (Jonsen 2003). Its chief creator and first director, the Dutch Roman Catholic obstetrician-gynecologist and fetal physiologist, André E. Hellegers, was a member of several Papal commissions and known by some as "The Pope's Biologist" (Baker and McCullough 2009). Hellegers and colleagues at the Kennedy Institute contributed to a number of areas of bioethical debates that involved the fetus and newborn infant (for instance, see Hellegers 1977, 1978; Hellegers and McCormick 1978), as well as other bioethical issues (Hellegers and Wakin 1978; Reich 1995, 1999). Most recently, the term has been redefined further as "bioscience ethics," to apply bioethics to technological/applied science (Pollard 2002, 2009). As one reviewer has pointed out, with the growing awareness of global healthcare issues, including access to care and outcomes, and ever-increasing technological advances, "... bioethics is becoming more responsive to a more global vision of health care ethics" (Ross 2010, p. 457).

In terms of the ethics on fetal research and human experimentation, the early 1970s were a time of ferment. In addition to investigations on human fetuses at the time of therapeutic abortion conducted in Scandinavia detailed above, several studies drew attention to this field of research. In an attempt to develop an "artificial placenta" for the treatment of prematurely born infants who would be anticipated to develop respiratory distress syndrome, in one study fetuses from 300 to 980 g (~17 to 26 weeks' gestation) were maintained on extracorporeal circulation (Chamberlain 1968). In another, the investigators demonstrated that attenuated rubella (German measles) vaccine virus administered to the mother could cross the placenta to infect the embryo/fetus at 7–15 weeks' gestation (Vaheri et al. 1972). In yet another, the authors established that at 15–17 weeks' gestation, the placenta was an effective barrier to ^{125}I-labeled glucagon crossing either from mother to fetus or in the reverse direction (Adam et al. 1972). Following a 1970 accusation by a British member of Parliament that live fetuses were being sold by abortion clinics for medical research (Anonymous 1970), a special advisory group of the Royal College of Obstetricians and Gynaecologists reported on the issues (Great Britain Dept. Health and Social Security 1972). This dozen member advisory group, chaired by the surgeon-gynecologist to the Queen, Sir John Peel (Anonymous 2006), and which included Geoffrey Dawes, considered "... the ethical, medical, social and legal implications of using fetuses and fetal material for research."

A major issue the panel addressed was that of fetal "viability," concluding the minimal limit to be 20 weeks' gestation (400–500 g). Included with a list of specific studies in which the use of fetal tissue had been reported, in their concluding "Recommended Code of Practice," the advisory group urged that research be limited to fetuses that were previable and living fetuses that weighed less than 300 g, that studies must be conducted in departments directly related to a hospital, that they not be conducted for more than ". . . two or three hours," that there be no monetary exchange, and that all studies be conducted only after specific sanction of the medical center ethical committee (Great Britain Dept. Health and Social Security 1972, pp. 1–15).

22.3 The Massachusetts Experience

In the USA, experimentation on living fetuses failed to attract the public attention until the early 1970s, although the NIH had established internal policy guidelines several years previously. Following the decision of the US Supreme Court "Roe vs. Wade," ruling that restrictive abortion statutes by states were unconstitutional (US Supreme Court 1973), both the Executive Branch via the Department of Health, Education, and Welfare (DHEW 1973) and the US Department of Health, Education, and Welfare addressed the issue of human experimentation in general and more specifically that of experimentation in the fetus.

In Massachusetts, several cases concerning fetal research resulted in legislation with wide-ranging implications. (See Dubow 2011 for an extensive review.) One of these involved four Boston City Hospital physicians accused of grave robbing. Because pregnant women often must be treated for intrauterine infection, to determine the extent to which the commonly used antibiotics, clindamycin and erythromycin, could be used in pregnant women allergic to penicillin, in 1971, these physicians commenced a study on the manner in which the antibiotics are metabolized by the pregnant woman and her fetus, compared to that in the nonpregnant individual. Women who were to undergo therapeutic abortion were given either a single or multiple doses of these antibiotics at various times in advance of the procedure. In addition to obtaining maternal blood, amniotic fluid and fetal tissues were sampled. The studies verified that pregnancy significantly alters metabolism of these antibiotics and that clindamycin crossed the placenta and was concentrated in liver of the fetus more readily than did erythromycin (Philipson et al. 1973). Publication of this study, which appeared in the *New England Journal of Medicine*, inflamed Boston "right-to-lifers" who were incensed about a study on women having therapeutic abortions, rejecting the ultimate medical benefits of the investigation. In April 1974, a Boston grand jury indicted the investigators for alleged violation of an 1814 Massachusetts law forbidding grave robbing. This case raised many of the same legal and social questions brought up by accusation and conviction of manslaughter against another Boston City Hospital physician who

previously had performed a therapeutic termination of pregnancy following appropriate hospital committee approval.

Similarly, a case was brought against a distinguished Harvard Medical School hematologist, David Gordon Nathan, in his attempt to identify hemoglobinopathies of the fetus in utero. Initial efforts in this regard were the detection of sickle cell hemoglobin (beta chain anomalies; Kan et al. 1972) and thalassemia (Cooley's anemia, failure to produce adequate beta chains; Kan et al. 1974) in fetuses from elective termination of pregnancy. Soon, these investigators used fetoscopy to sample fetal blood from the umbilical vessels to diagnose these and related disorders (Alter et al. 1976; Chang et al. 1974). In several reports, Nathan and colleagues have reviewed these contributions (Alter et al. 1977, 1980; Alter and Nathan 1978; Nathan 1975a; Nathan et al. 1975, 1979; Sankaran and Nathan 2010). In other essays, Nathan has considered the ethical issues of research on the fetus, and the dilemma of developing the biomedical technologies for more accurate and earlier diagnosis in those individuals most at risk (Nathan 1975b, 1976a; Nathan et al. 1975). For instance, in his "... Investigator's view" of fetal research in the *Villanova Law Review*, after surveying several aspects of the legal and social issues, Nathan stressed the benefits of such research in saving lives. After noting some of the obstacles and limitations, he concluded "... the work will move forward, and as a result, the health and welfare of pregnant women and their fetuses will be maintained and improved" (Nathan 1976b, p. 394). In his 1995 epic *Genes, Blood, and Courage...*, Nathan recounted the life saga of a young patient with thalassemia for whom he cared, the challenges of early diagnosis and treatment, and the manner in which advances in molecular diagnosis changed the nature of the ethical debate (Nathan 1995). Among his many other honors, Nathan was awarded the National Medal of Science in 1990 (Benz 2007).

A series of *news and comment* reports in the journal *Science* discussed in some detail the specifics of these cases (Culliton 1974a, b, 1975a, b), aspects of the Massachusetts Law, and its effect on fetal research (Culliton 1975c, 1975d, e). These essays also considered the response of a "National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research" (Culliton 1975f) and subsequent rulings by the Department of Health and Human Welfare (Culliton 1975g). In essence, the litigation and rulings of these cases, combined with public opinion and the rise of the "pro-life" movement, placed considerable restrictions on fetal research, evoked fear in the hearts of investigators, and virtually eliminated this line of inquiry, regardless of medical or social benefits. Major issues considered included definitions of "death," fetal "viability," the use of the dead fetus, and the use of living fetus whether "nonviable," "previable," or "viable." To a lesser extent, the ensuing dialogue/debates concerned issues of long-term social consequences, the scope of responsibility for decision making, the value of fetal life and "personhood," and others. These, with advances in neonatal intensive care, played a major role with development and advances in the field of bioethics, establishment of hospital-based Institutional Review Boards (IRBs), and a "brave new world" of complex cases and debate about their management (Motulsky 1974a, b).

22.4 Later Developments

With rapid advances in the field of neonatal intensive care, in the 1970s, a vital consideration was a series of ethical issues in the proper management of NICU infants, particularly those less than 24–26 weeks of age, those with severe congenital malformations, and those that had evidence of long-term disability. Beyond the immediacy of medical care per se, the optimal management of these infants raised intellectual and emotional challenges. In concert with the rise of the bioethics movements in general, Ross Laboratories, Columbus, OH, dedicated one of their Ross Conferences on Pediatric Research to a consideration of *Ethical dilemmas in current obstetric and newborn care* (Moore 1973). Over two dozen international leaders met for several days to concentrate, as the chairwoman Mildred Stahlman expressed it, "... on the questions to be asked rather than offering a variety of answers to questions that were assumed, inferred or poorly understood" (Stahlman 1973, p. 12). The conferees considered a number of aspects of the ethical problems and their limits, issues relating to specific diseases/conditions, and the process of decision making (Moore 1973).

Almost concurrently, a 1974 conference of theologians, ethicists, anthropologists, perinatologist obstetricians, and neonatologist pediatricians, many from the University of California San Francisco, considered a number of cases and scenarios associated with neonatal intensive care. These scholars proposed a *moral policy value*, based upon *responsibility*, *duty*, and *interest*, as moral fields of force to guide responsible actions (Jonsen et al. 1975). Later, Raymond Stanley Duff (1923–1996) of Yale critically emphasized the "doctor's dilemma" in assessing the importance of "close-up" ethics, relating to the immediacy of family feelings and specific circumstances, and religious and related social factors, as opposed to the dangers of "distant ethics," referring to relative abstract principles, moral ideologies, authority, and rules, in guiding the management of infants with poor prognosis (Duff 1987). As recognized by many, these infants present agonizing decision making regarding "quality-of-life" issues. Stahlman described these as "multifaceted, complex, and gut-wrenching for parents and care-givers alike" (Stahlman 1990, p. 169). In concluding her review of "The future of ethical issues in neonatology," Stahlman observed:

> Neonatal intensive care once demanded "caring intensively". The demand is still there, as imperative as ever, but caring has become old-fashioned and *passé* in the medical world of business. God help us and our profession if we have forgotten how to care!
>
> (Stahlman 1987, p. 273)

(For a more complete survey on these bioethical issues, see Duff and Campbell 1973, 1976; Hack and Fanaroff 1986, 1989; Nesbitt 1974; Pappworth 1967.)

In a provocative essay, John D. Lantos, currently the director of the Children's Mercy Bioethics Center, Kansas City, Missouri, has reflected upon the "hidden costs of success" of neonatal intensive care, financial, bioethical, and human (Lantos 2001). Lantos observed that this intensive care:

> . . . has confronted, clashed with, and in some ways rearranged our consciousness. By developing ways to save the lives of a whole population of babies who once were through too small to survive, it has changed the way we think about what babies demand from us as a society and about what we owe to them.
>
> (Lantos 2001, p. 235)

Lantos continued, pointing out the intensive care's limitations and the need to place increased emphasis on comprehensive, preventative care for mothers at risk by averting premature delivery. In addition, he noted the neglect of a society that allows one-third of children to grow up in poverty, in ". . . a moral environment that is both odd and compelling". He concluded, with the thoughtful challenge to ". . . think clearly about the choices we are making, and the choices we are thereby rejecting" (Lantos 2001, p. 240).

In commenting upon these observations, Jerold Lucey has emphasized the need for debate about neonatal intensive care, pointing out that in the USA 5000 "fetal infants" who weigh 400–500 g are born each year, of which an average of 12% survive (20–40% in some NICUs). He challenged the reader:

> The world has never seen so many of these infants. What are their long-term prospects? Sadly, we do not know. Everyone is worried about these so-called miracle babies. It's time for a major public debate on this subject.
>
> (Lucey 2001, p. 313)

Lucey concluded by asking if America should not follow the lead of European countries in setting a birthweight and gestational age below which such intensive care is not offered (Lucey 2001). Several years later, Lucey and colleagues reported results of the serious morbidity problems of 4172 "fetal infants" (birthweight 401–500 g) with 17% survival (Lucey et al. 2004). In an accompanying editorial, he considered some of the dilemmas presented by intensive care for these "fetal infants." Lucey asked, "What can be done? The admission of ignorance is the beginning of learning. . .. We should admit how little we know, explain the present bleak outlook for intact survival, and ask [parents] for their help." He continued, stressing the imperative to conduct randomized, controlled trials within an effective large network of major clinical centers, with long-term follow-up for 10–12 years. In conclusion, Lucey observed, "These infants are not 'miracle babies'. We are neither miracle workers nor 'techno crazies' . . . We need a new approach. If we don't, we will be asking the same questions 10 years from now" (Lucey 2004, p. 1819). For his innumerable contributions to pediatrics and to life, in 2009, Lucey was honored with the John Howland Award of the American Pediatric Society (Lucey 2010).

From mid-century onward, with increasing attention to bioethical issues in human research, it has become no longer possible to conduct nontherapeutic fetal studies even before therapeutic or elective abortion, and research on pregnant women became restricted to avoid potential injury to the fetus. Thus, many investigators looked to subhuman primates, particularly the Rhesus monkey, *Macaca mulatta*, as an appropriate "model" for experimental studies. In the USA, the National Center for Research Resources of the NIH in the early 1960s established

eight regional primate centers in a geographical distributed manner, each associated with a university. Although many obvious species differences exist between *Homo sapiens* and other primates, for many questions these centers proved to be of great importance for the ability to perform carefully controlled studies (For a review, see Cheek 1975). Although contributing greatly to an understanding of health and disease, investigative studies on primates have raised their own set of ethical issues, many of which are unresolved.

As is becoming ever more apparent, decision making has become more complex as clinical practice increasingly becomes directed by committee—mandated guidelines, outcomes research, and comparative-effectiveness analysis. Above all, by the use of the optimal medical judgment as investigators, physician-scientists, and caring clinicians, we must strive to treat patients and families with respect and concern for their individual needs.

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Chapter 23
Textbooks, Monographs, and Other Volumes on Fetal and Newborn Physiology

23.1 Volumes on Physiology of the Fetus and Newborn Infant

For many basic science and clinical investigators, Geoffrey Dawes' *Foetal and Neonatal Physiology* (Dawes 1968) has served as the *vade mecum par excellence*. A number of other such volumes have been published, latter ones of which were stimulated by Dawes' monograph. In 1920, the first work to appear on the subject, *The Principles of Ante-natal and Post-natal Child Physiology. Pure and Applied*, was written by William Moses Feldman (1880–1939), physician and lecturer on child physiology at the Infants Hospital, London (see Table 23.1). In the preface, Feldman observed that he prepared his work for "... students of physiology, and ... pediatric physicians, and all scientific persons interested in the study of children ... The task has not been an easy one, for I have had to travel far and wide in search of my material, and have had to cross numerous deep and uncharted oceans of literature..." (Feldman 1920, p. vii). In 41 chapters covering almost 700 pages, Feldman considered essentially every aspect of developmental physiology known at that time. Part I addresses conception, developmental biology, fetal nutrition, respiration, circulation, development of the nervous system, and the physiology of pregnancy. Part II considers the "natal stage" with the physiology of birth. In Part III, Feldman reviews the "postnatal" stage of life, physiology of the neonatal period, chemical composition of newborn tissues, the physiology of bone and the muscular system, infant metabolism, the circulation, and related topics. Part IV is dedicated to the physiology of the premature infant. Notable in Feldman's work was his use of mathematics, physics, and chemistry in considering physiological problems (Feldman 1920).

As noted above, in the USA the anatomist William F. Windle pursued questions relating to early development with emphasis on the circulation of the fetus, its oxygenation, and neural development. Based on his own work and early studies with Joseph Barcroft, in 1940 Windle published the first American work devoted to

L.D. Longo, *The Rise of Fetal and Neonatal Physiology*, Perspectives in Physiology,
https://doi.org/10.1007/978-1-4939-7483-2_23

Table 23.1 Volumes on fetal and neonatal physiology

Authors/editors	Year published	Chapters	Total pages
Feldman	1920	41	694
Windle	1940	25	249
Barclay, Franklin, and Prichard	1944	13	275
Smith	1945	13	312
Barcroft	1946	22+	292
Smith 2nd edition	1951	13	348
Smith 3rd edition	1959	13	497
Dawes	1968	17+	247
Stave	1970	35	1097
Stave 2nd edition	1970	40	851
Gevers and Ruys	1971	16	199
Hafez	1975	17	352
Smith and Nelson 4th edition	1976	14	771
Beard and Nathanielsz	1976	26	542
Jones and Nathanielsz[a]	1985	134	837
Battaglia and Meschia	1986	9	257
Gluckman[a]	1989	25	424
Polin and Fox	1992	190	1884+
Hanson, Spencer, and Rodeck (Vol. 1)	1993	19	438
Hanson, Spencer, Rodeck, and Walters, Vol. 2	1994	15	400
Thorburn and Harding	1994	36	468
Hanson, Spencer, and Rodeck Vol. 3	1995	13	353
Harding, Jenkin, and Grant	1995	42	358
Brace, Hanson, and Rodeck Vol. 4	1998	11	328
Polin and Fox 2nd edition	1998	228	2504+
Harding and Bocking	2001	12	284
Polin, Fox, and Abman 3rd edition	2004	192	1960+
Polin, Fox, and Abman 4th edition	2011	185	2038+

[a]Proceedings of Symposia

this field, *Physiology of the fetus: origin and extent of function in prenatal life* (Windle 1940). A student of history was familiar with the work of Thierry Wilhelm Preyer (1885). As noted in the preface, although he planned originally "... to produce a more comprehensive review somewhat similar to that of Preyer," he quickly came to realize "... the futility of doing so within a single small volume" (Windle 1940, p. v). Nonetheless, in 15 chapters, Windle surveyed a wide vista, including the fetal heart and its circulation, respiratory movements, blood, placental respiratory gas exchange, and other organ systems. In a comparison of textual material, it is clear that both Barcroft's later *Researches on Prenatal Life* (Barcroft 1946) and Dawes' *Foetal and Neonatal Physiology* owe a great deal to Windle's monograph, particularly in regard to their historical perspective.

Five years after Windle, the pediatrician Clement A. Smith, director of research on the newborn at the Boston Lying-In Hospital and Harvard Medical School, published *The physiology of the newborn infant* (Smith 1945). In his foreword, Frederick Carpenter Irving (1883–1957), Harvard professor of obstetrics, quoted a previously unpublished and undated poem by the man of letters and Harvard professor of anatomy, Oliver Wendell Holmes (1809–1894):

> So the stout fetus, kicking and alive,
> Leaps from the fundus for his final dive.
> Tired of the prison where his legs were curled,
> He pants, like Rasselas, for a wider world.
> No more to him their wonted joys afford
> The fringed placenta and the knotted Cord.
>
> (Smith 1945, p. vii)

Irving continued, "... never in the later life of man do such climatic changes occur in so short a time. The onset of puberty is gradual, the period of senescence consumes many years, even death itself for some may be a lingering event; a few seconds, however, suffice for the first breath, the adequate expansion of the lungs, and the adjustment of the circulation to pulmonary respiration .... Above all [obstetricians and pediatricians] should recognize that the newborn infant presents certain problems of its own and that it is not merely a very young baby" (Irving 1945, p. vii).

In this volume, Smith considered a number of aspects of respiration, the circulation, metabolism, renal function, and endocrinology from the standpoint of both the fetus and newborn infant. In his introduction, he noted his deliberate eschewing of a section on the nervous system, its knowledge being limited to the fetus as surveyed by Windle (1940). Smith's volume saw three subsequent editions (Smith 1951, 1959), the last of 1976 coedited with Nicholas M. Nelson of the Milton Snavely Hershey Medical Center and Pennsylvania State University College of Medicine (Smith and Nelson 1976). As noted earlier, Clement Smith developed one of America's earliest residency-postdoctoral programs for training investigative-minded neonatologists. Under his mentorship more than four dozen gifted neonatologists progressed to academic centers throughout the country. Sir Joseph Barcroft is said to have admonished Smith, "... remember that the essence of the missionary enterprise ... is to expel the people one by one" (Avery 1976, p. 857). In explaining why so many young pediatricians chose to train with him, Smith stated, "... if you were interested in babies and liked Boston, I was the only wheel in town" (Personal Communication to Nicholas M. Nelson).

Reflecting the increasing subspecialization of biomedical science, in 1970, Uwe Stave, of the University of Miami (and originally from the Universities of Hamburg and Marburg), published a multiauthored, two volume work with 35 chapters by 40 authors, *Perinatal physiology* (Stave 1970). Eight years later, Stave published a second edition, with 40 chapters by 48 authors (Stave 1978). These volumes also recognized the concept of "perinatal" biology and medicine, that is, the period extending from the last few months of gestation (24 weeks onward) through the first month of newborn life. As an aside, the term "perinatal," to

encompass the period from the last several months of pregnancy through the first month of life, appears to have originated in the 1930s (Reifarth 1934), although it did not come into common use until mid-century (Peller 1943, 1944; Yaffe 1966).

In 1971, Rudolf Hans Gevers and Jan Hendrik Ruys of Leiden University in the Netherlands published the papers presented 2 years previously at a Boerhaave conference on the *Physiology and pathology in the perinatal period*. With its focus chiefly on the fetus and newborn as patients, contributors considered the pathophysiology of hypoxia, asphyxia, hypoglycemia, and related problems (Gevers and Ruys 1971). The volume *The mammalian fetus* edited by Elsayed Saad Eldin Hafez of Wayne State University, Detroit, was the product of a December 1973 symposium and appeared in 1975 (Hafez 1975). With over 30 participants, the symposium considered "... recent advances in the control of fetal circulation, perinatal respiratory physiology, fetal behavior, lipid substrates and fetal development, perinatal energy metabolism ... biophysical techniques to study the fetus ... intrauterine detection of biochemical disorders and fetal malnutrition, embryological basis of abnormal development ... maternal health and fetal development, and fetal responses to asphyxia" (Hafez 1975; p. ix). The editor and authors concluded in their recognition of critical gaps in our knowledge, such as "... preventive mechanisms of intrauterine hypoxia, necessary nutrients for fetal growth, physiological and molecular mechanisms of intrauterine malnutrition and growth retardation, the effect and the extent of intrauterine malnutrition on postnatal physical and mental growth, the etiology of pregnancy toxemia, physiology of labor initiation, preventive mechanisms of premature labor, and genetic and embryonic manipulations to correct certain hereditary and congenital anomalies" (Hafez 1975; pp. ix–x).

The following year appeared the compendium *Fetal physiology and medicine, the basis of perinatology* by Richard William Beard (1931–2012) of St. Mary's Hospital Medical School, London, and Peter W. Nathanielsz, then at Cambridge University (Beard and Nathanielsz 1976). Twenty six chapters, each by an authority in his or her field, reviewed various aspects of fetal biology with emphasis on metabolism and endocrinology, particularly that of interest to perinatologist obstetricians and neonatologist pediatricians. In the preface, the editors emphasize the convergence of disciplines required for deeper understanding of the subjects. In commenting upon the rapidity at which the fields are advancing, they noted the need for "... frequent revision and reissue ..." (Beard and Nathanielsz 1976, p. v–vi). Alas, with Beard's death the latter goal was not realized.

A decade later in 1986, Frederick Camillo Battaglia and Giacomo Meschia, of the University of Colorado, published *An introduction to fetal physiology*. In nine chapters the authors guide the reader through intricacies of topics of their special expertise, e.g., growth of the placenta and fetus, metabolism of oxygen, carbohydrates, and amino acids, and some nuances of uteroplacental blood flow, placental exchange, and fetal respiratory and circulatory physiology. Foremost strengths of the work are the lucid presentation of major concepts, such as placental clearance and metabolic balance, and the description of various experimental techniques, such as the determination of metabolic fluxes of substrates across the placenta, and

the use of labeled microspheres to determine the distribution of fetal cardiac output and organ blood flows. The authors synthesized many isolated facts into a fairly coherent whole, placing these into a proper perspective as to biologic meaning. They also reviewed several caveats regarding potential errors of the various methods and the interpretation of results (Battaglia and Meschia 1986).

With the exponential expansion of the field of developmental physiology, and the virtual explosion of research and new information, in 1992, Richard Alan Polin, director of the Division of Neonatology at the Children's Hospital of New York-Presbyterian, Columbia University College of Physicians and Surgeons, and William Willis Fox of the Division of Neonatology, Children's Hospital of Philadelphia, and the University of Pennsylvania published *Foetal and Neonatal Physiology*, a 2-volume 1884+-page compendium of 190 chapters by 283 authors (Polin and Fox 1992). In 1998, an enlarged second edition included rapid advances in cellular and molecular biology, and their contributions to understanding the physiology and pathophysiology of the fetus and newborn infant. In 2004 and 2011, respectively, with Steven Herbert Abman, director of Pediatric Heart Center, The Children's Hospital, Denver, and the University of Colorado, the third and fourth editions were published (Polin et al. 2004, 2011). Without question, this work contributes greatly to both the fundamental science and the clinical implications of a broad array of topics in developmental physiology.

A further contribution was that of Mark Adrian Hanson and colleagues of the University College London, who edited a four volume series on fetal and neonatal physiology. By the editor's admission, these are intermediate between a textbook with breadth of coverage (which may lack critical analysis) and a group of review articles (that may lack balance because of the biases of the authors). In a cohesive set of reviews, the volumes address the circulation (vol. 1, Hanson et al. 1993), breathing (vol. 2, Hanson et al. 1994), growth (vol. 3, Hanson et al. 1995), and the body fluids and kidney function (vol. 4, Brace et al. 1998). Each volume addresses the subject in terms of physiology, pathophysiology, and clinical applications, with a number of authors and points of view for each subject area. The perinatal period, with its transition from fetal to neonatal life, is such a critical juncture that consideration of its multiple facets is most timely. Although one might confute the somewhat exclusive British perspective, of significance are the volumes' stress on, and illustration of, the considerable clinical applications of basic research.

An additional contribution of note was the 1994 *Textbook of Fetal Physiology* edited by Geoffrey D. Thorburn and Richard Harding of Monash University, Melbourne. This contains 36 chapters contributed by 57 authors. Planned as a textbook for undergraduate and graduate students, the volume served as reference work and a companion to the classical texts of physiology of the adult (Thorburn and Harding 1994). That same year (1994), Richard Harding and colleagues organized a symposium on Hamilton Island, Queensland, Australia, to honor Geoffrey D. Thorburn's contributions to developmental physiology. Rather than a textbook per se, the published volume comprises a series of papers on various aspects of this topic (Harding et al. 1995). Several years later, Richard Harding with Alan D. Bocking of the University of Western Ontario, Canada edited the volume

Fetal growth and development (Harding and Bocking 2001). The editors noted the purpose of this compilation was "... to provide an account of the major factors involved in the regulation of ... growth and development [of the human fetus] and to review the processes by which the fetus responds and adapts to a potentially stressful intrauterine environment" (Harding and Bocking 2001, p. ix). Presenting the material by organ systems, the editors rationalized this approach on the basis of "... the degree to which ... [systems] are important to the well-being of the fetus, the extent to which they enable the fetus to withstand adverse intrauterine conditions, and its ability to make the transition from intrauterine to extrauterine life" (Harding and Bocking 2001, p. ix).

Table 23.1 lists these volumes by year of publication. A number of other volumes have addressed the development, physiology, and pathophysiology of specific organs and/or systems (Assali 1968; Assali and Brinkman 1972; Faber and Thornburg 1983; Hytten and Leitch 1964; Jones 1988; Longo and Reneau 1978; Timiras 1972; Wolstenholme and O'Connor 1965). Their review, however, is beyond the scope of this essay.

23.2 The Josiah Macy, Jr. Foundation Conferences on Gestation

A worthy contribution to the development of the field of fetal and neonatal physiology, and reproduction in general, were the series of conferences held under the aegis of the Josiah Macy, Jr. Foundation of New York. Established in 1930 by Kate Macy Ladd (1863–1945) in memory of her father Josiah Macy, Jr. (ca. 1838–1876), who had died at an early age of typhoid fever, commencing at mid-century the Foundation concentrated its efforts on improving the education of physicians and other health professionals. In conjunction with that objective, and, in part, stimulated by the success of the Hixon Symposium on *Cerebral mechanisms* (Jeffress 1951) noted earlier, the Macy Foundation developed and supported an extensive program of conferences and publications. Specifically during the 1950s, under the leadership of its president Willard Cole Rappleye (1892–1976) and its medical director Frank Fremont-Smith (1895–1974), the Foundation organized and supported a number of focus groups which addressed topics believed to be compelling to the advancement of medicine. Annually for 2.5 days, each of these groups met over a 5-year period. The five conferences on *Gestation*, held at the Nassau Tavern in Princeton, NJ from 1954 to 1958, brought together two dozen or so (limit 25) leading investigators from a number of fields related to reproduction: embryologists, placentologists, anatomists, biochemists, pharmacologists, and physiologists, as well as obstetricians and pediatricians who worked in these fields. From widely different backgrounds, these individuals brought a multidisciplinary approach to the conference. Of the participants, a dozen and a half were "regular" members who attended all five annual meetings.

The others were "guests," invited for one meeting or more. Referring to themselves as the "Nassau Tavern Gestation Club," during the proceedings, only a half-dozen or so of the attendees would present a formal paper, these being limited to two or three per day. The majority of the time was devoted to discussion (Flexner 1955; Villee 1956, 1957, 1958, 1959).

In his opening remarks at a later conference, Louis M. Hellman (1908–1990) of the State University of New York, Downstate Medical Center, Brooklyn, recalled regarding the initial Macy Conference. As soon as the initial speaker, Louis B. Flexner, had read one sentence of his paper, "... the discussion began..., and nobody else ever read anything thereafter." Hellman also observed, "one of the most valuable rewards of these conferences is meeting people you never knew personally before. The influence that these people and their friendship may have on your lives is often striking" (Hellman, in Wynn 1965, p. 12).

A critical element to success of the Macy Conferences was the overriding philosophy of the spokesperson Fremont-Smith, whose "experiments in communication" encouraged the participants to experience a state of "free-floating security." Hopefully, in addition to the exchange of ideas, their interactions would stimulate creativity and collaborations. Trained as a psychiatrist with a background in cybernetics, Fremont-Smith held that in major ways scientific advance was limited because of a lack of communication, with the tendency for superspecialized scientists in the several disciplines to talk past one another. In his introduction to each conference, he stressed:

> We do not wish to compete with the scientific societies and journals which have established formats for the presentation of material in their respective fields; rather, our aim ... is to offer a very informal forum for the exploration of one another's views, feelings and attitudes and to encourage the exchange of methods, concepts, and difficulties in an atmosphere conducive to mutual understanding. Although the fertility of the multidiscipline approach has been recognized by the scientific societies and journals ... these organizations have not yet been able to establish adequate coverage of interdiscipline communication.
>
> (Fremont-Smith in Flexner 1955, pp. 7–8)

Fremont-Smith also emphasized the need to challenge "authority" in evaluating ideas, concepts, and data and the necessity of acknowledging unrecognized "blind spots" and prejudices in one's thinking. These he called "... hidden obstructions to communication" (Fremont-Smith in Villee 1956, p. 9). In addition to their benefit to individual attendees, the Macy Conferences gained a wider audience by publication of the proceedings. Following Louis B. Flexner editing transactions of the initial conference, Claude Alvin Villee, Jr. (1917–2003) of Harvard Medical School, edited the following four volumes. Striking for such volumes was inclusion of the often free-wheeling, spontaneous, and dynamic discussions among the participants. Joseph Dancis has written on the *Gestation*, series of Macy Conferences as "Classics Revisited..." (Dancis 1994).

23.3 New York Academy of Sciences Conferences on Fetal Homeostasis

Subsequently, Louis Hellman with Ralph M. Wynn of the State University of New York, Downstate Medical Center, Brooklyn, organized a series of four conferences on *Fetal homeostasis*. Also held at Princeton during the years 1964–1968, these were sponsored by the New York Academy of Sciences and its Interdisciplinary Communications Program (Wynn 1965). Following a brief stint at the American Institute of Biological Sciences, Fremont-Smith had moved to the Academy to head this program. Again in his opening remarks, as with the Macy Conferences, he stressed the need for communication among scientists. He emphasized that with new technologies and new insights, the frontiers of science were advancing in an almost exponential fashion, and the problem of individual scientists being separated and isolated by the formulated boundaries of departmental discipline. In addition to the goal of providing a forum for communication, Fremont-Smith emphasized the opportunity to thresh out problems and discuss them in depth and noted the privilege of developing friendships and collaborations among investigators who ordinarily might never meet one another. He pointed out that, although burdened by a severe information crisis with data overload, the various branches of science are branches of a single tree. Thus, while in our effort to understand the laws of nature, we may look through different windows, each of which permits passage of only certain rays of light, we must obtain our views through many windows (Fremont-Smith in Wynn 1965).

In addition to presenting introductory remarks at each of the conferences, Fremont-Smith published several essays in which he expounded upon his philosophy of the eminent need for interdisciplinary communication. For instance, in addition to the principles noted above, he stressed the need for scientists to engage in "conversation" and platonic dialogue, in contrast to presenting formal lectures (Fremont-Smith 1963). He also emphasized the critical role of philanthropic foundations in supporting such interdisciplinary conferences and scholarships, in addition to grants-in-aid for innovative research (Fremont-Smith 1964). In a letter to the editor of *Science*, following an earlier editorial challenging the mechanisms of large meetings for bringing scientists together (Abelson 1965), Fremont-Smith reviewed positive aspects of the small multiprofessional conferences he had orchestrated, emphasizing the virtues of "special microenvironments" for scientific exchange (Fremont-Smith 1965, p. 1669). In another essay, he considered the virtues of repeated "interruption" of a speaker, as a means of contributing to the scientific dialogue (Fremont-Smith 1971).

Although originally five conferences on *Fetal homeostasis* were planned, only three subsequent New York Academy of Sciences Conferences were held (Wynn 1967, 1968, 1969). It was during the third conference, that for embryonic, fetal, and newborn development, that the term "heterostasis" was suggested to be more fitting than "homeostasis," as a true steady state does not exist. A reviewer of the third volume observed:

> This book is joyful reading for the weekend, or after dinner while in a comfortable chair. There is so much fun in the midst of battle between participants! And what relentless, merciless interrogations by the participants, almost whenever a fact is claimed, or a new hypothesis or theory is suggested. It is far more exciting than fictional novels immersed in intrigue. This is a small convention of iconoclasts … The first formal speaker was to be M. C. Chang … About 2 minutes after Dr. Chang's presentation is underway, he is interrupted with the first of dozens of questions and comments. From this point until the end of the conference there are no longer any recorded attempts at formal presentations … Unlikely insights into the collective personality of the meeting, as well as into some of the participants, are clearly gained by the reader, who cannot help but feel seated at the conference table among the 25 obstetricians and reproduction scientists present. While the reading is easy and enjoyable, the caliber of the clinical and scientific insights presented are the very highest.
>
> (Fainstat 1969, p. 1290)

Regarding conferences such as these, an important consideration is that of their contributions to science in general, as well as to fetal-neonatal physiology, for their role in building relationships and communication among scientists with differing backgrounds, developing collaborations, and advancing the frontiers. Contemporary meetings of this sort, such as the Gordon Research Conferences (www.grc.org), Keystone Conferences (www.keystonesymposia.org), and groups such as the "No Name Society," are modern-day counterparts to these symposia and workshops from bygone era.

23.4 Essays in Perinatal Medicine

As noted earlier, although not a volume per se, the series of over one hundred essays on "Perinatal Lessons from the Past" by Peter M. Dunn, of the University of Bristol, is of relevance. These profiles of individuals and their contributions commence with the Holy Bible, Hippocrates (460–375 BCE), and Aristotle (384–322 BCE); extend to the sixteenth century royal midwife of France Louise Bourgeois (1563–1636), the master of British Midwifery William Smellie (1697–1763); and continue on to the Boston creator of modern neonatal medicine Clement A. Smith. Currently, Dunn is preparing these collected papers for publication. Regarding the background for these profiles, he has written:

> On [my] retirement from clinical practice in 1988, the Editor of the *Archives of Disease in Childhood*, Professor Malcolm Chiswick, invited me to prepare a series of biographical essays concerning contributions made by some of the great doctors and scientists of the past to the development of perinatal medicine. In the event, 108 biographical essays on distinguished men and women from 22 different countries were published in the journal between 1989 and 2009 …. Taken together in chronological order they provide 'stepping stones' in the history of perinatal medicine from the days of Hippocrates right up to the recent explosion of interest in this field.
>
> The series of profiles had a number of purposes. Above all, it was planned to emphasise that childbirth should not be divided into obstetrics/midwifery and paediatrics/nursing working independently and divided at the cutting of the umbilical cord. Rather, there was a need for continuous care of the fetus and the newborn by all the specialties working as a

team throughout. A second purpose was to draw attention to the truth of a remark made by Sir Robert Hutchison [1871–1960] in 1931 during his Harveian Oration at the Royal College of Physicians. He said: 'Look round this room in which we are met. It is a noble library indeed, but is it not also a mausoleum? And how many facts which men are at present hunting for, and theories which are even now being put forward as new, lie already buried in these shelves?' (Hutchinson 1931, p. 735). It gave me particular pleasure to draw attention to current 'discoveries' that had, in fact, been made previously in the distant past. The third purpose, subsidiary to the second, was to draw attention to certain themes that reappear a number of times throughout the series, such as the importance of maternal posture during childbirth, the management of the umbilical cord at birth, and the impact that rickets has had on obstetric practice. These themes have been interests of mine over the years. While either controversial or neglected by modern practice they remain important and I have derived encouragement from the knowledge that some of the great men of the past have also thought so. Our forefathers, unencumbered by modern technology and protocol, had more time to think and to watch nature in action. We can still derive benefit from the observations they made.

The choice of individual doctors, scientists and nurses about whom to write was not easy as there were so many from whom to choose. The decision was helped by creating some ground rules. First, the series would be based on western medicine. Second, an effort was made to cover broadly the whole period from classical times right up to 1950. The contributions of the last 60 years have been so numerous that they will require a second series in the future. Thirdly, special favour was given to those individuals whose contributions have only recently been credited or indeed still await recognition. Fourthly, I confess that being British and not a linguist, there was inevitably a strong bias towards those whose contributions were available in English. Apologies are due to the many, many distinguished individuals who have been omitted.

In order to bring the profiled individuals into sharper focus, their portraits have been included when available together with extracts from their works so that the reader may gain insight into the quality and style of their contributions. Some essays are shorter than others, not because of the importance of the person concerned but because at first I was only permitted one or at the most two pages of the Archives journal. In time, this limit was relaxed to three or even four pages. In my choice of authors I have not only attempted to cover the whole period of 2400 years but also to cover different aspects of maternal, fetal and neonatal medicine. Some of the people chosen may not be as well known as they deserve to be. It has been a pleasure to draw attention to their works.

The essays, originally published in random order in the *Archives* over the last twenty years, have now been put in chronological order based on the year of birth of each doctor or scientist. Many requests have been made by colleagues to publish the series in book form and this exercise is currently in preparation under the title: *Childbirth: perinatal lessons from the past*. Let me close with a quotation from Emerson: "...there is properly no History; only Biography."

(Letter from PMD to LDL, 23 February 2010)

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Chapter 24
Fetal "Breathing" in the 1970s and Fetal Heart Rate Analysis in the 1980s and Early 1990s

Progress in science depends on new techniques, new discoveries and new ideas, probably in that order.
(Sydney Brenner, attributed)

24.1 Early Studies of Fetal Breathing Movements

Since antiquity, mothers have been keenly aware of movements by the developing fetus. For instance, Rebekah wife of Isaac noted that "... The children [Esau and Jacob] struggled together within her" (Genesis 25:22[1]). Even before taking advantage of the chronically catheterized sheep preparation, Geoffrey Dawes rediscovered the fact that the fetus "breathed" in utero. He recounted later that it was, in part, because of "Mont" Liggins that he embarked upon this field of research. For it was in the spring of 1970 that Liggins spent a 3-month sabbatical at the Nuffield Institute. Several months earlier, in "acute" experiments in which the fetus still attached to the umbilical cord was placed in a warm saline bath, the Nuffield group had observed respiratory-like activity (Dawes et al. 1972a). Although initially planning to explore some aspects of the role of insulin in the regulation of fetal metabolic control, Liggins demonstrated to Dawes the value of the chronically catheterized, unanesthetized preparation to study this activity (Dawes 1989, p. 6). Concurrently, an American medical student, Harold Edward Fox from the University of Rochester, spent the academic year 1969–1970 working at the NIMR. In Rochester, Fox had done some work with Mortimer G. Rosen (1931–1992), studying the electrocorticogram (ECoG) of fetal guinea pigs and lambs. Fox who went on to become Chair of Obstetrics and Gynecology at the Johns Hopkins University (1997–2013) states that seeing Dawes' recordings of the periodic nature of fetal breathing movements was "... reminiscent of the high voltage slow/low voltage fast periodicity of the ... electrocorticogram of the fetal lamb." On several occasions Fox mentioned this to Dawes, who, however, showed "minimal interest" in this association. Later, at a Friday afternoon meeting in

[1]King James Version.

L.D. Longo, *The Rise of Fetal and Neonatal Physiology*, Perspectives in Physiology,
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Dawes' office for "sherry," they were discussing preparations for the following Monday's chronic preparation. Dawes invited Fox to prepare his electrodes to place in the fetal skull. "We did not have the sensitive biological amplifiers necessary for EEG recording"; however, working with Derek Wyatt over the weekend, they prepared a system for recording. Fox has recalled, "On Monday, I placed the electrodes and by Wednesday we were recording fetal electrocorticogram and correlating its activity to fetal respiratory movements. Subsequently, Dr. Dawes supported my proposal to record fetal eye movements as well and we embarked upon this several preparations later. I can remember Dr. Dawes' excitement when we sat down to review the polygraph records of fetal respiratory activity and fetal ECoG for the first time. Sharing in the excitement of discovery is infectious and I will be forever grateful." Fox continued, "My year at Oxford had a profound effect upon my career as did the mentoring of Dr. Dawes and Dr. Rosen" (Letter from HEF to LDL, 19 May 2009).

Previously, Dawes had recorded that near-term fetal lambs, delivered under maternal regional or local anesthesia onto a warm table with intact umbilical cord, did not normally breathe (Dawes 1968). About this same time, Dawes' Nuffield Institute colleague Derek Wyatt had developed a new flowmeter with near-zero drift that could be used to quantify fluid flow in the trachea, (Clark and Wyatt 1969), a technique used in later studies of blood flow (Wyatt 1984). With Fox, Liggins, and colleagues, in the acute preparation, Dawes reported bursts of irregular rapid shallow, breathing-like movements that occurred for 35–40% of the time from 40 dpc (0.27 gestation) (Dawes et al. 1972a). In the chronically catheterized preparation, particularly after 95 days (0.66 gestation), these breathing movements were associated with low voltage high frequency (LVHF) electroencephalographic activity and rapid eye movements (REM). In addition, he observed single, but infrequent, deep inspiratory gasps "augmented breaths" or "sighs" that occurred ~5% of the time (Dawes et al. 1972a; see also Dawes and Robinson 1976). Moreover, a prominent diurnal activity was evident with a peak in rapid, irregular breathing, tracheal volume flow, and LVHF ECoG state from 1800 to 2400 h. In the chronically catheterized preparation, this REM-associated breathing activity occurred almost solely in conjunction with LVHF ECoG and was absent during the high voltage low frequency (HVLF) state (Dawes et al. 1972a). In addition to negative pressures in the trachea that reached about ~25 mm Hg, in many instances, these respiratory movements also were associated with major fluctuations in heart rate and blood pressure. Despite the vigor of this respiratory activity, comparatively small changes were found in tracheal fluid flow or pulmonary volume. Also noted, fetal breathing was abolished by general anesthesia but not altered by transection of the vagus nerves (Dawes et al. 1972a). As an aside, although this report is Dawes' most highly cited publication, it had been rejected by *nature* for a "... lack of general interest" (Liggins 1998, p. 122), and similar findings were reported in guinea pigs (Dawes et al. 1972b).

Of relevance, in 1972 Edward James "Ted" Quilligan, at Yale University, published a report on their studies on the electroencephalogram of the near-term fetus, in which they described the cycling of high voltage low frequency activity

with low voltage high frequency, rapid eye movement sleep state (Jost et al. 1972). Regarding their early studies, Quilligan, more recently at the University of California, Irvine, has written:

> In the late 1960s, our lab at Yale was doing chronic preps to study the fetal heart rate and acid base balance of sheep in labor. Each medical student at Yale did a project and I had this bright young student Gilbert Jost indicate he wanted to study the fetal EEG. We devised a preparation which is exactly the same prep that Geoffrey [later] used. [Dawes] came to my lab in either 1968 or early 1969, saw the prep and was very interested in it. The study was finished in 1969, because I left and moved to the University of Southern California. Gill Jost wanted to publish the paper in a neurologic journal, but it got rejected by two journals, I believe, and thus was late being published in the *AJOG* (Jost et al. 1972). I believe from Dr. Fox's letter he joined the Nuffield group in 1969. This would be the time their lab started EEG recording and would be after the time Geoffrey had seen our preparation. I thought Geoffrey had mentioned this in a publication but cannot find that publication.
>
> (Letter from EJQ to LDL, 24 February 2010)

In reference to studies at the Nuffield Institute, Liggins has recorded that during his 1969 sabbatical with Dawes at the Institute, his ". . . adventure of the morning routine of carrying the sheep up the winding staircase. Some of the sheep were pregnant, some were not. The habit had been to make the distinction when the ewe was anesthetized and the abdomen opened, clearly wasteful of time and money. My legacy to the Institute was the demonstration of the art of abdominal palpation, whereby the presence of a foetus could be determined before the ascent up the stairs began" (Liggins 1998, p. 118).

As noted elsewhere, in 1970, the Nuffield Institute moved from the "Tower of the Winds" to its new quarters on Headington Road. Jeffrey Samuel Robinson, now at the University of Adelaide, Australia, recalls his first experiment with Dawes:

> My first fetal physiology experiment was conducted . . . in the new building of the Nuffield Institute. Every piece of equipment had been dismantled and reassembled . . .. All seemed to go well until Geoffrey came in to lead the experiment—an acute one with an exteriorised fetus in a saline bath. Geoffrey checked the temperature of the bath with his hand and was blown across the room by an electric shock as the current of heating element in the bath was going through the saline solution—the wiring had been connected incorrectly. Geoffrey recovered, the wiring was corrected and the experiment progressed well. It was one of the few experiments that he actually led while I was there. Geoffrey . . . later came to assist me with a chronic fetal sheep operation. He rapidly became bored with the surgery which had to be more careful as recovery and sterility were important. However, when we were short of people he came and assisted a few times.
>
> Computing was considered after he set me the task of analysing heart rate variability. When I said I could not do it from the printed records that ran for many days, he took over and locked the door of his room to analyse it himself. Three days later, he came into my office to agree and said that he had just taken the decision to purchase the Institute's first computer. I subsequently wrote the first program to record heart rate and blood pressure from fetal sheep. I was allocated 4k of memory for the program, recording and printing out the result. The data was then erased and the next set collected and printed out. Later analysis required re-entering the data by hand into statistical packages. Geoffrey progressively expanded the recording of heart rate leading to his program that is still used in Oxford.
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> (Letter from JSR to LDL, 17 April 2009)

Dawes' series of studies of fetal breathing movements confirmed the late nineteenth and early twentieth century findings of several German workers. Both Bernhard Sigismund Schultze (1827–1919) of the University of Jena (Schultze 1871) and Johann Friedrich Ahlfeld (1843–1929) at Marburg (Ahlfeld 1888) had observed fetal respiratory-like activity in women near term, as these breathing movements could be seen through the abdominal wall. Ahlfeld argued that because this activity was necessary to life following birth, it was reasonable that such activity precedes birth. As an aside, he noted that newborn infants swallow and suck their thumb, and he believed he saw evidence of this in the fetus, as well as kicking movements. One of Ahlfeld's students also published an early tracing of these in utero fetal movements transmitted to the maternal abdominal wall (Weber 1888). In a subsequent report, with a glass funnel placed on the mother's abdomen and a kymograph, Ahlfeld published remarkable records of fetal chest movements that averaged 54 min^{-1}. He also recorded periods of very rapid breathing followed by apnea, an observation he noted that also may occur in the newborn (Ahlfeld 1905). Soon, these movements were confirmed by others (Reifferscheid 1911) who with Ahlfeld maintained that fetal respiration was not associated with aspiration of amniotic fluid into its trachea and bronchi. Other observers, however, held that these movements were a consequence of external tactile or thermal stimulation, thus rejecting Ahlfeld's thesis (Runge 1905). In addition, the fact that radiopaque contrast material injected into the amniotic fluid later could be seen in the fetal gut, but not the lungs, caused some to discount the fact, or importance, of fetal respiratory-like activity. Ahlfeld also wrote on amniotic fluid and its formation, noting that it could not be accounted for by fetal urination, but rather was formed by secretion of the amniotic membranes (Ahlfeld 1911). Regarding these early studies, Robert Clair Goodlin, formerly of Stanford University and the University of California, Davis, has written:

> I tried to get Dawes to refer to Ahlfeld's multiple reports on human fetal breathing, but he declined to do so, claiming they were 'anecdotal'. The same with fetal heart rate beat-to-beat variability. He very much wanted to be 'Mr. Fetal Physiologist'. Given that I was working with Abe Rudolph, I found Dawes' demeanor hard to accept.
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> (Letter from RCG to LDL, 10 June 2009)

In his 1913 text, *Principles and Practice of Obstetrics*, Joseph B. DeLee of Chicago wrote that "the evidences of life of the fetus in utero form an interesting study ... The fetus moves its limbs and body from the earliest months, and the movements are audible and palpable from the fifteenth week ... The child has periods of sleep, of rest, and of activity." DeLee continued "another phenomenon, not so common, and more uncertain of diagnosis, is the respiratory action of the child in utero. If one carefully observes the umbilical region of a thin woman, pregnant near term, one may discover fine rising and falling movements of the abdominal wall. They occur 60 to 80 a minute, are intermittent, and are most pronounced in the region of the child's chest" (DeLee 1913, pp. 58–59).

In the mid-1930s, Franklin Faust Snyder (1897–1992), of the Department of Obstetrics, and Morris Rosenfeld (1906–1968), of the Department of Pharmacology

and Experimental Therapeutics, at the Johns Hopkins University, demonstrated that contrary to the idea commonly held that the fetus was apneic, fetal cats, guinea pigs, and rabbits showed considerable respiratory activity. Abolished by both hypoxia and hypocapnia, hypercapnia did not stimulate this activity significantly (Snyder and Rosenfeld 1937a). On the basis of the observed responses to hypoxia, the authors concluded that carotid body chemoreception and activation were unimportant during fetal life, a fact subsequently confirmed by Dawes (1972a) and others (Jansen et al. 1981). In an associated study, Snyder and Rosenfeld demonstrated similar respiratory-like activity in the human fetus near term and suggested that fetal aspiration of tracheal fluid contributed to dilatation of the future air passages, with development of the lungs and their alveoli. They also noted that in some instances cells, meconium, and other debris could be found within the potential air spaces, a possible route for the introduction of infection (Snyder and Rosenfeld 1937b). In yet another study, these authors demonstrated that India ink injected into the amniotic cavity soon appeared within the lungs of those fetuses that displayed respiratory movements, but not in those that were quiescent. Again they stressed that such activity plays an important role in the dilatation of the future air passages (Snyder and Rosenfeld 1937c).

Joseph Barcroft, in his observations in premature, exteriorized fetal lambs, concluded that from 40 to 60 dpc, although fetal breathing-like activity normally was not present, it could be elicited by tactile stimulation of the face. He reported that from 60 dpc onward, the animals showed no response, this respiratory activity commencing only at the time of birth (Barcroft 1946, p. 260). In his *Physiology of the Fetus*..., Windle dedicated a chapter (VI) to breathing-like activity in the developing fetus, noting the possibility that this represented "practice" in preparation for breathing after birth. Nonetheless, Windle held, as did Barcroft, that for the most part, such respiratory movements were initiated by hypoxia and/or hypercapnia stimulating the brain stem respiratory center, following its maturation during the latter part of gestation (Windle 1940, p. 78ff). In his *Physiology of the Newborn Infant*, Clement A. Smith summarized some historical aspects of fetal respiratory movements (Smith 1945), much of which was based on a previous review (Bonar et al. 1938). For several species, Davenport Hooker (1887–1965) of the University of Pittsburgh also has given a valuable account of the genesis of respiratory and other movements in the developing fetus (Hooker 1952). Following his analysis of the interrelations of structural (neuronal pathways) and function (the several types of movements), Hooker concluded:

> ... it is evident that each class of vertebrates, perhaps each order, genus, and species, exhibits characteristics in the development of behavior which belong to that subdivision of animals alone. Nevertheless, each form of activity shown by any fetal organism is a step in normal development, hence a step in preparation for postnatal behavioral capabilities. Furthermore, there is a tendency for voluntary acts, where and when they appear, to develop in a sequence based upon the earlier reflexogenic sequence of prenatal life. This is particularly well demonstrated in the case of human behavior. The frontiers of research in the development of fetal activity are by no means closed.
>
> (Hooker 1952, p. 120)

Several decades later, Michael J. Purves and colleagues at Bristol University explored aspects of neural respiratory activity in the fetal and neonatal lamb (Bystrzycka et al. 1975; Ponte and Purves 1973).

In contrast, with diaphragmatic electromyographic recordings, others soon demonstrated that in association with diaphragmatic activation, tracheal pressure decreased, and a small volume of tracheal fluid flowed inward (~0.5 ml per breath). Additionally in sheep, during the latter third of gestation from 100 dpc to term, the pattern of respiratory activity was shown to change, with a significant increase in the relatively slow (1–2 min^{-1}) mode of regular breathing (from ~6% to ~17% of time), with a concomitant decrease in rapid breathing (~60 min^{-1}) (from ~19% to 3% of time) (Maloney et al. 1975). In humans, by the use of continuous Doppler ultrasound instrumentation, the Nuffield Institute group soon demonstrated fetal breathing movements (Boyce et al. 1976). This discovery gave rise to the promise of the clinical application of FBM in assessment of fetal well-being. Dawes and Robinson concluded that near term, fetal breathing movements with the LVHF ECoG state to be present about 55 and 70% of time in sheep and humans, respectively (Dawes and Robinson 1976).

In a later review of this topic, Dawes reported unpublished observations from his laboratory on several phenomena (Dawes 1980, p. 5). These included almost continuous diaphragmatic electromyography activity, with only intermittent contraction of intercostal muscles prior to differentiation of the diaphragm. He also stated that following fetal ECoG differentiation, diaphragmatic EMG activity became intermittent, correlating with transthoracic pressure changes. Dawes concluded that the mechanisms which regulate fetal breathing develop progressively and concurrently with general growth and development. In terms of the ontogeny of respiratory-like and diaphragmatic activity, these workers reported that from mid-gestation onward to ~110 dpc (0.75 gestation) in the sheep, breathing movements were almost continuous, becoming more periodic from 130 dpc to term, as reflected by diaphragmatic electromyographic activity (Bowes et al. 1981). Subsequently, the Dawes group demonstrated three distinct age-related patterns of the interrelations of breathing movements, ECoG activity, and rapid eye and neck movements. For instance, from 95 to 106 dpc, FBM, LVHF ECoG state, and ocular and nuchal activity were relatively continuous. From 107 dpc, FBM became episodic, in association with LVHF, rapid eye movement, and nuchal muscle activity. Then from 120 dpc onward, the ECoG was differentiated clearly into LVHF and HVLF states, with FBM and rapid eye and nuchal movements occurring almost exclusively during the former (Clewlow et al. 1983). Dawes also confirmed that these activities become episodic, and with development of cyclicity of the electrocorticogram, breathing movements occurred solely during the LVHF state (Dalton et al. 1983).

To determine the mechanisms of regulation of fetal respiratory-like activity, the Oxford group and others used several approaches. For instance, they demonstrated in sheep during the last third of gestation that breathing activity was inhibited significantly or abolished by hypoxia but was stimulated by hypercapnia. Concomitantly, hypoxia reduced the proportion of time occupied by LVHF ECoG activity,

while this increased in response to hypercapnia (Boddy et al. 1974a, b). Also in sheep, fetal breathing-like activity occurred during rapid eye movement LVHF ECoG state, but not during HVLF. With the use of a double-wall plexiglass window, these associations also were demonstrated visually (Rigatto et al. 1986). Further, because E-type prostaglandins (PGs) used to maintain *ductus arteriosus* patency in newborn infants with certain forms of congenital heart disease were associated with apneic spells (Olley et al. 1976), it was postulated that prostaglandins were involved in regulating respiratory-like activity. Indeed, in near-term fetal sheep, intravascular infusion of a prostaglandin synthase inhibitor was associated with an increase in breathing movements from 38 to 69% of the time, including during the HVLF state. In addition, prostaglandin inhibition showed an increase in respiratory amplitude (Kitterman et al. 1979), and such prostaglandin-mediated inhibition also stimulated respiratory movements independent of sleep state (Jansen et al. 1984). In a further study in the near-term fetal sheep, these workers demonstrated that with infusion of PGE_2, FBM decreased to 10% of control. In contrast, infusion of $PGF_{2\alpha}$ or endoperoxide analogs resulted in breathing movements decreasing only to 64–69% of control (Kitterman et al. 1983). In addition to confirming the role of PGs in inhibiting FBM in brain stem-sectioned fetuses, Brian John Koos, working as a graduate student with Dawes, demonstrated that the PGs act centrally in the medulla or lower pons to modulate breathing (Koos 1985). Koos also showed that despite their abolition by acute hypoxia, in animals maintained chronically hypoxemic for 6 days, breathing-like movements returned to normal (Koos et al. 1988). Geoffrey Thorburn argued that the placenta and its secretion of PGE_2 alter fetal peripheral chemoreceptor activity in response to arterial blood gas values and thus modulate breathing movements. He also suggested that in the newborn, it is the rapid fall in PGE_2 concentrations following delivery that plays a critical role in both the initiation of breathing and reabsorption of lung liquid (Thorburn 1995).

Breathing movements also were shown to be relatively unaffected by peripheral arterial chemoreceptor input (Dawes et al. 1972a), and not essential to the establishment of breathing at birth (Jansen et al. 1981). In an effort to elucidate the central regulatory mechanisms of FBM, another approach was to examine the association of LVHF ECoG activity with breathing movements following transection of the brain stem (Dawes et al. 1981c, 1983). Several years previously, Liggins had shown that following fetal spinal cord transection at C2–C3 (above the phrenic nucleus), lung development was reduced significantly (38% decrease in weight and 55% decrease in distensibility) (Liggins et al. 1981). With a focus on higher center, Dawes and colleagues demonstrated that fetal brain stem section above the pons, at the level of the superior colliculus, was followed by continuous FBM, with dissociation of "breathing" from electrocortical activity. In contrast to the decrease in FBM rate and amplitude seen normally in response to hypoxia in control intact fetuses, FBM increased following suprapontine section. When sectioned more rostrally, this dissociation between breathing movements and ECoG persisted, and the baroreflex sensitivity was increased. These experiments established that higher medullary centers exert an inhibitory influence on FBM (Dawes 1984; Dawes et al. 1983).

24.2 Fetal Breathing in Humans

These findings in sheep opened the way for studies of fetal behavior both in sheep and humans. For instance, Dawes in his 1987 James Alexander Frederick Stevenson (1918–1971) Memorial Lecture at the University of Western Ontario, Ontario, Canada, stressed the increasing complexity of neural functional integration during the course of development, synthesizing relevant studies in neurobiology (Fitzgerald and Koltzenburg 1986), with the evidence for fetal perception of pain, diurnal rhythms, sleep states, breathing-like activity, and body movements (Dawes 1988, 1994). During the following decade, studies on fetal breathing patterns and their associations with related physiologic phenomena including behavior, neurophysiology, pulmonary mechanics, growth of the lung and other organs, and cardiovascular dynamics would occupy much of his energy. In humans, the use of Doppler ultrasonography and linear-array real-time imaging to detect fetal breathing and body movements gave hope of its use in diagnosing fetal hypoxia/asphyxia; however, this promise remains to be fulfilled (Boddy et al. 1974b, 1976; Boddy and Dawes 1975; Boyce et al. 1976; Chapman and Dawes 1978; Dalton et al. 1977; Dawes 1977a, b, 1979, 1984; Dawes and Robinson 1976; Duenhoelter and Pritchard 1977; Patrick 1980; Patrick et al. 1978; and others). In the humans, in addition to breathing-like activity, fetal body movements soon came to be appreciated as an index of fetal well-being (Timor-Tritsch et al. 1978a, b; Walters 1964). By use of ultrasonography in humans, Heinz Friedrich Rudolf Prechtl at the University of Groningen, the Netherlands (currently at the University of Graz, Austria), reported on several aspects of the patterns of fetal breathing and body movements to define distinct behavioral states (Prechtl 1974, 1984).

John Elgin Patrick (1942–1989), of the University of Western Ontario, one of Dawes' postdoctoral fellows, was particularly active in quantifying fetal activity in the human. For instance, in the healthy fetus at 34–35 weeks' gestation, both FBM and body movements were shown to increase several hours following meals, presumably reflecting plasma glucose levels (Patrick et al. 1978). In patients near term, FBM occurred ~26% of the time, but in association with labor decreased to ~8% during latent phase, and was essentially absent (~1% of time) during active labor ($p < 0.001$). Although both FBM, in association with body movements, and heart rate variability occurred during a control period, during the first stage of labor, the latter persisted despite absence of FBM (see below; Richardson et al. 1979). Near term, a circadian pattern of FBM activity was observed, with the peak between 2100 and 0100 h (Patrick et al. 1982). Such a circadian rhythm also occurred for heart rate acceleration patterns (Patrick et al. 1984). In 1980, Patrick reported on several aspects of fetal breathing in the human (Patrick et al. 1980; Patrick and Challis 1980). In addition, he edited a special issue of the journal *Seminars in Perinatology*, which summarized aspects of fetal breathing movements in human pregnancy, including their measurement by various techniques, their relations to blood glucose and oxygen levels, and related factors (Patrick 1980).

In perspective, a greater measure of the work by Dawes and his associates on fetal breathing and body movements was the impact to stimulate the efforts by others. In toto, findings suggest an important role for this movement activity on fetal growth and development, serving as an illustration of development of an activity-dependent mechanism in the central nervous system. As a demonstration of their importance, fetal behavioral states also have been demonstrated in the guinea pig (Umans et al. 1985), rhesus monkey (Martin et al. 1974), and baboon (Grieve et al. 1994). With hypoxia and/or asphyxia, the alterations in behavioral state are a function of gestational age, the severity and duration of hypoxia, and its association with progressive academia (Bocking 1992). In response to hypoxia, attenuation of fetal activity may be important in conserving oxygen consumption and energy expenditure. Clearly, gestational age, time of day, relation to maternal food intake, uterine activity, and other factors are important in the evaluation of fetal movements. Thus, clinical assessment of the "nonstress test" (Evertson et al. 1979) and "biophysical profile" (Manning 1999; Manning et al. 1985, 1993) in which fetal movements are evaluated needs to be interpreted carefully in high-risk obstetrical patients, with the appreciation that the loss or attenuation of this activity may represent impending catastrophe.

In 1973, a new dimension was brought to the Nuffield Institute, when Geoffrey Thorburn, on sabbatical leave from the University of Melbourne, and who had been working for a year there under the aegis of a Medical Research Council Program grant, joined the Institute staff. A gifted endocrinologist, Thorburn, was joined by John Challis and Jeffrey S. Robinson. During a 5-year period at the Institute, this trio was to revitalize many aspects of an understanding of fetal endocrinology, particularly that of the hypothalamic-pituitary-adrenal axis. As noted in regard to fetal breathing, Thorburn conducted a series of studies demonstrating the role of placental prostaglandin E_2 (PGE_2) and also adenosine, in inhibiting fetal breathing movements (see Thorburn 1995 for review).

In recalling Thorburn's years at the Nuffield Institute, Jeffrey Robinson noted:

> Both Geoffreys were amazing people to work for, but their style was very different. Geoffrey Thorburn was known as the "Big G". He was always speculating and designing new experiments. Our sessions in the White Hart, the local pub could solve any problem in fetal physiology and each time we seemed certain that we had got the final answer. The Big G was very interested in the trigger for parturition. He was talking on the topic at a Ciba Symposium, as was my other co-worker of the time, John Challis. I was the junior back in Oxford. I was testing if administration of arachidonic acid or prostaglandins given locally into the uterus. Our hypothesis was that adding arachidonic acid would lead to parturition—Geoff was anxious the present the "success" of this experiment. Therefore there were a series of phone calls each day for an update of the state of the pregnant monkeys—they did not go into labour and a manuscript was rapidly re-written!
>
> The pace of fetal physiology seemed to be very rapid in those days, even if each experiment took a long time to complete. It's just that there were many experiments going on simultaneously and one or two were reaching completion.
>
> (Letter from JSR to LDL, 17 April 2009)

In commenting upon some aspects of this work, Dawes reflected, "I set out to speak about revolutions and cyclical rhythms in prenatal life. There certainly has

been a revolution in thought in the recognition that the physiological systems of the fetus are exercised, though discontinuously. It will now be interesting to try to design experiments to determine whether this activity serves a useful function in development, as seems likely" (Dawes 1973, p. 970). Almost a decade later, Dawes remarked, "... progress since 1970 has been swift. The broad outlines of physiological control ... is understood, but not by any means the details of all the mechanisms involved. It has proved more complex than at first supposed. Smaller may be more beautiful[2], but evidently not simpler" (Dawes 1980, p. 5).

In another reflection regarding his investigations of fetal breathing movements, he stated:

> These studies have developed logically and irrevocably, from the observation of episodic fetal breathing arrested by a mild degree of isocapnic hypoxia. The contrast with control mechanisms in the adult was obvious. I had hoped, even expected, that physiologic controls in the fetus would be built on a simpler plan than in the adult. With the passage of years I have learnt the hard way that it is not so. The fetus is very complicated, and the mechanisms by which its physiologic functions are integrated, to use BARCROFT's phrase, are still a profitable subject of enquiry.
>
> (Dawes 1985, p. 29)

Dawes' son, Nicholas William, has written regarding the rediscovery of fetal breathing:

> Pa recorded a ... scan of [my son] Simon Dawes in utero, the first healthy human baby control during the discovery of fetal breathing in Oxford. This ... was taken on 27 March 1974 when Simon was 14 weeks gestation. [My wife] Sue Dawes ... then gave birth to Simon on 21 September 1974 ... Geoffrey's grandson, is now a doctor and team leader of hospitalists at the Peter Lougheed Centre in Calgary [Alberta, Canada].
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> (Letter from NWD to LDL, 18 January 2009)

24.3 Early History of Fetal Heart Rate Monitoring

The fourth movement of Dawes scientific *quadrivium* was that of electronic fetal heart rate (FHR) analysis. Following invention of the stethoscope in the early nineteenth century by the French physician René Theophile Hyacinthe Laennec (1781–1826) (Laennec 1819), several workers used abdominal auscultation of the fetal heart rate (FHR) to diagnose fetal well-being (Kergaradec 1822; Pinkerton 1969). Although this is not the place to review in extenso auscultation and/or electronic monitoring of the fetal heart, several aspects are of relevance. In his *Observations on Obstetric Auscultation...*, Evory Kennedy (1806–1886) of the Rotunda Hospital, Dublin, was one of the first to recognize that abnormal fetal heart rate was a sign of compromised fetal well-being with impending death of the fetus in utero. He described auscultation with the stethoscope to aid in the diagnosis of "evidences of pregnancy." After noting its normal rate of "about 130 or 140 [beats]

[2]This is reference to Ernst Schumacher's book, *Small Is Beautiful* (1973).

in the minute," Kennedy recorded the variation in fetal heart rate, "becoming suddenly more or less frequent, and then returning to its natural state without any apparent reason. . .. The external cause, which we shall find most frequently. . . is uterine action, particularly when long continued, as in labour" (Kennedy 1833, pp. 90–91). He also noted the effect of compression of the fetal head or "funis" (umbilical cord) on heart rate decelerations and recorded an instance of fetal death following deep deterioration with disappearance of its heart rate (Kennedy 1833, pp. 92–93). Kennedy stressed the value of *mediate* auscultation in the diagnosis of twin pregnancy and the fetal positions, location of the placental soufflé, and as an aid to distinguish true from pseudopregnancy. Kennedy concluded this tract by considering both the advantages and difficulties of auscultation, stating "We would merely beg, that those who have an opportunity, will give it a fair and impartial trial. As to the result we feel perfectly satisfied. And in conclusion, . . . if its application be properly understood, it will afford us as satisfactory and unerring signs, as any diagnostic means relied on in medical practice" (Kennedy 1833, p. 260). In an Appendix, Kennedy considered legal issues such as ascertaining the presence of pregnancy in the case of a woman "convicted of a capital felony," who pleads for a stay in execution because she "is quick with child" (Kennedy 1833, p. 261ff; see Pinkerton 1969, 1984). Goodlin has reviewed the contributions of other workers who auscultated the fetal heart rate and some aspects of its variation, during the latter nineteenth and early twentieth centuries (Goodlin 1979).

Early in the twentieth century, an electrocardiogram of the human fetus first was obtained (Cremer 1906); however, as noted earlier, continuous intrapartum electronic fetal heart rate monitoring as an alternative to traditional auscultation was not introduced clinically until shortly after mid-century (Hon 1958, 1959, 1960; Larks 1961). In several reports, Edward Harry Gee Hon (1917–2006) who had a background in electronics (Yeo and Edward 2000) and "Ted" Quilligan, of Yale University, described changes in electronic fetal heart rate monitoring (EFM) baseline variability and the patterns of bradycardia in association with uterine contractions (Hon and Quilligan 1967, 1968). In particular they attempted to quantify FHR patterns of bradycardia in response to uterine contractions on a rigorous basis, viz., "early deceleration" being associated with head compression, "late deceleration" being associated with uteroplacental insufficiency, and "variable deceleration" being associated with umbilical cord compression (Hon 1968; Hon and Quilligan 1967, 1968). During the 1970s and 1980s, with the use of fetal scalp or other internal electrodes, this technology became widely used, a practice of care that has continued to the present. The attraction of this well-intentioned modality was the perception of many that by continuous recording and with supposed "objectivity," fetal monitoring with detection of bradycardia would provide almost immediate evidence of intrapartum hypoxia. In turn, this would permit the attending physician to intervene, thereby avoiding the severe acidosis and/or asphyxia that could eventuate in attendant sequelae (Hammacher 1962, 1966, 1967; Kubli et al. 1969) including respiratory distress syndrome (Hon et al. 1975). In one of the early studies on high-risk patients at the Los Angeles County General Hospital, Hon, Quilligan, and colleagues reported on a number of aspects

of fetal heart rate monitoring. In consideration of the particular benefits of electronic FHR monitoring in the premature infants in the 1000–1500 g weight range, they admitted that a well-controlled trial with long-term infant growth and development studies was required (Paul and Hon 1974).

In turn, Roberto Caldeyro-Barcia (1921–1996) of the University of Uruguay, Montevideo, another pioneer in electronic fetal heart rate monitoring, referred to the first two of these patterns of bradycardia as "type I" and "type II dips," respectively (Caldeyro-Barcia et al. 1966; Schwarcz et al. 1974). These latter workers also studied aspects of the fetal electrocardiogram tracing in detail (Figueroa-Longo et al. 1966). Later, Caldeyro-Barcia wrote an account of the evaluation of his thinking in regard to EFM patterns in human labor, their relation to uterine activity, and to simultaneous blood gas measurements from the fetus (Caldeyro-Barcia 1985). In this essay he described some aspects of a meeting he organized devoted to the topic "Effects of labor on the fetus and newborn" in Montevideo in October 1964. Here, James, Quilligan, Saling, and others participated in a joint study. Caldeyro-Barcia recorded that after the meeting, the group worked together to monitor the fetuses of three patients in labor, recording electronic FHR, uterine contractions, and sampling fetal scalp blood. This was the first time these methods had been used simultaneously (Caldeyro-Barcia 1973, 1985).

With the use of microballoons, Caldeyro-Barcia and colleagues also were the first to record intramyometrial pressures of pregnant women in labor. By placing these in different parts of the uterus, they studied the origin and characteristics of pacemaker areas with spread of the wave throughout the organ (Méndez-Bauer et al. 1961). In an attempt to objectify the regularity of uterine contractions, in early reports, these workers measured the "coefficient of variation" of the contraction peak-to-peak intervals, as determined from amniotic fluid pressure, and then calculated the "index of uterine arrhythmia." They then applied this index as a measure of uterine efficiency in cases of uterine inertia and in response to drugs, distinguishing between true and false labor (Effer et al. 1969). In further studies, in which they defined uterine activity as the product of contractile intensity multiplied by frequency (e.g., mm Hg per 10 min, or "Montevideo unit"), Caldeyro-Barcia and colleagues determined that in normal labor near term, uterine activity ranged from 100 to 250 Montevideo units (Krapohl et al. 1970). As noted earlier, these workers also used this methodology to measure hydrostatic pressures in the placental intervillous space of the rhesus monkey (Reynolds et al. 1968).

Despite efforts to objectify rigorously the fetal electrocardiographic tracings, some have argued that, for the most part, this analysis has remained relatively subjective (Goodlin 1977, 1979; Wulf 1985). A number of noncontrolled, retrospective analysis supported the view that continuous monitoring would allow the caregiver to detect fetal hypoxia and/or acidemia, although this resulted in significantly increased rates of cesarean section. As noted earlier, in North America in addition to continuous EFM monitoring per se, clinicians challenged the fetal heart rate response by inducing uterine contractions (contraction stress test, CST, or oxytocin challenge test, OCT) (Pose and Escarcena 1967; Ray et al. 1972). As exemplified in the work of Dawes and his group, others emphasized the importance

of FHR variability. This foreshadowed the introduction of the relatively popular nonstress test (NST), a "reactive" test being characterized by two accelerations (of 15 beats·min^{-1} minimum amplitude lasting for a minimum of 15 s) within 10 min (Lee et al. 1975, 1976; Nochimson et al. 1978; Schifrin et al. 1979; Trierweiler et al. 1976). A problem with these tests was their relatively high rate of false positive (e.g., not truly abnormal) results (Parer 1991).

In large part because of the fear of adverse fetal outcome during the course of labor, electronic fetal heart rate monitoring has achieved near universal acceptance. Nonetheless, two large prospective, randomized trials in high-risk pregnancies at the University of Colorado failed to demonstrate the hoped-for benefits in infant outcomes (Apgar scores, umbilical venous or arterial blood gases, neonatal morbidity or mortality) (Haverkamp et al. 1976, 1979). A later comparative study by this group, in which the infants later were assessed at 9 months of age, showed no significant differences in growth, development, Bayley Scales of Infant and Toddler Development, or Milani Comparetti tests (Langendoerfer et al. 1980). In yet another follow-up at 18 months of age, no significant differences were seen in psychomotor development scores, and the incidence of cerebral palsy was slightly greater (but not significantly) in the electronically monitored group (Shy et al. 1990). Not all authors have agreed, however. In at least two large studies with ultrasound measurement of the fetal risk assessment by the "biophysical profile" (see above; breathing movements, gross body movements, muscular tone, reactive heart rate (acceleration associated with body movements) and amniotic fluid volume; Manning et al. 1980; Platt et al. 1983), considering both short- and long-term indices of fetal condition, with FHR monitoring, the corrected perinatal mortality rate was reported to be decreased significantly (Manning et al. 1985, 1993; Platt et al. 1983). Frank Arthur Manning, at the Albert Einstein College of Medicine in the Bronx, has reviewed the clinical value of the biophysical profile and its scoring in the prediction and prevention of perinatal morbidity and mortality (Manning 1999).

In response to several queries regarding the development of fetal heart rate monitoring, "Ted" Quilligan responded:

> My initial interest was in maternal physiology [of pregnancy]. Charles Hendricks (1917–2010) had recruited me to work with him and Leo Sapirstein (1919–1969) on maternal cardiac output in pregnancy. They felt that cardiac output could be determined on a continuous basis using a formula developed . . . utilizing arterial pulse pressure and heart rate (Remington et al. 1948). We correlated the pulse pressure model with a dye-dilution standard and found the correlation to be quite good in the young healthy pregnant patient (Sapirstein et al. 1954). We looked at the effect of uterine contractions, position of the patient and various anesthetic agents on cardiac output (Hendricks and Quilligan 1956). These studies were done at Ohio State University during the first two years of my residency.
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> We then moved to Cleveland, Case Western Reserve University. At this time my interest turned toward the fetus and its oxygenation. I did some studies on intervillous space blood oxygen content and amniotic fluid oxygen content. The latter was an attempt to correlate amniotic fluid oxygen and fetal arterial oxygen levels (Quilligan et al. 1960). The correlation was poor. While I was doing these studies I attended a meeting at Western Reserve organized by Edward Hon and Hendricks, in which Ed Hon presented his

pioneering studies on recording the fetal heart. I felt these finding could be enhanced by measuring fetal cord blood acid base status which I was doing. Ed was very gracious in helping me get started in fetal heart recording. I visited him several times when he was at Yale and later when he moved to Loma Linda. I did the correlation studies and there was excellent correlation between severe variable decelerations and late decelerations with metabolic acidosis (Quilligan et al. 1965).

Somewhat later in 1965 I went to Yale as Chair of the Department of Obstetrics and Gynecology. Ed Hon had returned to Yale and was responsible for my recruitment. We worked together in New Haven, and then in 1969 we together moved to the University of Southern California. Ed and I and everyone who worked in this area had two objectives in mind, reduce the rate of fetal death in labor and reduce neurologic damage to the fetus during the course of labor. We were optimistic on both counts, because both human and animal studies had excellent correlation with the pathologic patterns and oxygen deficit and acidosis. Perhaps we were too optimistic. The optimism was based on the original description of cerebral palsy by Little (1861/Little 1862). He believed the majority of cases were caused by birth injury. This has been shown to be incorrect in many studies (Pschirrer and Yeomans 2000). The prevailing thought is that about 7 to 10% of cases of cerebral palsy in the term infant are due to hypoxia. In theory, intervening in the labor when a hypoxic pattern was detected should reduce the incidence of cerebral palsy in the term infant. While some studies indicate that the incidence of hypoxic ischemic encephalopathy is lower (Cyr et al. 1984) there is no evidence that cerebral palsy in the term infant has decreased (Clark and Hankins 2003). In addition, the incidence of cesarean section due to "fetal distress" has soared. This has led some to feel that fetal monitoring has had a negative impact on obstetrical care.

The high rate of false positives has been reported from the earliest studies of fetal monitoring. When an abnormal pattern was seen in the last 30 minutes of labor, Hon was able to predict a low Apgar score with only a 50% accuracy (Hon and Petrie 1975). Several recent studies have shown the low predictability of abnormal patterns and a low pH or high base excess (Graham et al. 2014; Larma et al. 2007). The forecasting becomes much worse when attempting to predict cerebral palsy. Does this poor predictability rule out fetal monitoring as tool in obstetric management? (Nelson et al. 1996). In my opinion, the answer is NO. Fetal heart rate monitoring is a screening tool. In the vast majority of cases the tracing will be normal and the fetus normal, a low false negative rate. How can we improve the false positive rate? We need more refined methods to evaluate the fetus during labor, and as a suggestion fetal cardiac output or fetal blood flow in the brain might further define that fetus truly in distress. Pursuing this line of thought, I recently conversed with Yuji Murata who has done pioneering work in fetal monitoring using both the sheep and monkey models. It was his observation using a sheep model and cord compression that fetal brain damage occurred only in those fetuses who had a decrease in blood pressure during the prolonged cord compression. It seems intuitive that when the fetus can no longer compensate for the decreased oxygen available brain damage will follow shortly.

(Letter from EJQ to LDL, 7 November 2014)

24.4 Subsequent Studies on Electronic Fetal Heart Rate Monitoring

In the majority of Geoffrey Dawes' studies of fetal breathing activity, he also had recorded the fetal heart rate. Soon, he came to appreciate the limitations and frailties of visual assessment of the electrocardiographic tracings obtained and, following the introduction of personal computers, commenced to use this new

digital technology to analyze the mass of data collected. In addition, although not widely quoted, it is clear that a critical review of fetal monitoring by Robert Goodlin, then of the University of California, Davis (Goodlin 1979), influenced other workers in the field. Dawes probably was one of these. Thus, beginning in the late 1970s through the 1980s and until the end of his life, Dawes with colleagues worked to measure heart rate variability in a more objective and quantitative manner. To this end, they employed the power of numerical analysis, including fast Fourier power spectral analysis, to study heart rate, its variability, and the implications of changes as an index of well-being. In an early, highly cited study in fetal sheep, the Dawes group demonstrated several rhythms in short-term variability, which increased as the fetus matured beyond 130 dpc and also increased in response to hypoxia, despite the concomitant arrest of breathing movements (Dalton et al. 1977). With neuromuscular blockade by gallamine to abolish breathing movements, the heart rate variability was significantly reduced (Dalton et al. 1977). (It must be noted, however, that gallamine also has a vagolytic action.) In humans, the Dawes group confirmed this increase in FHR variability with gestational age, showing that during the last trimester, it was not the presence of accelerations, nor the absence of decelerations, nor overall variability per se that best reflected fetal state but rather the presence of intermittent episodes of both high and low variability (Dawes et al. 1982a, b). In lambs, Dawes and colleagues demonstrated an attenuated relation of baroreceptor sensitivity and activity (by relation of FHR to arterial blood pressure) to heart rate variability in both fetus and newborn, as compared to adult, and demonstrated that this variability was not associated with changes in ECoG state (Dawes et al. 1980). In a related report, in response to physiologic changes in humans, the beat-to-beat variations were shown to alter in a complex manner, independent of either breathing or body movements (Dawes et al. 1981a).

With the obstetrician-gynecologist Christopher Willard Redman of Oxford, with whom he was to collaborate during the remainder of his career, Dawes worked to develop further reliable methods to assess heart rate and its variability as indices of fetal well-being in the human. In the first of their collaborative studies, they developed an algorithm to quantify record quality (signal-to-noise ratio) to correct for signal error loss, which could be excessive during episodes of high heart period variability. They then used this approach to explore the effect of fetal body movements and breathing on FHR variability and establish the value of these measures as an index of fetal well-being (Dawes et al. 1981b). By simultaneous measurements of FHR variability with use of both direct EFM and ultrasound, during the latter half of gestation in humans, they showed a reasonable correlation of these methods, with a lower percent failure time of Doppler ultrasound after 28 weeks' gestation, compared to the prior 12 weeks. In this study, they also determined normal variations in pulse interval averaged over 4 s epochs, demonstrating that an accurate measure of accelerations and decelerations could be obtained from the ultrasound records (Dawes et al. 1981b). Because it was believed that administration of the opiate-like agonist pethidine (Demerol) to women in labor resulted in loss of FHR variability, Dawes assessed this effect, showing that

although FHR accelerations of >10 beats per min were reduced significantly (46%), the reduction in overall variability was only 20% (Wheble et al. 1988). By the use of a microprocessor at the patient's bedside, Dawes and colleagues attempted to determine the extent to which fetal compromise by asphyxia might be diagnosed on the basis of reduced heart rate variability to warn of impending death. Following a decade of studies, in 1989 a commercial instrument (System 8000) was developed, for use by clinicians to measure accurately FHR and its variation as well as fetal movements (Dawes et al. 1991b; Dawes and Redman 1993). For this innovation, in 1990 Dawes received the British Design Award. Ms. Sue Halson-Brown, who worked for Oxford Sonicaid Ltd. and who collaborated with Dawes and colleagues in developing the System 8000, recounts that she became close friends with Geoffrey and his wife Margaret, often enjoying afternoon tea in their garden. Further she noted that the British Design Award was given "... for technical innovation in an emerging field," and the ceremony was followed by "... a marvelous lunch at the Savoy" (SHB letter of LDL, 13 December 2011).

Dawes' studies demonstrated that heart rate variability was a more reliable measure of fetal deterioration than decelerations per se or the velocity of umbilical artery waveforms determined by ultrasonography (Dawes et al. 1992a, b). These workers also showed variability to be reduced in instances of severe anemia as a consequence of rhesus alloimmunization (Economides et al. 1992). In addition, they presented evidence that, compared to mean epoch-to-epoch variation in the normal near-term fetus of 50 ms and <20 ms in cases of chronic hypoxia, below 3 ms was associated with metabolic acidosis and risk of intrauterine death (Street et al. 1991). Dawes also demonstrated the requirement for 1 ms accuracy of beat-to-beat measurements, if short-term variability, and thus the state of fetal well-being, was to be assessed reliably (Dawes 1993). Dawes and his colleagues also demonstrated a significant increase in short-term FHR variability following dexamethasone administration (Dawes et al. 1994). They also stated that the normal range of basal FHR to equal 120–160 beats per min (Dawes et al. 1996). Dawes with Redmond and others continued to fine-tune this methodology throughout the remainder of their careers (Dalton et al. 1983; Dawes et al. 1981a, b, 1985, 1990a, b, 1991a, b, 1993, 1996; Dawes and Redman 1987, 1992, 1993; Henson et al. 1983, 1984; Lawson et al. 1982, 1983, 1984; Pello et al. 1991; Smith et al. 1987, 1988; Spencer et al. 1987; Visser et al. 1982).

While Dawes appreciated that heart rate analysis was of great value for antepartum assessment of fetal well-being, he had hoped that it also would be of value in intrapartum assessment. Unfortunately, at that time, that was not to be the case. An analysis of the FHR tracings of 394 women in labor at or near term disclosed a wide range of heart rates with diversity of patterns. In addition, epidural anesthesia was identified as a confounding variable that affected FHR patterns (higher heart rate with less variation), despite having no apparent influence on the infant at birth (Pello et al. 1991). In his last published paper, with his son Nicholas, a D.Phil. physicist-computer scientist, Dawes reported significantly greater heart rates in female than male fetuses during labor (but not prior to the onset of labor), a difference exaggerated by epidural anesthesia. In view of this finding, he stressed

the requirement to consider the multiple variables in this regard (Dawes et al. 1999). "Mont" Liggins has given some insights into the controversy this approach initially engendered, especially the requirement to measure with great accuracy the fetal heart beat-to-beat interval (Liggins 1998, pp. 117–118). Others have expressed their skepticism as to the fundamental value of these particular studies by the Nuffield group, stating that this latter decade and more of Dawes' life occupied by this work were "wasted years" (Several Personal Communications to LDL).

24.5 Some Contemporary Developments

As is widely appreciated, many hold that the presence of asphyxia is a major cause of intrauterine death or hypoxic ischemic encephalopathy with impaired neurologic and cognitive development in survivors (Low 2004; MacDonald 1996). In view of this belief, a number of investigators have sought to discover the Holy Grail of a diagnostic tool to diagnose fetal asphyxia accurately. The variation of the fetal heart rate appeared to offer a possibility in this regard, as FHR variability was believed by many to be of value in determining the state of fetal well-being and impending asphyxia. In the years during and following Dawes' studies, a number of individuals have contributed to this field.

Perhaps surprisingly, it was not until the mid- to late 1970s that randomized controlled trials of electronic FHR monitoring first appeared. These failed to demonstrate dramatic benefits (Thacker et al. 2006). In 1996, a Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology reviewed the value of heart rate variability in healthy adults as well as those with various cardiovascular and non-cardiovascular disorders. The analysis included aspects of heart rate variability and the relation to function of the autonomic nervous system, including sympathetic and parasympathetic outflow, physiologic phenomena such as respiratory activity, and other functions. This report concluded that considerable caution was required in the interpretation of variables such as heart rate variability, the electrocardiographic R–R interval, and other functions (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). Nonetheless, in the obstetrical literature, mounting evidence suggested a strong correlation between prolonged hypoxia and asphyxia and loss of baseline variability, bradycardia, or other abnormal FHR patterns, with subsequent neurologic damage (Parer 1988, 2003; Siira et al. 2005), and further the association of non-reassuring FHR patterns and intrapartum stillbirth (Parer 1979). Thus, because of the non-specificity of FHR decelerations per se, intra- and interobserver variations, and other methodologic variables, the recognition of FHR variability with non-reassuring patterns came to be of critical importance. Stressed by many was the absence of objective, observer-independent measures of the association of FHR variability to asphyxia. For instance, a NICHD research planning workshop included participation of a dozen and a half of the leaders of the field. They could reach no consensus of agreement on

guidelines of management, other than to stress that a "normal" FHR tracing showed normal baseline rate and moderate variability, with the presence of accelerations and absence of decelerations (Parer and Quilligan 1997). More recent reviews have endorsed this assessment (Malcus 2004; Parer 2003). In 2001, the Royal College of Obstetricians and Gynecologists published ". . . Evidenced-based . . . guidelines" on the use of electronic FHR monitoring that included a review of the literature on the topic, and recommended fetal blood sampling in instances of two or more abnormalities in heart rate (Royal College of Obstetricians and Gynecologists 2001). As an aside, although fetal pulse oximetry and its correlation with both electronic FHR and intrauterine pressure measurements have gained some popularity in Europe, this has not been the case for the USA; and an NICHD-supported clinical trial showed that this failed to reduce cesarean section rates (Bloom et al. 2006).

In a 2003 survey of electronic fetal heart rate monitoring, Julian Thomas "Bill" Parer of the University of California, San Francisco, concluded:

> . . . to justify our continued use of EFM, we need to clean up our house. We must come to some agreement on a national level about interpretation and management. We must teach EFM more appropriately. We must carry out surveys to determine accurately the relationship between FHR patterns and newborn academia. We must define the realities of logistics involved in emergent intervention so that hospitals with less comprehensive facilities can continue to serve their local communities without fear of legal vulnerability. Only then can this potentially valuable screening test for newborn acidemia be appropriately evaluated, and then either be discarded if results are negative for efficiency or accepted with an aura of scientific decency if results are positive.
>
> (Parer 2003, pp. 562–563)

In further consideration of the guidelines promoted by several groups, Parer observed that ". . . we have found them to be of limited use" (Parer and Ikeda 2007). In 2008, in partnership with the American College of Obstetricians and Gynecologists (ACOG) and the Society for Maternal-Fetal Medicine, the NICHD convened another workshop to reassess and update the 1997 definitions and guidelines and to standardize the terminology and interpretations employed by various workers. The participants adapted a three-tier category classification: viz., I, normal tracing with moderate baseline variability; II, intermediate, nonclassical; and III, abnormal tracing with bradycardia, absent variability, and sinusoidal pattern. They also reaffirmed that a tracing is a poor predictor of those infants that will develop long-term neurological sequelae (Macones et al. 2008). The following year, a *Practice Bulletin* of ACOG reassessed the definitions of EFM pattern categorization and reviewed classification systems (ACOG 2009). Although recommending electronic monitoring in instances of high-risk pregnancy and continued use of the three-tiered system of categorization, the committee acknowledged a number of deficiencies including problematic tracings without additional data and poor inter- and intraobserver reliability (ACOG 2009).

Because the intermediate (tier II) category consists of a heterogeneous array of patterns with variations in baseline fetal heart rate, variability, and decelerations, an interdisciplinary group from Canada and the USA has developed specialized software to analyze and categorize EFM traces, with a five-tier color-coded classification to aid in the interpretation of tracings and to be predictive of adverse

outcomes. They present a high correlation of their predictability with outcome (Elliott et al. 2010; Parer and King 2010). In a test of the correlation of interpretation of abnormal FHR tracings by use of the five-tier color-coded framework with that of PeriCALM computer analysis, five expert perinatologists achieved remarkable agreement both on the FHR tracing itself (89% within one tier) and with that of the computer (Parer and Hamilton 2010). A meta-analysis of 12 randomized, controlled, clinical trials suggested the chief significant benefit of FHR monitoring to be a reduction of neonatal seizures (Thacker et al. 1995), and several reports reaffirm the validity of FHR variability as an appropriate index of asphyxia (Frasch et al. 2009) and lowered infant mortality (Chen et al. 2011). Nonetheless, some skepticism has continued regarding evidence for the effectiveness of routine EFM, its role in minimizing the occurrence of hypoxic ischemic encephalopathy, and concerns as to its costs, as opposed to its benefits (Banta and Thacker 1979a, b, 2001; Goodlin 1977; Shy et al. 1990; Thacker et al. 1995). A contemporary report suggests that internal tocodynamometry during either induced or oxytocin-augmented labor demonstrates no advantage over that of external monitoring in FHR pattern recognition or neonatal outcome (Bakker et al. 2010).

A retrospective cohort study of 2004 singleton births and infant mortality in the USA (1,732,211 live births in the study population, 42% of the total), among which 89% were monitored electronically, reported significant reductions in low Apgar scores, neonatal mortality, and incidence of neonatal seizures. In turn, the rates for operative deliveries increased (Chen et al. 2011). As noted by others, problems remain in the interpretation of FHR patterns and the assessment of fetal well-being. These include the use of Apgar scores and/or neonatal seizures as surrogate markers for hypoxic-ischemic-induced neurologic injury and other caveats as noted above (Devoe 2011b).

Based on observation that a biphasic or depressed electrocardiographic ST segment usually was associated with fetal hypoxia, during the 1980s, structured ST waveform analysis (STAN) was developed. This methodology included improved signal processing, computerized calculations of several electrocardiographic variables (T:QRS ratio, ST-segment depression), and clinical guidelines for applying this information for patient management (Rosén and Lindecrantz 1989). From the Universities of Lund and Gothenburg, Sweden (Amer-Wåhlin et al. 2001), a 2001 randomized trial of 4966 near-term women in labor reported that STAN combined with cardiotocography resulted in significantly lower rates of umbilical arterial acidosis and operative delivery for fetal distress, as compared to cardiotocography alone (Amer-Wåhlin et al. 2002). On the basis of another multicenter trial, the sensitivity and specificity of this analysis for fetal hypoxia and academia were soon established in Scandinavia (Arulkumaran et al. 1990). In the USA, one non-randomized cohort study established the agreement for clinical management between clinician-users and STAN experts (Devoe et al. 2006). With the use of this technique, a decision tree model has predicted a significant reduction in the occurrence of cerebral palsy with increase quality of life and lifetime cost savings (Heintz et al. 2008). In 2005, the US Food and Drug Administration's Obstetrics and Gynecology Advisory Panel voted to approve the STAN®

fetal heart rate monitor to assist clinicians to identify infants at risk (http://www.obgyn.net/print.asp). A more recent Swedish prospective clinical study on the use of STAN, conducted over 7 years in almost 13,000 term pregnancies, demonstrated a major reduction in fetal metabolic acidosis and increase in neonatal outcome, with a stable rate of vaginal delivery (Norén and Carlsson 2010). In contrast, an international meta-analysis of four randomized trials concluded that fetal ECG plus ST segment analysis did not reduce the risk of metabolic acidosis, although it did lessen the need for instrumental vaginal delivery (Schuit et al. 2013).

Lawrence Daniel Devoe of the Medical College of Georgia has placed many of these relatively recent developments in perspective (Devoe 2011a). This review notes that, while STAN has been used successfully in Europe and has largely achieved some of the goals initially promised by EFM to reduce perinatal morbidity and unneeded operative interventions, similar outcomes have yet to be demonstrated in the USA. To this end, the NICHD charged the Maternal-Fetal Medicine Unit network to undertake a prospective randomized controlled trial of STAN. This study, which included a number of network centers, aimed to determine whether US clinicians, trained in the use of the STAN system, could achieve clinical outcomes comparable to those of the original Swedish randomized trial. This trial, which included over 11,000 patients with a singleton pregnancy attempting vaginal delivery at >36 weeks' gestation, concluded that "fetal ECG ST-segment analysis ... did not improve perinatal outcomes or decrease operative-delivery rates" (Belfort et al. 2015).

In terms of technologic innovations, a new monitoring system, the Monica AN 24, has been developed that utilizes advanced signal processing to derive fetal heart rate signals from fetal ECG and maternal uterine activity from obtained via transabdominal transducers for prolonged periods of time (Graatsma et al. 2009; Jacod et al. 2010). This system, currently being marketed in the USA, in addition to using wireless transmission, is purported to have improved signal capture in obese patients. Large prospective clinical trials are still awaited to determine whether the Monica AN 24 will result in improved outcomes with decreased morbidity and mortality when compared to standard external monitors. Finally, through the use of smartphones or tablets, applications are being developed to bring EFM recordings with their corresponding patient data to the obstetrician, regardless of location. The purported advantage of data immediacy is intriguing and eliminates the problem of bedside staffs communicating a visual pattern verbally to a distant provider. As yet, we await the results of trials demonstrating such improved patient care and outcome.

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Chapter 25
Dawes' Contributions to Symposia and a Summing Up

> *The poetry of history lies in the quasi-miraculous fact that once, on this earth, once, on this familiar spot of ground, walked other men and women, as actual as we are to-day, thinking their own thoughts, swayed by their own passions, but now all gone, one generation vanishing after another, gone as utterly as we ourselves shall shortly be gone like ghost at cock-crow. This is the most familiar and certain fact about life, but it is also the most poetical…*
>
> (Trevelyan 1949, p. 13)

In addition to his scientific contributions per se, and helping to inspire the small army of leaders to be that came of age under his tutelage, Geoffrey Dawes had a special and amazing gift for synthesizing information and ideas. Many of his overviews of various issues were given as invited lectures, keynote addresses, and introductions to various proceedings and conferences. For instance, in what was to be the first of several symposia devoted to the "baffling problem" of sudden infant death syndrome (SIDS), held in 1963 in Seattle, WA (Wedgwood and Benditt 1965), with his background in physiology and pharmacology, Dawes brought valuable insights to the discussion. In his presentation on cardiovascular pulmonary reflexes, he emphasized the relation of his studies with asphyxia in newborn and young lambs to the enigma of SIDS. Additionally, in terms of casual mechanisms, he stressed the necessity to consider alternative hypotheses (Dawes 1965). A decade later at a Bethesda, MD, symposium devoted to this topic (Bosma and Showacre 1975), Dawes participated in the discussion of almost half of the formal presentations. In concluding comments, he challenged the participants on their many assumptions and the supposed "facts" presented; "I would be careful about this evidence," he cautioned (Dawes 1975, p. 266). Importantly, Dawes formulated a number of ideas relating to SIDS and outlined research questions that required exploration (Dawes 1975). Many of these remain unanswered to the present day (Kinney and Thach 2009). Along this line the hippocampus is a key component of the forebrain-limbic network that modulates respiratory regulation via interactions with brainstem neuronal centers. A provocative study has reported that a marker of hippocampus dentate gyrus abnormality, food granule cell

L.D. Longo, *The Rise of Fetal and Neonatal Physiology*, Perspectives in Physiology,
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bilamination, was present in 41% (47 of 114) of SIDS newborns whereas this abnormality was apparent in only 8% (3 of 39) controls (Kinney et al. 2015). This may serve as a morphologic marker for underlying brain vulnerability.

25.1 Ciba Foundation Symposia

A particular contribution to fetal and neonatal physiology in which Dawes played a key role was the series of symposia sponsored by the Ciba Foundation. Table 25.1 lists these symposia, in which Dawes participated or organized. Following World War II, in 1949 Ciba Ltd. (currently Novartis) of Basel, Switzerland, established a foundation in London to promote international cooperation in medical and chemical research. Its first (and for three decades) director was Sir Gordon Wolstenholme (1913–2004). These symposia, each held on a topic of general interest to medicine and biomedical science, were held at its headquarters, Ciba House, 41 Portland Place, London. Recognizing the barriers that exist between biomedical scientists

Table 25.1 Ciba foundation and other symposia on fetal-neonatal development

Year/title	Editors	Chairman	Participants	Presentations	Pages
1961 *Somatic Stability in the Newly Born*	Wolstenholme and O'Connor	Robert Alexander McCance (1898–1993)	30	20	393
1967 *Development of the Lung*	De Reuck and Porter	P. Hugh-Jones	25	20	408
1969 *Foetal Autonomy*	Wolstenholme and O'Connor	Geoffrey Sharman Dawes	25	16	326
1974 *Size at Birth*	Elliott and Knight	Geoffrey Sharman Dawes	27	15	408
1977 *The Fetus and Birth*	Knight and O'Connor	Geoffrey Sharman Dawes	26	18	481
1981 *The Fetus and Independent Life*	Elliott and Whelan	Geoffrey Sharman Dawes	25	15	372
1985 *The Roots of Perinatal Medicine*	Rooth and Saugstad	Rooth and Saugstad	15	17	132
1990 *Fetal Autonomy and Adaptation*	Dawes, Borruto, Zacutti and Zacutti Jr.	Geoffrey Sharman Dawes	17	14	186

and healthcare specialists, the goal of these was to be "... not a laboratory for mixing compounds, but a laboratory for mixing scientists" (Wright 2004). The Foundation's motto *Consocient gentes* [let the people come together] exemplified this objective. Sir George White Pickering (1904–1980) has detailed several aspects of the Ciba Foundation series of symposia. He noted that perhaps the most important contribution of these conferences has been the discussions that followed each presentation, "... which often begins lifelong friendships between scientists who have met for the first time" (Pickering 1979, p. 98). The first of a half-dozen Ciba symposia devoted to the developing organism considered the topic *Somatic Stability in the Newly Born*. Thirty scientists from various disciplines gathered under the chairmanship of Robert A. McCance, to consider the lung and its development, numerous aspects of fetal and newborn metabolism, thermogenesis, and the issue of homeostasis. Dawes presented his most recent work on oxygen consumption and hypoxia in the newborn lamb. In the concluding papers, Clement Smith of Harvard Medical School and Sir John Hammond (1889–1964) of Cambridge University considered the human clinical and veterinary implications, respectively, of these topics (Wolstenholme and O'Connor 1961). Several years later in 1967, under the chairmanship of Philip Hugh-Jones (1917–2010) of King's College Hospital Medical School, the Ciba Foundation Symposium was devoted to *Development of the Lung* (De Reuck and Porter 1967). At this conference, consideration was given to the phylogeny and ontogeny of lung development, the physiology and biophysics of the pulmonary gas/liquid interface, respiratory gas exchange in the fetus, and initiation of breathing in the newborn. Again, Dawes reviewed his work and that of others on oxygen consumption of the developing fetus and placenta and contributed to the discussion of essentially every presentation, asking probing questions and challenging assumptions (Dawes 1967).

Several of these symposia followed the discovery in sheep that the fetal adrenal glands play a key role in determining the onset of parturition. No doubt because of the vigorous role he played in contributing to previous symposia, Dawes was asked to organize and chair the symposium *Foetal Autonomy* (Wolstenholme and O'Connor 1969). Again, two dozen investigators from around the world considered issues of implantation and the maternal recognition of pregnancy, the fetus as an allograph, fetal endocrine autonomy, fetal metabolism, circulation, and neuromuscular function, the role of the fetus in the initiation of parturition, and so forth. In his introduction to the gathering, Dawes stressed the importance of fetal homeostasis, e.g., that despite the embryo/fetus passing through a number of critical transitions, "...at implantation, during organogenesis, at the time of imprinting the sexual character on the nervous system, at birth, and after birth during the establishment of behavioral patterns—periods which pass never to return. Failure to make the transition at the right time is crippling or lethal." Further, he called attention to the paradox that, despite their exploring several aspects of fetal biology, to a great extent they were separated by both discipline and geographical distance, and they published in the journals of different learned societies, probably not read by one another. He noted, "There ... remains a large, almost unexplored territory between the embryologist and the foetal physiologist. The gap between the experimental

immunologist and the obstetrician or pediatrician is only slightly less." Dawes stressed the joint goal, to explore important gaps in knowledge and to bridge connections between the many different branches of the subject (Dawes 1969b, p. 1). In his main address at this symposium, Dawes reviewed the topic "fetal blood gas homeostasis," emphasizing its stability being dependent upon proper circulatory regulation. Drawing upon his background in pharmacology, he reviewed his and others' work on the role of the systemic arterial chemoreceptors in this regulation. He called attention to the fact that, beginning at 0.6–0.7 gestation (85–100 dpc) onward, fetal sheep show evidence of central nervous system regulation of the circulation with this playing a key role in its blood gas homeostasis. Additionally, he noted that although section of the carotid nerves (from the carotid body and sinus) had little effect on fetal responses to hypoxia, section of the cervical vagus or aortic nerves resulted in vasodilatation with blood pressure decrease and abolition of the hypoxia-induced rise in blood pressure (Dawes 1969b). At the conclusion of this symposium, Dawes reminded his colleagues of the apparent paradox of the developing fetus that, despite its immaturity, many aspects of its cellular and organ system functions were quite adequate and appropriate, raising the question, by what criteria should one define "... immaturity in different organ systems?" He continued with the challenge to gain an understanding of details of systems the group had not addressed, such as immunological competence, mineral metabolism, and enzyme development. Dawes concluded, "... the present solution to the problem of mammalian viviparity is not merely compatible with but is directly dependent on a high degree of foetal autonomy" (Dawes 1969a, pp. 315–316).

For the 1974 Ciba Foundation Symposium, *Size at Birth*, again over two dozen investigators considered issues such as: What factors determine the size of a newborn mammal? What consequences follow being born either abnormally small or much too large? How does one diagnose accurately the genesis of variations in size? What are the possibilities of correcting abnormal growth rates? To what extent can postnatal compensatory measures prevent deficits in ultimate intellectual and physical development in the severely intrauterine growth retarded infant? In his introduction to this volume, as he had at earlier symposia, Dawes emphasized the critical role antenatal influences play in development and life of the newborn. Asking, "what are our long-term objectives," Dawes stressed not only the ability to diagnose variations and abnormalities in the size of the fetus but the biologic basis for it being either "... too large or too small, ... and to discover whether [such aberrations could] be prevented, and whether [they] can be cured..." (Dawes 1974, p. 1). Again, in the discussions that followed each of the over a dozen presentations, Dawes stimulated the dialogue and posed challenging questions (Elliott and Knight 1974).

Several years later, a Ciba Foundation Symposium again considered issues regarding the role of the fetus in the timing of its birth, *The Fetus and Birth* (1977). The two dozen scientists from far and wide explored potential mechanisms by which the fetus determined when it would be born. These included the roles of the fetal hypothalamic-pituitary-adrenal axis, estrogens and/or progesterone

withdrawal, prostaglandins, and the extent to which these differ among the several species. As conference chair, in his introduction, again Dawes posed questions to be discussed with particular reference to the discoveries during the previous decade. Importantly, he emphasized the critical necessity to attempt to resolve issues of contention and to probe more deeply into the cellular and biochemical pathways of the mechanisms involved. For example, he challenged the concept that the fetal control of the timing of parturition is explored to best advantage in ruminants as the sheep or goat. He also raised a number of questions regarding the role of fetal adrenal steroids, that of prostaglandins, and the uterine and cervical responses to hormonal stimulation (Dawes 1977).

The last of the Ciba Foundation Symposium which Dawes would chair was that of 1981 on *The Fetus and Independent Life*. Again, the two dozen scientists, including many who participated in prior symposia, dissected the maturation of various organ systems of the fetus and the newborn infant. Topics included development of the hypothalamic-pituitary-adrenal axis, the placenta and its hormone production, fetal growth factors, metabolism, central nervous system transmitter systems, and development of the lung. In addition to considering the central regulation of fetal breathing movements, in his introduction, Dawes reviewed a number of questions regarding the ontogeny of various fetal systems, basic issues of fetal growth, and the possible role of circadian rhythms. He challenged the participants:

> This symposium is designed to follow a somewhat different path from those previously organized by the Ciba Foundation that were directly related to the basic sciences of perinatal medicine. . .. The range of disciplines of the participants is unusually wide. The purpose is not just to rehearse recent advances in specialist aspects of the development of the mammalian fetus towards independence, but to identify and discuss large areas of research in relation to the control of integrated physiological functions. Can we begin to see a coherent pattern in the stages of fetal development? Is that pattern determined by the selection of broad physiological mechanisms which serve an essential purpose in survival to birth and independently thereafter? Or is it an accident of phylogeny?
>
> (Dawes 1981, p. 3)

25.2 The Barcroft Centenary Symposium

In 1972, on the one hundredth anniversary of Sir Joseph Barcroft's birth, Cambridge University hosted a symposium, *Foetal and Neonatal Physiology*. Held at the Physiological Laboratory, this was the first such large international gathering since the Cold Spring Harbor Symposium held two decades earlier (Demerec 1954). The *Proceedings*, edited by Kenneth W. Cross, included contributions from over 100 developmental physiologists and other scientists (Cross 1973).

In commenting later upon the Barcroft Symposium, Dawes attributed the considerable increase in interest in the field to several factors. These included the fact that the physiology of the fetus and newborn, "... was one of the few branches of systems physiology in which major advances were being made" (Dawes 1984,

p. 259). As examples, he noted the discovery of pulmonary surfactant and the rapid recognition of its clinical significance in respiratory distress of the prematurely born infant (see above). Dawes also noted the important recognition of "critical periods" as being essential to normal development, citing the work of Hubel and Wiesel (noted above). A second factor Dawes acknowledged for popularization of the study of fetal and neonatal physiology was that of technological advances such as the chronic catheterization of the fetus (Meschia et al. 1965), which allowed measurements of respiratory blood gases and pH and various hormones and other biochemicals under physiologic, steady-state conditions. In conjunction with these advances were those methods employed to produce fetal growth retardation experimentally in a number of species (see Elliott and Knight 1974). Dawes advanced a third critical factor, the appreciation by clinicians that knowledge of basic physiologic principles often was required to affect appropriate interventional and/or therapeutic measures of clinical problems in obstetrics and neonatal pediatrics. In addition to respiratory distress syndrome in the premature newborn infant, examples included problems such as maintaining newborn body temperature and maintaining the concentrations of blood glucose and other metabolites. Dawes also emphasized the urgent need to gain an understanding of the genesis of congenital malformations (Dawes 1984).

A decade later, in a satellite symposium *Fetal Physiology and Behavior*, held in Melbourne, Australia, in conjunction with the 1983 International Union of Physiological Sciences meeting, Dawes addressed the "changing direction" of fetal physiology during the previous three decades. He credited the increased interest in this field to the main factors to which he had referred previously: this was one of the few branches of physiology in which major advances were being made at the systems level, important technical innovations allowed studies of many systems in the unanaesthetized "physiologic" preparation, and clinical problems in perinatology and neonatology demanded exploration of fundamental mechanisms and contributions to understanding. He concluded, detailing several differences between laboratory experimental animals and humans, stressing the challenge to understand these differences at a deeper level (Dawes 1984). A decade later, Dawes re-reviewed these issues (Dawes 1994).

25.3 The "Dawes Symposium" and Others

In 1984, to honor Dawes for his four decades of inspiring young investigators to excellence and other contributions to life, and prior to closure of the Nuffield Institute, Colin T. Jones, of the Nuffield Institute, and Peter Nathanielsz, of Cornell University, Ithaca, NY, organized a conference, *The Physiological Development of the Fetus and Newborn*, held at St. Catherine's College, Oxford (Jones and Nathanielsz 1985). As a testimony to Dawes and his influence on the field, over 300 investigators many of whom had worked at the Nuffield Institute gathered for 6 days to share their discoveries and give "... tribute to the influence of Geoffrey

Dawes and an inspiration to convince present and future generations of the challenge that studies on development provides" (Jones and Nathanielsz 1985, p. xix). In his closing essay, "Perinatal Physiology, the Past, Present, and Future," Dawes reviewed a number of contributions to understanding the complexity of development. In particular, he stressed the unsteady state in which the fetus develops, being perturbed by transient changes in behavioral state, ECoG activity, and hormonal release (Dawes 1985).

In remembrance of this meeting, David Mark Olson, of the University of Alberta, has written:

> . . . as a young scientist in 1984, I traveled to St. Catherine's College, Oxford to attend my first international fetal physiology symposium. The meeting was timed to coincide with the retirement of Professor Dawes from his directorship of the Nuffield Institute. I was amazed as I looked around at all the well-known fetal physiologists from the United Kingdom, United States, Australia, New Zealand, The Netherlands, Canada, and elsewhere who were in attendance. Why, I asked, were all these people here? Simple, I was told. Most of these individuals either trained or spent a sabbatical with Dawes at the Nuffield. Indeed, virtually an entire generation of fetal physiologists at one time or another performed research in Oxford, many with Dawes. And from these scientists a second generation of fetal physiologists were being trained or established in their careers. Many were still investigating important questions explored by Dawes. Others had moved into new fields of inquiry. All carried the legacy of Dawes.
>
> (Olson 1997, p. 1382)

Also in conjunction with this meeting, Arne Jensen of the University of Bochum, and at that time a fellow at the Nuffield Institute, recalls:

> At the end of my stay I was honoured to witness one of GSD great moments: At the occasion of the Meeting on 'The Physiological Development of Fetus and Newborn', held in . . . his honor . . .. Finally, GSD was in a position to prove to the scientific community that his theory concerning the governance of the fetal breathing reflex was correct. How important this moment of personal triumph over the reviewers was for GSD, I became to sense in our uncountable daily rehearsals—since the onus was on me to present his precious data on the meeting. He did not want to leave anything to chance. His caution climaxed when he pre-determined a question to be asked in the discussion after my talk and even the person who should ask it. The smile on GSD's face could not have been brighter when things turned out as hoped for. Several months later he stepped down from his position as Director and the Institute was closed.
>
> (Letter from AJ to LDL, 15 April 2009)

As noted earlier, in his introduction to the symposium to honor G.C. Liggins (Gluckman et al. 1989), Dawes reviewed "Mont" Liggins' many contributions to science and to life, including studies on the role of the fetal hypothalamic-pituitary-adrenal axis in the timing of parturition, the role of glucocorticoids in development of the lung, the value of antenatal glucocorticoid therapy in lung maturation in women with impending premature labor, and others (Dawes 1989)

In what would be his last of such endeavors, the ever peripatetic Dawes also helped to organize and moderate the 1989 symposium *Fetal Autonomy and Adaptation* held at the lovely *Villa Marigola* in San Terenzo di Lerici, Italy (Dawes et al. 1990). Promoted as a two-decade follow-up to the 1969 Ciba Foundation Symposium *Foetal Autonomy*, the participants reconsidered topics discussed at that

gathering such as the fetal cardiovascular function, the role of the fetus in the initiation of parturition, fetal immunology, and its hormonal milieu. In his introduction, Dawes stressed the continually changing fetal physiology during developmental maturation and its transformational environment. As was typical, following each presentation, he raised provocative issues to consider in regard to the autonomy of the fetus (Dawes et al. 1990).

With the goal of having his research be ever relevant to clinical problems, his gift for synthesis of ideas, and clarity of presentation, it is not unexpected that Dawes could be a popular and highly regarded keynote speaker at various clinical conferences and society meetings. In addition to those already mentioned, such presentations included the Donald Paterson lecture delivered at the University of British Columbia, Vancouver, BC, Canada (Dawes 1973); "The Roots of Today's Perinatal Medicine" Symposium held in 1984 in Zurich, Switzerland; the James A.F. Stevenson Memorial Lecture at the University of Western Ontario, London, Ontario, Canada (Dawes 1988); his 1995 special lecture to "The Society of Foetal Physiology" (forerunner of "The Fetal and Neonatal Physiological Society) in Malmö, Sweden (see below); and others.

25.4 A Summing Up by Dawes

In a thoughtful retrospective, Dawes recalled his early years in developmental physiology, of having to bend his own glassware to cannulate blood vessels, building "... his own amplifiers, stimulators and oscilloscope time bases, mainly with war surplus items ... home built camera...," and his purchasing the "... first programmable electronic calculator ... in 1967, the first microcomputer in 1972." In addition he emphasized that until 1970, funding from non-Nuffield Institute sources was "trivial."

Regarding the field of fetal physiology, Dawes observed:

> When I first started working in this field I hoped that physiological mechanisms would prove to be simpler in the fetus than in the adult. We would be free of the complexities of postnatal life, without the need for temperature control, independent breathing, feeding, exercise or weight-bearing, with limited visual, auditory or tactile stimuli. I hoped that with one deft movement it might be possible to split open the oyster and find the single pearl of truth at its centre. We've all found that oyster to be an onion. As you carefully peel away one layer of truth you find another one beneath. The complexities are built in at the beginning, to preserve each and every single cell; upon this is superimposed the integration of physiological functions. With its recognition as a complex discipline, fetal physiology has yet more to offer.
>
> (Dawes 1984, p. 262)

He continued:

> So what, in this competitive physiological world, has perinatal physiology got that those other organ or tissue oriented fields have not? I think its weakness and much of its strength lies in the degree to which it is still oriented to the integration of function, in whole animals and man. As an international group we have specialized in the relationship of cellular

> physiology to the whole animal, rather than concentrating on the cell itself. Of course there are fashions in our subject. In my younger days we looked, with some cynicism, at the attempts to describe vascular changes solely in terms of adenosine or of histamine, Kallikrein, substance P (which has survived), bradykinin, serotonin or angiotensin. To these we may now add the prostaglandins, the intestinal hormones, opiates and the other neurotransmitters acting directly or indirectly. It is a nice problem to pick out which to study in relation to our favorite pastime, the subject area we chose or had thrust on us by a supervisor, in which we have made a big investment and from which it may not be easy to escape. It is the interplay of relationships, the insight which comes from looking at the results in related fields (even those concerned with monoclonal antibodies) which makes the subject so fascinating. Its weakness is in the degree to which analysis of cellular mechanisms must be foregone in the pursuit of its strength, the integration of functions.
>
> (Dawes 1984, pp. 262–263)

Dawes then continued, pointing to several specific areas of cell biology that demanded attention. His examples included the function of placental and fetal tissue cell membranes, the mechanisms of chemo- or baroreceptor signal transduction, vascular smooth muscle signaling mechanisms, and the neurobiology of the developing brain (Dawes 1984). In conclusion, Dawes observed that "the behaviour of the fetus, like that of the adult, not unexpectedly differs in sheep and man. But a deeper understanding of the one helps that of the other, in both directions. It is a topsy-turvy situation ..." (Dawes 1984, p. 263). Later, he wrote the ". . . more that is learned about the general features of fetal physiology, the problems have become more complex, not simpler." In a concluding soliloquy, he stressed the importance of discovering knowledge of the physiology of the fetus, to achieve an understanding of clinical problems such as premature birth, cerebral palsy, and other conditions. Commenting on the link between perinatal physiologists and clinicians, Dawes noted, "Though their primary interests may differ, there is a broad area of common knowledge and concern that deserves fostering" (Dawes 1994, p. 5).

In 1985, the Nuffield Institute was commencing its sixth decade of existence as a bastion of activity and a beating heart of academic life. Rather than celebrations, however, because of limited funding, Oxford University officials elected to shutter the institute and abolish the position of director (Anonymous 1985). (As an aside, despite a number of inquiries, I have been unable to unearth details regarding this decision.) Thus, at age 67, and 37 years after being appointed director, Dawes was forced to retire from the Nuffield Institute. Its research program then became focused on molecular biology. His restless mind not allowing the ease of a sedentary life, Dawes became Director of Medical Research at the Charing Cross Sunley Research Centre at Charing Cross Hospital, London (subsequently, the Sunley Research Centre was incorporated into "The Mathilda and Terence Kennedy Institute of Rheumatology Trust" (http://www.kirtrust.org). In his position at Charing Cross, Dawes continued his work on computerized analysis of the fetal heart rate and breathing movements. He strove continuously to foment "A Revolution at the Bedside".

Dawes' bibliography contains 238 published scientific papers, (abstracts not included). According to the ISI Web of Knowledge, 259 published items (including some abstracts) in their database have been cited 11,532 times (November 2012).

Table 25.2 Dawes' awards and honors

Year	Award
1963	Max Weinstein Award, USA
1966	Gairdner Foundation Award, Canada
1969	James Spence Medal, British Paediatric Association Fellow, Royal College of Obstetricians and Gynecologists
1971	Fellow, Royal Society, London
1972	Fellow, Royal College of Physicians
1974	Fellow, American College of Obstetricians and Gynecologists
1976	Fellow, American Academy of Pediatrics
1981	Commander of the British Empire Blair Bell Gold Medal, Royal Society of Medicine
1982	Honorary DMed University of Gothenburg, Sweden
1990	Osler Memorial Medal, Oxford University British Design Award

As evidenced by his bibliography, Dawes collaborated with at least 143 colleagues/coauthors. As detailed in Chap. 26, Tables 26.1, 26.2, 26.3, 26.4 and 26.5 list the graduate students, postdoctoral fellows, and others with whom Dawes collaborated with by decade. Table 26.6 lists those individuals with whom he most frequently coauthored papers, with Christopher W. Redman and Joan C. Mott heading that list with over 30 publications apiece. Seventy-three of Dawes' publications were single authored.

Recipient of numerous awards and honors, among others, Dawes received the following: James Spence Medal, British Paediatric Association (1969); fellow, Royal College of Obstetricians and Gynaecologists (1969); fellow, Royal Society of London (1971); fellow, Royal College of Physicians (1972); Commander of the Order of the British Empire (1981, the motto of which is "For God and the Empire"); Blair Bell Gold Medal, Royal Society of Medicine (1981); Osler Memorial Medal, Oxford University (1990); and British Design Award (1990) (see Table 25.2)

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Chapter 26
Dawes as a Mentor: Reminisces of Former Graduate Students, Postdoctoral Fellows, and Associates

> *The greater the man, the more he is soaked in the atmosphere of his time; only thus can he get a wide enough grasp of it to be able to change substantially the pattern of knowledge and action.*
>
> (Bernal 1954, pp. 57)

For many of us, academic medicine is one of the highest callings that one can pursue in life. To chair, develop, and direct a major institute at a great university shares that unique privilege. Among academic leaders in developmental physiology and the reproductive sciences, few have surpassed Geoffrey S. Dawes, one of the outstanding leaders in the field. Many terms could be used to describe Dawes: celebrated administrator, doyen of physician-scientists, illustrious, innovative, and creative investigator, distinguished world authority in the reproductive sciences, author of a foremost volume on developmental physiology, outstanding role model, and friend.

A term that, I believe, encapsulates his career is mentor. As you recall from *The Odyssey* by Homer, when he left Ithaca for the Trojan War, Odysseus chose as a guide for his son Telemachus his friend *Mentor*. Like *Mentor* of Greek mythology, Dawes dedicated his career to the development of young reproductive endocrinologists and investigators both clinicians and basic scientists. Without doubt, to a great extent it was through the commitment, dedication, and perseverance of Geoffrey Dawes that the field of developmental physiology came into its own. In helping to educate and transform the lives and careers of a generation of young scientists, his leadership helped to mold the field into one of the most academically exciting.

As noted by John Challis in his Foreword, Geoffrey Dawes, the demiurge behind the creative contributions at the Nuffield Institute, served as a wise and trusted teacher and inspiration to a large number of protégés, graduate students, postdoctoral fellows, and other colleagues. Many of these went on to become highly productive, scientists and leaders in their own right. For over four decades, life at the Nuffield Institute embodied a halcyon era. Tables 26.1, 26.2, 26.3, 26.4, and 26.5 list these individuals by decade, with the number of publications coauthored

L.D. Longo, *The Rise of Fetal and Neonatal Physiology*, Perspectives in Physiology,
https://doi.org/10.1007/978-1-4939-7483-2_26

Table 26.1 Mentor for graduate students, postdoctoral fellows, and other collaborators—1950s

Investigator	Publications
G.H. Acheson	1
E.C. Amoroso	1
Gordon M. Ardran	1
Gwenda Barer	–
Gustav Victor Rudolf Born	5
Julius H. Comroe Jr.	1
Kenneth W. Cross	1
J.J. Handler	1
Joan C. Mott	23
Marjorie M.L. Prichard	1
Barbara R. Rennick	3
Samuel Robert Means Reynolds	1
Heather J. Shelley	1
John R. Vane	2
John G. Widdicombe	10
Derek G. Wyatt	2

with Dawes in parentheses. Table 26.6 lists those collaborators with whom he published most frequently.

Clearly, Dawes possessed a number of strengths and passions. Although at times he could be quite "difficult," yet he glowed with an intellectual sheen. One of his major gifts was his upholding of "translational" or clinical applications in the forefront of his thoughts, such that a number of his contributions to physiology proved to be of considerable importance to perinatologists and neonatologists. Truly, his was a life well-lived.

Personally, Dawes was focused, scientifically rigorous, uncompromising, logical, energetic, and often generous in praise to others. In the mid-1960s, I first met him as a fellow participant at one of the Princeton *Fetal Homeostasis* conferences. From thence and for over three decades of interacting at international meetings together, and with several visits to the Nuffield Institute, I always was struck with his breadth of knowledge and abilities as a polymath. As a discussant at scientific meetings, truly remarkable was his capacity to expatiate with confidence across a remarkable spectrum of biological science. Following a presentation he would rise and cut to the heart of the topic under consideration, expounding at length about many aspects of the issue, considering alternative hypotheses for accepted paradigms, and concluding with one or more critical questions to consider. He thrived in the hot house of focused discussion, new ideas, and debate. Upon learning about some exciting new work, often a beatific smile would spread across his face. Over the three decades of our interacting at meetings, we corresponded frequently. Often he would inquire about some aspect of a study we had reported and request a copy of the offprint for his file. Not infrequently, in commenting upon the work, Dawes would conclude with "Good Work!" "What Fun!"

Particularly impressive was Dawes' enthusiasm for, and commitment to, the young people who worked with him at the Nuffield Institute. As evident from the

Table 26.2 Mentor for graduate students, postdoctoral fellows, and other collaborators—1960s

Investigator	Publications
Karlis Adamsons	2
Richard E. Behrman	2
A.J.M. Campbell	6
Sidney Cassin	2
H.J.H. Colebatch	1
F. Cockburn	2
Kenneth W. Cross	3
S.S. Daniel	3
M.J. Dawkins	1
Alfred P. Fishman	4
J.W. Goodwin	1
E. Hibbard	1
A.I. Hyman	6
H.N. Jacobson	2
G.B. James	1
L. Stanley James	5
C. Koford	1
B.V. Lewis	3
C.L. Merlet	2
G. Mestyan	1
J.E. Milligan	2
Joan C. Mott	7
R.E. Myers	1
R.A. Nadeau	1
W. Niemann	1
J.B. Owen-Thomas	2
H.B. Parry	1
H.M. Perks	1
J.T. Reeves	2
Margot R. Roach	1
H. Rodriguez de Curet	1
B.B. Ross	6
Heather J. Shelley	4
A. Stafford	2
L.B. Strang	1
N.S. Talner	1
William F. Windle	2

reminisces recorded earlier, he could be magnanimous in spirit and generosity. He also was keen to support those who returned to their home institutions, helping to launch them on a successful career. In observing Dawes and his interactions with those under his tutelage, philosophically, he was what is sometimes referred to as an "educational perennialist." That is, one who strives to teach that which one deems

Table 26.3 Mentor for graduate students, postdoctoral fellows, and other collaborators—1970s

Investigator	Publications
P. Bailey	1
Kenneth Boddy	3
E.S. Boyce	1
R.L. Chapman	1
J.B. Clegg	2
K.J. Dalton	2
R.L. Fisher	2
Harold E. Fox	2
J.D. Gough	1
Bernard M. Leduc	1
Graham C. Liggins	1
C.L. Merlet	1
J. Nash	1
John E. Patrick	2
K. Pearce	1
S. Pinter	2
E. Robin Poore	1
Robert T. Richards	3
Jeffrey S. Robinson	5
Geoffrey D. Thorburn	2
D.J. Weatherall	2
W.G. Wood	2

to be of everlasting importance for life, to stress principles rather than specific facts, to help develop an individual's mind to reason scientifically, and thus to stand out as a true leader in the world of investigators and academic medicine.

Regarding some aspects of Dawes' mentorship, the late John G. Widdicombe of London, who worked at the Nuffield Institute in the 1950s, wrote:

> In his early professional days at Oxford, Geoffrey was nicknamed 'the Bishop'. This had a grain of truth in it. His demeanour did at times seem serious and pontifical, but this hid a great sympathy and affinity with his audience. He certainly took great care of his 'flock' of young scientists, both scientifically and socially. His home in Belbroughton Road, North Oxford, was famous for the parties he and his wife Margaret gave, at which we were all made to play childrens' games.
>
> (Letter from JW to LDL, 20 March 2009)

In regard to his research, Widdicombe recalled:

> [My] work on reflexes from the airways was nearly a nonstarter. In ... [the early 1960s] it took eight weeks to get a licence to do animal research (now it takes eight months) and, to fill the time, Geoffrey advised [me] to read all about the oesophagus ('the most neglected tube in the body') as a preparation for research for a D. Phil. After eight weeks [I] told Geoffrey that [I] found the oesophagus dull and the bronchi fascinating; ever positive and encouraging Geoffrey agreed that the bronchi should be the basis of [my] research.
>
> (Letter from JW to LDL, 20 March 2009)

Table 26.4 Mentor for graduate students, postdoctoral fellows, and other collaborators—1980s

Investigator	Publications
K.J. Anand	1
O.S. Bamford	5
Richard Belcher	3
Carlos E. Blanco	4
R.L. Chapman	1
F. Clewlow	3
P.M. Cotes	1
K.J. Dalton	1
R. Denny	1
W.N. Gardner	2
J.G. Gianopolus	1
M.D.G. Gillmer	1
Jonathan D. Goodman	7
Keith R. Greene	1
Mark A. Hanson	2
R.A. Harkness	1
D.J. Henderson-Smart	1
G.L. Henson	2
G.J. Hofmeyr	2
T.A. Howlett	1
C.R. Houghton	2
Arne Jensen	1
Barbara M. Johnston	5
Brian J. Koos	–
G.W. Lawson	3
D.H. Levine	3
H. Lilja	1
H.B. McCooke	2
Renato Natale	1
M.J. Parks	1
John E. Patrick	1
L.C. Pello	1
Christopher W. Redman	14
L.H. Rees	1
K.G. Rosen	1
Dan W. Rurak	1
J.H. Smith	4
J.A. Spencer	1
G.S. Sykes	1
I. Thaler	1
G.H. Visser	9
J.I. de Vries	1

(continued)

Table 26.4 (continued)

Investigator	Publications
David W. Walker	7
R.A. Ward	3
A.M. Wheble	1
P.J. Wickham	1
P.L. Wilds	1
H.J. Zeelenberg	1

Table 26.5 Mentor for graduate students, postdoctoral fellows, and other collaborators—1990s

Investigator	Publications
O.S. Bamford	1
P.J. Bowell	1
G. Conoscenti	1
Nicholas W. Dawes	1
P.J. Ferguson	1
M.O. Lobb	2
I.Z. Mackenzie	1
L.A. Magee	1
G.P. Mandruzzato	2
Y.J. Meir	1
M. Moulden	16
L.C. Pello	2
G. D'Ottavio	1
Christopher W. Redman	18
S.K. Rosevear	2
O. Sheil	1
P. Street	1
M. Selinger	1
V. Serra-Serra	1
T. Wheeler	2

Widdicombe also noted Dawes' thoughts regarding collaboration with clinicians:

> From the outset Geoffrey had the wisdom to foster relations with clinicians (including the thoracic surgeon Phillip Allison and the physician Hugh Cairns) at a time when many physiologists considered clinical departments unworthy of scientific acknowledgement. [I] had wanted to start physiological research immediately after his science degree, but Geoffrey was firm. 'Get medical degrees first, and I will keep a place for you'. His advice was, as always, very sound. Geoffrey initiated Nuffield research fellowships for visiting physicians, who came from all over the world. This led to many fruitful links between basic research and advances in clinical practice.
>
> (Letter from JW to LDL, 20 March 2009)

Table 26.6 Top coauthors with Geoffrey S. Dawes

Investigator	Publications
Christopher W. Redman	32
Joan C. Mott	31
M. Moulden	17
John G. Widdicombe	10
G.H. Visser	9
O.S. Bamford	8
Jonathan D. Goodman	8
David W. Walker	7
A.I. Hyman	7
Jeffrey S. Robinson	7
A.J.M. Campbell	6
L. Stanley James	6
B.B. Ross	6
Barbara M. Johnston	5
Gustav Victor Rudolph Born	5

From the University of British Columbia, the 16th Baron of Corcomroe, Anthony Manning Perks has written of working with Dawes at the Nuffield Institute in the mid-1960s:

> I greatly enjoyed my years with Geoffrey in Oxford. He was a kind and supportive friend. I left the Nuffield Institute with great regrets, but my wife, from Pennsylvania, hated Oxford, in part because the University turned down her application for graduate study: I wanted to save my marriage (it did not). Vancouver was a great compromise between England and the States. Geoffrey looked disappointed when I gave him my resignation, but I think he had anticipated it: I had been there as his "permanent" endocrinologist. My new post, in Zoology at the University of British Columbia, gave me wide latitude in research, and I enjoyed my task of teaching medical subjects on Campus, in the Faculty of Science. Geoffrey came through in those early days, to open the new Research Division in Obstetrics and Gynaecology, a division started by two of my own students, including ... Dan Rurak, who had gone from a Masters with me to do a D.Phil with Geoffrey. Geoffrey was enthusiastic about our work on the amnion.
>
> (Letter from AMP to LDL, 23 April 2010)

Baron Perks continued with other personal reminisces:

> As to Geoffrey Dawes himself, he was a wonderful boss, kind and considerate, and always interested in what you were doing. However, one personal memory keeps coming back. During surgeries he would suddenly disappear below the surgery table. No-one said anything. Geoffrey suffered from asthma, and he was taking a puff from his inhaler—but he never wanted people to see him doing it. Also, at those times, Geoffrey would deliberately drive Alfred P. Fishman [(1918–2010)] mad. He would ask what should be done next; Dr. Fishman would give an entirely sensible suggestion. Geoffrey would immediately reject the good suggestion, and do something entirely different. This did not lead to blows (but someone like me did wonder about it). You see, Geoffrey knew the results they had found the previous year; Fishman did not!
>
> (Letter from AMP to LDL, 23 April 2010)

Eugenie Ruth Lumbers, of the University of Newcastle, Australia, wrote regarding her time at the Nuffield Institute working with Joan Mott during the late 1960s and early 1970s:

> Joan was one of the most generous people I have ever met and I was very fond of her. She organised a fellowship for me at Wolfson College which resulted in some of the most exciting experiences in my life. Oxford was for both of us (Bill, my husband and I; I am not sure about the 2 very young children) one of the loveliest times of our lives and it was largely due to Joan Mott. The Nuffield Institute was a very interesting environment. Geoffrey Dawes was in his prime and research wise it was growing as we generated the technologies for studying the fetus in utero (Joan would never accept foetus—it is a mixture of Greek and Latin and fetus is 4th declension Latin as I assume you know). I remember Geoffrey and Joan having an argument over using fetus as both singular and plural vs fetuses. But it was a meeting place of international research, of fun (singing American Pie/or was it Hi Hi Miss America Pie? With the crowd from the Nuffield) and many many other happy memories. Then there was the international visitors who we met through the Nuffield . . ..
>
> To me Nuffield was my introduction to the international world of research, something you guys from the USA or from the UK would never appreciate to the same extent as a woman (shock, horror) coming from the Antipodes. I was fond of Geoffrey but concerned about his attitudes to women like Joan and Heather Shelley. At the same time I feel that his contribution to fetal physiology was great, as great as . . . others who contributed so much to establishing this discipline.
>
> Now when the fetal origins of adult disease have assumed their significance in terms of the study of human health and disease to an extent that we can see its role in the aetilogy of end stage renal disease in indigenous Australians and I hope we can set in place measures that will reduce the incidence of this shocking epidemic in our indigenous populations and raise their life expectancy to that of Caucasian Australians, I realize that Geoffrey Dawes, and others laid the foundation stones of a discipline that can make a magnificent contribution to human health and I haven't even begun to talk about the improvements in neonatal care—the knowledge base is just too big thanks to you pioneers.
>
> (Letter from ERL to LDL, 16 September 2009)

Later, Lumbers sent a photo of her at the Nuffield several decades earlier.

> Yes, I am younger here and not so wrinkly BUT I have a good excuse, apart from the Australian sheep who is beautiful, the gentleman in the background is Andrew Stevens. Andrew worked for many years at the Nuffield under Geoffrey and especially in the chronic sheep work so many old Nuffielders will remember him. Andrew came to Australia (he had married Denise Madill who also worked at Nuffield) and joined my fledgling research effort. Together we got my chronic sheep work going- we had sheep in offices, in sheds etc. until we finally were recognized and got some space of our own. Andrew did a PhD under my supervision. He is now retired and lives on the South Coast of New South Wales. Denise has retired but has quite an outstanding reputation in textiles and weaving. I am meant to be retired but am involved in four research projects ranging from neonatal piglets to human studies. My biggest interest is trying to work out the role of the intrauterine renin angiotensin system in pregnancy- in particular the prorenin-renin receptor system. Having described prorenin in human amniotic fluid many many moons ago I would like to find out what it does before I fall of the perch.
>
> (Letter from ERL to LDL, 21 September 2009)

Gordon Gilbert Power of Loma Linda University remembers:

> Often, in the early sixties, Geoffrey was a lonely pioneer at lung meetings trying to interest pulmonary investigators in his studies of the placenta and oxygenation of the fetus. It was

an uphill battle, to say the least, but he was persistent and articulate. His 1968 book, of course, had a great impact in legitimizing the field of fetal physiology and establishing it as an independent discipline.

I recall that at one International Conference, Geoffrey and I were alone in a garden outside a convention center. There, in private, we fell into conversation and he said, "Well, do you have any new ideas for your research?" I responded, "Well, lets see, we're thinking that perhaps carbon dioxide plays a major role in the regulation of fetal cardiac output—it forms bicarbonate and pulls in water from the mother by osmotic force to increase fetal blood volume." Geoffrey replied, "Well, no, I don't think that's right. You're on the wrong track. And besides, I thought after my work there weren't any new ideas left to explore anyways." He prided himself for being the leader in the field, and he was sensitive if this was not always duly recognized as such.

(Letter from GGP to LDL, 28 November 2011)

In addition to his Foreword, John Challis, Professor Emeritus of the University of Toronto and President and CEO of the Michael Smith Foundation for Health Research, Vancouver, British Columbia, writes on fetal research in Oxford:

In the mid 1970s Oxford was one of "the" places for fetal physiology research. Later some called it the Camelot period of research in our specialty. We had the availability of the chronic fetal sheep preparation, the ability to measure small concentrations of hormones using sensitive radio-immunoassay, sympathetic funding agencies, a public that was interested in the development of the baby, and in Oxford an extraordinary collection of great minds, superb mentors and enthusiastic fellows and young faculty assembled together at the same time. The proximity of the Nuffield Institute for Medical Research (NIMR) with the John Radcliffe Hospital and the Nuffield Department of Obstetrics and Gynaecology created the environment for interaction between basic scientists and physicians and the application of basic research to problems of clinical importance.

In 1974 there were three main research groups in fetal physiology at Oxford, headed by Geoffrey Dawes, who was Director of NIMR; Alexander Turnbull (later Sir Alexander Turnbull), the Nuffield Professor of Obstetrics and Gynaecology, and Geoffrey Thorburn. Geoff Thorburn had earlier been a sabbatical visitor with Geoffrey Dawes and had been persuaded to stay in Oxford, with salary provided through the British Medical Research Council (MRC). Geoff Thorburn's role was to help facilitate the transfer of knowledge between NIMR and the hospital. Jeffrey Robinson and I worked with him, supported through an MRC program grant. Our labs were in the hospital, but our animal studies conducted in the Institute. The corridor between the two, affectionately known as the "umbilical cord", was a conduit for great ideas although at times could be rather cool, in different ways.

The other two groups also had Medical Research Council program grants in addition to individual grants. Geoffrey Dawes of course held a major program grant at NIMR, where the staff included C.T. Jones, Joan Mott, Derek Myatt, John Bassett and a group of highly enthusiastic overseas fellows, Knox Ritchie, David Walker, John Patrick and Dan Rurak a Canadian DPhil. student with Geoffrey. Across the bridge, Alec Turnbull's group included Anne Anderson and Tony Flint, a young DPhil. student Murray Mitchell, senior academics including Mostin Embry and John Bonnar, and a string of enthusiastic young clinical trainees including Andrew Calder, Ian McKenzie, and ... Chris Redmond. There was unquestionably an intense, if good spirited, rivalry between our three groups. That helped our individual research, ensuring that it had been subjected to intense internal scrutiny before presentation outside the Institute, but it also brought us all together in collective pride. This was so evident in circumstances of competition with Cambridge and the team of Robert Comline, Marion Silver and the young Peter Nathanielsz, and with San Francisco, with Abe Rudolph, Michael Heymann and Joe Kitterman.

Geoffrey Dawes had reported on the occurrence and regulation of fetal breathing movements in the sheep (a paper with "Mont" Liggins as co-author), and continued outstanding studies on fetal cardiovascular control mechanisms. The acute fetal sheep preparation was still used at the Institute, but the development of the chronic fetal sheep prep had opened up a whole new field of opportunity. We knew that we were achieving a very high success rate with this preparation, attributable to the skills of the surgical teams and the meticulous attention to sterile technique and monitoring, but also to the superb work of Harry Elvidge the Chief Technician in the animal care facility at NIMR. There were three venues for the scientific discussion and academic discourse. The most formal were clinical rounds in hospital, where Geoffrey would often attend to ensure that young obstetricians were aware of the fundamental scientific principles underlying their clinical practice and that they practiced evidence-based medicine to the extent possible, and well before that term acquired broad usage.

The second forum for discussion was the large round table situated in the open area at the end of the umbilical corridor to the hospital. At morning coffee, Geoffrey Dawes would hold forth about new ideas and new avenues of research and often launched into a tutorial on various aspects of fetal physiology. Thirty years of being a Senior Oxford Don had molded his character. Pushing back his mop of hair he was a powerful debater and at times a fearsome teacher. I had arrived in Oxford via a PhD in Cambridge (with Brian Heap, now Sir Brian Heap) and a post-doc in San Diego and at Harvard with the late Kenneth J. Ryan. I had aspirations of becoming an endocrinologist which in Geoffrey's mind was 'okay', so long as I did not consider myself to be a real physiologist. Soon after my arrival at Oxford he asked me across the large round table and in front of the assembled group of morning coffee drinkers if I could explain some of the physiological responses of the fetus to hypoxemia. It was clearly a test and a challenge. Fortunately I must have muttered a sufficiently acceptable response that I was allowed to stay. When we made the first measurements of prostaglandin concentrations in the circulation of the fetal sheep and showed that there were extraordinarily high concentrations of PGE2 which rose at the time of labour I had sufficient courage to show these to Professor Dawes. Actually I made the mistake of showing him the raw data coming off the scintillation counter. The counts went down as the concentration of PGE2 was going up. I could see he was unimpressed until it occurred to him that these changes might have something to do with the decline in fetal breathing movements at the time of labour. Our relationship was fine after that.

Our third venue for discussion was of course the pub at lunchtime. I laugh that we now have to create a formal setting for lab meetings at a prescribed time each week. In Oxford in the mid 70s there was a lab meeting everyday; at coffee time, and at the "White Hart" at lunchtime. I became great friends with John Patrick in the course of those "lab meetings". The three Geo(Je)ffrey's (Dawes, Thorburn and Robinson) were inevitably part of the group. There was often a vigorous, sometimes furious exchange of views about the day's experiments, speculation about results and their interpretation, and a rich abundance of new ideas and new directions. I learned quickly to respect and indeed be amazed by Geoffrey Dawes' extraordinary breadth and depth of knowledge across different areas of physiology and admired his ability to pull in pieces of apparently unrelated information to generate a coherent story.

As I was leaving Oxford to move to Montreal, Canada, I had a final formal meeting with Geoffrey Dawes in his office. It had been a difficult decision for me to leave Oxford but ironically the richness of the environment and one's continuous exposure to new ideas left a need to demonstrate, at least to oneself, an ability to be sure that one could create one's own original thoughts. Without saying so, I think that Geoffrey understood. He passed on two messages. "John", he said staring at me with those penetrating little eyes, "you should be sure to learn and publish a new major technique every year" then he added, "and John, publish less." It took me some time to understand what he meant by that last remark but then I remembered that I was an endocrinologist and in Geoffrey's view endocrinologists

were enthusiastically applying the newly evolving radio-immunoassays to every tissue and fluid available. In his view, we needed to think more and strive to publish the highest quality papers in the very best journals.

Finally I must comment on another side of Geoffrey Dawes. Professionally, he had acquired the reputation of a brilliant experimentalist with extraordinarily high standards and quality of research. But he could be ruthlessly demanding, and critical if others did not meet those same standards. There was also a softer, gentle kindness to Geoffrey Dawes as well. I remember a dinner party at Geoffrey's house in north Oxford where this very famous man became a caring and obviously loving husband to Margaret and was at her side throughout the evening. He had enormous respect for Geoff Thorburn and when Geoff was ill, Geoffrey showed great concern and compassion. He helped me, I am sure once I had got the hypoxia question right, win a Junior Research Fellowship at Wolfson College and I have no doubt that his supportive hand was there unsolicited and undisclosed at other times. Finally, it was of course through Geoffrey Dawes and Geoff Thorburn that I had the privilege of meeting the young John Patrick. Geoffrey Dawes held John in very high regard. Here was a young obstetrician who was clearly a thinker, a leader with unrivalled enthusiasm to take on the experimental approach and to pursue the results of animal studies in human subjects. By 1980 both JP and I were working together in London, Ontario. Geoffrey Dawes would always visit us if at all possible during his trips to North America. I recall one time standing at the airport in London, Ontario with John Patrick waiting for Geoffrey to arrive. His plane was late and the SGI abstract deadline was fast approaching. John and I sat down and wrote our abstract on the back of a brown paper bag from Tim Horton's Coffee Shop. We laughed and showed it to Geoffrey when he finally arrived. The research concerned diurnal rhythms of steroid hormones in pregnant women. It was not exactly Geoffrey's area of interest, but he made a couple of suggestions and said he thought it might make a reasonable presentation. To our great surprise the paper was selected for the President's Plenary Session at the Annual Meeting of SGI the following March. Geoffrey became aware of this, and took time to send us a very kind note of congratulations.

Geoffrey Dawes was a great visionary who helped to open up a new field of research and a new foundation to clinical practice. He was a great teacher, and underneath that tough exterior was a sensitive and compassionate man. His legacy of course is not just his science, but his impact on the succession of colleagues and young trainees who were influenced by him.

(Letter from JRGC to LDL, 8 January 2010)

Also from Canada, and the Mount Sinai Hospital, University of Toronto, James Whiteford Knox Ritchie wrote of the early 1970s.

The whole era was, of course, very exciting and established the scientific evidence for much of what we do today. Oxford of that time has been described as a "Camelot". There were so many great names [that] came through the department and so many young people came for scientific training and went on to become significant investigators—or chairs [around] the world.

I was there for 2½ years from 1973–1975. I remember being interviewed by Geoffrey for the position—then we went to the pub. There, out of the blue, he looked at me and asked why the fetus breathes intermittently. Having read his book on the way to the interview I thought I had missed something important, and not knowing the answer would be the end of the job. Little did I know that no-one knew at that time—he was just looking for some intelligent reply which I don't think I managed. He could be quite intimidating until you got to know him!

(Letter from JWKR to LDL, 1 February 2010)

Following completion of his residency in obstetrics and gynecology at Harvard's Boston Lying-In Hospital, Brian John Koos, of the University of California Los Angeles, spent 3 years at the Nuffield Institute. He recalls:

> In 1979, I began a research fellowship under the auspices of the NIH with Geoffrey Dawes at the Nuffield Institute for Medical Research at the strong recommendation of my mentor Lawrence D. Longo. Thrilled to be able to work with such an internationally acclaimed authority on the fetus, I aspired to earn a D.Phil. under his tutelage. While understandably reticent to support such a commitment, Geoffrey eventually endorsed my matriculation at Oxford University, but there was one condition—that I ride a bicycle like the other students.
>
> In the first year, my family and I lived Woodeaton, a hamlet a little more than three miles from the Nuffield Institute. Every morning I set out just before 7, pedaling down a winding lane through paddocks to Marston Lane and then up a hill via Headley Way to the Institute. On many occasions, as I strained to pump my ancient, rusting bike up the hill, Geoffrey would pass me in his shiny new Saab, honking and waving with a wide grin. I have often thought that Geoffrey was quite pleased with himself for making an American physician ride a bicycle!
>
> (Letter from BJK to LDL, 23 December 2009)

Also from the late 1970s to early 1980s, Gerard Hille Adriaan Visser of Utrecht, the Netherlands, has written:

> I arrived in August 1978 to spend a Royal Society Fellowship year at the Nuffield Institute. During my first meeting with Geoffrey, he carefully managed to situate me facing the light, some strategic planning that appeared to be a consistent part of his behaviour, even in situations where he had to maneuver himself in some dark corners, opposite to chairs that were at easy access. Some power play; it was obvious that he was the boss. My first name, Gerard, was too Victorian according to him. So I became Gerry, all over the world, except for the Netherlands.
>
> Two years earlier Geoffrey had gained interest in the human fetus. Before that time it was mainly sheep. Fetal breathing and numerical analysis of fetal heart rate (FHR) patterns were his focus at that time and I could join the team, with Chris Redman (obstetric physician) and Jonathan Goodman (Senior Registrar Obstetrics and Gynaecology and ultrasound expert). The fact that human fetuses did not behave like sheep fetuses frustrated Geoffrey. A sheep fetus does not breathe during quiet sleep (High voltage low amplitude EEG.) and it demonstrates an increased heart rate variability during hypoxaemia. A human fetus continues to breathe during quiet sleep and demonstrates a reduction of FHR variation in case of (chronic) hypoxaemia. The latter difference with sheep can partly be explained by differences between acute and chronic hypoxaemia, the first appeared to be a frustrating species difference.
>
> It was amazing how Geoffrey, he must have been 60 at that time, managed to start writing computer programmes on prehistoric devices to tackle antenatal FHR patterns. That was not easy given the presence of shifts in baseline FHR, and different implications of periodic ... accelerations and ... decelerations. This hampered the use of SD as a measure of FHR variation with time. No, first baseline recognition, and subsequently analysis of variation after exclusion of decelerations. The antepartum FHR program developed in that period is still ... in clinical use in obstetrics. He did not succeed in developing a similar program for intrapartum FHR monitoring, and no one else has managed to do so since then. Geoffrey was a skilled mathematician, a trait inherited from his father.
>
> Geoffrey was a good teacher, especially to us foreigners. After writing a paper and discussing it with him, he commented once, 'so now the real problem starts'. That appeared strange to me, proud as I was that he had approved my intellectual efforts. But of course, each result contains a new problem: why, what is the patho-physiological background, what

if..., etc. etc. This has stayed with me ever since, just as his clear style of writing. Quoting Chris Redman in an obituary on Geoffrey (either from the Times or Independent) "He had a terse synoptic style of writing, clear and economical; sometimes he had to be reminded that his readers' mind were not as quick and logical as his own and so be persuaded to insert what he considered to be unnecessary elaboration and explanation". From the same obituary: "He retained astonishing vigour, openness to new ideas, a precise and detailed memory and an unremitting dislike for thoughtlessness and ignorance. His encounters with the latter stimulated his asthmatic wheeziness, so it was a familiar signal of his mood when he angrily had to use his inhaler". Yes, that was Geoffrey.

The Nuffield Institute for Medical Research was the center of fetal and neonatal physiology in those days. Either you were working there, with an extremely little desk in the lecture hall, or you visited the place, gave lectures and had individual talks with the research gang. The continuous intellectual challenge has stimulated many of the young researchers to continue in the field of perinatal medicine. Peers were and remained those present in Oxford beforehand or during those days.

In 1974 the first fetal breathing conference was organised by Geoffrey and the Swedish obstetrician [Gerhard] Bo Gennser. Yes, the fetus was breathing, which was new after Ahlfeld had suggested the presence of fetal breathing in 1903 and was therefore expelled from his German society. I remember Geoffrey demonstrating the Doppler noises of fetal breathing at the European Congress of Perinatal Medicine (ECPM) in Uppsala in 1976 (the Fifth Congress). Later he got the maternity prize of the ECPM. The fetal breathing conferences brought together obstetricians, neonatologists and physiologists, especially the young ones and preferably those who had an Oxford history. Since you could not earn your money with fetal breathing the conference was renamed the Fetal Breathing and Other Measurements Conference in 1979 (and saw the first presentations on fetal Doppler blood flow velocity measurements) and changed its name later in to: Fetal and Neonatal Physiology Society, a name better suited to the contents of the meeting. This society is still flourishing, first being chaired by Geoffrey, than by Bo Gennser, than by me, and thereafter by Marc Hanson, Bill Parer, Laura Bennett and now by Jan Nijhuis. During all those years 100-140 mainly young scientists came together annually to present their preliminary work on fetal and neonatal medicine; no official abstracts, but brief presentations and especially lively discussions, with Geoffrey—when he was still around—as the mild Godfather, critical but also very stimulating. Rather unique of this annual conference was and is the sporting event. Over the years the different countries have been competing on rowing, punting, sailing, beach volleyball, spitting, football, croquet etc. In 1989 the final at the Great Barrier Reef was played between Geoffrey and Mont Liggins (croquet). Mont won, which was difficult for Geoffrey. The Society is still very much awake and has been able to continue to attract young people working in the field of perinatal medicine. Its 39th annual meeting will take place in Utrecht, the Netherlands (my home town) in July 2012.

The ones of us who came regularly to Geoffrey and Margaret's house at Belbroughton road, saw a different man. A real gardener and family man, helpful in the kitchen and a very good host. If you wanted to see his CBE, than he took you to his bedroom, pulled a suitcase from under the bed and showed it to you. Margaret is almost 100 years old now and writes her memoires about her early days in Malaysia. She has remained witty and intelligent. She and the four children have always been the real driving force behind Geoffrey.

(Letter from GHAV to LDL, 16 January 2012)

Danny Rurak, of the University of British Columbia, Vancouver, who worked at the Nuffield Institute in the mid-1980s, testified to Dawes' importance to his career. Rurak summarized many of the features that helped to make Dawes' mentorship and the Nuffield Institute a life changing experience.

I worked in the NIMR under Geoffrey's supervision from 1972 to 1977, first as a DPhil student and then as a post doctoral fellow. Without doubt is were those years spent in Oxford that were the most important in terms of me becoming established as an independent investigator. I am extremely grateful to Geoffrey and for the opportunity to work in the NIMR.

I first met Geoffrey in the fall of 1971, when I and some friends were traveling in Europe. I had learned of him from Tony Perks, who was my MSc supervisor at UBC and who had worked in Oxford at the NIMR for a while in the 1960s, along with Sid Cassin, who he continued to collaborate with for many years. I wrote to Geoffrey before leaving for Europe and arrange to visit him. I and one of the friends I was traveling with stayed for a night at Geoffrey's house on Belbroughton Street and I had a tour of the NIMR. I think that was when I first met Colin Jones, Joan Mott and Fiona Broughton Pipkin. When touring Joan's lab, she described her work on the renin-angiotensin system in the fetus and mentioned the JGA [juxta-glomerular apparatus]. Geoffrey than asked me what JGA stood for and fortunately I was able to provide the correct answer. In retrospect, I think that it was my correct answer that led Geoffrey to accept me as a DPhil student.

In the years I spent at Oxford and also after leaving, I have thought a lot about what is was about the NIMR that made being there be such a rewarding and valuable experience. I think that the following factors were important.

1. Geoffrey himself, who was extremely bright in the Oxbridge way. He was an excellent graduate student supervisor, in the sense that he left it mainly up to the student to develop the research proposal and conduct the experiments. That certainly was the case with me.
2. The amazing collection of clinical and basic science students, fellows and visitors who worked in the NIMR. Over the time that I was there that included: Bob Bradley, Charlotte Mistretta, Ken Boddy, Geoff Robinson, John Patrick, Kevin Dalton, Frank Manning, Colin Mantel, Geoff Thorburn, John Bassett, John Challis, Knox Ritchie, Roger Chapman, Lea Wilds, Richard Robinson, Fiona Broughton Pipkin, Barbara Johnston, Richard Harding, Paul Johnson, David Walker, Graham Jenkin and Eugenie Lumbers and many others whose names I have forgot. Working with them over the years was very stimulating and rewarding and most of them have remained friends and colleagues to this day.
3. The camaraderie that was present among the "visitors". Oxford for someone that comes from outside, particularly from the "colonies" is initially a somewhat intimidating place and this certainly applied to the NIMR. I found that you have to work there for a year or so, before you were accepted or even acknowledged by 'Old Guard"—the permanent faculty. As all of the visitors were in the same boat, we joked about it and kept together as a group that also included the technical staff that worked there, particularly Andrew Stevens and Nigel Brooks, who worked on the sheep projects. I remember the lunches and Friday nights that were spent at the White Hart pub, which was in Headington, close to the NIMR and Morris and Cynthia Jacobs, who ran the pub at that time. Most of the scientific discussion that I had with other NIMR folks occurred in the White Hart pub. There were also cricket matches, volleyball and other sporting events on the lawn in front of the NIMR, at least until the John Radcliffe Hospital was built.
4. The common open lab that was at one end of the second floor of the NIMR. I know that open labs are common now, but back then they were not. I had a bench in the lab, for my AVP bioassays, and also in the lab were Barbara Johnston, Fiona, Eugenie and Joan Mott and Richard Harding. We got to know a lot about each others' research by being in the same lab, although there were sometimes disadvantages. Barbara was working on the effects of hydralazine on cardiovascular function in rabbits and did the experiments right next to my bench. One day a carotid artery catheter came out and blood from the rabbit spurted over me and some papers I was reading. I still have some of these papers with spots of dried rabbit blood. Another time, a rat that I had in a cage escaped and

disappeared although there was some evidence that he was still in the lab. A month or so later, the paper feed on the polygraph recorder that Richard was using stopped working. Ken Bolton came up to look at it and found that the rat had made a nest in the recorder using the polygraph paper for the nesting material. He blew it out with compressed not realizing that the rat was still in there.

5. The research infrastructure that was present in the NIMR, which included electronic and mechanical shops, photography and histology services, with the latter overseen by Majorie Prichard, who had worked with Barclay and Franklin, before Geoffrey became Director. This made it possible to have specialized equipment and apparatus made quickly in house and this definitely benefited me during my doctoral and post-doctoral work. As much as the services provided, it was the characters that worked in the shops—Harry Elvidge, Ken Bolton, Stan Ashington and others, that made it very interesting and enjoyable. Ken was responsible for keeping the Schwarzer polygraph recorders working and I still remember his repeated comment when the calibration procedures worked—Perfectus Jubilatus. He has served in the British Army in India during the war and had lots of Indian words that he used. Harry served in North Africa.
6. Another important resource was Derek Wyatt, a medical physicist who was one of the NIMR faculty. He had developed the electromagnetic flowmeters that Geoffrey had used in his early fetal cardiovascular studies and when I was there Derek was still working to improve the flowmeters. I think they were the best flowmeters ever developed but I do not think that they were ever commercialized. Derek also contributed to the design of many of the other devices that were developed, particularly those used for fetal HRV studies and the early work on computerized analysis of fetal breathing. I got to know him quite well as we both worked at night and his lab was just around the corner from the open lab. Every once and awhile he would come in and chat. Like most of the faculty and staff in the NIMR, he had seen military service in the war—in his case as a midshipman on the battleship King George V and would talk about his experiences, as well as other more contemporary subjects, during these evening chats.
7. The weekly seminars that occurred in the NIMR. Sometimes they were given by outside visitors but more often were presented by folks in the NIMR. In the latter case they were more informal, involving discussion of current or plans for future research.

(Letter from DR to LDL, 22 December 2009)

Mark Hanson, of the University of Southampton and who worked at the Nuffield Institute during the 1980s, also recalled Dawes' rigor:

> Geoffrey of course could be a harsh critic, partly because he insisted on high standards in science. He was not averse to poking fun at those whose results he thought were weak. I remember a large clinical meeting in London where a young clinician was presenting some blood gas results from cordocentesis samples. During the discussion after his paper it appeared that he had not measured pH, nor did he seem to think that this was an important consideration. Geoffrey rose to his feet and said that the paper, and the approach of the young researcher, reminded him somewhat of a mistake recently made by his secretary in typing a paper. The phrase she had meant to type was "buffer the pH". However as on the standard keyboard the g is next to the f it is very easy to make a mistake. So what she typed, and what reminded Geoffrey of the young man's approach ....
>
> But perhaps my favourite reminiscence of Geoffrey is a story which he told ... against himself, and to remind the audience of the importance of skill, patience and keeping a cool head—qualities which he attributed to Mont Liggins in particular .... Geoffrey and Mont were keen fishermen and one day, somewhere in the world, were in a boat, on a lake, fishing from the early hours of the morning. The day passed without either of them catching anything, or even getting a single bite. When the evening drew on and they were just about to give up Geoffrey saw in the distance the characteristic ripples of a fish rising. In great

excitement he leapt to his feet in the boat and shouted "Look Mont, look there's a fish!". "I know Geoffrey" remarked Mont dryly, "it's on the end of my line."

(Letter from MAH to LDL, 12 March 2009)

From the University of Nottingham, Fiona Broughton-Pipkin who worked at the Nuffield Institute in the early 1980s has recalled some aspects of Dawes' mentorship:

He was . . . very strong on everyone talking to everyone else at coffee- or tea-time, and that is also an excellent training. Listening, arguing, asking for advice, picking up different ideas, in an informal setting works very well, and it strengthened the team spirit among that group of marked individuals.

One of the things I particularly remember about Geoffrey was his insistence that no-one from [the Nuffield Institute] should read any presentation which they made at a research meeting. This initially terrified me, but the point that, if you couldn't talk about your own work without notes, you didn't know it very well, was entirely fair. Once I began to supervise my own students, I insisted on the same thing, and have continued to do so.

He probably didn't realise that he inadvertently taught me something of man-management too. One day I asked him if I could try a particular set of experiments, having, I thought carefully, explained why. He said "No", with reasons which I wasn't sure about. A few weeks later, he said "You really ought to be thinking about looking at . . .." (the experiments I had wanted to do). Being well brought-up, I bit my tongue, didn't say "But I asked you about doing that a couple of weeks ago", and just got on with them. A little later much the same thing happened again, and again I bit my tongue. I had, however, learned, and used the technique reasonably successfully quite often thereafter. The gestation period was usually 2–3 weeks!

I remember with great gratitude an intervention from him at the very first external research meeting at which I spoke, the Neonatal Society meeting in Newcastle upon Tyne. They are a friendly Society, but one's first meeting is something of a nightmare. The actual presentation went reasonably well, though the long pointer in my hand was swaying like a pendulum, but someone (I can't remember who) asked a question which made no kind of sense to me. I thought that I must have misheard, and asked for it to be repeated. Still no understanding—but at that point Geoffrey twisted round in his seat and said something along the lines of: "X—that's not at all the area Fiona's working in. We can talk about it afterwards". I could have hugged him!

(Letter from FB-P to LDL, 9 June 2009)

In March of 1981, Dawes was honored by the Queen, Elizabeth II, as Commander of the Order of the British Empire (CBE). Fiona Broughton-Pipkin recalls:

He was, as you may remember, given a CBE by the Queen. This is the highest of our civil "British Empire" Orders other than a Knighthood. We were all delighted by it, and Worcester College, of which he was a Fellow, threw a very good party for him. It was in the Fellows' garden, on a glorious summer evening (we do have them sometimes!), and was very Oxford.

(Letter from FB-P to LDL, 9 July 2009)

David Walker of Monash University, Melbourne, Australia, also contributed some thoughts on Geoffrey Dawes and the Nuffield Institute during the 1980s.

What did we call him?

I think we all ended up calling him 'GSD', but I remember being surprised when I first got to Oxford that many in the lab addressed him as 'Sir'. Not Sir as in Sir Geoffrey, but Sir as one would address a revered school master. As a naïve colonial boy I remember being

rather puzzled by this. Most of those calling him Sir were Canadians, and I wondered if this was something they always did back home in relation to senior colleagues. It didn't seem to matter to Geoffrey—I don't think it mattered particularly what you called him as long as you weren't rude or disrespectful. In my case I went some months (a year, perhaps) before I felt comfortable in moving from 'Dr. Dawes' to Geoffrey. It was sometime after I came to Oxford in 1974 that he officially became Professor Dawes.

Listening

GSD was a quiet man and not someone who needed to proclaim his opinion at any opportunity. At times he seemed to be quite remote. Mostly, he was a good listener, and whenever he said anything you listened carefully. Because he was so famous and we were all striving to become known, it wasn't unusual for someone to take the high ground in order to impress him. This often took place at a round table in a common area at the top of the stairs in the Nuffield Institute where everybody gathered for morning or afternoon tea—a quaint ritual that has simply vanished. There was even a tea lady who made a big urn of tea in readiness, and there were biscuits. People smoked, and GSD famously had a pipe with wonderfully aromatic tobacco. Somebody would hold forth on something or other—it was almost always to do with physiology or medicine and rarely about politics even though the UK was in turmoil at that time with fuel shortages and union strikes, and anyone could have been forgiven for venting their spleen over the current situation. Anyway, Geoffrey always did the speaker the courtesy of listening carefully. Often he looked quite inscrutable, and if he said nothing or little in reply that was as good as letting you know you didn't really know what you were talking about. But when he did offer an opinion—and again it was rarely about anything other than the work we were all trying to do—then you had to listen carefully because it was always important.

Fishing was another matter—he was always happy to talk about fishing.

Mentoring

This is probably too modern a word to apply to Geoffrey. I don't think he ever thought it was his responsibility to "mentor" any of us in a formal way, and I can imagine him "Hrrmp-ing" at the idea that you might need to be formally trained to do it, as is the fashion in universities today. His attitude was more like "jump in and learn to swim," and I remember him saying something like that about somebody, to the effect that he thought they were at risk of drowning! I don't remember ever receiving direct advice. But what I do remember is that if you had made an observation and wanted to talk to him about it, he would always listen, prompt again and again about the circumstances, ask you what you thought about it, what did you know about the topic relevant to this, and slowly the excitement would build, and then you had all his attention, and all his support, and all his knowledge.

Progress

I don't remember GSD ever calling a meeting specifically for everybody to report to him on the progress of their work. Usually, the way it worked was that when you had a finding (was it a real "discovery" or not?) you would try to catch him during one of his peregrinations through the Institute or at morning or afternoon tea. When you were telling him what was happening, he became very still and looked at you very quietly but intensely, and then he'd ask you what you thought it meant. Now, this was difficult because you were always at the limit of your knowledge, but after you had stumbled through some sort of explanation, he would then tell you what it probably meant, that someone else had published on this before and you would be well advised to read it, but "Well done" and, "Now, what are you going to do?"

Grant Writing

Somewhere in my files, I have copy of a grant application that GSD submitted to the MRC. This would have been about 1978, and it is only 3 or 4 pages in length. The copy I have is probably the third or fourth carbon copy, because this just pre-dates the arrival of office computers with word processors and was typed on an IBM golf-ball typewriter by GSD's secretary. It is a request for funding to continue work on fetal breathing movements and outlines in the simplest terms what he had already achieved, and why it was important to continue this work. There are no graphs or figures or tables in this document, no detailed research plan, costings, or mention of how the data would be analyzed, but it is utterly convincing and most likely was successful because we continued this work for the next 5 years or so.

That is all I can say about how the activities of the Nuffield Institute were supported. It is a mark of GSD's organizational ability that we—the workers from many different places in the world—never had to worry about the realities of research funding except at the personal level of ensuring that our salaries from our home countries were covered. When my initial salary support from the Nuffield Dominions Scheme ran out, GSD provided me with a MRC Research Fellowship, and I continued not even having to worry about salary support for several more years.

There was a "downside" to all this of course, because reality hits home rather forcefully a few years later when one really did have to find all your funds for research. But what I want to say is that, while I don't really know how the entire Institute was funded year in, year out, for many of us, we had several years not having to worry about this side of things at all, and we were free to immerse ourselves wholly in the demands of research.

Seminars and Conferences

We were expected to participate in the Oxford meeting of the Physiological Society, and with the advent of the FNPS, we were all excited about being able to stand up and deliver the new discoveries that we thought we had made (usually much less novel and complete than initially imagined). Even with these exercises, which I expect were quite important to GSD, and the reputation of the Institute, there was virtually no pressure from GSD for people to participate or perform. But if you wanted to, then he was very, very good at getting you ready. He taught us how to speak slowly, to say one thing at a time, and emphasized that the audience would totally forget what you had been staying unless you tried to leave them with one (at most, two) important points to take home. It was probably at these times that I had the most intimate contact with GSD—he taught and I learnt.

Fetal Physiology: During and After GSD

When I got to the Nuffield Institute in 1974, although Liggins had made an impact with fetal breathing and fetal sleep, and Thorburn's influence with fetal endocrinology was increasing, the major legacy of GSD's work on the fetal circulation and the placenta was still very dominant. There was a palpable friction between GSD and Thorburn, and as an Australian, I think I was expected to fall into the Thorburn camp, but I resisted this and placed myself with GSD. I was still in the thrall of his book *Fetal and Neonatal Physiology* which I carried everywhere and read constantly. I would have liked to work on primates and was hoping that another trip to Puerto Rico might come up, but instead I was paired with Colin Jones and asked to work on catecholamines. This was nearly a very great disaster because Colin was a very poor teacher, I didn't know anything about HPLC, and the equipment they had was appalling and wouldn't have been able to do the job. In the event I had to make myself an expert in fetal surgery so that I could find the fetal adrenal gland (I had never seen one before this!), and I am eternally grateful to Jeffrey Robinson and Knox Ritchie for their patience and kindness. The work eventually went well and was done mainly by Barbara Johnston and myself. We managed to adrenalectomies and devised a method for injecting formalin into the middle of the adrenal to effectively produce a

de-medullated gland. To this day I have no idea where Colin got the catecholamines assayed, but they were not done at the Institute. During all this time, GSD was very interested and thought the work important but never sought to direct it—except of course, by somehow allowing it all to proceed without us having to worry about where the funds were coming from.

Colin Jones brings me to thoughts about GSD's possible successor. There was no Deputy Director of the NIMR—at least, not that I knew about. I think Colin thought he would be the next Director, but there was nothing GSD ever said or did that confirmed this to me. Joan Mott and Heather Shelley never discussed the idea, but I remember John Bassett proposing that Geoff Thorburn would eventually take over the Institute. I think Paul Johnston, though very clever, was considered too erratic to be trusted with a directorship. So—all the time I was there (1974–1982), GSD was the director, and there was never any serious common room talk about what was going to happen next.

Then the story starting circulating about what GSD was supposed to have said to an Oxford University committee, to the effect that when he retired everything of importance in fetal physiology would have been achieved. I won't say who put this about or who said it was true and an absolute fact, but it had a very destabilizing effect. This would have been 1980 or 1981, but I remember that Thorburn was already back in Australia so perhaps it is the later date. There was a feeling that there wasn't much future for the NIMR and that with the further development of the John Radcliffe hospital site, there were many people who wanted to see it go. Certainly, a great many people left after this time.

For the record, I don't think GSD said anything like that. As remote and withdrawn as he could sometimes be, and sometimes acidly critical of some of his peers with whom he had to complete for MRC funds (e.g., Robert Comline and Michael Purves would never forgive him for apparently excluding them from MRC funding), he was not arrogant to the point of believing that fetal physiology rested only on his shoulders. His 1968 book is a testament to his academic generosity. I think he could well have said something like—"by the time I retire I will have achieved everything *I* set out to do." He was very aware of all the new developments taking place in molecular biology, and David Barker's work was known to him at this time. He had actively promoted all the work being done by Jeffrey Robinson on fetal growth retardation, and he was vigorously pursuing the computer-aided analysis of fetal heart rate monitoring. In short, he knew that greater collaboration with clinicians was going to be very important. He may have recognized that the type of work to be done in the near future would be different, and that there were significant forces at work within Oxford University that would make it difficult for the NIMR to stay as it was. Whatever it was going on in his mind at that time, it is perhaps typical that he didn't discuss it openly or draw the NIMR staff together to let them know what might happen in the near future. I think the only person that might have been drawn into his confidence would have been Jeffrey Robinson, but if memory serves me correctly, he had also already departed to Adelaide.

That is all I can remember in any detail. It doesn't seem enough, but I can say that I have no regrets about the time I spent in Oxford. Mark Twain [(1835–1910)] wrote (more correctly, dictated) an autobiography that he stipulated was not to be published until after his death. At the end of the Preface (*As From The Grave*) he writes:

"*It has seemed to me that I could be as frank and free and unembarrassed as a love letter if I knew that what I was writing would be exposed to no eye until I was dead, and unaware, and indifferent*."

Well, I'm not dead yet and I have written very few love letters in my life, but I came to have a genuine love for Geoffrey and remain very much in his debt. I wish I had known him better.

(Letter from DW to LDL, 30 January 2010)

Regarding Geoffrey Thorburn, the Nuffield Institute, and Dawes, Richard Harding of Monash University, Melbourne stated, "you may know that Geoff[rey]

Thorburn was not healthy, in fact at times was seriously ill, during his period in Oxford. However he accomplished a lot in spite of this and in spite of Dawes' attempts to keep an "upstart Aussie" under control. There was quite a bit of rivalry; both Dawes and Thorburn were similar personalities. Dawes was apparently incensed when he found that Thorburn had been approached as Dawes' successor after Dawes' retirement" (Letter from RH to LDL, 1 March 2009).

Arne Jensen, of the University of Bochum, worked at the Nuffield Institute during the academic year 1983–1984. He has recalled:

> Dawes attended the meeting on Fetal Heart Rate Monitoring—Clinical Practice and Pathophysiology in Rauischholzhausen near Giessen, Germany, in summer 1983. He presented his data from chronically prepared fetal sheep on breathing movements that were dissociated from the ECoG pattern after brainstem transection, demonstrating that higher centres are responsible for the inhibition of fetal breathing during high voltage ECoG activity. The other intriguing observation was that in normal fetal lambs a minor degree of isocapnic hypoxia causes arrest of fetal breathing movements. This reflex is abolished when the brainstem is sectioned in the upper pons. Then the fetuses are sent into continuous breathing during isocapnic hypoxemia, an effect that is unrelated to chemoreceptor activity, because the effect is still present when the carotid nerves have been cut. A possible explanation was, that section of the brainstem in the upper pons or above may damage the blood supply to the medulla and hence to the respiratory centres residing there, so that blood flow is unable to increase sufficiently to maintain normal metabolism in hypoxia.
>
> This point was raised by the reviewers when GSD submitted the paper for publication, and the onus was on him to prove that local hypoxia due to the transaction-procedure—hence an artefact—was not involved. This criticism was not easy to disprove in the absence of a method to measure organ blood flow in distinct parts of the fetal brain.
>
> [At] the same meeting on Fetal Heart Rate Monitoring our group presented data from chronically prepared fetal sheep on circulatory changes during asphyxia and those in organ blood flow—particularly to the brain—using the radioactively labelled microsphere technique. This method, adopted from ... Rudolph and ... Heymann, was modified by us to observe dynamic changes in organ blood flow in a short period of time. After my talk GSD approached me, ... saying: "I believe, it wouldn't do your career any harm, if you come to Oxford, would it?" ...and off he went. It almost took my breath realizing that the author of the book *Foetal and Neonatal Physiology*, the bible of fetal physiologists, had just invited a young investigator like me. I was thrilled.
>
> By this time GSD knew already that I was bound to do my post-doc fellowship at the CVRI at UCSF with ... Rudolph, his great rival. His intention was clear, he wanted to get hold of the microsphere technique, to prove the point that local hypoxia after brainstem transaction was not the cause of continuous breathing during hypoxia, rather the disruption of the neural pathway that blocks fetal breathing in the normal fetus when oxygen is at short supply. And—beyond this scientific reason—he loved the idea to 'snatch' me on my way over to San Francisco before I would leave in summer 1984 to work with ... Rudolph. Thus, to my knowledge, I had the unique privilege to work with both protagonists. ... I was very proud to be invited and overwhelmed by the scent of academic life in Oxford. I had such great pleasure in imagining being part of it, that I intended to undertake a degree at Oxford University, but GSD talked me out of that, saying: "Arne, I have seen so many people 'dripping from degrees'. Don't be overly impressed—they never get things done".
>
> It became quite clear during the visit that for GSD it was a matter of utmost importance to make the point that after brainstem transaction the functional integrity of the spinal medulla below pons was unambiguously proven to support his theory. Hence, my main task for my stay in the Department was to establish the microsphere technique, validate and use

it in a study on the effects of brainstem transaction and on isocapnic hypoxemia. To provide a personal project for myself, a study on fetal organ blood flow changes during high and low voltage ECoG activity in normoxia and during hypoxemia was designed . . ..

Being a clinician amongst basic scientists was a heavy burden, particularly since I had to set up a fairly complex new method. Even more scepticism arose, when it became obvious that the historic Packard Gamma-Counter that had not been in use for ages required major repair, because the crystal had a crack and produced erratic count rates during the standardization procedure in various parts of the spectrum. Raised eyebrows followed the service bill of 26,000—Pounds Sterling, including a replacement of the crystal, but GSD defended the project even though not a single measurement was accomplished as yet. In the meantime GSD set out to write the programme in Basic on his own computer, me sitting aside of him in his study, trying to explain the algorithm laid out in formulae that were fairly complex. We chose to use five different isotopes. Hence, the spill-over from one spectrum into the next affected the count rate and had to be mathematically accounted for. . . . GSD was not amused, pulled his hair and bluffed: "What the flaming hell are you trying to tell me, Arne. Would you mind stopping confusing me, please!" But in the end after 4 weeks of programming and eliminating computational bugs he was delighted. "Congratulations, Arne, we've got it done!" This joy was even topped when after six weeks the first real organ blood flow results came out of the printer. He took the print out, two meters in length, and waved it through the air going from one laboratory to the other to present the success. He obviously was relieved that the unexpected enormous hardware investment . . . in a junior German clinician had eventually paid off.

Being a novice in scientific writing my manuscripts were lengthy, extremely verbose, redundant, and unclear for uninitiated readers. This evoked GSD's pity. Obviously in reward for my engagement in setting up the microsphere method, he offered his editorial help and sat down with me to streamline my manuscripts word by word, jotting down his corrections with his distinct handwriting in pencil. He was a purist and, among other things, hated poorly defined expressions in written or spoken English, e.g. STRESS [see attached Figure].

GSD's manuscripts were pieces of art in scientific writing and subjected to scrutiny before submission for publication. I once was heavily criticized in a typed letter for submitting a manuscript in a 'pre-final' version and he added the following post scriptum written by hand: "I hope you will not feel too hard about this letter. Ultimately what you, and I, are judged on is not speed but accuracy, care, excellence." This paternal and personal tutorship of GSD was the most enlightening and fruitful experience in my scientific career, which made me utmost grateful.

One of the most impressive personal experiences was when we were invited to his home at Belbroughton Rd. 8 for a round of Croquet followed by dinner. There we came to realize that GSD besides being a world renowned scientist and Director of the Nuffield Institute, also took a lot of domestic responsibilities, as his dear wife Margaret was blind. This was the most memorable afternoon. He introduced us to the art of rose gardening—his favorite hobby besides flyfishing.

(Letter from AJ to LDL, 15 April 2009)

In regard to Dawes' administrative style, several correspondents commented upon his total dedication, with single-minded focus on the task at hand. Pierre Foex, at that time on the Governing Body of Worcester College, recalled that during a discussion of academic visitors and the work entailed in their being hosted, Dawes noted that ". . . with visiting scientists, there is a strict rule: one day or one year, nothing in between!" (Letter from PF to LDL, 3 November, 2009).

Nonetheless, Dawes was not always focused and could be rather aloof from his surroundings. From a visit to Auckland in the early 1980s, Ross Howie recalled:

> I remember meeting him only once personally, and then briefly, when Mont Liggins brought him into our neonatal unit ... I had looked forward to meeting Dawes—there was so much I thought we could have talked about—but he gave me the impression that could have been the first time he had visited a neonatal unit anywhere. He was a great physiologist but seemed not much interested in the clinical applications of his work. In Oxford the heads of both pediatrics and O&G (Tizard and Stallworthy) were friends of mine but there seemed to be very little interaction between their departments. That could have applied to physiology as well. Having been used to good collaboration in Auckland that came as a surprise to me.
>
> (Letter from RH to LDL, 3 December 2011)

From the Oregon Health Sciences University, Portland, Kent Lu Roy Thornburg recalled:

> I met Geoffrey Dawes when he came to Portland in the early 70s. I had used his 1968 monograph as a textbook for a fetal physiology course in my graduate program a few years earlier. I was anxious to meet him. During our visit, he became intrigued with our data on the electrical potential in the placenta, and invited me to present the work at the Physiological Society meetings in Cambridge. He took me under his wing and allowed me to stay in his home. I also stayed for a short time with Richard Harding who was in the Dawes laboratory at the time. I remember one warm evening when we sat in Geoffrey's back garden, he offered milk to a hedgehog with whom he had evidently developed a long-term relationship. Geoffrey made kissing sounds to entice the little creature to the milk saucer.
>
> Geoffrey supported my work. He was especially happy when we countered the view he expressed in his book that the two fetal ventricles are alike. I was worried that he would be upset with our findings. He was at the meeting on Vancouver Island when I gave a paper on the topic. I remember saying that unlike the words in the fetal bible written by Professor Dawes which side with William Harvey by saying that the fetal heart ventricles are "... of much the same shape and size, like the twin kernels of a nut ..." (Dawes 1968), the ventricles are actually very different in shape, size and function. During the discussion period that followed, he walked to the microphone and jokingly made the comment that from time to time the "Fetal Bible" has to be revised. He took no offense. At a later time, however, he suggested that I should not try to publish one of our papers on fetal lung function in the *Journal of Physiology* because it contradicted work that he had previously published in that journal. He thought it would be confusing to the reader. More to the point, I think he was convinced that our data would stand the test of time.
>
> Geoffrey and I corresponded from the time we first met, and I visited him from time to time thereafter. In 1989, he invited me to participate in a meeting on fetal physiology in Liguria. He offered an enticing note by saying that he wanted to introduce me to a David Barker who "... has gathered evidence which suggests that defective perinatal growth may be associated in adult life with the liability to hypertension and ischaemic heart disease..." (from Dawes 1990, p. 4). The proceedings of the meeting became a monograph. David Barker and I solidified our friendship at that meeting and we have from that time forward been soul mates in our quest for understanding the fetal origins of adult disease. We are grateful to Geoffrey for bringing us together.
>
> (Letter from KLT to LDL, 3 May 2010)

As editor for many years of the journal *Obstetrics and Gynecology* published by the ACOG, Roy Macbeth Pitkin, Emeritus Professor at the University of California, Los Angeles, recalls Dawes' contributions as a reviewer of manuscripts:

> His reviews were always detailed, objective, and in all other ways perfect. Additionally, they always came back very promptly. The last I sent was returned by his wife with a note saying Geoffrey had died suddenly (a stroke, as I recall) several weeks earlier. She added

> her thanks for my sending papers for him to review, saying it had meant a great deal to him to be asked to perform such academic duties. What a scholar!
>
> (Letter from RMP to LDL, 7 January 2010)

Several correspondents emphasized the role Joan Mott played in Dawes' studies, in essence being his "girl Friday." Although she was capable and clever in designing original approaches for their joint studies, yet she remained "underappreciated" for her abilities and intellect. One correspondent stated that, although Mott worked independently in her studies of the role of the renin-angiotensin system and the regulation of blood pressure in the developing mammal (Mott 1978), for the most part, her role was that of a high-class technician as Dawes' "spear-carrier." In his obituary of Mott, Dawes barely refers to her role as a Nuffield Institute fellow scientist and collaborator (Dawes 1994).

In preparing this review, I contacted almost 100 individuals who either worked with or were contemporaries of Dawes. Somewhat surprisingly to me were the number (over a dozen) who expressed reservations or overt hostility, to the extent that they declined to comment or stated frankly that they did not wish to contribute in any manner. As noted, Dawes had his "rough side." Early on, he disagreed with the statutes of the Nuffield Institute and had some conflict with Gordon Ardran concerning the importance of radiologic studies. Among those who would discuss Dawes or their relationship with him, the major reservations regarded his at times self-aggrandizement, rather authoritarian management style, frequent failure to acknowledge other contributors to a given field, often marmoreal aloofness, offering of animadversions, and on rare occasion overt hostility and argumentativeness. Several stated that he ran the Institute in a most "paternalistic" and "authoritative" manner. Apparently, staff meetings were seldom held, there often was disagreement and conflict at those Institute meetings that were held, the committee minutes were kept secret, and he had no tolerance for what he perceived as insubordination. One individual stated that, he could be the "Genghis Khan" of British developmental physiology, who fostered a "cult" following among those working, or who had worked, at the Institute. Another wrote of him as an éminence grise [gray eminence, powerful advisor], mentioning "... the insecurity in which he found himself as the nominated pioneer of a field he was not really able to master." Liggins has referred to some of these issues (Liggins 1998, pp. 114–116).

As noted, although quite supportive of young people and their work, Dawes could be cool or even hostile to anything that smacked of mediocrity or carelessness. From the mid-1960s onward, I participated with Dawes in about two dozen international meetings of various types. On several occasions was I taken aback as he engaged in hostile and bitter debate with individuals with whom he differed. As I understood the conflict, it was because the individual with whom he had a difference of opinion was somewhat glib, careless, or cavalier in his presentation or their findings contradicted his own work. Also on two of these occasions, the confrontations precipitated a severe attack of status asthmaticus. These experiences left in his wake a train of considerable angst. Among respondents to my queries, several stated that Dawes was a "complicated person," and despite them having worked

with him at the NIMR, they never became truly well acquainted. Others were downright negative.

Dawes' son, Nicholas William, recalled his father's interest in people as well as science:

> He would stop and make time to listen to the difficulties of running a small fishing shop in rural Ireland or the problems of a carpenter in getting hardwood for making furniture. It was the knowledge that people shared with him that he found fascinating. Their expertise and experience in widely varying backgrounds was always of interest. He would take as much delight and pride in understanding, and practicing, the mechanics of smoking fish as he would the variability of fetal heart rate. He smoked his own Adwell-caught trout, with sawdust he bought from the local arboretum. It was delicious.
>
> (Letter from NWD to LDL, 18 January 2009)

Nicholas also reminisced about his father's plans for the future:

> Pa always had long term plans. Even in January 1996 he had a five-year rolling plan and would explain it with great energy. This was one of the reasons for his extraordinary success at research, since the short term held for him the steps needed to move towards the long term goal. Even the choice of five years as a horizon had been carefully thought out.
>
> (Letter from NWD to LDL, 18 January 2009)

In regard to any successful group, the question arises, what are the major factors that account for their achievement? In the social sciences, a potent concept of "network theory" is that individuals become imbedded in webs of social interactions and relations to succeed and endure. That is, the theory attempts to explain a myriad of social phenomena, from the creativity of an individual or group to the profitability of a corporation (Newman et al. 2006). Applied network analysis, with determination of the "social fabric" or degree of "centralization" versus "decentralization" of structure and interrelations, is believed to account for the topology of ties and the extent to which power and influence determine a given outcome (Borgatti et al. 2009). In the case of the Nuffield Institute group, many would agree that a "centralized" mode would describe best its operation, with Dawes as the "sun" in a solar system of talent. Of former Institute members, a number recall with affection tea time and the stimulus of sitting around the large circular antique table at the entrance to the Observatory. Here they would discuss and debate critical issues for research (e.g., see Robinson 1997). With his commitment to clinical relevance, Dawes was one of the founders of the "Neonatal Society of the United Kingdom." With the literal diaspora that occurred of bright young investigators in both basic science and clinical translational research, the influence of the Institute has extended beyond the UK to the USA, Canada, the European Continent, Australia, New Zealand, and the World.

In the memorial service held for Geoffrey S. Dawes on 19 July 1996 at the University Church of Saint Mary's, his long-time friend and collaborator Christopher W. Redman observed,

> And what was the essence of the man?
>
> He was strong-willed; some might have called him stubborn. There was a streak of ruthlessness within him. He rarely failed to finish a project that he had started, and had the

ability to hussle things along if they began to lose their momentum. Here his phenomenal reserves of energy—physical and mental—his toughness and resilience, were essential. For a man qualified to practice medicine he was unusual in his mathematical skills; where most clinicians flounder out of their depths he reveled in the precision of numeric analysis. His logic was that of the chess player; he moved forward in methodical stages. He took a position after careful appraisal of its validity and strength, then did not look back; his thinking had a forward momentum with an end-game always in mind.

Was he a humble man? Well yes and no. He never lacked in self esteem but was not vain. He had a straight forward estimate of his worth and saw no reason to hide it but neither did he call attention to it. As important, he was always quick to appreciate the worth of others and was generous in his praise of their achievements.

Was he a tolerant man? Well yes and no. He saw good in all those who, as was he, were honest, rigorous and careful in what they did. But thoughtless modishness, careless or sloppy thinking he could not abide and quickly said so.

His strong will was allied to a strength and vigour of mind that made him such a successful man. But he was totally without deviousness. Which did not necessarily make him always an easy colleague because he knew what he wanted and could be single-minded in its pursuit. . ..

So no longer will there come a light tap at my office door. And no longer will he appear with his pale blue eyes twinkling above half moon spectacles, below a shock of thick white hair, slightly hunched but nimble even in his late seventies, to slip into my room with the announcement: "May I disturb you. I have something absolutely fascinating to tell you." And it was true; he always did, some interesting further development in his thinking or analysis to reveal for discussion. He delighted in discovery, logically made, rigorously tested and proven to be robust. He made discovery exciting; our discussions were punctuated with explosions of his laughter, long peals of mirth marking pleasure in some humorous aspect that could always be shared. He would hunch his shoulders, rub his hands happily and gleefully chuckle "Isn't this fun!"

(Redman 1996a)

In a later obituary notice, Redman recalled Dawes' love for, and commitment to his wife Margaret and his two sons and two daughters. Redman concluded, "Of formidable intellect, great integrity and questioning spirit, he was also a kind and humorous man" (Redman 1996b, p. 18).

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Chapter 27
Early Years of the Society for Reproductive Investigation (Formerly Society for Gynecologic Investigation), the Fetal and Neonatal Physiological Society, and Several Other Groups

> *Another feature of the scientific attitude is organized skepticism, which becomes, often enough, iconoclasm. Science may seem to challenge the "comfortable power assumptions" of other institutions, simply by subjecting them to detached scrutiny.*
> *Most institutions demand unqualified faith; but the institution of science makes skepticism a virtue.*
>
> (Merton 1957, pp. 547)

Until the mid-twentieth century, academicians in obstetrics and gynecology concentrated their study primarily on the treatment of women's reproductive maladies and pregnancy. It is difficult to conceive of how elementary and empirical the craft was, even these few years ago. Despite the responsibility for care of the fetus as well as the pregnant mother, there was little interest in the basic biology of reproduction. Since that time, however, the specialty has matured as an academic discipline, joining departments of medicine, pediatrics, surgery, and others as a field making a serious commitment to both basic and clinical investigation of problems in its purview. By increasing the understanding of normal reproductive function and its regulation at the cellular and molecular levels, advancing the technology for diagnosis and treatment, and developing new approaches to therapy, academic obstetricians and gynecologists have dedicated themselves to the improvement of health care of women and their infants. In concert with clinical investigation, basic research has increased enormously our understanding of the regulation and interrelations of the maternal-placental-fetal complex and the relations of these functions to optimization of the course of pregnancy and to the life and health of the newborn infant.

L.D. Longo, *The Rise of Fetal and Neonatal Physiology*, Perspectives in Physiology,
https://doi.org/10.1007/978-1-4939-7483-2_27

27.1 Beginnings of the Society for Gynecologic Investigation, Now Society for Reproductive Investigation

In the USA, to a great extent, investigators on the cutting edge of these developments have been members of the Society for Gynecologic Investigation, whose motto is "science in the service of women's health." Founded in 1953 by a handful of American academic obstetrician-gynecologists interested in "Toxemia of Pregnancy" (preeclampsia and eclampsia), from initial studies of blood pressure, renal function, and water balance in the pregnant women, the Society has grown to a membership of about 1000, a third of whom are basic scientists. Rather than representing scientists from the USA alone, the Society has become international in scope, including many investigators from Canada, the UK, Europe, Australia and New Zealand, and the Far East. Although including reproductive scientists of all varieties, a large contingent of the membership are fetal and neonatal physiologists. From its simple beginnings, the Society has grown into a group of the world's leaders in the basic biology and clinical investigation in essentially all aspects of research that relates to women. Currently, in the modern era of molecular medicine, studies conducted by the members rely heavily on cloning, exploring gene regulation, the use of transgenic animals, tentative excursions into gene therapy, and related technologies of a brave new world of reproductive science. With the dawn of a new century and millennium, members of the Society have striven to keep pace with the rapid advances taking place in biotechnology, information technology, and an ever enlarging global scientific community. Of importance to academic medicine, Society members have contributed greatly to the building and development of centers of excellence in many of the University-based departments of obstetrics and gynecology and pediatrics, as well as to departments of physiology, biochemistry, cell biology, and others. In turn, individuals in these centers of research ferment have contributed greatly to the life of the Society.

Founders of the Society noted that it was organized "... to stimulate, encourage, assist, and conduct fundamental gynecologic research and to provide opportunities for investigators ... to enter into free exchange of ideas to the end of increasing knowledge and techniques in this field." It was intended primarily for those individuals who were essentially full time in teaching and research. Nicholas S. Assali, of the University of California Los Angeles, cofounder of the Society and its third president, has written:

> I saw it as a dynamic young society—and one that would remain so—where a presentation could be thoroughly discussed and dissected. Such an organization would serve as a forum to educate the young researcher and show him not only the good points of his data but also the deficiencies.
>
> (Assali 1979, p. 148)

It was the intention of the founders to keep the Society limited in size and youthful, as one member observed "... if the free-for-all discussion ... is to be maintained the group will have to be kept small, certainly under 100 and ideally

under 50. Then everyone will be well enough acquainted to speak freely and ask for suggestions on contemplated or unfinished projects" (Longo 1983, p. 34). Within the first 5 years, the membership had almost quadrupled, within a decade, there were about 100 members, and within two decades almost 200.

In the Society's original Constitution, the status of members was defined to keep the group young and vigorous.

> Each member of the Society shall, upon reaching his fifty-fifth (55th) birthday, automatically become an Emeritus Member of the Society. Emeritus Members shall be entitled to all of the rights and privileges of other members of the Society, except that they shall not be entitled to vote at any regular or special meeting of Members, nor to hold any elective or appointive office in the Society. Each Emeritus Member shall, upon reaching his sixty-fifth (65th) birthday, automatically become an Honorary Member.
>
> (Longo 1983, p. 39)

As has been noted elsewhere, several members of the Society played a critical role in the 1962 founding of the NICHD, meeting with the then Director of the NIH James Shannon and members of Congress (Longo 1983, p. 47ff).

In the summer of 2013, the SGI Executive Council commenced a strategic planning initiative regarding its future. Representatives from both SGI members and nonmembers were interviewed on their views. In addition, a survey was sent to the entire membership. One of the most common retorts was that SGI had an identity crisis and the name failed to describe "who we are." The strategic planning committee came up with several name variations and "Society for Reproductive Investigation" won by a unanimous vote. The official name change occurred on 1 July 2014. The premier scientific organization in obstetrics and gynecology, the now SRI, has almost 1000 members throughout the world. About one-third are basic scientists, and two-thirds are clinical investigators. SRI members conduct basic, translational, and clinical investigation in essentially every aspect of the reproductive sciences and women's health (http://www.sri-online.org/about-sri/strategic-plan).

27.2 Journal of Gynecologic Investigation/Reproductive Sciences

In 1955, the SGI Council first considered the publication of an official journal for the Society; however, little came of that suggestion. Nonetheless, with continued agitation for a journal of its own, almost a decade and a half later (1969), Walter L. Herrmann (1922–2012), then at the University of Washington, reported to Council that he had been in contact with S. Karger A. G., Basel, Switzerland, regarding a journal to be entitled *Gynecologic Investigation*. This would replace *Gynecologia*, a clinically oriented publication, and would serve as the official organ of the Society. Following approval by Council, and the members at the Spring 1970 meeting, *Gynecologic Investigation* became the Society's official publication (Longo 1983, p. 54).

Although Volume 1, Number 1 of *Gynecologic Investigation* carried a 1970 date, it did not appear until the following year. In this inaugural issue, the editor Herrmann asked a critical question and reflected upon the literature in the specialty. Because this editorial addresses ageless issues, it is reproduced below.

Who Needs a New Journal?

Discovering this latest addition to the already diverse array of gynecological journals may very well prompt the compulsive consumer of professional literary offerings to sigh, asking "who needs it?"—and move up to a large size waste basket. It is estimated that a new medical article is published every 26 seconds in the world literature. This would mean that in every 24-hour period an additional 3323 articles go into print. Fortunately, only a portion of these articles relate to obstetrics and gynecology. Nevertheless, a significant number of publications dealing with data of interest to the obstetrician are currently available, and this editor admits, along with many of his colleagues, that his hope of perusing and assimilating the material is usually greater than actual accomplishment. Furthermore, an admittedly cynical attempt to prove that (paraphrasing the *N.Y. Times*) not all the news fit to print is news, revealed some rather amusing facts. During the past 5 years there have been at least 78 published articles on Clomid, 542 on pregnancy tests, 543 on estriol, and 1228 on oral contraceptives. During the first 3 months of this year, 96 articles on oral contraceptives have already been published.

These facts alone perhaps would have discouraged us from the ambitious plan of remodeling *Gynecologia*, the Methuselah (1895) of gynecological literature with its modest circulation, into a first-rate gynecological journal. Some searching and introspection are required to justify this undertaking. With all due respect for the clinical practice of our specialty and the usefulness of statistical clinical studies, case reports, applied pharmacology, etc., we must recognize, along with other specialties, that experimentation in the basic sciences provides the key to progress. At the risk of being redundant, let me say that investigative aspects of medical science cannot be accepted as being "way over the head" of practicing physicians and particularly not for the physicians in residency training. But such an objective requires a recognizable forum for dissemination, for discussion and for criticism.

No such forum identified with Ob-Gyn is available at this time. Our scientists tend to publish the results of their better efforts in sub-specialty journals, inviting the challenge of highly discriminating, sophisticated, and rigorous editorial reviews, all for good reason: far more academic credit is given by peers, prestigious societies, and promotion committees for a paper published in the *Journal of Clinical Investigation* than one appearing in our more clinically oriented periodicals. Having had some papers accepted and others turned down by a variety of editorial boards, I can assure the uninitiated that there is a significant difference, with some of the following consequences: scientifically speaking, obstetricians and gynecologists are seldom recognized on equal footing with colleagues of some other clinical specialties; our residents too often shy away from reading anything that does not have the "practical" appeal; gynecologists loyally restricting their reading time to "their" journals are not aware of the contributions of their most distinguished colleagues. Surely there is the throw-away, the condensed, simplified, in plain-language communication for those who prefer a medical *Reader's Digest* instead of digesting and evaluating an original paper. Such "shorties" merely allow one to remember the name of the author and his conclusions. Whether such conclusions will hold water under the rigorous scrutiny of editorial review in the strict scientific tradition, or better, whether the data and analysis will meet your own criteria of validity, cannot be evaluated.

There is still another story. Curriculum revision in many schools has left Ob-Gyn largely as elective material. The interpretation of "Core" material (obstetrics for non-obstetricians) has reduced the educative process to one not too far removed of one

befitting a trade school. Here, too, identify of the specialty as a predominately scientific discipline is needed.

By the way, who in your school knows about the Society for Gynecologic Investigation? And where is there a uniform source of material for study for the resident preparing himself for the in-service examinations now being contemplated or for a better, more logical preparation for the Boards?

For an answer to the foregoing, admittedly with a fair dose of optimism—here is the proposition for the *Journal of Gynecologic Investigation*: scientific reports in reproductive biology, and related sciences. To be published in English only, we nevertheless plan for it to be an international journal, with an editorial board providing a true peer review and criticism, acceptance, or rejection of submitted material, to be determined by at least two referees and one of the editors.

Finally, for those who after all this still object to a new journal: in the spirit of the general concern with the population increase, before starting this one, we have buried another. We have only replaced ourselves.

(Herrmann 1970, pp. 1–3)

The first volume of *Gynecologic Investigation* published 25 papers, 23 of them from SGI members. However, during the following years, the number of manuscripts published per volume varied widely, and despite the editors' prodigious efforts, relations between the Society and the journal never went very well. Some of this discontent resulted from transatlantic delays in receipt of the bimonthly issues, ever-increasing subscription costs, and delayed publication. In retrospect, much of the dissatisfaction on the part of the members is difficult to define however. In the autumn of 1975, editor Herrmann notified the Society's Publications Committee of his intended resignation, and this was accepted at the Interim Council Meeting. Following this, the editors of the *American Journal of Obstetrics and Gynecology* agreed that two issues of that journal, to appear at six month intervals, would be devoted to SGI papers and that the manuscripts would be rigorously reviewed by the editorial advisory staff. Daniel Randolph Mishell, Jr., of the University of Southern California, became chairman of the SGI Editorial Advisory Committee, and the first SGI issue of the *American Journal of Obstetrics and Gynecology* appeared 15 May 1979 (Volume 133, Number 6) (Longo 1983, p. 54ff). Commencing in 1986, the Journal changed its publication cycle from two to one issue per month, with one of the monthly issues devoted to papers from Society members. Also at that time, Rogerio Arnaldo Lobo assumed responsibility for editing the SGI issue of the *American Journal of Obstetrics and Gynecology*.

Despite the efforts of editors, the years of this collaboration saw disagreements over the number of pages/issues devoted to Society papers, perceived delays in publication, a requirement that *Journal* subscriptions be mandatory for all Society members, and so forth. In 1988, the President Daniel Mishell appointed a committee to explore the feasibility of establishing a new and official Society journal. At the March 1989 Business Meeting, Frederick Naftolin presented his committee report. He noted that several issues justified the Society taking such action.

(1) The Society members need to publish their scholarly contributions more rapidly; (2) The *Journal* would enable the SGI to better fulfill its role as a flagship society representing the best of scholarship in the reproductive sciences. This point was believed to be critical as subspecialization in clinical and basic disciplines fragments research in

> reproduction and the recognition of health care for women as a discipline; (3) The *JSGI* would serve as an important public forum for the Society to express its point of view in academic and public debates; (4) The *JSGI* could be an important source of income for the SGI; (5) By allowing publication of non-members' papers introduced by SGI members, the *JSGI* would foster interest in reproductive research throughout the national and international scientific community; (6) The *JSGI* would serve as a timely site of publication for the high quality studies presented at the Annual Meeting; and (7) The *JSGI*, as a publication of the stature of the *Journal of Clinical Investigation*, would serve as an important yardstick of peer review for academic appointments and promotions.

Thus, at that 1989 meeting following almost four decades of hesitation, debates, discussion, and poll taking, the members finally voted to have their own journal. In January 1994, almost two decades following the cessation of *Gynecologic Investigation* (and during most of which time papers from the Annual Meetings appeared in the *American Journal of Obstetrics and Gynecology*), the *Journal of the Society for Gynecologic Investigation* first appeared, published by Elsevier Science Inc. (Longo 2000, p. S90).

In his celebratory essay "A Journal at Last!" in the inaugural issue, January 1994, Nicholas Assali effused:

> ... this decision is wise for the following reasons: 1) It definitely transforms the organization into "a year-round Society"; 2) it provides the membership, particularly the young, with an opportunity to read and examine some of the full papers that had been presented in abstracts, the experimental techniques of which have great teaching value; 3) it permits the Society to publish pertinent announcements and other matters of interest to the membership; and 4) it liberates the Society from arbitrary decisions taken by other publishing companies ....

Assali concluded:

> For the membership at large and nonmembers who are interested in the field of reproduction, their obligation consists of providing maximum economic, scientific, and moral support. This support is badly needed during the early part of the Journal's life. In addition, patience and understanding are essential from authors who submit manuscripts to be considered for publication .... May I wish you all good luck.
>
> (Assali 1994, p. 2)

In this premier issue, Editor-in-Chief Rogerio Lobo set forth the new journal's purpose and mission.

> The JSGI provides an opportunity to bring the field together by publishing seminal papers in reproductive biology. In so doing, the Society hopes that JSGI will become a primary resource in academic medicine, reproductive biology, and obstetrics and gynecology in particular.
>
> The mission of JSGI is to publish cutting-edge research in all aspects of reproductive biology. This will primarily be from papers presented at our Annual Meetings, but is not limited to this forum. Other quality papers from members and nonmembers will also be published. It is also our intention to publish important review papers in reproductive biology as well as occasional editorials and position papers. In our first year we will publish quarterly, and the number of volumes will increase in the years that follow.
>
> (Lobo 1994, p. 1)

In an editorial, Frederick Naftolin and Tamas Horvath pointed out the role of the new journal in "Providing a 'Noah's Ark' for Research in the Reproductive Sciences by Fostering Young Investigators: A Role for Our Journal." They wrote:

> For the past decade, the Society for Gynecologic Investigation has focused increasingly upon the development of young investigators in the reproductive sciences. The *JSGI* should do the same. We can identify those manuscripts whose authors performed their work under the support of young investigator programs, such as foundation fellowships, the Reproductive Scientist Development Program, the Clinical Investigator Development Award, and the National Institutes of Health First Award (R29) Program. We can develop a Young Investigators' Forum. We can serve as a clearinghouse for professional opportunities and support mechanisms. We can debate in print, and thus help develop the future structure of research and funding strategies in our discipline. There is much that can be done to stimulate research in the reproductive sciences until the rising tide of adversity recedes.

Naftolin and Horvath concluded:

> Now is the time to foster the young investigator and encourage progress and excellence in the reproductive sciences. We are privileged to have vehicles such as *JSGI* and the Society for Gynecologic Investigation to use in carrying this mission forward. Let us use them to their full extent.
>
> (Naftolin and Horvath 1994, p. 246)

Unfortunately, because of limited funds, this support for the RSDP lasted only a decade. With the assumption of Hugh Smith Taylor of Yale University as editor in January 2007, in addition, the name of the Journal was changed to *Reproductive Sciences*.

In conclusion, the Society for Gynecologic Investigation was conceived through the enthusiasm, drive, and sense of mission of a handful of investigative-minded clinicians and several basic scientists. In the main, they were associated with university departments of obstetrics and gynecology in the American Midwest. Their explorations of the "frontiers" of the specialty initially focused on the hypertensive disorders of pregnancy and rudimentary questions in reproductive endocrinology. To that end, beginning in 1953, each year, this coterie of "Young Turks" spent a day gathered around a table presenting their work in progress. Over the past half-century, the Society has evolved into a premier group of the leaders in the science of reproduction, growing into an international membership of basic scientists, physician-scientists, and clinical investigators from many disciplines. To my mind, the Society has developed and maintained a "Heritage of Excellence" in the reproductive sciences. Maintaining this heritage, the assemblage now numbers almost a thousand respected research workers who gather to present sophisticated, relatively complete work on the cutting edge of biomedical science. A significant proportion of these studies of "science in the service of women's health" concern fetal and neonatal physiology.

Several other societies have worked to advance the field of fetal and neonatal physiology. In the USA, this includes the Society for Pediatric Research (founded in 1929 as the Eastern Society for Pediatric Research), and of which several accounts have been written, including publication of the journal *Pediatric Research* (Bellanti 1980; Weil 1996). In 1959, the "No Name Society" was organized by

Charles Henning Hendricks (1917–2010) then at the Western Reserve University (now Case Western Reserve University) in Cleveland, OH. The Society is distinguished by having no members (attendance at meetings is by invitation only from that year's organizer, and all attendees must present provocative unpublished data for discussion), no constitution, and no bylaws (a framed blank piece of paper). The key element is unlimited discussion with stimulating new ideas. In its formative years, clinician-scientists comprised the majority of the two dozen or so invitees, while presently most are basic scientists in various disciplines of the reproductive sciences. In 1970, the beginning of the Perinatal Research Society (PRS) saw a group of about 150 rather evenly divided, with one-third each physician-scientists in perinatology and neonatology, while the remaining third being in the basic sciences. In the UK, the Paediatric Research Society serves a similar function (Cosgrove, http://www.prs.nhs.uk). At a global level, the World Association of Perinatal Medicine held its *First World Congress* in 1991 in Tokyo (Sakamoto and Takeda 1992). This gathering of several hundred obstetrician perinatologists and pediatric neonatologists from various countries meets every 2 years or so at major centers, the most recent *Tenth World Congress* was held in 2011 in Punta del Este, Uruguay.

27.3 The Fetal and Neonatal Physiological Society

Because it is to Geoffrey Dawes that the Fetal and Neonatal Physiological Society (FNPS) owes its existence, it is only appropriate to consider its origin. As an outgrowth of his studies, in 1974, Dawes organized the first "Conference on Foetal Breathing" in Oxford, UK, which included a group of only about a dozen individuals interested in this physiologic function. Over the next six years, meetings were held in Oxford, and on the Continent at Malmö, Sweden, Nijmegen, the Netherlands, Paris, France, and again in Oxford (Tables 27.1 and 27.2). As the number of obstetricians and pediatricians with an investigative bent increased, they joined developmental physiologists to the group's ranks, as they studied factors that influenced fetal breathing movements such as the incidence, periodicity, and role of maternal smoking and hypoxia, low blood sugar, and others. Although it became clear that the rate of fetal breathing decreased significantly in response to these and other stressors, the mechanism(s) of the regulation of these movements, under either normal or abnormal conditions, eluded explanation. By recording fetal brain electroencephalic activity and eye movements, it became clear that during breathing movements, the fetus alternated between states of low voltage, high frequency EcOG during which the rapid eye movements and breathing movements were predominant and high voltage, low frequency state during which breathing and eye movements were suppressed. Still later, it was demonstrated that limb (arm and leg) movements accompanied these other activities. Thus, Dawes and the Oxford group developed the idea of "sleep states" or "behavioral states" for the developing fetus. In addition to the associations noted above, these behavioral "states" changed as the fetus became more mature from 16 to 40 weeks gestation.

Table 27.1 Conference on Foetal Breathing

Year	Venue
1974	Oxford, UK
1975	Oxford, UK
1976	Malmö, Sweden
1977	Oxford, UK
1978	Nijmegen, the Netherlands
1979	Paris, France
1980	Oxford, UK

Table 27.2 International Conference on Foetal Breathing

Year	Venue
1981	Maastricht, the Netherlands
1982	London, Ontario, Canada
1983	Malmö, Sweden

Their geographic base represented by attendees also expanded. In 1981, the group changed its name to "International Conference on Foetal Breathing" meeting over several years in Maastricht, the Netherlands; London, Ontario, Canada; and Malmö, Sweden (Tables 27.1 and 27.2).

In regard to the 1982 Meeting at the University of Western Ontario in London, Ontario, Mark A. Hanson has recalled regarding Dawes:

> . . . as someone who worked closely with him (although I was never directly employed at the Nuffield Institute in Oxford) my memory is of an incredibly kind and supportive person who did much to encourage young investigators such as myself to pursue contemporary questions in fetal physiology. When I first showed Geoffrey some of our fetal chemoreceptor data, he urged me to present it at the FNPS in London, Ontario. I explained that I would love to go but unfortunately my research grant was relatively small and did not include a travel allowance. He immediately got out his cheque book and wrote me a personal cheque to cover the airfare. Thus began my real association with FNPS.
>
> (Letter from MAH to LDL, 12 March 2009)

At the 1984 meeting in Oxford, the topics of interest expanded beyond fetal breathing activity to include endocrine, cardiovascular, neurobiological, and other aspects of fetal and neonatal growth and development. In view of its enlarged scope, the group again changed its name to the "Society for the Study of Foetal Physiology." Over the following 4 years, meetings were held at Haifa, Israel; Banff, Alberta, Canada; Groningen, the Netherlands; and Cairns, Australia. At the 1989 meeting in Reading, UK, a new feature was introduced, that of inviting an outstanding leader in the reproductive sciences to present a plenary lecture. It should be noted, however, that this was not accepted without some controversy. Many members believed that the meetings should consist wholly of short, 15 min free communications of cutting-edge research by attendees, including postdoctoral fellows and graduate students, rather than a "canned" lecture by some supposed notable. Despite such difference of opinion, the first of these special lectures was by Elizabeth Anne Linton of the University of Reading, on corticographic releasing factor in pregnancy. Over the next few years, this special lectureship was continued (see Table 27.3).

Table 27.3 Society for the Study of Foetal Physiology

Year	Venue	Speaker	Title
1984	Oxford, UK	–	–
1985	Haifa, Israel	–	–
1986	Banff, Alberta, Canada	–	–
1987	Groningen, the Netherlands	–	–
1988	Cairns, Australia	–	–
1989	Reading, UK	Elizabeth A. Linton University of Reading	Corticotrophic Releasing Factor in Pregnancy
1990	Asilomar, Pacific Grove, California, USA	Betsey Rasmussen	Reproductive Chemical Communication in Elephants, Other Mammals and Fish
1991	De Eemhof, the Netherlands	Dick F. Swaab University of Amsterdam	Development of the Human Hypothalamus in Relation to Birth
1992	Niagara on the Lake, Ontario, Canada	Alan Bernstein University of Toronto	The Doctor's Dilemma: Molecular Biology and the Future of Biomedical Research
1993	Plymouth, UK	Euan Brown Stazione Zoologica Anton Dohrn, Napoli	Squid, Sex and Science in the Southwest: Marine Biology with a Medical Slant in Plymouth
1994	Palm Cove, Australia	–	–
1995	Malmö, Sweden	Geoffrey S. Dawes Oxford University	Control of Fetal Behaviour

Among those who did not work with Dawes at the Nuffield Institute but fell under his spell was Dino Antonio Giussani, of Cambridge University. Giussani has recalled some details of the 1991 meeting of the FNPS:

> . . . one of my fondest and most cherished memories . . . was at my first FNPS meeting in *De Eemhof* The Netherlands in 1991, soon after completing my first year of PhD training. Being South American, I remain convinced to this day that one of the main reasons for Mark Hanson employing me was because of my footballing skills, as in *De Eemhof* the traditional sporting event was to be football. Having been introduced to the 'ins and outs' of the Society by endless anecdotes told by the then Post-Docs of the lab . . . you can imagine that I looked forward to participating at these social events so impatiently. I always wondered what would be the effect of putting several driven scientists at the same time in the same sporting field and, needless to say, often pondered whether that would be such a good idea. Well, at 5 o'clock in the afternoon of May 8th 1991 at Center Parcs *De Eemhof*, one of the main organisers of that meeting, Dr Jan Nijhuis, placed a whistle around Geoffrey Dawes' neck and asked him to Referee THE football match. I cringed having realised that I was placed on the same side as Mark Hanson and Carlos Blanco, both of whom wore rather high-cut running shorts and certainly did not look the part! I also remember that the 'opposition' was particularly ominous-looking, with players such as Bill Gilbert, Edu Mulder, Hugo Lagercrantz, Bob Brace and Gerry Visser. The game started and rather than a sport, the activities of both teams better resembled a very poor effort of trying to illustrate the application of the chaos theory to fetal heart rate variability. Almost 20 minutes into the game, John Spencer on our side crossed the ball with unexpected

accuracy into the opposition's penalty area. Mark Hanson dove hopelessly head-first into the vicinity of the area pretending to make contact with the ball, only to be met by the boot of Hugo Lagercrantz which he had raised in Bolshoi-style well above the height allowed. Geoffrey blowed the whistle with deafening volume, Mark ended up with a double rib fracture and Hugo was concussed for a while. Five minutes later, the game carried on. It was at that precise moment that I knew I had made the right choice of subject and that fetal physiology would remain part of my entire life. Letter from DAG to LDL, 20 July 2009)

At the August 1994 21st Annual Meeting in Cairns, Australia, the first of the meetings that I attended, although unable to be present, Dawes wrote a letter to the attendees on origins of the Society. This is given as follows:

Between 1970 and 1975 one of the interests of the Nuffield Institute for Medical Research, Oxford, was the exploitation of the rediscovery of normal fetal breathing movements in utero, in sheep and man. Acute hypoxia caused an arrest of fetal breathing, in contrast to the hyperventilation seen in normal adults. Was this helpful in identifying sick fetuses? Could the technology be improved to record breathing movements more directly, or more accurately, using ultrasound? On April 25, 1975 we held a one-day meeting, to which visitors with skills in ultrasound technology or in clinical obstetrics contributed, as well as the laboratory staff, about three dozen all told. The discussion proved useful, and it was decided to hold another meeting on 3 October 1975, the day before a meeting of the Blair Bell (obstetric research) Society was due to be held in Oxford to obtain the views of more people, especially from overseas. The discussion again ranged widely over the physiological, pathophysiological, clinical and technical problems of recording fetal breathing movements. The introduction of real-time ultrasound was about to revolutionise the clinical scene and reveal the fetus in utero for all see. But alas, no record was kept of the discussion, and I do not even have a list of the participants of these two meetings.

As a consequence Gerhard Gennser and Karel Marsal organized a further meeting "The Third Conference on Fetal Breathing" in Malmö, Sweden on 8 June 1976. In their report of the proceedings they quote Lord Kelvin [First Baron Kelvin, Title of William Thomson (1824–1907)] "I often say that when you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind; it may be the beginning of knowledge but you have scarcely, in your thoughts advanced to the stage of science, whatever the matter may be" [(Thompson, Letter to the Institution of Civil Engineers, 3 May 1883)]. Gennser and Marsal go on to say that "The significance of the present Conference, the third to be held. . . lies, in our minds, in emergence of techniques yielding valid results and in the clinical application of the measurements. This is no minor achievement since less than 5 years has passed since Boddy and Robinson published the first ultrasound recording of human fetal breathing."

The meetings settled at once into an annual pattern, within Europe. The first outside Europe was in London, Ontario, Canada (1982) with which there were close connections to Oxford through many exchanges of visiting scientists. [I now have a red-headed grandson studying there, in the University of Western Ontario and hope thereby to keep contacts with old friends]. There was, from the beginning, pressure to widen the scope of the meetings, especially from Geoff Thorburn. By the eighth meeting, at Maastricht (Holland) in 1981, it had become an international conference "on fetal breathing and other measurements." By 1984 it was a meeting of the Society for the Study of Fetal Physiology. There were 34 oral communications and 26 posters at this meeting in Oxford ten years ago, covering a very wide range of fetal studies in animals and man. Nevertheless the fetal applications of molecular biology were slow to appear. Will they have a revolutionary effect? You must tell me.

Indeed I am desperately sorry to miss this meeting, the first I have not attended, the 21st in the 20th year. Hurrah for the Fetal and Neonatal Physiological Society I shall try to come next year.

> Now to more serious things. Tom Kees Anton Bonifacius Eskes (1933–2011) and Lawrence Longo have recently selected a group of classic contributions of "innovative papers"[1] But they have omitted all reference to a transient and fascinating art from, which illumined the hearts and minds of all good men, that is, you and me. Some of our early meetings were enlivened by elegant draughtsmanship, on the outer cover of the volume of Proceedings. That in Malmö (1976) had the human fetus, in 5 stages of development, discussing its situation in Latin. By 1979, in Paris, it had broken free of the mother and was happily snorkeling in the amniotic fluid. Was this The Concept of Fetal Autonomy? A year later, in Oxford a thoughtful ewe was sitting on her rump, listening through a stethoscope to the fetal heart, taught no doubt by Jeff Robinson, who can do anything with sheep. There are other ideas to conjure with, now we know that the mature fetus can hear all that a mother says. I plead with you not to let this form disappear. What should we be thinking of next year? The younger and the more irreverent the draftsman the better. Don't we also need a LOGO? And a shortened title, such as International Fetal and Neonatal Physiological Society or INFANTS. That word is happily derived from the Latin, and means "inability to speak."
>
> *Au Revoir* Geoffrey Dawes (Personal Communication)

At the 1995 Malmö meeting, a year before his death, Geoffrey Dawes reviewed his and others' work on the control of fetal behavior. It was at the 1996 meeting in Arica, Chile, the first in South America, that the designation of the Society was changed again. This time to the more inclusive "Fetal and Neonatal Physiological Society." A mission statement was adopted that included the following:

1. The Fetal and Neonatal Physiological Society encourages research and dissemination of knowledge in the field of fetal and neonatal physiology, broadly defined.
2. The Organizational Coordinator will be selected by the Organizational Committee and shall serve for 3 years.
3. The Organizational Committee shall consist of representatives from Africa, Asia, Australasia, Canada, Continental Europe, South America, the UK, and the USA and shall be selected by the Committee.
4. The annual meeting will be held in Europe, North America, and the Southern Hemisphere, in approximately equal proportions, in June–September, as determined by the Organizational Committee.
5. The membership list of the Society shall consist of those participants registered for any of the past three meetings, and this shall serve as the mailing list for the subsequent meeting.
6. Any residual funds from prior meetings shall be passed on to the Coordinator of the next meeting. Audit will not be required if the residual funds are <US $10,000.
7. The Organizing Committee shall have the right to solicit funds in the name of the Society from organizations, for the purpose of providing financial support for students and fellows-in-training to attend meetings.

Following the Africa meeting, annual gatherings were held in Italy, the USA, and the Netherlands (see Table 27.4). It was at the 1998 meeting, 2 years following

[1] Here Dawes refers to Eskes and Longo, 1993.

Table 27.4 The fetal and neonatal physiological society Geoffrey S. Dawes lecture

Year	Venue	Lecturer	Title
1996	Arica, Chile	Peter W. Hochachka University of British Columbia	Deep, Prolonged Diving in Large Seals: Metabolic Marvels and Metabolic Mysteries
		Alan H. Jobe Harbor-UCLA Medical Ctr	The Future of Surfactant and Surfactant Research
1997	Santa Margherita Ligure, Italy	Alberto Piazza University of Turin	A History and Geography of Human Genes
1998	Lake Arrow-head, California, USA	Jack L. Feldman University of Califor-nia, Los Angeles	Pre-Botzinger Complex: the (proposed) Site of Respiratory Rhythmogenesis
1999	Vlieland, the Netherlands	Christine L. Mummery Netherlands Institute for Developmental Biology	Embryonic Stem Cells and Cloning in Humans: Present and Future
2000	Southampton, UK	David J.P. Barker Univ. Southampton	The Biological Origins of Coronary Heart Disease
2001	Auckland, New Zealand	Ruud Kleinpaste New Zealand Entomologist	Bugs in the System
2002	Prague, Czech Republic	Anthony M. Carter Univ. Odense	Phylogenic Aspects of Placental Development
2003	Banff, Alberta, Canada	Peter D. Gluckman Univ. Auckland	Fetal and Perinatal Brain Injury: The Development of Therapeutic Approaches
2004	Tuscany, Italy	L. Angelo Vescovi Univ. Milano- Bicocca	Functional Properties of Neural Stem Cells and their Therapeutic Potential
2005	Glenelg, Australia	Jeffrey S. Robinson University of Adelaide	The “Fatal Fetus”
2006	Cambridge, UK	Stephen O’Rahily Univ. Cambridge	Human Obesity and Insulin Resistance: Lessons from the Extremes
2007	Sendai, Japan	Hitoshi Oshitani WHO Western Pacific Regional Office	Avian Influenza and Pandemic Threat
2008	Maastricht, the Netherlands	S.E. Buitendijk TNO Netherlands	Measurement of Perinatal Mortality in the European Context
2009	Lake Arrow-head, California, USA	Lawrence D. Longo Loma Linda University	Geoffrey S. Dawes and the Rise of Fetal and Neonatal Physiology
2010	Winchester, UK	Colin Sibley Univ. Manchester	Placental Physiology in the 21st Century: Using the Knowledge
2011	Palm Cove, Australia	Richard Harding and David Walker Monash University	From Oxford to Melbourne, Two Extraordinary Contributions
2012	Utrecht, the Netherlands	Gerard H. A. Visser UMC Utrecht	An Exciting Time for Obstetricians

(continued)

Table 27.4 (continued)

Year	Venue	Lecturer	Title
2013	Puerto Varas, Chile	Anibal J. Llanos and Maria Seron-Ferre Universidad de Chile	A Lecture *Al AlimÓn* on Low Oxygen and Light-Melatonin
2014	Saint Vincent, Italy	Maria Lodovica Gullino University of Turin	Emerging Problems in Plant Pathology: Alien Species and Human Pathogens
2015	Vancouver, Canada	Stephen Lye University of Toronto	The Initiation of Labour—New Understanding from an Old Hormone

Dawes' death, that Brian J. Koos of University of California, Los Angeles, who had organized the meeting, elected to use funds collected in excess of expenses to establish a keynote address, designated the "Geoffrey S. Dawes Lecture." The first of these presentations was by Jack L. Feldman of University of California, Los Angeles. At the 2002 meeting, held in Prague, Czech Republic, the mission statement was modified somewhat, to state, "The FNPS stimulates discussion and exchange of ideas between physiologists, obstetricians and neonatologists. The FNPS considers an informal gathering and presentation of new and preliminary data, especially by investigators in training, essential to achieve goals."

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Chapter 28
The Reproductive Scientist Development Program and Related Programs

The pursuit of science can be organized. . .in no other manner than by granting complete independence to all mature scientist. They will then distribute themselves over the whole field of possible discoveries, each applying his own special ability to the task that appears most profitable to him. The function of public authorities is not to plan research, but only to provide opportunities for its pursuit. All they have to do is to provide facilities for every good scientist to follow his own interest in science.

(Polanyi 1951)

The Republic of Science shows us an association of independent initiatives, combined toward an indeterminate achievement. It is disciplined and motivated by serving traditional authority, but this authority is dynamic; its continued existence depends on its constant self-renewal through the originality of its followers.

The Republic of Science is a Society of Explorers. Such a society strives toward an unknown future, which it believes to be accessible and worth achieving. In the case of the scientist, the explorers strive toward a hidden reality, for the sake of intellectual satisfaction. And as they satisfy themselves, they enlighten all men and are thus helping society to fulfill its obligation toward intellectual self-improvement.

28.1 Introduction

In comparison with several major specialties—internal medicine, pediatrics, surgery, and others—historically basic, translational, and clinical research in obstetrics and gynecology has been severely lacking. This is evident in terms of NIH-funded grants for both research and training (Longo 1992). In an effort to correct this deficiency, in 1988 the Reproductive Scientist Development Program (RSDP) was initiated as a joint venture of the NIH and several professional societies in the specialty. The goal of this program was to support young obstetrician-gynecologists

L.D. Longo, *The Rise of Fetal and Neonatal Physiology*, Perspectives in Physiology,
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for 2 or 3 years of basic science education in cell and molecular biology, working under a world-class scientific mentor. This experience, followed by a 3-year period to establish their laboratory research program as a junior faculty in one of the departments of obstetrics and gynecology, was conceived to help prepare them for a productive career in academic medicine.

28.2 The Reproductive Scientist Development Program

The immediate objectives of this program, the RSDP, as it was later termed, were to increase the awareness and attractiveness of a career in investigative obstetrics and gynecology. Originating objectives were also to facilitate the research career development of obstetrician-gynecologists in approaches and techniques of contemporary cell and molecular biology, to foster the productivity of scholars by guaranteeing their placement as faculty members in medical school departments, and to inculcate in the scholars the value of hard work, perseverance, self-reliance, and respect for others engaged in basic, translational, and clinical research endeavors. The overall goals were to strengthen the field of obstetrics and gynecology for all those professionals involved, whether their emphasis be on clinical patient oriented or basic research, and to improve health knowledge and care for all women and their children.

From a historical perspective, innovative developments and advances in obstetrics, gynecology, and the reproductive sciences have resulted from research in the basic sciences. Therefore, the chief objective of the program was to emphasize education in biochemistry, biophysics, cell biology, immunology, and molecular biology through direct and intimate involvement in ongoing research with outstanding investigators, complementary formal course work, and a combination of seminars and conferences.

The program plan emphasized the education of the most promising independent scholar-investigators who could develop into leaders in the reproductive sciences. Program scholars were to seek an environment in which creative thinking about fundamental problems is encouraged and innovative approaches to developing hypotheses and experimental solutions are fostered. As is the ideal in science, the approach has been to inculcate a “problem,” rather than a “method” orientation. The RSDP also fostered an understanding of fundamental mechanisms of function, rather than merely a descriptive or phenomenological accounting of data.

Phase I consists of 2 or 3 years of basic research education in cell and molecular biology with relevance to reproductive medicine. During this period, the scholar was required to work full time in the laboratory. Scholars in the program have worked and are working, in some of the most distinguished scientific laboratories in the world. Several have worked with Nobel laureates and many with members of the US National Academy of Sciences.

This full-time basic science phase was to occur in any well-established research laboratory with a nationally recognized productive scientific leader. Originally, we

believed that for optimal career development, the scholar should work in a laboratory/department different from his/her home institution. As it worked out, because of spouses' employment, children's schools and many other nonscientific factors, few scholars moved from their mother institution. Nonetheless, the quality and depth of this research experience is the essential feature. As noted, during this initial basic science phase, support has been provided through the NICHD and matching donor organizations.

Phase II followed this 2 or 3 years of intensive basic research. During this second phase of 3 years, the young physician-scientist assumes a junior faculty position in a Department of Obstetrics and Gynecology. The sponsoring department provides full salary and guarantees that the individual will devote at least 75% of his/her time to research. The remaining time can be spent in teaching and clinical service. To optimize efficiency, however, this should be structured to least encroach on time in the laboratory. Of course, this phase requires that the scholar be provided adequate space, equipment, and technical support. In addition, the scientific advisor during Phase I and/or other outstanding investigators should continue to mentor the scholar. At the end of Phase II of career development, many of the scholars are in an excellent position to compete for further funding from NIH and/or from private and public foundations to continue his/her research endeavor. The Program also provides continued career guidance and periodic review of the scholars' progress. Additional support may be sought by the scholar and the department from NIH sources such as a Clinical Investigator Award, First Award, or traditional Research Project Grant.

28.3 Some Personal Reminisces

In this volume, I have been careful to be self-effacing, not interjecting my person other than attempting to place various contributions in the context of their time and commenting on their significance. Because of my very deep personal involvement and commitment to the RSDP, perhaps I may be forgiven for this exception. As noted, a major issue in academic medicine in America is the relatively small numbers of individuals seeking careers as physician-scientists. Almost each of us in medicine wants to be a healer of the highest caliber. Because of pressures of economics and clinical practice, other than the occasional MD-PhD, relatively few MDs make the commitment to advance the frontiers of medical service, spending the years required to become a competent and competitive biomedical scientist. In academic obstetrics and gynecology, the situation is particularly grave. Although considered to be one of the major fields of endeavor—perhaps second only to internal medicine, pediatrics, and general surgery in numbers of specialists—in comparison with those other specialties, few obstetrician/gynecologists have the background and expertise to serve as physician-scientists.

In great measure, it was because of this disconcerting lack of young academicians well trained in contemporary biology that a small group of us initiated a

program in an attempt to correct this deficit. At the September 1986 meeting of the American Gynecological and Obstetrical Society (AGOS) at The Homestead, Hot Springs, Virginia, six of us (Robert B. Jaffe, Chairman of the Department of Obstetrics and Gynecology and Reproductive Sciences at the University of California, San Francisco; Warren H. Pearse, Executive Director of the American College of Obstetrics and Gynecology (ACOG); Edward E. Wallach, Chairman of the Department of Obstetrics and Gynecology, the Johns Hopkins University School of Medicine; James C. Warren, Chairman of the Department of Obstetrics and Gynecology, Washington University, St. Louis; Gerson Weiss, Chairman of the Department of Obstetrics and Gynecology, Medical College of New Jersey; and I) met together over breakfast. Our goal was to develop a scheme to educate some scientifically based academicians for the future. To support the program, Bob Jaffe suggested that I be chosen to develop the idea, and to prepare the grant application for submission to the National Institutes of Health. (Although this was a compliment and an honor, I was probably elected to do this as I was perceived as the one of the group not having a "real job.")

The following several months, I spent in intense thinking, analysis, and writing. From time to time, I circulated a draft of the proposal to my fellow colleagues. My bias was that the education (training) of reproductive scientists should be an intense immersion of 2 or 3 years in the laboratory of a world-class cellular/molecular biologist. In part, this was because of my role at Loma Linda University in helping to formulate the new molecular biology-based Medical Scientist Training Program for medical students who wished for a career in academic medicine. The centerpiece of this Medical Scientist Training Program was 3–4-year education in cell/molecular biology, in addition to their 4-year medical course, so that the students would obtain a doctorate (PhD) in addition to the MD and become physician-scientists. Over a period of several months, particularly with the help of Fred Naftolin, I fine-tuned the RSDP application, focused it, and prepared it for submission to NIH by the 1 July 1987 deadline.

In addition, in discussions with the NICHD Director, Duane F. Alexander, M.D., he had made it clear that the only way the Institute would fund such a program would be if we obtained "matching funds" from non-NIH groups. Thus, it was that I traveled around the country attending meetings of the American Gynecological and Obstetrical Society, the Association of Professors of Obstetrics and Gynecology (APGO), the American College of Obstetricians and Gynecologists, the Society for Gynecologic Investigation (SGI), and several other societies, meeting with their Boards of Directors, and requesting help to support the program. Our idea was that if we could get these professional organizations to each contribute $25,000 toward the support of a scholar, with $50,000 per scholar from the NIH, the $75,000 would support salary ($45,000), laboratory supplies ($10,000), their medical insurance, etc.

A major problem was that we had no official sponsor for the program. In contrast to the academic pediatricians, who had the organizational clout of the American Society of Pediatric Chairmen, Inc. (AMSPEDC), none of the professional societies in obstetrics and gynecology wished to assume responsibility for such a fledgling

(and many thought illusory and quixotic) program. As a corollary, and the "Catch 22," was that we had no budget. Thus, I had to make a tough decision. Did I believe in this idea enough to pay my own way to fly around the country trying to convince the various professional groups of the importance—and long-term value to the specialty—of this program? The decision was really a "no brainer." I bit the bullet and decided to give it a shot, in addition to the financial commitment (on nine trips back and forth to Washington, DC, New York, Hot Springs, VA, New Orleans, and Chicago). Not being genetically programmed to be a fund-raiser, in a sense I felt somewhat like the traveling salesman Willy Loman (in Arthur Miller's *Death of a Salesman*) pleading my case before my rather tough, skeptical peers, hat in hand, going from one group to another, making my pitch, trying to justify 2- or 3-year laboratory training/education for individuals who had completed a residency in the specialty, and in most instances, subspecialty fellowship. It wasn't easy.

In retrospect, with considered naiveté I had anticipated that the leaders in the specialty would greet this idea with enthusiasm. That was not the case. Several individuals on these committees recounted how they had worked night and day to support themselves for their graduate education and postgraduate training, so why should it be any different for someone who "decides to become a physician scientist." Several suggested that I was, in fact, a bit loony. As one individual expressed it, "no one in their right mind would want to subject themselves to the kind of laboratory training you are proposing after they had completed four to seven years of clinical specialty/subspecialty residency/fellowship. If they are that crazy, they should see a Psychiatrist." Others suggested that the program I outlined was "elitist" and had no place in a democratic society. Several maintained that obstetrician/gynecologists should be doing what they are trained to do, taking care of women and delivering babies, rather than trying to be bench scientists. Overall, it was an edifying, although somewhat depressing, experience.

Nonetheless, three of the groups signed on—each agreeing to support one of the scholars for their 2 (or 3) years of Phase I laboratory experience. Another of my most loyal and helpful supports along this line—bless his soul—was Warren Pearse. After meeting with the ACOG board, Warren committed the College to support one scholar with matching funds. Every month or two, I would call Warren to apprise him on where I was with the fundraising. He continued to remind me that we were doing the right thing and encouraged me to stay on track. Another individual who was most supportive was William A. Sadler, PhD, Chief of NICHD's Reproductive Sciences Branch (RSB). Bill Sadler was frank to admit that while he did not have great enthusiasm for the program or its long-term viability, nonetheless, he would be supportive. This is because "The Director wants this to happen." Following Dr. Sadler's departure from the NICHD to become a Dean at Howard University, Michael E. McClure became Chief of the RSB. He also proved to be of enormous help in keeping our Program—and the scholars—on track.

In October 1987, several months after submitting the proposal, an NIH administrator Scott Andress, PhD, called to state that an ad hoc committee would meet several weeks later in Bethesda to review the application. He requested that I, with

several colleagues, come to the meeting for a "reverse site visit." Thus, it was that in early November at our own expense, four of us, Fred Naftolin, Warren Pearse, Gerson Weiss, and I, went to Bethesda for the interview process. The reverse site visit with the committee was a total disaster, or at least I perceived it to be so. The 13 member committee consisted of a mixture of pediatricians, psychologists, molecular biologists, and an experimental pathologist (there was only one obstetrician gynecologist). They were anything but friendly. They wanted to know why we wished to initiate this type of program. Who did we think we were in believing that obstetrician-gynecologists should be educated in basic research, rather than delivering babies as was their forte? "Isn't science in obstetrics and gynecology an oxymoron?" one committee member asked. A major concern that required resolution was the issue of oversight. How were we going to insure that the scholars would really work in the lab and not spend most of their time engaging in clinical work? A related issue concerned having the trainees (as they were called initially) work in various universities, rather than being centered at the NIH? Why did we wish to organize the Program in such a fashion? Why did not we have more explicit commitments from some of the basic scientists—potential mentors whom we had contacted—regarding their role in the program? Why did we have the *chutzpah* to think that world-class scientists would want—or allow—an obstetrician-gynecologist to work in her/his laboratory? Why did we seek to use an NIH training mechanism (K12) intended for university departments separate from individual physician-scientist training grants (K08) to support the program? An additional criticism was that we had "confused the funding" and "politicized" the application by obtaining the commitments for matching funds from the several professional organizations (ACOG, AGOS, and APGO) that had "representatives" on the Executive Committee. In fact, this was what we had been directed to do; and I tried to reassure the reviewers that we believed that because these individuals were leaders in the specialty, they could maintain their objectivity while still fulfilling their role as a representative.

The reviewers did not appear to be convinced. There were several other questions about the proposed Executive Committee. How would these individuals, who in some cases represented the sponsoring organizations, maintain their independence from those groups? The essence of the concern was that there be "no strings" attached to the support given by the several groups. There also was a criticism that we had failed to include specific plans to recruit women and minorities into the Program and that the Program would not appeal to gynecologic oncologists. In addition, several reviewers expressed concern about how during Phase I of laboratory experience the scholars could fulfill the requirements of the specialty and subspecialty Boards for a given number of patient cases within the strict time limits following completion of clinical training. (That turned out to be a difficult issue and was only resolved after 4 or 6 years. Basically, the Board of Directors decided that the RSDP scholars would be in a "time cocoon" during Phase I and/or Phase II, so that "the clock would not start to tick" until they had resumed some clinical responsibilities.) Another major criticism (and one with which we all agreed) was

that we had no sponsoring body. We were simply an ad hoc group of quasi-entrepreneurs without official sponsorship. The program would succeed or fail depending upon us!

The "star chamber" examination/grilling went on for almost 3 hours. Finally, Dr. Andress excused us so that the committee could deliberate upon the matter. The four of us had a cup of coffee together to commiserate over the fiasco and then went our separate ways returning home.

Upon returning to Loma Linda, I drafted a letter to Dr. Andress expressing my misgivings about the hostile review we received. At the outset, I pointed out that I was writing with much hesitation. I had been the recipient of an NIH fellowship, special fellowship, research career development award, several individual (RO1) grants, a program project (P01) grant, and a training grant. In addition, I had served on several NIH committees including the Maternal and Child Health Training Committee (1971–1975). I also had served on the Public Affairs Committee of the American Physiological Society (1971–1977), had testified before five Congressional Committees, and had played an active role in lobbying for increased NIH funding by Congress. In addition, on several occasions I had met with the Presidential Science Advisor and individuals from the Office of Management and Budget to lobby the Executive branch. During all of these years, I had been a staunch supporter of the peer review process. Nonetheless, I believed strongly (and was supported by the three colleagues who accompanied me) that the reverse site visit was grossly unfair and that much of the discussion concerned issues that were irrelevant to the application. I pointed out some of the lines of questioning which we believed were inappropriate. I noted that "this was, in fact, an application for an institutional physician scientist award per the expressed instructions of the NICHD Director. Also I noted that I was specifically instructed by the Director to obtain matching funds from private sources, hence the "confused funding" from the several professional societies of obstetricians and gynecologists. As outlined in the proposal, the three abovenamed academic organizations committed themselves to partial funding ($25,000 each) of four fellowship positions for the 1988–1989 academic year. I also pointed out that I had sought support from other groups including several pharmaceutical firms but was informed that they were not interested in supporting education in basic research with little or no perceived clinical application. In addition, I noted that I had solicited support from the March of Dimes Birth Defects Foundation but was told that their commitment "...was to Pediatrics, not Obstetrics and Gynecology." Thus, despite accomplishing what frankly I had thought was rather unlikely, i.e., obtaining matching monies; when I did accomplish that feat, it was turned against us as a "political ploy." I noted to Dr. Andress that some of the questions regarding the proposal indicated to those of us "testifying" that few of the reviewers had actually read the proposal. "Quite frankly," I wrote. "I found it shameful and a cause for embarrassment for the committee that many of those who voted to give the application a low priority score had not taken the time or effort to read it. Again, as a former member of this committee, how in good conscience can members make critical decisions without knowing the proposal's contents and understanding its goals." I was so furious; I

couldn't even conceive of cogent arguments as to why the committee were biased against the proposal. Fortunately, I placed the letter in a drawer and never sent it.

About a month later, I was rather shocked when Andress called me to state that the program would be funded, to commence 1 July 1988! When I asked how that could occur in the face of the rather hostile review, he replied, "First, the critique was more positive than you might think, and besides, the Director believes in this program and wants to support it."

Thus it was, that in July 1988, with financial commitments from the NIH and five professional organizations (ACOG, the American Fertility Society, AGOS/American Association of Obstetricians and Gynecologists Foundation, The Council on Resident Education in Obstetrics and Gynecology, and several pharmaceutical companies) the first three embryonic reproductive scientists/scholars, Karen P. Beckman at Washington University, Setsuko K. Chambers at Yale, and Thomas J. Musci at University of California, San Francisco, entered the program. They were exceptional individuals and very brave souls to embark on this experiment with no real assurance of long-term support.

28.4 A Meeting of the Selection Committee

Sitting across the table from the eight applicants for the RSDP was like déjà vu all over again. Four were women and four were men. They appeared bright, polished, and eager to get on with the job at hand. Following their graduation from medical school, each had completed a 4-year residency in obstetrics and gynecology. Each also had completed or was completing a 2- or 3-year subspecialty fellowship in genetics, gynecologic oncology, maternal-fetal medicine, or reproductive endocrinology. They were graduates of the finest universities in the country—Harvard, Johns Hopkins, Stanford, UC Berkeley, UCLA, UC San Francisco, or Yale. Several had been valedictorian of their graduating class; almost all were *Phi Beta Kappa* and *Alpha Omega Alpha*. They were the *crème de la crème* of young obstetrician-gynecologists.

Now, each was subjecting her- or himself to yet another hurdle in the obstacle course that is the path to a career in academic medicine. They had applied to become a scholar in the Program. This entailed a commitment to another 5 or 6 years of education and hard work, but this would be unlike anything they had ever experienced before. As noted, the first 2 or 3 years of the Program (Phase I) would be in a research laboratory—not just any laboratory, but in the laboratory of a world-class scientist—learning the myriad of details of cell and molecular biology. In starting all over again, they would go back to "square one." Although they might be highly skilled and polished gynecologic surgeons and obstetricians used to long hours of hard work, they would enter a new world of 12–15 h days (or more) in 6 or 7 days of the week. Rather than making critical decisions regarding a woman and/or her developing infant, whose life hung in the balance in surgery or an intensive care ward, they would be a "lab rat," learning how to separate DNA fragments or

proteins on an electrophoretic gel, how to amplify a given nucleotide sequence by the polymerase chain reaction, how to quantify protein expression, how to transfect a cell and to sequence or clone a gene, and many of the other high-powered technologies vital to the new priesthood of cell and molecular biology. In short, they were embarking on a difficult and tortuous journey into the most fundamental aspects of the basic sciences. Their goal would be to discover the innermost secrets of how normal cell growth and replication are regulated and how via signal transduction information is transferred from a cell membrane to its nucleus to initiate a process—whether it be turning a given gene on or turning it off. Hopefully, the data from these studies could be applied to problems concerning women's health and a specific disease or problem. After a few years of such toil and experience, they would have to compete for research grant monies with highly skilled and focused PhD basic scientists, who work at an accelerated pace and with no clinical responsibilities to divert them from concentrating on and thinking about their research.

The task ahead would not be easy. It was, in fact, rather sobering to contemplate. Why, in heaven's name, would anyone in their right mind, much less bright young highly qualified clinicians such as this—with the world at their feet—want to set their education, training, and rich experience as highly qualified healers aside, to embark on this path up a treacherous mountain with an uncertain future?

Each of the aspirants had prepared a 20-page application. This included her or his personal statement regarding their background, motivation, short-term goals and long-term goals, and career objectives. The heart of the application was the research proposal itself. The key element was the importance of the problem, the hypothesis to be tested, and the specific aims of the project. The application also included a literature review on the background of the research problem, with appropriate references. If applicable, the proposal described preliminary studies performed by the applicant her- or himself. The section on experimental design, spelled out in considerable detail, included the specific protocols and methods to be conducted. For each proposed study, the applicant was expected to discuss the anticipated results and their biological significance. In addition, if they were prudent, they would consider potential problems and their alternative strategy if a given experiment did not work out as planned. In effect, these were pre NIH grant applications.

Each application had to include letters from the scientist who would serve as their mentor, directing them during the first 2 or 3 years of their laboratory work. The scientific mentor also had to include an abbreviated version of their curriculum vitae, listing awards, honors, research grants, and recent publications. The application also included a letter from the chairman/chairwoman of the parent Department of Obstetrics and Gynecology which would serve as their sponsor. An essential requirement of the process was that each scholar should have a departmental "home" to which he or she could return upon the completion of the 2 or 3 years in the laboratory. Thus, a vital component was a commitment by the departmental chair to protect the applicant's time, so that he or she could devote at least 75% effort to research during the second phase of the Program. The application also had to include a letter from the dean of the medical school, indicating institutional

support, as well as letters of recommendation by two or three other individuals who knew and had worked with the applicant.

All in all, the applications themselves gave a fairly good picture of the aspiring Scholar, her or his soundness of mind in picking an important scientific problem, judgment in choosing a world-class mentor with whom to work, ability to think and to articulate ideas and present them in a logical fashion, grasp of the elements of a good project, and facility in showing the basic scientific importance, as well as clinical relevance of the problem which they planned to solve. It was not a trivial task!

When we started the program, I had asked Fred Naftolin of Yale to chair the Selection Committee. This he did in an outstanding manner. Fred organized an "advocate" system for this process. That is, from members of the Selection Committee, two advocates will be assigned to each candidate. Prior to the meeting, after reviewing the application, the advocate would call the candidate to discuss the proposal, the rationale for certain aspects of the experimental protocols, and explore other areas. This is an attempt to obtain a clear picture of the individual and the overall project. The advocate also would speak with the scientific mentor, to obtain a flavor for that individual, her or his work, laboratory, number of predoctoral students and other postdoctoral fellows, and commitment to the applicant and her or his project. In addition, they would call the chairman/chairwoman of the sponsoring Department of Obstetrics and Gynecology to ascertain their commitment to the applicant and her or his research career. All of these preliminary contacts would be made in an effort to minimize the number of unresolved issues at the time of the committee meeting/selection process.

In retrospect, it was, I believe, a stroke of genius to have asked Fred to chair the Selection Committee. A brilliant neuroendocrinologist, in addition to being a visionary leader of one of the finest departments of obstetrics and gynecology in the country, he is one of the most understanding, considerate, and fair people I know. Fred and I had grown up together so to speak (he a resident and I a fellow) at UCLA during the early 1960s, the "golden years" of academic medicine. In addition to being friends, somehow our umbilical cords had become intertwined. We were soul mates on the same wavelength of life.

Early in the evolution of the program (~1990), several problems arose. Two of the trainees experienced difficulties with the progress of their research and needed a third year of Phase I. Each of their projects was a technical *tour de force*. Although the NICHD position was that a third year would be supported only under unusual circumstances, fortunately, NICHD administrators and members of the Executive Committee agreed that for these trainees a third year was vital, and this was approved.

Also, about this time we held our first RSTP "forum" at the meeting of the SGI. Second year fellows presented their work at a plenary session of the Society meeting so that the scientists and others present could evaluate in their research progress. Eli Y. Adashi of the University of Maryland organized these sessions; almost without exception the work presented was cutting edge and exciting. I believe that these presentations also helped to generate interest in the Program

and to inspire others to greater things. In the evening following the "forum," I hosted a reception for all committee members and scholars at the meeting. I also invited the SGI president, members of Council, and other distinguished scientists. These served as a good venue for people to interact and get to know one another better and, I believed, helped to widen enthusiasm for the Program.

As noted, an issue which always caused concern for those of us running the Program was the lack of official sponsorship. Despite my efforts to persuade leaders of the AGOS/AAOGF, APGO, and SGI to assume that role, I failed. In 1990/1991 the Council of University Chairs of Obstetrics and Gynecology (CUCOG) was organized; however, they too saw no light in assuming responsibility for the program.

In the spring of 1991, NICHD instituted a new grant mechanism, the Small Grant Program (R03), to support the research of RSDP trainees during Phase II. Also at this time, because of concerns that the Program was for career development rather than "training," NICHD suggested that we change the name to the Reproductive Scientist Development Program.

About that time (in early 1991), Duane Alexander asked to meet with members of the Executive Committee. Thus in July, we met together in Rockville, MD, with half a dozen or so NICHD staff. I started off by mentioning that I had just reviewed the original application to NIH and the three progress reports to that time. I was struck with our audacity to think that the program would fly but also by the excitement of the scholars' progress and scientific contributions. In addition, I noted how prescient we were, as the need for the program was so much greater than we originally conceived. We faced a paradox, however. We had conceived and given birth to a program that is perhaps the most important activity with which we could be associated. At the same time, it all had a very uncertain future. We had no official sponsor; the K12 grant was coming up for competitive 5-year renewal, and we had no assurance of long-term funding. I concluded that we must not rest on our laurels but had to face the challenge of keeping the Program afloat and continue to help superb clinicians become first-rate reproductive scientists.

Duane Alexander then reviewed some historical aspects and potential problems of Physician Scientist Program awards. He also discussed the philosophy of NICHD support as regards matching monies and "whole-body" versus "in part" matching. To our delight, Dr. Alexander stated that for Phase I of the Program, the Institute was committed to support fully four positions per year for 2 years. Also, these funds could be allocated among more than four individuals if commitments for sufficient matching funds were obtained. In addition, upon recommendation by the RSDP Executive Committee, he stated that NIH would support up to four third year positions. As a group, we agreed to the official name change (to RSDP) and outlined the explicit functions of the Executive, Selection, and Evaluation Committees.

By 1993 I had organized and directed the Program for its first 5 years. Although I had no plans to retire, I believed it was time for someone younger and with fresh ideas to take over. Apparently, the NICHD staff thought so also. Thus, with the start of the second 5-year grant period, Robert B. Jaffe, at UCSF, who had worked with me in the initial period of development, became Director of the Program, and I

served as co-director. This was an appropriate time for a change as the Director of the companion NICHD—orchestrated Pediatric Scientist Development Program—was at UCSF and its administrator, Ms. Monica St. Geme, was moving to San Francisco from Denver to continue in her role. Thus, it made sense for her to serve as administrator for both programs. In September 1991, NICHD staff called a meeting at UCSF of the principals involved to work our administrative details of the two programs.

During these first few years, in discussing the Program with potential applicants and others, questions would arise such as candidate eligibility, the expectations from the scientific mentor, the sponsoring department chairman/chairwoman, the school, etc. In addition, there were a series of questions that almost always arose, such as what should one look for in a scientific mentor, could one conduct "clinical" research rather than that in cell/molecular biology, could one accept other awards in addition to that of the RSDP, what about fulfilling requirements for the specialty and subspecialty Board examinations, how does one keep from losing their clinical skills during the 2 or 3 years of scientific training or Phase I, and so forth.

Recognizing the need for a Handbook to describe the Program and answer questions such as these, in October 1992 several of us met at the ACOG headquarters in Washington, D.C. The meeting was quite productive, and with drafts of several sections from NICHD program staff, Michael McClure and Donna L. Vogel, we came up with a good outline for a Handbook.

During the course of our meeting, the question arose as what to call participants in the Program. As NIH administrators did not regard this as a training program, "fellows" or "trainees" were not appropriate as that appellation usually applies to subspecialty trainees. I had recently completed a survey of training programs in the Reproductive Sciences for a committee of the Institute of Medicine—National Academy of Sciences. (I was not a member of that group.) In that analysis I reviewed the John and Mary R. Markel Scholar Program which did so much to educate young academicians in the mid-century. The Markel scholars accomplished much to strengthen academic departments of medicine, surgery, psychiatry, and so forth (only a few scholars were appointed from obstetrics and gynecology). At any rate, recalling that Program and its success in educating leaders in American medicine, I suggested that we call those in our Program RSDP "scholars." Everyone seemed to think this was a great idea.

At the Selection Committee meeting on the evening before we met the candidates, Bob Jaffe and I joined the Committee members to review the candidates and to consider their project, their scientific mentor, and other aspects of their application packet. For each applicant, the primary and secondary reviewers analyzed a number of issues. In most instances, the reviewers had spoken by telephone with not only the applicant but also the proposed mentor and their sponsoring departmental chairman or chairwoman. In a few cases, these conversations revealed important auxiliary information of relevance which was not apparent from the application.

The following morning Bob Jaffe, Fred Naftolin, and I met with the applicants to introduce the Program. After his welcome, Dr. Jaffe reviewed the Program's objectives and reassured them that we were here to help them. He also outlined

some aspects of the operation of his office and that of the Program administrator. Fred Naftolin detailed the selection process. He emphasized that the Selection Committee nominates candidates on the basis of intellectual ability; motivation; commitment to an academic career; adequate clinical training as demonstrated by having been a bright, committed, superior resident; scientific research plan; quality of the scientific mentor's laboratory, mentor's training record; and appropriate sponsorship by a department of obstetrics and gynecology. He outlined the "advocate" system used by the Selection Committee to assess the candidates' qualifications.

I then spoke briefly on my perspective of the Program. I noted that for me the Program could be described by three words: commitment, excellence, and excitement. By commitment, I referred to the commitment and responsibility on not only their part but also on the part of their scientific mentor and by their departmental chairman. By excellence, I referred to the RSDP watchword, whereby every aspect of the Program must excel, the scholars, their scientific mentors, the research, and their future productivity. By excitement, I referred to the exhilaration and passion they would experience as they discovered new aspects of how cells work and how genes function, with important implications both for understanding the basis for cell function and for contributing to better understanding of women's health problems, patient care, and the elimination of disease.

We then were joined by the other members of the Selection Committee for a continental breakfast. Following breakfast the Committee's four teams of two members each dispersed to different rooms to commence the interviews. Each candidate spent one-half hour with three of the four pairs of interviewers. Thus, each candidate was interviewed by six of the eight committee members. Although I did not participate in interviewing the candidates, from the questions raised during the previous evening's meeting to go over the applications, I knew pretty much what would be covered. Questions of motivation, why an applicant had chosen a particular scientific supervisor with whom to work, the rationale for a given project, or some aspect of the methodology would be explored. Other questions are related to the candidate's focus or to project priorities, if the project seemed overly ambitious. One of the proposals was for purely applied technology. Obvious questions arose as to whether the applicant had a hypothesis to test or specific question to answer. (It happened that this applicant wanted to work in her husband's laboratory; although because they had different last names, this did not become apparent until the time of the interview. In this case, the issue of nepotism was raised, in addition to that of low-level science.) All in all, each examiner had the opportunity to probe beneath the surface and ferret out otherwise obscure details and to gain some insights into what made a given applicant "tick." Also, during the hour and a half of interviews, each applicant had the opportunity to clarify possible misunderstandings, amplify areas of confusion or voids in the proposal, or otherwise attempt to improve her or his chance of being selected.

At eleven o'clock the committee reconvened to rate the applicants. The Chairman, Paul McDonough, asked the primary and secondary advocates to summarize their interviews and note any changes in their assessment. After each of the

advocates had given their score, he opened the discussion to others who had interviewed the applicants. The discussion covered almost every possible aspect of the candidate, proposal, mentor, and related topics. At the same time, Paul insured that the discussion remained focused on the application under consideration. The discussion of each candidate consumed about half an hour and considered issues such as the applicant's apparent motivation, their reasons for wishing to enter the program, to what extent they were "self-directed," and their knowledge of the field and related techniques. For two candidates who also had the PhD degree, the issues were whether they were perennial students who could not decide what they wanted to do in life and the extent to which the program would further their careers.

In regard to the scientific mentors, the major issue was that of their experience both as a creative scientist and in training young investigators. A scientist without a large, active laboratory with neither too many nor too few postdoctoral fellows and graduate students was not looked upon with favor. In addition to their research per se and extramural research funding, a critical issue included the journals in which they published. An overriding issue was whether the candidate would truly be immersed in learning the methodologies of cell and molecular biology, not just as techniques but for problem solving. For the most part the study, design had to be hypothesis driven and the approach that of "having mechanisms on the mind."

After each proposal had been considered and received a score, we tallied the numbers and ranked the candidates. The top four candidates scored from 1.1 (outstanding) to 2.0 (excellent). There was then a clear break with the other four scorings between 3.2 and 4.5 (acceptable but not great). The scoring clearly separated the candidates into two groups. On some of the previous occasions, the results were not so clear-cut. Because of limited monies of the program, we could only accept four candidates. Sometimes, five or six would be outstanding. It always caused us considerable distress to have to draw the line at four. In fact, that was one of the few disagreeable aspects of the selection process.

These nominations were then forwarded on to the Executive Committee, which would determine the appropriateness of the recommendations and choose the scholars who could be supported by the financial resources of the Program. Scholars then would commence the Program the following 1 July.

By this time it was 1:00 p.m. The hotel catering department rolled in a bountifully laden table with our luncheon (a bowl of crème of mushroom soup and a cold plate with cheese, turkey, black bread, and bagels). The candidates joined us. I sat between two of the applicants, a man and a woman. After a few pleasantries, I inquired as to what suggestions they might have for improving the application and selection process. To my disappointment, neither had much to say. Was it the fear that they might say the wrong thing or in some other way jeopardize their chances? I do not know. I tried to draw them both out, but the woman seemed suspicious of my intentions, and the guy was pretty uptight. Although both candidates had scored fairly high and would be accepted into the Program, I could not indicate this to them at that time. Our conversation was rather strained. Thus, the luncheon was a bit of an anticlimax to what should have been a great and rewarding experience.

28.5 Further Reminisces

In March 1996, a year and a half following that meeting, I was in San Diego at the SGI meeting viewing the "posters." Here, postdoctoral fellows and other young investigators showed off the results of their investigations on posters. As I was making my way down one of the long rows from which emanated the results of both brilliant and rather pedestrian studies, a young woman stopped me. "Dr. Longo," she said, "You don't remember me, but . . .." She went on to tell me about her view of the RSDP selection process that day in Washington, DC. She agreed that the interview/advocate system was about as fair as it could be. However, she felt that several members of the Committee were overzealous in their attempt to separate the "wheat from the chaff" and probe the nuances of the specific experiments and line of inquiry she had planned. A specific criticism she made was that following the interviews themselves and the Committee's meeting to pick the scholars, the moment she came into the room with everyone for luncheon, "I knew I had lost out." She recounted how the Committee members were affable and warm to the four applicants selected, but as she expressed it, "I felt that I had leprosy."

We talked for a while. I expressed my regrets over the deficiencies in the process and the fact that she believed herself to be singled out as not "up to snuff" as she had expressed it. After discussing her research, I attempted to reassure her that, despite that setback, her future as a reproductive scientist appeared to be assured. She shook my hand and thanked me, and I moved on down the aisle between posters and their discussants. But it made me wonder. How much difference does it really make in the career of someone who is highly motivated whether or not they are chosen as a scholar in the Program? It would be a fascinating study to follow the careers of all applicants to the Program and examine the careers of those who were accepted and those who were not. We would like to think that our support for those scholars in the Program made a great difference in their careers. Perhaps it is not as great as we would like to think!

28.6 The Naftolin Excellence in Mentorship Award

In March 2004 in Houston, Texas, at the fifty-first annual meeting of the SGI, I was given a newly established award, "The Frederick Naftolin Award for Excellence in Mentorship." My remarks of acceptance follow.

Thank you President Strauss, President Gabbe, past President Naftolin, and members of the Society. It is an enormous honor to receive this accolade. I am awestruck by its significance, and I accept it on behalf of all of us involved in the Reproductive Sciences Development Program. It is doubly an honor to be on this platform in Fred's honor. We grew up at UCLA together, in what some of us regard as the "golden age" in medicine. Having somehow gotten our umbilical cords entangled, our lives have been interconnected in one way or another since that time.

It is said that "Mentorship is like motherhood—It is for life." Please allow me to make three points about the Reproductive Sciences Development Program and its genesis. For rather than "Minerva-like springing from the head of Zeus," this program came into being from the efforts of several of us who are now more senior members of the Society—Bob Jaffe, Fred Naftolin, Ed Wallach, Jim Warren, Gerson Weiss, and others.

First, in some ways it was an accident, perhaps not like that of an unplanned pregnancy, but nevertheless not entirely thought out. Two decades ago in the mid-1980s, the problem as we perceived it was that from an investigative standpoint; academic departments of obstetrics and gynecology was in deep trouble. For my part, I was preparing a report on the state of NIH funding to departments of obstetrics and gynecology. Whether one looked at R01 Project awards, Program Project Grants, Research Career Development Awards, Research Training Grants, or whatever, in comparison to other major academic departments—Medicine, Surgery, Pediatrics, Radiology, and Neurology/Psychiatry—in each category Obstetrics and Gynecology received the lowest levels of funding, only "scrapings from the very bottom of the barrel." (Later, that analysis became part of a report by the Institute of Medicine "Strengthening Research on Academic Ob/Gyn Departments" Washington, D.C., National Academy Press, 1992).

To consider how to meet this challenge, those of us I have mentioned met together at one of the major meetings to discuss the problem and what to do about it. To help correct this deficit, we decided to approach the NIH for a multi-institutional, multi-departmental training grant, in which bright young people could be well prepared as physician-scientists for an academic career in the specialty. Rather than merely more subspecialty training, we elected to emphasize education in cutting-edge approaches in cellular and molecular biology as applied to the reproductive sciences. The other individuals on our ad hoc committee were all chairs of major departments. As I was not, I probably was viewed as being without a "real job," and thus was "elected" to write the proposal and do the necessary leg work to get it underway.

Second, there was a paradox, however. Despite the fact that we were convinced that we had a great idea, we had no official organizational sponsor. Thus, it was totally an ad hoc effort. Here, two individuals played key roles in helping to promote the program. To his credit, NICHD Director, Duane Alexander, bought into the concept. He authorized his associates William Sadler and Michael McClure, successive Chiefs of the Reproductive Sciences Branch, with Charlotte Catz, to help guide us through the labyrinth of NIH rules and regulations. Nonetheless, Dr. Alexander cautioned us that to receive Institute funding, we must obtain "matching" financial support from the specialty's major professional societies. Here, Warren Pearse, Executive Director of the American College of Obstetricians and Gynecologists, played a critical role. For it was Warren's broad vision for the specialty that helped us convince leaders of ACOG, the American Gynecological and Obstetrical Society, the Association of Professors of Obstetrics and Gynecology, and the American Fertility Society (later named the American Society for Reproductive Medicine) of the vital necessity for such a program. Somewhat later,

this Society, the SGI, also became a co-sponsor. On one hand, as I approached the Boards of these societies, I am afraid that I was viewed as a cross between a used car salesman, hat in hand asking for a handout, and some kind of visionary lunatic. On the other hand, fortunately, we also convinced executives from several of the major pharmaceutical companies of the importance of educating young academic obstetricians and gynecologists in reproductive science.

There were other problems. Paradoxically, some of the "leaders" of our professional societies resisted the idea, with statements such as, "obstetrics is a mechanical specialty, how do you think that you're going to make it a science?" or "why not let Ph.D.'s do the basic research, and let the ob/gyns take care of the patients?" In Bethesda at the reverse site visit before the NIH review committee, Doctors Naftolin, Pearse, Weiss, and I were grilled in a rather hostile manner as to the need for, or practically of, such a program. One reviewer challenged us, "Is not science in obstetrics an oxymoron?" We also were challenged on our ability to attract into the program women, minorities, and gynecologic oncologists. Finally, one reviewer asked "What's your problem? Obstetricians should be delivering babies—not trying to be scientists." In a way, this reflected my own experience when applying for a postdoctoral position in physiology. During the interview, my mentor to be (who later was elected to a membership in the National Academy of Sciences) asked about my subspecialty in internal medicine. Upon my replying that I was not an internist—but rather an obstetrician gynecologist—his jaw dropped and he asked in amazement, "My God! Can you read and write?" But that was the perception of our specialty.

Third, a *caveat*. As you know, presently because of our attracting outstanding scholars and having them work with some of the finest scientists in the country, many view the Program as a great success (Longo et al. 1999). In numerous ways, it far transcends a "program"—it is a family. Nonetheless, despite this achievement, let us not be lulled into tranquility. We are far from being out of the woods. As you may recall, it was 25 years ago that James Barnes Wyngaarden, then Director of the NIH, sounded the tocsin of "The clinical investigator as an endangered species" (Wyngaarden 1979). Wyngaarden's warning remains even more the case today. We do not need a meta-analysis to disclose that study after the study report the declining numbers of physician-scientists in our academic departments. Many departments are little more than high-class practice groups that help to teach medical students. Even within many of the major academic departments, sociocultural forces are tending to separate clinical and research activities and stress academic departments as a business. Thus, the challenge remains for us as investigators and for us as a society to preserve the physician-scientist in obstetrics and gynecology.

In summary, the Program was somewhat of a fortuitous accident; a number of obstacles had to be overcome in bringing it to fruition, yet today we remain faced with major challenges. I cannot adequately thank my colleagues who contributed so much to develop this program—Fred Naftolin who chaired the selection committee and established guidelines to select the most promising candidates, Bob Jaffe,

Warren Pearse, Gerson Weiss, and the many others. We have come far beyond what any of us might have anticipated or hoped.

In closing, I would ask all of the past and present RSDP scholars to stand. Also the AAOGF, Society of Maternal Fetal Medicine, Berlex, and other scholars, as, I believe, for the most part, it was members of this Society who worked to develop these programs. It is these scholars who are the future of our Society. They are the future of academic obstetrics and gynecology and the future of women's health issues.

You may recall that in the early eighteenth century, it was proposed to build a monument to Sir Christopher Wren (1632–1723) the creative architect who, following the great fire of London in 1666, designed St. Paul's Cathedral and many other churches and fine buildings. Standing in St. Paul's, Wren rejected this idea. He said, simply, "Look about you—this is my monument."

So, this afternoon fellow members of the Society, I say look about you; these scholars, these terrific young investigators are our monument; they are your monument. Again, thank you Fred. Thank you Presidents Strauss and Gabbe. Thank you all. The challenge is let us continue to build and persevere! (Longo et al. 1999; Longo and Jaffe 2008).

28.7 Perspectives of Several RSDP Scholars' Reports

Since its initial funding in July 1988, over 100 scholars have completed the RSDP program, almost all of whom have remained in academic medicine. In an attempt to capture some of their experience, passion, and success, brief reports from three follow.

One of the early and successful scholars, Kelley Moley is the James P. Crane Professor of Obstetrics and Gynecology and Vice Chair for Basic Research at Washington University School of Medicine. She is also Director of the Division of Basic Science Research within the Department and a practicing clinician and Director of the Reproductive Endocrinology and Infertility fellowship program. Dr. Moley is an internationally recognized expert in the field of glucose transporter biology and highly regarded for her work in gamete biology, preimplantation embryogenesis, and implantation in mammalian models of maternal metabolic diseases.

> My major achievements in the area of biomedical sciences cross the disciplines of reproductive medicine, metabolism, and cancer. I identified the molecular mechanisms by which maternal diabetes and obesity adversely affect preimplantation development by linking glucose utilization and insulin signaling to the cellular homeostatic processes of apoptosis and autophagy. I also was one of the first to develop single cell metabolomics for cleavage stage embryos and oöcytes. My work supports the hypothesis that environmental and metabolic insults to maternal oöcytes have long lasting and possibly irreversible effects on the resulting embryos and offspring via mitochondrial and epigenetic mechanisms, thus contributing to our understanding of development origins of adult diseases. Outside of reproductive medicine, I was the first to clone and identify GLUT8 and GLUT9, two novel

facilitative glucose transporters. I first demonstrated that in select tissues GLUT8 is insulin-regulated and is responsible for non-alcoholic fatty liver disease under conditions of metabolic syndrome. More recently, I determined that GLUT9 is not only a hexose sugar transporter, but is also a high capacity uric acid transporter predominantly expressed in liver, kidney and gut. In humans, polymorphisms in this gene are associated with hyperuricemia, hypertension and gout. In addition, recently I linked maternal high fat diet to the generation of hyperproliferation in the prostate glands of male offspring. This extends the concept of the developmental origins of adult diseases potentially to cancer, and currently I am investigating the mechanisms by which this may occur. In this manner, I have attempted to be an interdisciplinary physician scientist bridging not only the clinical and basic areas of reproduction but also the disciplines of obstetrics and gynecology, metabolism, and oncology.

I serve key leadership roles both at Washington University and internationally. I am Co-director of the Institute of Clinical and Translational Sciences at Washington University. In this capacity I run several key programs within the institutions including the Operations Committee and the Program Committee, which awards over $1 million per year in funding. Currently, I serve as PI and Director of the Reproductive Scientist Development Program, a K12 program awarding Career Development awards to Ob/Gyn physicians across the country seeking a career in basic science. This program has been in existence for almost three decades and is considered the premier training program for the field. I also am the immediate past president of the Society for Reproductive Investigation, the foremost society in academic Obstetrics and Gynecology that promotes science in the service of women. Finally, since 1993, I have been funded by NIH continuously and in 2013 was the 8th highest NIH funded individual in Obstetrics and Gynecology and 2nd highest Reproductive Endocrinologist. I also have served as a full time member of NIH standing study sections and Chairperson of Pregnancy and Neonatology (PN) study section for the last two years. This past year I was elected to the Institute of Medicine of the National Academy of Sciences.

(Letter from KM to LDL, 26 January 2015)

Another former scholar, Errol R. Norwitz, Louis E. Phaneuf Professor of Obstetrics and Gynecology, Tufts University School of Medicine, Boston, MA, has written,

As a founding investigator of the Mother Infant Research Institute (MIRI) at Tufts Medical Center, which operates under the Executive Directorship of my friend and colleague, Dr. Diana Bianchi, my research interests include the genetics of adverse pregnancy outcome and the molecular regulation of parturition, both at term and preterm.

I trained initially at the University of Cape Town, South Africa and then at Oxford University in the UK before moving to Boston to pursue my Obstetrics and Gynecology Residency at Harvard University. In 1995, I attended the 42nd Annual Scientific Meeting of the Society for Gynecologic Investigation in Chicago and heard the RSDP investigators present their research. Although only a third year resident, I decided then and there that I wanted to be an RSDP fellow. At the end of the talks, I launched myself at Drs. Lawrence D. Longo and Robert B. Jaffe. They introduced me to the program administrator, Monica St. Geme, whom I emailed relentlessly over a period of more than two years until I was finally old enough to be offered an interview. I started the RSDP program in 1997 during the second year of my maternal-fetal medicine fellowship.

What appealed to me most about the RSDP at the time was: (i) the guaranteed protected research time that I was able to secure for the first 2 years (Phase I) of the program; (ii) the commitment from the sponsoring institution to a 5-year faculty appointment; (iii) the requirement that the research be done in a laboratory outside of obstetrics and gynecology (I worked with Dr. William Chin in the Department of Genetics at Harvard investigating the molecular mechanisms of gonadotrophin-releasing hormone receptors (GnRH) gene

regulation in the mouse); and (iv) the promise of a long-term 'academic home' for like-minded physician-scientists. There is no doubt that the RSDP changed the course of my career. It helped lay the foundation for my research and for the funding I have been able to secure moving forward, and it helped to establish a wide network of colleagues and collaborators who have supported me as I ascended slowly up the academic ladder.

The RSDP has evolved and matured over time, all for the good. When I commenced in 1997, for example, having a PhD was considered an exclusion criterion for RSDP funding. After all, the program was designed to take physicians and teach them how to do research. If you already had a PhD, the argument went, you presumably knew how to do research and didn't need further training. This has changed and many current RSDP fellows are MD/PhD graduates. In addition, when I started in 1997, you could begin your RSDP tenure during your clinical fellowship. This is what I decided to do. As a result, I lost at least one year and possible two years of protected research time, which was guaranteed by my fellowship. This has changed and it is now recommended that you start your RSDP after your fellowship. And, in 1997, you could not hold another NIH-sponsored K-award along with your RSDP. I was therefore forced to choose between my K08 and K12. This too has changed and it is now possible to hold a K08 concurrent with the RSDP. While some of the details have changed, the primary goal of the RSDP to train physician-scientists in the reproductive sciences has remained the same. When I started, the RSDP was in its fifth year. It has now been in existence for over 20 years and has trained almost 100 Scholars, many of whom have risen to positions of influence in major academic medical centers around the country as well as at the NIH. And several of us now sit on the RSDP Executive Committee, giving back to the program that has given us so much.

To a large extent, the RSDP has achieved the goals laid out more than 20 years ago. For this, we have many people to thank, not least Drs. Longo and Jaffe for their vision and perseverance in getting the program started. So what does the future hold? What must we do to keep this training program alive and thriving? With NIH funding levels continuing to fall, it's time to become more creative. Having secured a full 5 years of funding for all of our new RSDP fellows, we need to turn our attention to bridge-funding for junior faculty until they can achieve independence. And it is up to those of us who have benefited from such training programs to support the next generation of reproductive scientists. I remain optimistic. While we may well lose some promising candidates to the private sector with the assurance of higher salaries and a more secure lifestyle, I am confident that we will continue to attract a small elite cadre of Ob/Gyn residents committed to a career as a physician-scientist. The lifestyle is more challenging, but the rewards are enormous.

(Letter from ERN to LDL, 8 October 2014)

A third scholar who has enjoyed considerable success is James H. Segars, for many years Program Director of the Clinical Training Program in Reproductive Endocrinology sponsored by NICHD. Currently, he is Professor of Obstetrics and Gynecology and chief of the Division of Reproductive Research Science at the Johns Hopkins University School of Medicine, Baltimore, MD.

To summarize my experience is a very difficult task because the impact of the RSDP has been immense and the consequences are so far-reaching. That is, the program helped to launch my career in academic medicine, has provided lasting friendships and collaborations, and has helped me to train others who will continue to conduct research on women's reproductive health—the next generation of scientists!

Research is a life theme that now, in hindsight, began in middle school, but was not pursued in earnest until fellowship in reproductive endocrinology at Vanderbilt. Then, with the support and mentorship of Dr. Anne Colston Wentz, I applied for the Reproductive Scientist Training Program (RSTP, as it was then known). After one unsuccessful application, I was accepted into the program and distinctly recall the phone call congratulating me,

but with the admonition that "this was a 20 year commitment" and "we'll be following your career". It was a rather daunting thought.

Beginning the RSDP training at NIH under the mentorship of Dr. Keiko Ozato, I was amazed that the questions were so profound and the implications so vast. Her laboratory had cloned a gene that interacted with estrogen-responsive DNA elements, and the protein I worked on proved to be the retinoid X receptor beta. After Phase I of the RSDP, I joined the NIH intramural program in 1992 and ever since my research has focused on the molecular mechanisms of hormone action. I've lead a small group to explore the *in vivo* role of the Brx oncogene, a gene we cloned (aka, AKAP13). The targeted loss-of-function mutation of the gene in mice revealed an essential role for the protein in cardiac development. To further examine function of the gene, we created a Cre-Lox model to examine targeted deletion in the heart, ovary, and bone. In brief, these studies led to a new understanding of the function of this gene as a protein capable of integrating hormone action with mechanical signaling—thus leading to a new understanding of the dynamic nature of reproductive tissues; tissues that are characterized by remarkable changes in tissue plasticity on a monthly basis and during pregnancy (a photo of our laboratory group is attached).

A second major research focus has concentrated on uterine leiomyoma. Through fruitful collaborations, our group was one of the first teams to apply microarray technology to uterine fibroids and our laboratory has been the first to emphasize the crucial role of mechanical signaling in fibroid growth and the maintenance of the "fibroid cell phenotyple". Studies by the group have emphasized the production of an altered structure and content of the extracellular matrix in uterine leiomyoma cells and normal uterine smooth muscle leading to a new understanding of the condition. We are actively pursuing new and novel therapies for this common condition based on the results of our research.

In 1995, I was appointed the Program Director for the Clinical Training Program in Reproductive Endocrinology sponsored by NICHD. As a Director of this graduate medical education training program, it has been my pleasure to provide clinical and surgical training for 44 young clinical investigators and mentorship for these fellow trainees. Several fellows now serve as faculty and leaders in the field of reproductive endocrinology and two now are RSDP graduates; Drs. Bo Yu and Erin Wolff. Thus, the RSDP has influenced not only my career, but those 44 fellows and another 20 pre- and post-doctoral fellows in reproductive research.

All along the way there have been trials and tribulations, as is typical of research and life in general. Many higher-ups at NIH and USUHS have provided key backing; including Drs. Duane Alexander, Owen Rennert, Igor Dawid, William Haffner and Alan DeCherney. RSDP colleagues such as Kjersti Aagaard-Tillery, Elizabeth McGee, Kim Leslie, Aleks Rajkovic, Deb Driscoll, John Yeh, Lisa Halvorson who have collaborated and helped in many ways over the years. Likewise, the RSDP leadership has provided support and encouragement that has been inspirational. The wisdom of the RSDP leadership, Drs. Longo and Jaffe, and now Dr. Moley, have served as "true north" bearings throughout the years; their message: persevere! I will soon be leaving NIH for a professorship at Johns Hopkins. My role there will be to establish a Division of Reproductive Research Science under the leadership of the chair, Dr. Satin. In that venue as well, I plan to continue research to understand fundamental issues of women's reproductive health.

(Letter from JHS to LDL, 23 December 2014)

28.8 Other Education Awards in Obstetrics and Gynecology

The Building Interdisciplinary Research Careers in Women's Health (BIRCWH) is a mentored Career Development Program. It connects junior faculty, known as BIRCWH scholars, to senior faculty with shared research interest in women's health and sex differences research. Since this NIH-supported program was created in 2000, 77 grants to 39 institutions supporting more than 542 junior faculty have been awarded by the Office of Research on Women's Health (ORWH) and BIRCWH program co-sponsors.

The BIRCWH awards are a trans-NIH collaborative effort. The most current round of BIRCWH Programs is supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), the National Cancer Institute, the National Institute on Aging, the National Institute of Arthritis, Musculoskeletal and Skin Diseases, the National Institute on Drug Abuse (NIDA), and the National Institute of Mental Health. ORWH and NIDA provide programmatic oversight for these BIRCWH Programs, and NICHD provides the grants management oversight for most of the programs. To be eligible for the BIRCWH Program, junior faculty must have recently completed clinical training or postdoctoral fellowship and must plan to conduct interdisciplinary basic, translational, behavioral, clinical, and/or health services research relevant to women's health. Most BIRCWH scholars move on to obtain independent NIH grant funding following their participation in the BIRCWH Program.

The Women's Reproductive Health Research (WRHR) Career Development Program was initiated in 1998 to provide the opportunity for obstetricians/gynecologists who recently completed postgraduate clinical training to further their education and experience in basic, translational, and clinical research. Program sites provide obstetrics and gynecology departments with an opportunity to create a talented pool of junior investigators with expertise in women's health research. The WRHR program is funded by the NICHD, through its Gynecology Health and Disease Branch (GHDB), and the NIH Office of Research on Women's Health using the NIH Mentored Research Scientist Development Program Award (K12) mechanism.

Currently (2015), there are 17 active WRHR sites in departments of obstetrics and gynecology throughout the nation. The primary goal of these sites is to provide Ob/Gyn junior faculty with state-of-the-art training in Women's Reproductive Health Research in an academic setting and to increase the research capacity of clinically trained Ob/Gyns. WRHR scholars represent a diverse group of physician-scientists from several subspecialties and emerging areas of obstetrics and gynecology; they pursue a broad range of basic science, translational, and clinical research topics and are often appointed to faculty positions. As of May 2013, 215 Ob/Gyn junior faculty have been appointed to the WRHR program.

The scope of the WRHR program encompasses all areas of obstetrics and gynecology research. The focus is on scientific topics relevant to general obstetrics

and gynecology and/or its subspecialties: maternal and fetal medicine, gynecologic oncology, reproductive endocrinology and infertility, and female pelvic medicine and reconstructive surgery (urogynecology). Related fields such as perimenopause and adolescent gynecology are also of interest. Senior investigators from established research programs, which address a broad range of basic and applied biomedical and biobehavioral science, in obstetrics and gynecology and collaborating departments form an intellectual and technical research base for mentoring WRHR scholars.

28.9 The Pediatric Scientist Development Program (PSDP)

A companion to the RSDP, the PSDP provides a training environment in which talented young pediatricians address central problems in child health with the most current scientific tools. Ultimately, these pediatric scientists, dedicated to continuing careers in basic, translational, and clinical research, will translate research advances to improvements in clinical care for children. Thus, the PSDP provides career development support for pediatricians committed to careers in academic medicine.

The PSDP is an intense, full-time experience in basic, translational, or clinical research training. Fellows conduct research in eminent laboratories in Canada and the USA. Up to three years of research training are funded by the Program after the completion of the clinical fellowship years. A sponsoring pediatric department supports the clinical fellowship apart from no patient care or clinical duties being allowed during the first 2 years of PSDP-supported training.

The PSDP is sponsored by the Association of Medical School Pediatrics Department Chairs (AMSPDC). In a unique collaborative arrangement, the program is funded by grants from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) and numerous private agencies and foundations, including the American Academy of Pediatrics, the American Pediatric Society, the Sick Kids Foundation (based in Toronto), the Paediatric Chairs of Canada, and the March of Dimes Birth Defects Foundation.

The PSDP provides 2–3 years of support for pediatricians who will devote themselves to academic careers with a strong research component. Candidates must be nominated for the PSDP by the chair of the department in which their residency training is being completed (nominating chair). The chair of the department into which the candidate has been accepted for clinical fellowship training must also contribute a letter of support (sponsoring chair) affirming that the candidate will have no clinical or patient care responsibilities for the first 2 years of PSDP funding.

PSDP trainees pursue the following career path:

- One year of clinical fellowship training in a subspecialty area that will lead to sub-board eligibility. This training is funded by the Fellow's sponsoring

department. Subspecialty programs, such as cardiology, critical care, emergency medicine, and neonatology, may cause fellows to extend their clinical training requirements to 18 to 24 months. In such cases, more than 1 year of clinical fellowship training may be required.

- Two to three years of PSDP-supported research training, typically in a laboratory outside the sponsoring department of pediatrics, in a field applicable to a pediatric subspecialty. These are the only years of training funded by the PSDP and, in most cases, take place at the sponsoring institution. Ideally, trainees will be mentored by senior investigators in basic science departments, epidemiologists, statisticians, or health policy experts. The PSDP requires that the first 2 years of research training be devoted to full-time work in the laboratory, uninterrupted by clinical or patient care duties. Candidates proposing patient-oriented research or translational research that requires patient contact (not patient care) may request up to 10% patient contact time in years 1 and 2. This request must be incorporated into the initial PSDP application. Candidates selected for a third year of funding may commit 15% time to clinical work including patient care within the sponsoring institution.
- Two years of continued support, after PSDP training, in a faculty position that provides at least 75% time for research. Funding for these two faculty years can be provided by the nominating institution, by the sponsoring institution, or by any other department of pediatrics that commits to 75% protected time for research. The nominating department must guarantee its best efforts in identifying such a position, but the PSDP trainee is free to consider competing offers from other departments, including the sponsoring department. Time not spent in research (25%) is available for teaching and clinical service.

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Chapter 29
Epilogue

If we are to vindicate the claim to be seeking the nature of things, then it must be extensive. The broad picture must be sought, outworn detail discarded, and all help possible derived from simplifications and generalizations. . . . This is no doubt more easily said than done, but [1]whoever believed that our task is an easy one? Nor is our responsibility lessened by the need . . . of guarding against the superficial and the inaccurate, of deriving full benefit from the intensive approach and from special disciplines, and of combining the survey of the small-scale map with the detailed scrutiny of the large-scale insets.

(Sir Cyril Hinshelwood 1954, p. 307)

29.1 The Adventure of Science

As noted in the introduction, the Oxford chemist and Nobel Laureate Sir Cyril Hinshelwood defined science as ". . . an imaginative adventure of the mind seeking truth in a world of mystery" (Hinshelwood 1954, p. 301). In the lines quoted above, he cautioned that the search is not without its challenges. Science constitutes a boundless and persistent quest for knowledge and wisdom. Unique among fields of mental enterprise, the process requires curiosity, creativity, and dedicated work and is characterized by communalism, universalism, disinterestedness, originality, and skepticism. Science possesses an informal quality assurance system of peer review, publication, and independent replication. As noted earlier, this essay might be viewed as a case study in the manner in which a special field of biomedical science has emerged and continues to evolve, and the way in which individuals, their ideas, and social forces critically interact in that development. As with much of contemporary biomedical science, fetal and neonatal physiology is based in general on the "Galilean-Harveian" hypothetico-deductive method, with the careful analysis of observed phenomena, generation of a testable hypothesis, designing and recording of experiments to test these hypotheses, and the further wholesale collection of observations and data to explore a more refined hypothesis. These are generated in sufficient detail to extend beyond mere empirical observation and allow the

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innovation of quantitative reasoning to establish a proposed general principle and to enable reproducibility.

Because the approach to scientific investigation is such a critical aspect of fetal-neonatal physiology, it may be of value to consider its several approaches. As noted earlier, in his efforts to understand the living organism, William Harvey was the first investigator in biology to employ the methods of science as presently conducted, that is, observation, hypothesis, deduction, experiment, reformulation of hypothesis, and further observation. This path follows neither scholastic Aristotelianism nor the Baconian (named for the visionary English philosopher and essayist Sir Francis Bacon) laborious accumulation of data, its manipulation by the complex tables of the *Novum organum*, and its inductive method (Bacon 1620a, b, 1623; see Whewell 1837). During the late nineteenth and early to mid-twentieth centuries, as with related fields of biomedical science, physiology moved from being descriptive and phenomenological to hypothesis-driven experimentation. For instance, in his *Introduction à l'étude de la médicine expérimentale* [Introduction to the study of experimental medicine], Claude Bernard presented the basic principles of physiologic investigation as applied to the life sciences (Bernard 1865). Included in this approach is the increasing dominance of reductionism, the concept that complex biological systems can be understood only by dismantling them into their constituent components, and to explore each in isolation. Implicit is the idea that experimental observations should be made to support or attack a given hypothesis of a phenomenon or mechanism of action. In this paradigm, straightforward observation/phenomenology for its own sake is of limited value. Further, as noted, the rigor of scientific investigation has included the concepts of blinded, randomized design, repeatability of experiments to establish their validity, peer-review evaluation of manuscripts for publication, and independent confirmation from other laboratories. Based on empirical a priori experience, over the past half century, these standards and approaches have served the biomedical community well, witness the revolutions in cellular and molecular biology, genetics, neuroscience, endocrinology, immunology, and the multitude of advances in related disciplines. As a consequence, the complexity of biology has grown by orders of magnitude. At times delving into it seems as though one were entering a Mandelbrot [named for Benoît Mandelbrot (1924–2010)] set of complex dynamics and abstract mathematics, e.g., that space determined by a relatively simple equation, but as one approaches its fractal boundary, ever more intricate patterns of complexity are revealed (Mandelbrot 1977, 1982).

Philosophers of science have supported, challenged, attacked, or otherwise attempted to modify this hypothetico-deductive approach over the years, stressing rather the metaphysical, epistemological, semantic, or other aspects of science. For instance, in the early twentieth century, the polymath Michael Polanyi (1891–1976) advanced the idea of the social construction of science. That is, science proceeds by something other than strict rationality or algorithmic procedure, arising rather from a *mélange* of social processes that no purported-scientific method could capture. In his *Science, Faith and Society* (Polanyi 1946), *Personal Knowledge* (Polanyi 1958), and *The Tacit Dimension* (Polanyi 1966), Polanyi spelled out his opposition to a

positivist account of science, claiming it to be a relativistic discipline, and that absolute objectivity is a false ideal. As noted in the Preface, following Polanyi's role in initiation of the "science wars," another to challenge the triumphal tales of upward and onward scientific processes was the philosopher of science Thomas Kuhn. In his *The Structure of Scientific Revolutions* (Kuhn 1962), Kuhn furthered the idea of the sociological construction of science, denying the concept that science progresses in a linear, continuous manner. Rather, he postulated that science progresses by periodic "paradigm changes" or shifts that revise dogma and open new vistas of understanding. Kuhn also argued that rather than being established by objective criteria, the notion of "scientific truth" often is defined by consensus of the scientific community. A 50th anniversary fourth edition of Kuhn's work includes a perceptive essay that places it in the context of contemporary scientific, historical, and philosophical thought (Kuhn 2012).

Healthy skepticism is of course an essential element of the scientific process. The philosopher of science Sir Karl Raimund Popper (1902–1994), of the London School of Economics, Fellow of the Royal Society, and 1992 awardee of the Kyoto Prize in Arts and Philosophy, contended that the central problem in science is to distinguish science per se (e.g., that based on observation) from non-science (e.g., logic, metaphysics, and so forth); that is, a theory of demarcation or "critical rationalism," as he called it. Popper argued against the Baconian insistence on the primacy of observation per se, noting that all observation is selective and theory incumbent and that rather than the espousal of any unique methodology, science consists of problem-solving. In Popperian epistemology, a central requirement of science is to falsify hypotheses (Popper 1959, 1999; Thornton 2011). Popper cautioned that if we respect truth, we must search for it by persistently searching for our errors. So arose the Popperian thesis of falsification, e.g., that a given hypothesis cannot be proved, only shown to be false. Among contemporary philosophers of science, a rather extreme extension of this view holds that a hypothesis can neither be proven nor disproven (Atkinson 1985; Kinraide and Denison 2003; Lipton 2005; Weimer 1979).

In his Jayne Lectures to the American Philosophical Society, the 1960 Nobel Laureate in Physiology or Medicine, Sir Peter Brian Medawar (1915–1987) agreed, in part, with this view, observing:

> Ask a scientist what he conceives the scientific method to be, and he will adopt an expression that is at once solemn and shifty-eyed: solemn, because he feels he ought to declare an opinion; shifty-eyed, because he is wondering how to conceal the fact that he has no opinion to declare. If taunted he would probably mumble something about "Induction" and "Establishing the Laws of Nature", but if anyone working in a laboratory professed to be trying to establish Laws of Nature by induction we should begin to think he was overdue for leave.
>
> (Medawar 1969, p. 11)

As articulated by Bacon, Medawar also emphasized that although much original research begins with experimentation, it must progress beyond that. His exegesis critiqued both the Baconian-inductive approach to science, the Harveian "hypothetico-deductive" scheme of thought, and the Popperian "falsifiability." He noted further:

> Scientific reasoning is an exploratory dialogue that can always be resolved into two voices or two episodes of thought, imaginative and critical, which alternate and interact. In the imaginative episode, we form an opinion, take a view, make an informed guess, which might explain the phenomenon under investigation. The generative act is the organization of a hypothesis . . . (which) we can expose to criticism, usually by experimentation.
>
> (Medawar 1969, p. 46)

Medawar stressed the requirement by a combination of intuition and good sense, to move beyond compiling an inventory of factual information, to arrive at ". . . a logically articulated structure of justifiable beliefs about nature" (Medawar 1969, p. 59). Further, some contemporary philosophers of science would argue that science proceeds optimally by a combination of a more rigorous hypothesis-based deduction to derive the particulars and refine the hypothesis, combined with Baconian-induction from particulars to formulate general principles and theories (for instance, see Nabel 2009; Theocharis and Psimopoulos 1987).

This quasi-metaphysical background to the philosophy of science having been given, we must recognize that the decades encompassed in the present essay were in many regards another era. Late early- to mid-twentieth century saw the flowering of systems physiology, by which scientists gained considerable insights into various organ systems with their individual tissue functions, regulation, interrelationships, and feedback loops. Initial endeavors along this line pursued problems in the physiology, biochemistry, and pharmacology of the adult organism. Slowly, dawned the realization that the developing fetus and newborn do not constitute small adults, the field generated its own set of problems to explore with hypotheses to test. Currently, the opportunities in science are unbounded. For physiology, the past half century has been a golden age, with creation of a new geography of science.

As noted, it was Claude Bernard who in the latter nineteenth century presented a rigorous outline of the scientific method in experimental physiology (Bernard 1865). It was at mid-twentieth century that the study design for clinical investigation changed from retrospective to prospective, from observational to experimental, from uncontrolled to randomized controlled experiments. Laboratory investigation of cellular and molecular biology emerged from this chrysalis. As all-encompassing disciplines, which in bringing fresh insights invigorated every field, by the end of the century dominated biomedical thinking. With the convergence of diverse disciplines fueling the expansion of research, and with the underpinning of ingenious and complex contemporary tools, emerged genomics, transcriptomics, epigenomics, proteinomics, metabolomics, and other "omics," coupled with bioinformatics and information technology. These disciplines have accelerated the pace of biological discovery and expanded the boundaries of the worlds to be explored. Even the past decade has witnessed fundamental shifts, with the experimental landscape changing exponentially as the rate of discovery accelerates. Remarkable technological advances in molecular genetics, advanced optical laser scanning confocal, atomic force, and electron microscopy, novel approaches to fluorescently labeled proteins with ever more sensitive imaging capabilities of subcellular localization, protein–protein interactions, nanotechnology, live cell dynamics,

high-throughput genomic and proteomic approaches, and the ability to manipulate proteins and other modalities are leading to the elucidation of the most basic mechanisms of cellular functional biology. Discovery of nuances of membrane receptors, transmembrane trafficking, genome organization, and gene regulation allow the imaging of specific subcellular signaling molecules their pathways and networks, and their location/movement within subcellular organelles, and details of cytoskeletal dynamics. These also permit a better understanding of transcription and translation regulatory mechanisms including that of gene expression and thus biological function by small noncoding RNAs. Revealing the role of these small microRNAs, with the histones and other constituents, now allows a new level of description of physiologic regulation of the classic dogma of DNA → mRNA → protein. Coupled with other major conceptual breakthroughs and "shifting paradigms," these advances promise to have an enormous impact on both understanding in the basic sciences and on the role of physiology in integrating the findings of reductionism into clinical/translational research in the study of disease. Within the past decade in the reproductive sciences, including embryonic development and fetal and neonatal physiology, these methodologies have come to the fore, contributing greatly to our understanding.

In a sense, this period is similar to the late fifteenth and sixteenth centuries, with exploration and discovery of new lands, previously unknown peoples, and a profound paradigm shift in concepts and understanding of the world. Indeed, presently we are in the midst of a second Renaissance, a vibrant *Zeitgeist* [spirit of the age] and culture of discovery. It is a most exciting time in biomedical science, and we can be allowed to possess great hopes and expectations. Although we face challenges, for physiologists, there are enormous opportunities to take advantage of these new methodologies and approaches and thus to gain increased understanding of fundamental biologic mechanisms of high relevance. As recognized, the ever-rising tide of microarray genomics and other "-omics," demonstrates a shift from a deductive hypothesis-based approach, to questions of natural phenomena, to data-driven "discovery." This is not to attempt to transcend reductionism, as with such discovery one can pose important hypotheses to test and unravel fundamental mechanisms. Physiologists then, as "integrative" and regulatory biologists, form the bridge between cellular and molecular discovery, working to understand fundamental mechanisms of bodily function, the pathobiology of disease, and the challenges of patient care. For fetal and neonatal physiologists, as with all other biomedical scientists, the goal is to continue to pursue and uphold the core values of science: objectivity, independence, self-critical thinking, and a relentless urge to explore the most important problems within our ken, with unwavering commitment to fulfill these ambitions.

As noted, Sir Isaac Newton and others before him, observed that if today we see further, beyond the hills and rivulets of our predecessors, it is because ". . . we stand on the shoulders of giants" (Merton 1965). Bertolt Brecht (1898–1956), the German dramatist and poet, observed in his *Leben des Galilei* [Life of Galileo] "Science has

only one commandment: contribution" (Brecht 1938–1939). This constitutes part of the life and vitality of science; it is the essence of progressivism in the life of the mind. In an interview with the TV personality and commentator Charlie Rose, Jeff Bezos cofounder of Amazon Books observed, "Our aim is to Invent the Future" (Bezos, 26 February 2009). Despite almost exponential increases in our knowledge and understanding, in many ways our situation is analogous to that of Sir Isaac Newton, as we are but picking up bright pebbles by the seashore, while a vast horizon stretches ahead.

We must beware of hubris, however. Among caveats to consider are our timidity to think "outside the box," to speculate and envision beyond our data and limited information. In his essay "Speculation beyond speculation," Julius Comroe addressed this issue:

> One problem here may be not what's *in* the published article, but what's *not* in it. In the myriads of articles listed monthly in *Index Medicus*, what is usually *not* included is speculation—an author's ideas or suggestions reached by contemplation, reasoning, conjecture, or surmise but not yet supported by sufficient hard data to merit separate publication. I speculate that we might speed the advance of medical science by inviting all authors to include some speculation, labeled clearly as such in their scientific articles, to follow Introduction, Methods, Results, and Discussion—and to precede and not to be confused with the author's well-fortified Conclusions.
>
> (Comroe 1977, p. 343)

In addition, we face other challenges. In his 1967 lecture at the University of Newcastle upon Tyne, the 1953 Nobelist in Physiology or Medicine, Sir Hans Adolf Krebs (1900–1981) spoke on "The Making of a Scientist." He emphasized the importance of working under and being mentored by great teachers, the critical role of centers of excellence with cross fertilization of ideas among colleagues, and the importance of having adequate time for research with reflection (Krebs 1967). These issues, vital to science, continue to face us today. As a corollary, despite recognition of multiple serious problems in the reproductive sciences, and the monumental advances we have (made much of which has resulted from support of governmental agencies, the MRC, NIH, and others), the amount spent on research for the diseases of women and their fetuses, infants, and children is miniscule compared to that for waging war with its deadly toll. This, despite the importance of the problems presented, and the incredible opportunities that lie before us.

In terms of the vital need for creativity in science, more than a century ago, the French mathematician Jules Henri Poincaré (1854–1912) wrote:

> To create consists precisely in not making useless combinations and in making those which are useful and which are only a small minority. Invention is discernment, choice . . . Among chosen combinations the most fertile will often be those formed of elements drawn from domains which are far apart . . . The true work of the inventor consists in choosing among these combinations so as to eliminate the useless ones. . .
>
> (Poincaré 2000, pp. 87–91)

In terms of questions to explore, the mathematician David Hilbert (1862–1943), of the University of Gottingen, once said that the vigor of a science can be measured by the number and quality of the unsolved problems it addresses. To demonstrate the viability of mathematics, at the 1900 International Congress in Paris, he formulated a dozen (later expanded to 23) "Problems of Mathematics" that he believed to be of fundamental importance. Although several of these were solved within a short time, some continued to engage mathematicians throughout the twentieth century (Weyl 1944). A challenge in the new century is to identify and explore the physiologic problems and enigmas of most fundamental and general significance to life.

For fetal and neonatal physiologists, as well as all scientists, an essential requirement is to be as rigorous as possible in designing and conducting experiments. Several decades ago, the biophysicist John Rader Platt (1918–1992) considered the basis on which some fields of science such as molecular biology and nuclear physics experience rapid progress, while other fields languish. As he outlined with a Popperian view, we must formally, explicitly, and regularly have multiple working hypotheses; design crucial experiments to exclude, e.g., falsify one or more of these; and conduct our experiments so as to obtain clear results. This path of "strong inference," with multiple working hypotheses, must then be reformulated into a branching logic tree to refine the possibilities and hypotheses (Chamberlin 1965; Hiebert 2007; Platt 1964). With its systematic use of the scientific method, this approach is of inestimable value in terms of economy of time, money, and experimental resources (Davis 2006; Kinraide and Denison 2003; Wenner 1989). Despite certain limitations with the Popperian-Platt approach, in terms of being able to prove or disprove a given hypothesis (Kinraide and Denison 2003), the correction of competing hypotheses and rationale has transformed science and our understanding of the world. Also a consideration in the concept of multiple working hypotheses is that of Bayesian inference (named for the English cleric of Dissenters, Thomas Bayes (ca. 1702–1761); Bayes Bayes 1763, 1958). That is, by repeated experiments and updating of the probability estimate for a given hypothesis, one can approximate, with greater accuracy, the value of a given function (Howson and Urbach 2005; Jensen 1996). A cautionary note, however, is not to have one's perspective distorted by the patina of precision of some statistical methods and be blinded by the "Haze of Bayes" (Feinstein 1977). In this regard, as physiologists, we do well to not ignore the ontological parsimony of Occam's Razor, named for the English Franciscan friar William of Ockham (ca. 1288–1348); in theory always opt for an explanation in terms of the fewest possible causes, factors, or variables. In his consideration of "Emergent phenomena. . .," the Canadian physiologist Peter T. Macklem (1931–2011) stressed the vital role of physiology in obtaining a deep understanding of life and its secrets (Macklem 2008). In its essence, the seeking of understanding the mysteries of our world's living things is what biomedical science is all about.

29.2 Fundamental Research, Clinical Medicine, and the Role of the Physician-Scientist

In addition to gaining an understanding of fundamental mechanisms per se, of critical importance so as to minimize morbidity and mortality is the need to apply these insights and understanding to clinical problems in medicine. In his consideration of the manner in which basic research has contributed to a number of advances in clinical medicine, the 1962 Nobel Prize Laureate in Chemistry, Max Ferdinand Perutz (1914–2002), of the MRC Laboratory of Molecular Biology, Cambridge, reflected:

> These concepts and techniques were developed by scientists who set out to interpret fundamental biological processes in physical and chemical terms. Generally the problems were so complex that it took very many years to solve them. At the outset our ignorance was too profound for us to foresee the relationship of our work to human disease, and it became apparent only afterwards. Although we know more now than when we started, molecular biology is still too young a science for it to be clear exactly where it will pay off, whence we may do best if we spread our efforts over a wide field. Since research is the art of the soluble, it is often more profitable to study a basic problem in microorganisms where it can be solved, rather than in mammalian cells which are so much more complex. One of my examples has shown how such an indirect approach has helped to unravel pathogenic events in man. It would be a mistake if all molecular biologists switched to mammalian cells as has recently become the fashion, or as they are being forced to do by policy decisions of supporting agencies. There is a unity of life at the molecular level which implies that anything found to be true in *E. coli* may also hold in man.
>
> (Perutz 1976, p. 453)

The same year as the appearance of Perutz' paper, Julius H. Comroe, Jr. and Robert Dunning Dripps (1911–1973), the latter of the University of Pennsylvania, published the results of an exhaustive study in which they examined about 4000 scientific papers to identify those that contained critical information necessary for the treatment of the "top 10" cardiovascular or pulmonary disorders. These included hypertension, coronary insufficiency, cardiac resuscitation, congestive heart failure, and cardiac and vascular surgery. In consultation with over 100 academic physicians, basic scientists, and other consultants, Comroe and Dripps identified 20–30 essential bodies of knowledge required for each clinical advance. As an example, for open heart surgery these included electrocardiography, cardiac catheterization, angiocardiography, anesthesia, specific drugs, and so forth. Of the 529 key scientific articles judged essential for these clinical advances, 41% were in basic science. That is, of reports critical to the advancement of the care of patients, at the time of their publication, four of ten contributions were directed to fundamental methodologies or understanding of mechanisms not believed to be of clinical relevance. Comroe and Dripps concluded that in addition to the need for investigation to be conducted on the nature of research per se, much greater support was needed for biomedical research and its development (Comroe and Dripps 1976). In a preparatory report "Ben Franklin and Open Heart Surgery," these authors had outlined their plan for this study, in which they stressed the importance

of basic, non-targeted, or mission-oriented research in laying the foundation for translational-clinical advances (Comroe and Dripps 1974).

In his 1979 presidential address to the Association of American Physicians, then National Institutes of Health Director James Barnes Wyngaarden warned of the clinical investigator becoming an "endangered species." With considerable insight he noted, "The physician-scientist has a very special role both in posing relevant medical questions and in applying new knowledge to the investigation of disease . . .. The future of clinical science depends on the quality and the numbers of new leaders in the field" (Wyngaarden 1979, p. 1259). In a later address to the Association of American Medical Colleges, Wyngaarden expanded upon the view that ". . . the application of scientific advances to maintain good health and to prevent and treat diseases, ultimately is the responsibility of the physician. The trained clinical investigator is the critical link between the laboratory and the health care provider. In the face of the explosive growth of basic knowledge in the biomedical sciences . . . the shortfall in training of clinical investigators assumes additional significance" (Wyngaarden 1984, p. 159). In addition to Wyngaarden (1983), the critical need for physician-scientists in maintaining the biomedical-research enterprise has been noted by many others (for instance, see Cadman 1994; Neilson et al. 1995; Rosenberg 1999; Schechter 1998).

More recently, Nobel laureates Joseph Leonard Goldstein and Michael Stuart Brown, of the University of Texas, Southwestern, in Dallas, have emphasized the urgent need to reinvigorate clinical investigation, the "intellectual core of academic medicine." They note that the clinical scholar with analytic insight is the limiting factor in this process of revitalization (Goldstein and Brown 1997). As we commence a new century, those of us in academic medicine need to evaluate carefully where we are going in terms of advancing research in the basic sciences, in the reproductive sciences, and in fetal and neonatal physiology.

Also not to be overlooked, by educating a generation of physician-scientists, advances in the field have contributed greatly to the development of academic departments of obstetrics and gynecology, pediatrics, and other fields. As part of their training, such "bridge builders" have cultivated the "investigative mind" and, in addition to their research contributions, have served as mentors and role models for students, residents, and fellow faculty (see Longo et al. 1999; Longo and Jaffe 2008). A contemporary problem of concern is that of the physician-scientist as beyond being an "endangered species," but rather as with the passenger-pigeon and many other species, near extinction. As Wyngaarden noted, the ". . . trained clinical investigator is the critical link between the laboratory and the health care provider" (Wyngaarden 1984, p. 159).

29.3 Fetal and Neonatal Physiology and Its Relation to Physiology in General

As noted in the introduction, physiology as "the Queen of the Biomedical Sciences" has a glorious history. However, as with so many aspects of its development, the field of fetal and neonatal physiology has been a relative latecomer to this Renaissance in contemporary science. Only toward the *fin d'siècle* has it taken full advantage of the powerful tools of cellular and molecular biology, to gain a deeper understanding of the functions in the several areas of interest. Concurrently, development of this discipline has played a central role in the advancement of physiology, in general, and allied sciences. Both from the standpoint of the basic sciences and from that of translational/clinical applications, these investigations have contributed enormously to an understanding of life and the betterment of humankind. In terms of fundamental science, advances have proceeded at essentially every level: system, organ, tissue, cellular, subcellular, and molecular. In many instances, this advanced understanding has contributed to topics such as the hormonal regulation of physiologic systems; pulmonary surfactant, cellular effects of abnormal oxygenation (hypoxia or hyperoxia), asphyxia, and ischemia, and the function(s) of essentially every organ/system in the body. Although some would argue that systems and organ physiology is *passé*, we would be reminded that despite the enormous advances and insights that cellular and molecular biology has garnered, a number of vital questions in terms of function only can be addressed by integrative, translational physiology. Thus, rather than an "either-or" approach to science and its support, we must continue to advance integrative physiology/biology in a vertical approach at every hierarchical level possible.

From the standpoint of clinical care, these advances in understanding of the fundamental basis of functional mechanisms have led to enormous advances. For instance, the mid-twentieth century survival rate of premature infants weighing ~1 kg was ~5%. Today, it is 95% or higher. Many other indices of progress in lessened morbidity and mortality could be cited. Despite these achievements, however, we are faced with challenges for the future. As early as in his 1968 Presidential Address to the Society for Pediatric Research, Norman Kretchmer (1923–1995), then at Stanford University, observed that research in perinatology must become a full-fledged science of human development and that this constitutes both its definition and its challenge for the future (Kretchmer 1968). A decade later, as Director of the NICHD, Kretchmer reiterated this view, stressing the paradox of the new methodologies presenting a "burst of technological advance," accompanied by the simultaneous limitations in funding for cutting-edge research with "escalation of costs." Being ". . . charged with the responsibility of protecting the health of tomorrow's children as well as the health of today's children," Kretchmer noted the two functions of scientific research, both to discover new findings and to identify areas of ignorance. He concluded, ". . . as clinicians we have learned that we cannot apply our present knowledge to the care of the patient without simultaneously applying our present ignorance" (Kretchmer 1977, pp. 992–993). In a 1970

Symposium *Horizons in Perinatal Research. Implications for Clinical Care*, Kretchmer endorsed the power of the idea of considering the mother and fetus as a unit, investigation providing knowledge of details of which will lead to ever greater reduction of fetal, infant, and maternal morbidity and mortality (Kretchmer and Hasselmeyer 1974).

Another who considered the role of scientific research in contributing to health and combating disease was Lewis Thomas (1913–1993), former Dean of the Schools of Medicine at both Yale and New York University. Thomas made the prescient observation:

> If our society wishes to be rid of the diseases, fatal and non-fatal, that plague us the most, there is really little prospect of doing so by mounting a still larger health-care system at still greater cost for delivering essentially today's kind of technology on a larger scale. We will not do so by carrying out broader programs of surveillance and screening. The truth is that we do not yet know enough. But there is also another truth of great importance: we are learning fast. The harvest of new information from the biological revolution of the past quarter-century is just now coming in, and we can probably begin now to figure out the mechanisms of major diseases which were blank mysteries a few years back as accurately and profitably as was done for the infectious diseases earlier in this century. This can be said with considerable confidence, and without risk of overpromising or raising false hopes, provided we do not set time schedules or offer dates of delivery. Sooner or later it will go this way, since clearly it can go this way. It is simply a question at this stage of events of how much we wish to invest, for the health-care system of the future, in science.
>
> (Thomas 1977, p. 46)

We are blessed to live in a world in which biomedical science continues to make enormous progress. Virtually, every issue of *Cell*, *Nature*, *Science*, and *Proceedings of the National Academy of Sciences* announces new triumphs in the accelerating gain of new knowledge. Nonetheless, despite these accomplishments, our ignorance remains vast. As has been pointed out by others, in science, the full fruition of our studies depends upon three factors, the passion to know and to seek understanding, the initiative to study and discover, and the awareness and ability to apply these. "Highly specialized conditions have to be fulfilled before the plant will bloom, before the flower will fruit and before the fruit will ripen" (Hinshelwood 1960, p. 424). As this essay gives evidence, advances in fetal and neonatal physiology, with breakthroughs in related fields of science, have helped to lessen the gap between fundamental and clinical science, basic and translational, and the theoretical and the practical. Nonetheless, a vast gulf, a "no-man's land," or terra incognita remains. Our task therefore is clear; it is to persevere! By our efforts may knowledge, one of the greatest instruments of highest ends, "advance, ... mastery over environment increase, drudgery be abolished, sickness healed, the people fed and life made happier" (Hinshelwood 1960, p. 429). As biomedical scientists, we seek a vision of nature, which may be expressed and communicated in an approach to understanding. With the ever-expanding mass of data, and the potential for further achievements in diagnosis and therapy, who can predict the future advances to be achieved? We must not ignore the admonition of T.S. Eliot, poet, playwright, and critic, "Where is the wisdom we have lost in knowledge? Where is the knowledge we have lost in information" (Eliot 1963, p. 147).

29.4 Fetal-Neonatal Physiology and the Future

One author has divided scientists into two groups, those who make it *possible* and those who make it *happen* (Calder 1951). Geoffrey Dawes and many of the other investigators considered in these pages were influential in both of these roles. Rather than simply enthusiasts of reductionism, with an historical view of scientific knowledge and as determined scientists, they offered a distinctly self-critical, analytic view of scientific investigation, one that challenged orthodoxy. With sound mind and robust enthusiasm, their passion for science and for life was unbounded. In addition to furthering basic science, many were particularly mindful of the clinical implications and relevance of their studies. In general, this nuanced, irenic approach avoided the polemics of much of the ill-willed and ill-conceived contention, in terms of the commonly accepted paradigm or set of assumptions, held by some in science. With a broad vision of the experimental terrain, they carefully chose the questions to explore. With a lucid pen, the writing styles of many were clear and succinct. Among this group, Dawes became a doyen of developmental physiology and its exposition. With the many investigators who "came of age" at the Nuffield Institute, they left a legacy to be honored. As noted by John Challis in his Foreword to the first edition of this book, the names of Dawes' students and collaborators form a stellar roster of those who proved to be leaders in developmental physiology/pharmacology and perinatal/neonatal medicine. As one who sought to understand the regulation and integration of physiological processes in developmental physiology, with multiple feedback systems at successive levels of organization, along with his predecessors, Dawes was a seminal figure. Without question, he left an enormous legacy, both in terms of science per se, as well as in dedicating his career to educate and inspire young basic scientists, and physician-scientists to become the future leaders in investigation and education.

A fundamental tenet of science is its international scope. Its advances and achievements cannot be considered only in terms of contributions by a particular organization or given country. Although discoveries that open new lines of investigation are made by individuals, almost without exception others have expanded upon and developed these further. Quite obviously, such achievements depend upon the ideas and creativity of gifted individual investigators. Their fruition, however, is a function of opportunities, intellectual environment, the availability of technological resources, financial support, and other social-cultural forces. Under Dawes' direction, the Nuffield Institute was in many respects an Arcadia (Jacopo Sannazaro, *Arcadia* Sannazaro 1504), a model of combined individual brilliance, collaboration, and competition among its members. With expansion and transfer of "offspring" to other centers, the Institute contributed to the advancement of research and scientific progress throughout the world. With his fine mind, being bright and well-read with an awesome memory, Dawes was blessed with a passion for excellence. With an infectious enthusiasm and basic goodness, he was a first-rate mentor and role model. Some have said that "physiology is not so much a subject as a point of view" (Franklin 1953, p. 344). Dawes and many of the others

exemplified this thesis. Those with an astrological bent might view these events as a conjunction of the stars; the time was right for the opening of a new field of science; the individual was right in possessing many gifts, being focused, and having a platform at one of the world's great universities from which to work and expand his influence.

In recalling the eulogy noted earlier by F. J. W. Roughton for Sir Joseph Barcroft, the same words may be said of Geoffrey S. Dawes. In one of his brief essays on the history of fetal and neonatal physiology, presented at a special symposium to honor him and his life contributions, Dawes observed, "To discuss the past too long is neither profitable nor interesting. To discuss the present is not easy …. To discuss the future is another matter altogether. If one could see far ahead, the word discovery would be inappropriate." Then in an allusion to the writings of St. Paul, he continued, "We see through a glass, darkly.[1] We have consistently underestimated the complexities of life before birth" (Dawes 1985, pp. 829–830). In 1946, Dawes commenced his scientific life with a question, how to understand cardiorespiratory reflexes and responses to stress. Five decades later, his final contribution also was a question, how will our work, research, and new knowledge benefit human welfare?

29.5 What Lessons Are to Be Learned?

In closing, several individuals have inquired as to what lessons, if any, I have learned in surveying the literature and discussions with fellow scientists in preparing this essay. Among those who learned of my efforts on this endeavor, I must admit that several projected a dubious air, "who needs history?" They argued that the universe of science is changing to such a great extent and, so rapidly, that history can contribute little to our understanding. "The past is another world, and that world is gone," one stated. In line with this argument, that philosophy which some would call "presentism" appears to be not uncommon among many scholars who question a historical perspective as being misleading, irrelevant, or even unknowable.

If for nothing else, it is hoped that the present synthesis will be of value to young workers in the field, to satisfy their curiosity, and with the core of references, to give them an appreciation of from whence we have come and the opportunity to pursue in more depth some of the issues presented. Of course, history is more than "firsts" dates and the lives of those who have gone before. I would suggest that an anchor to the past provides a foundation for getting things right, i.e., replicable answers to important questions, as well as for tracing influences and perceptive interpretations of society and culture to shape our current thinking. A historical perspective helps to restore focus and enables us to place our lives in the context of those individuals

[1] I Corinthians 13:12, King James Version.

whose works have shaped our thinking. By presenting another dimension, it helps us to understand the present, to appreciate our limits, and to know ourselves in a more meaningful way. It offers the opportunity to reflect upon the complexity of life and appreciate that despite how much we know, there exist a vastness that we do not understand. Of the many uses of history, one is to inspire the present and, hopefully, use this appreciation of the past to guide our future.

In an interview following publication of her blockbuster *Silent Spring* (1962), Rachel Carson (1907–1964) is said to have observed that sometimes the writer doesn't choose the subject, rather the subject chooses the writer (Carson 1962). Perhaps that is the case in the present instance. Myths fill our lives and are transmitted from generation to generation. An historical view can fill us with a sense of humility. Humility is appreciating how much we owe to those that have gone before, and the recognition of how much we have yet to learn. No one could claim that historical synthesis produces definitive answers for all time; it is a process, an evolution of sorts. As Samuel Reynolds observed, history traces the "many slender threads" of our heritage (Reynolds 1978). Hopefully, the tapestry woven will help to present a cogent story of discovery as well as inspiration to others, a vision of challenges that lie ahead, and of possibilities for further refinement.

Thus, among the questions we may ask are: For what kind of world of biomedical science should we strive, and how can we create it? Given our individual abilities and the multitudinous technologies available, what are the most critical questions that we can ask? How can we work so that, beyond being mere "stamp collectors," our discoveries be of utmost value, not just in gaining a better understanding of fundamental mechanisms but as importantly in contributing to more meaningful lives for pregnant mothers and their infants and children? Within this context and for the extent to which one can learn lessons, I would like to consider several major issues.

First, I have come to appreciate more than ever before our rich heritage. In truth, and as exemplified in many examples given, research in the basic sciences has made enormous contributions in the endless war against disease and to the well-being of mothers and children. As an illustration of "translational" research at its best, one could hardly have a more lucid and noteworthy example than fetal-neonatal physiology. The many cases cited are foundations upon which contemporary care of the pregnant mother and her newborn infant rest. Can we do more? Of course, that is the challenge and opportunity for the support of bright young investigators to invent the future.

To be a part of academic medicine is one of the most noble and rewarding life experiences that one can imagine, and the opportunity to work with bright students, gifted postdoctoral fellows, and passionate colleagues is unsurpassed. For me, with the exception of Huggett and Sir Joseph Barcroft, I have known essentially all of the key contributors to this discipline: Edward Adolph, Virginia Apgar, "Dr. B" Donald Barron, Roberto Caldeyro-Barcia, Sid Cassin, John Clements, Robert Comline, Kenneth Cross, Nicholson Eastman, Louis Flexner, Stan James, Ted Quilligan, Elizabeth Ramsey, Sam Reynolds, Millie Stahlman, Leonard Strang, Geof

Thorburn, Wilfred Widdas, William Windle, and of course Geoffrey Dawes. Indeed, these are some of the giants of basic, translational, and clinical research upon whose shoulders we stand.

If I may be allowed a brief anecdote, it was in late 1962 or early 1963 that, as an embryonic faculty member at UCLA, I spent 3 days squiring Professor Nicholson Eastman of the Johns Hopkins Hospital around the Los Angeles area. He had come to the campus to serve as a Visiting Professor, and my departmental chairman, Daniel Green Morton (1903–1980), requested that I meet his airplane on arrival and chauffer him to the several hospitals and venues at which he would be speaking. As background, Dr. Eastman had spent a year at the Rockefeller-funded Peking Union Medical College in China. He lit up when he learned that I had spent a year and a half in China as a US Army medical aid during the latter part of World War II and thereafter (1945–1946) and also that I had returned recently from spending 3 years in Nigeria, West Africa, trying to "save the world" (which I didn't). Notable for me during this 3-day immersion was the manner in which Dr. Eastman quizzed me. In particular, several times each day he would confront me with what I viewed as the major challenges facing the pregnant mother and her newborn infant, "what are you going to do to improve maternal and child health?, or "what were my plans to advance the specialty?" Regrettably, I do not believe that I ever gave him an answer that satisfied his roving mind. Those 3 days changed my life forever.

Second, the Greek philosopher Democritus (fl. 500 BCE) is credited with saying, "I had rather discover one true explanation than be the King of Persia." Nothing is more exciting or breathtaking than making a new discovery. As has been said, ". . . life is not about how many breaths you take, but what leaves you breathless." I am struck that, in many respects, knowledge and understanding grow most rapidly in the border zones between the sciences. In conjunction is the appreciation that with the rapid, exponential advances in science and technology, one of our challenges as investigators is that we must be reinventing ourselves continuously to remain current. And this is, in part, what makes it so exciting. Daily, we are learning and expanding our horizons. Certainly for Geoffrey Dawes, this was the case. Among his achievements was to have developed an ambitious agenda, to have "sowed many seeds of erudition," and with that to have made discoveries with a profound resonance for their time. Within the Nuffield Institute, he built a valuable edifice notable for its scientific rigor and clarity.

Research, research, research, that is the key to unlocking the mystery of disease and entering a paradise of health. As investigators, we are limited not so much by existing technology but by our curiosity and imagination. Importantly, we must remember to "tie ourselves to the mast," Ulysses-like, to remain true to the ideals of science. We must avoid excessive enthusiasm for technology and instrumentation as ends in themselves, rather than as tools to advance understanding. At the same time, another of the challenges, and sometimes the most difficult question a scientist must address, is the uncertainty of which of innumerable problems to pursue, what is the most important question or innovative idea of which we are capable of addressing. Of course, conducting research has its own challenges and difficulties. As a postdoctoral fellow when I expressed frustration with the way

things were going, my mentor Robert E. Forster II at the University of Pennsylvania would respond, "If research was so easy, everybody would be doing it." It is never a "breeze" to conceive of the great ideas or hypotheses to test. Performing the experiments is not without unanticipated difficulties. Interpreting the results is not always straightforward. And writing up the results and getting them published in a first-rate journal is not without its challenges. As is too well known, in many respects, the process is painfully slow and fraught with hurdles.

Somewhere, I have heard that there are "Ten Commandments" of what it takes to be a successful scientist. These include being reasonably bright, being curious and having a passion for discovery with commitment to the work ethic, perseverance, having lots of drive (not being complacent), choosing one's mentor with care, pursuing a problem that is important, being creative, reading widely and being open minded, remaining focused, not overreaching (choosing a project that is doable), and being lucky! (the more the better).

Third, one cannot be but awed and humbled by the manner in which the field of fetal-neonatal physiology has progressed. As noted, tracing its development is to follow as a case study the evolution of biomedical science from organs and systems to the most subtle detailed and reductionist alchemy of subcellular mechanisms of cellular and molecular biology. Some have likened this to a Rorschach ink blot test, which while it possesses a definable shape, is recognized by different individuals in terms of unique patterns and unifying themes. It is a challenge of immense complexity and is truly multidisciplinary, with the common link of achieving an understanding of developmental processes that binds investigators together. As noted in the introduction, during the past several decades, the genomic revolution has somewhat overshadowed classical physiology. Despite a tsunami of data, however, it remains for physiology (systems biology, functional genomics, or whatever) to interpret and understand the implications and how the organism develops and interacts with its environment. Along this line, one must acknowledge the enormous debt we owe to animal-based research, from mice, rats, guinea pigs, to sheep and other ruminants, and then to primates. Quite obviously, if one is to understand mammalian biology, one must study mammals.

Fourth, the community of scientists is vast, perhaps one of the truly global communities. From genesis of the "big bang" of the new galaxy of investigation that emerged at Oxford and Cambridge, a closely interconnected chain of small research groups joined Britain in an "invisible college" that stretches across the USA, Canada, and Australia. In contrast to neuroscience, cell and molecular biology, and other fields, an examination of research centers of fetal-neonatal physiology discloses that they are comparatively few. Some examples in no particular order include in the USA, Cincinnati, Ohio, Denver, Colorado, Gainesville, Florida, Madison, Wisconsin, Portland, Oregon, San Antonio, Texas, and Loma Linda, Los Angeles, and San Francisco, California, London, Ontario, Canada, and Melbourne and Sydney, Australia, and perhaps a few others. As is perhaps self-evident, for the most part, the rise and fall of these centers has depended upon a scientific and often charismatic leader.

Fifth, in this regard, mentorship by experienced scholars is vital to the development and maturation of young scientists. A great mentor resembles being a parent, "like motherhood, mentorship is forever." It requires a commitment to listening, helping with problem-solving, mutual respect, trust, and assisting in every way possible the advancement of the mentee's career. The handing down of information, approaches, and values is encompassed to some under the rubric of tradition and bonding in a shared remembrance. By example and the enhancement of idealism, one learns to spurn the path that is straight and smooth, and to pursue the scholarly and contemplative life. With such dedication and commitment, one becomes a first-rate scientist—and something more.

Sixth, cutting-edge science is fast moving and requires collaboration of individuals with widely different training, experience, and expertise. Science has become social with a distinctive culture. Because the disciplines of fetal and neonatal physiology have become broad and multifaceted, the former paradigms of the "solo scientist" and "rugged individualism" are less applicable today. In addition to developmental aspects of essentially every organ system, this includes vertical integration of the regulation of membrane trafficking, cytoskeletal dynamics, signal transduction pathways and networks, protein-protein interactions, nuclear organization, gene regulation, cell division, cell death, and so forth. In the search for truth, advantage must be taken of every opportunity to learn and to grow by interacting with the most able minds available. In light of the major issues before us, we are well advised to strengthen connections for interdisciplinary investigation with scientists and institutions beyond our own universities and our borders. In terms of interdisciplinary investigation, we need to include physical scientists, bioengineers, mathematicians, information technologists, and others sharing ideas and working together to answer the most challenging questions.

Along this line is the meritocracy of science: that original and important ideas and experimental studies originate from investigators in all quarters of the globe. Fundamental is the importance of collaboration of basic scientists and physician-scientists to advance the frontiers. As noted earlier, with the roots of biomedical science in understanding natural phenomena, in view of the enormous complexity of life, collaboration among those of different disciplines will be required for the harvest is in implementing discoveries and that knowledge to effect solutions that solve health problems to benefit individuals, and the world in which we live. In terms of physician-scientists, this "endangered species" of individuals who can bridge disciplines must be preserved in the exploration and search for increased understanding and the answers to serious problems. Given the urgent need to confront major issues and problems of diseases of the fetus and newborn about which we know little, our goal must be to advance the frontiers of basic research and the search for truth, and its applicability to clinical medicine. Among great challenges we face is to identify the most urgent research questions and explore the most effective ways to attack and solve them. As in the past, it is most probable that individual scholars or small groups will determine the most pressing questions and seek their solutions with success. As leaders and innovators, we must maintain a vision to advance the frontiers of science and the good of humankind.

Seventh, it is clear that the practice of biomedical science and the quest to understand how living organisms function has been transformed dramatically. To a great extent, technological advances drive scientific discovery and its application to healthcare. In the present age, investigation is expensive with many technologies requiring costly instruments best suited to a core laboratory (advanced multiphoton laser scanning confocal microscopy and other imaging, DNA, RNA, and protein sequencing, mass spectrometry, oligonucleotide microarray analysis, and so forth). In large part, the future of research and discovery in developmental physiology lies with "next-generation" genomic, RNA, and protein analysis, advances in imaging, tissue engineering, computational biology, mathematical modeling, and many other areas.

Eighth concerns the issue of funding to support one's studies. In the advancement of science, one must appreciate the vital role fellowships, training grants, scholarships, visiting professorships, sabbaticals, and other mechanisms play in promoting first-rate science and scientists and in obtaining a fresh viewpoint on life. By most standards, the funds required to support these endeavors are relatively small, however, their payoff and benefit for the good of humankind have been, and can be, immense. An immensely complex business, in science, one can never know enough.

As is appreciated, unpredictability is the fabric of discovery. In much of present-day academia curiosity-driven research is not looked upon favorably. Rather, grant applications must include specifics of "preliminary studies" and the most minute details of the work already having been done, with anticipated "relevance" to human health. Investigators spend increasing amounts of time or fine-tuning their proposals, chasing diminishing funding. Thus, their available time for "day dreaming" and creative thinking is all but obliterated. Coupled with increasing University administrative responsibilities, compliance with new healthcare rules, and decreasing indirect support for research efforts, one wonders to what extent a young Barcroft, Barron, Windle, or Dawes would be attracted to or be able to survive the current environment.

Sobering in this regard is an appreciation of the number of instances recounted here in which an investigator, who later would become world-renowned, experienced difficulty in gaining funding for their research, and/or presenting their work at a national meeting, and/or getting their studies published. One is reminded of the poignant words of Nobel Laureate Albert Szent-Györgyi (1893–1986), pioneer in studying bioenergetics and oxidative phosphorylation, who, several times having been denied grant support, decried the process of applying for external funding. In reflecting upon his life experiences, he wrote:

> One ... which has filled my whole scientific life with agony has been writing project proposals ... the situation is not ... simple because research means going out into the unknown with the hope of finding something new. ... If you know in advance what you are going to do, or ... to find there, then it is not research at all ... it is only a kind of honorable occupation ... all my life [I] have had to fill up page after page of my ... proposals with untruths. There was no way out. The only alternative would have been to give up research.
>
> (Szent-Györgyi 1971, pp. 1–2)

Szent-Györgyi also wrote further on the funding quagmire and frustrations faced by young investigators (Szent-Györgyi 1974).

Nonetheless, I must confess that for the scientific officers and staff members of the National Institutes of Health and other funding agencies who help to make all of this possible, I have the highest regard. Over the many years, I served on several different NIH study sections as well as other review groups. My contacts with NIH staff began with a Postdoctoral Fellowship in 1964, and first R01 research grant in 1967. The ensuing decades have also included a training grant, two program project grants, and other awards as Principal Investigator. Some of the finest people in public service that I know have been at the NICHD. I think and have wonderful memories of working with Duane Alexander, Charlotte Catz, Joseph Hwang, Jehu Hunter, Michael Knecht, Norman Kretchmer, Michael McClure, Samuel Moss, Tonse Raju, William Sadler, Sumner Yaffe, and many others. Without exception, these bright, dedicated, wonderful people were and are committed to first-rate science and improving the health of mothers and children. An historian could make a great contribution to life by documenting the many, many contributions by those in this Pantheon of administration.

Finally, in preparing this volume, I have been humbled and honored for the opportunity to renew friendships with many of the leaders and "masters" of medicine. A few of these include Sir Robert and Richard Boyd, John Challis, John Clements, Richard Harding, "Mont" Liggins, Mildred Stahlman, Philip Sunshine, David Walker, John Widdicombe, Maureen Young, and many others.

29.6 Conclusion

To draw to a close, one of fisherman Geoffrey Dawes' favorite authors was the English man of letters Izaak Walton. In his 1653 work, *The compleat angler. Or, the contemplative man's recreation*, Walton wrote that, "... it is an art, and an art worthy the knowledge and practice of a wise man," "may be said to be so like the mathematics that it can never be fully learnt," and "somewhat like poetry—men are to be born so" (Walton 1983, pp. 155–163). In concluding his essay, Walton wrote,

> My rod, and my line, my flote and my lead,
> My hook, and my plummet, my whetstone and knife,
> My Basket, my baits, both living and dead,
> My net, and my meat, for that is the chief;
> Then I must have thred and hairs great and smal,
> With mine Angling purse, and so you have all.
> (Walton 1983, p. 155)

And further,

> Welcom pure thoughts, welcome ye silent groves,
> These guests, these Courts, my soul most dearly loves;
> Now the wing'd people of the Skie shall sing

My cheerful Anthems to the gladsome Spring;
A Pray'r book now shall be my looking glasse,
In which I will adore sweet vertues face.
Here dwell no hateful looks, no Pallace cares,
No broken vows dwell here, nor pale fac'd fears,
Then here I'l sit and sigh my hot loves folly,
And learn t'affect an holy melancholy.
And if contentment be a stranger, then
I'l nere look for it, but in heaven again.
(Walton 1983, pp. 162–163)

Such is a fair description of the Geoffrey Dawes that we knew.

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