Reviews of

90 Physiology, Biochemistry and Pharmacology

With 31 Figures

Springer-Verlag
Berlin Heidelberg New York 1981

ISBN 3-540-10657-X Springer-Verlag Berlin Heidelberg New York
ISBN 0-387-10657-X Springer-Verlag New York Heidelberg Berlin

Library of Congress-Catalog-Card Number 74-3674

Offsetprinting and Binding: Konrad Triltsch, Würzburg
2121/3130-543210

Contents

Indexed in Current Contents

Feodor Lynen (1911–1979)

Nur wenige Wochen nach seiner Wahl zum Präsidenten der International Union of Biochemistry, am 6. August 1979, verschied Feodor Lynen an den Komplikationen eines operativen Eingriffs. Es war ihm nicht vergönnt, die Pläne zu verwirklichen, die ihm seine ungebrochene wissenschaftliche Dynamik und seine jugendliche Lebensfreude für die Zeit nach seiner

Emeritierung vorgaben. Am 29. Februar 1980 versammelten sich weit über tausend Schüler, Freunde und Kollegen aus aller Welt im Münchener Herkulessaal, um in Anwesenheit des Bundespräsidenten in einer Akademischen Feier von diesem großen Forscher, begeisternden Lehrer und liebenswerten Freund Abschied zu nehmen. Mit ihnen betrauerte die internationale biochemische Gemeinde den Verlust eines ihrer bedeutendsten und produktivsten Mitglieder. In einem kleinen Friedhof, auf einem Hügel nahe des Starnberger Sees gelegen, der an schönen Tagen einen eindrucksvollen Blick auf die von ihm so sehr geliebten Alpen bietet, liegt Feodor Lynen zur letzten Ruhe gebettet.

Feodor Lynen entstammt einem seit Jahrhunderten im Raum Stolberg (Rheinland) ansässigen, erfolgreichen Fabrikantengeschlecht, in dem das Streben nach Pflichterfüllung und Leistung ein beherrschender Zug war. Sein Vater, Wilhelm Lynen, wurde im Jahr 1901 als Ordinarius für Maschinenbau an die Technische Hochschule München berufen und lebte dort bis zu seinem Tod im Jahre 1920. Seine Frau Frieda, geb. Prym, schenkte ihm neun Kinder, von denen Feodor als siebentes am 6.4.1911 zur Welt kam. Die Stadt München und ihr oberbayerisches Umland wurden ihm zur echten Heimat, die er nie für längere Zeit verlassen sollte. Sie hat mit ihrem lebensfrohen, barocken Wesen nachhaltig seinen Lebensstil geprägt. Seine Liebe zu den Bergen und dem Skisport bescherte Feodor nicht nur viele, in vollen Zügen genossene Stunden der Entspannung und Erholung; sie hat auch den Verlauf seines Lebens nachhaltig beeinflußt. Denn eine schwere Knieverletzung, die er sich bei einem Skirennen in Kitzbühel zuzog, und die ihn kurz nach Studienbeginn für mehrere Monate ans Bett fesselte, bewahrte ihn vor militärischer Ausbildung und dem Dienst in einer NS-Organisation und ließ ihn den 2. Weltkrieg in der Heimat überstehen. Selbst ein erneuter Skiunfall im Jahr 1952, der das bereits angeschlagene Knie vollends versteifte, hinderte ihn nicht daran, auf Kurzskiern seinen Mitarbeitern bei hochalpinen Skitouren rasant voranzufahren. Die unnachgiebige Beharrlichkeit, die er in der Ausführung seines geliebten Skisports zeigte, spiegelte sich auch in seinem wissenschaftlichen Arbeitsstil wider.

Feodor Lynen war, in den Worten Konrad Blochs, einer der großen Architekten der klassischen Biochemie. Er vereinigte das Gefühl des Chemikers für die Substanz und ihre Reaktionsweisen mit dem Verständnis des Biologen für die besonderen Bedingungen und Möglichkeiten des zellulären Stoffwechsel. In dieser glücklichen Verbindung erwies er sich als würdiger Schüler seines akademischen Lehrers Heinrich Wieland und des von ihm hochverehrten Altmeisters der Biochemie, Otto Warburg. Das wissenschaftliche Werk Feodor Lynens, seine Zielsetzungen und sein methodisches Vorgehen spiegeln deutlich die Einflüsse seiner Lehrzeit als Student und Doktorand wider. Er hatte das Glück, in einer Zeit an der Universität München Chemie zu studieren, in der die Naturwissenschaftliche Fakultät

mit hervorragenden Wissenschaftlern besetzt war. So gehörten der Physiker Walter Gerlach, der Physikochemiker Kasimir Fajans, der Botaniker Karl von Goebel, der Anorganiker Otto Hönigschmid und der Organiker Heinrich Wieland zu seinen Lehrern. Bei Geheimrat H. Wieland, dem Nobelpreisträger für Chemie des Jahres 1927, fertigte er eine Dissertation „Über die toxischen Inhaltsstoffe des Knollenblätterpilzes" an und kam so mit einem stark biochemisch orientierten Arbeitskreis in engste Berührung. Nach seiner Promotion im Jahr 1937 heiratete er Eva, die Tochter seines Doktorvaters.

Frau Eva Lynen hat das Forscherleben ihres Mannes tatkräftig unterstützt. Sehr oft unter Zurückstellung eigener Interessen und Pläne wußte sie sich ganz auf ihren „Fitzi" einzustellen. In den schwierigen Kriegs- und Nachkriegsjahren, in denen ihre fünf Kinder der Betreuung bedurften, hielt sie ihm den Rücken frei für den vollen Einsatz in seiner Wissenschaft. Durch ihre offene und humorvolle Art trug sie wesentlich zur Festigung vieler Freundschaften mit in- und ausländischen Kollegen bei und vermochte ihr Haus in Starnberg zu einer vielgerühmten Stätte entspannter Geselligkeit zu gestalten.

Lynens Wirken als Forscher fiel in die Periode, in der die Biochemie aus dem deskriptiven Stadium heraustrat und in die Phase der exponentiellen Zunahme von Erkenntnissen und Methoden über den Zellstoffwechsel überging. Sein Hauptinteresse galt zeit seines Lebens den enzymatischen Reaktionsmechanismen. Als er Ende der dreißiger Jahre seine wissenschaftliche Laufbahn mit den Studien über die Pasteur-Reaktion in Hefezellen begann, entwickelte F. Lipmann das äußerst fruchtbare Konzept der „energiereichen Verbindungen" und stellte die von K. Lohmann 10 Jahre zuvor entdeckte Adenosintriphosphorsäure in den Mittelpunkt des zellulären Energiestoffwechsels. O. Warburg hatte, nach der Entdeckung des sauerstoffaktivierenden Atmungsferments (Zytochromoxidase), in den Pyridinnukleotiden die primären Wasserstoff- und Elektronenüberträger gefunden und damit die Voraussetzung dafür geschaffen, daß die von H. Wieland und T. Thunberg entwickelte Dehydrierungstheorie mit der Sauerstoffaktivierung zu einem einheitlichen Konzept der Zellatmung verschmolzen werden konnte. Die oxidative Phosphorylierung begann durch die Arbeiten von V. Belitzer, A. Lehninger und H. Kalckar Gestalt anzunehmen; H.A. Krebs erkannte, daß der Vorgang der biologischen Endoxidation der Nahrungsstoffe in einer zyklischen Reaktionsfolge (Zitratzyklus) abläuft.

Als Stipendiat der Deutschen Forschungsgemeinschaft (1937–1942) wandte sich F. Lynen dem Problem des Energiestoffwechsels von Tumorzellen und, beeinflußt von den Arbeiten Warburgs über die aerobe Glykolyse der Tumoren, der Pasteur-Reaktion zu. Diese besagt, daß Zellen unter aeroben Bedingungen weniger Zucker verbrauchen als in Abwesenheit von Sauerstoff. Die Arbeiten, die bereits unter den erschwerenden Bedingungen

des Kriegszustands durchgeführt wurden, bescherten Lynen einen ersten großen Erfolg, den Nachweis der regulatorischen Rolle des anorganischen Phosphats für den Zuckerabbau und die Formulierung unterschiedlicher Phosphatkreisläufe in atmenden und gärenden Zellen. In einem 1969 verfaßten Rückblick auf sein wissenschaftliches Leben bekannte sich Lynen mit Stolz zu diesen Frühwerken, die auch der Gegenstand seiner Habilitationsschrift (1941) waren. Daß sie ihm zunächst nicht die gebührende internationale Anerkennung brachten und im Jahr 1944 vollends für mehrere Jahre unterbrochen werden mußten, ist eine unmittelbare Folge des 2. Weltkriegs. Nach der Zerstörung des Chemischen Staatslaboratoriums in München (Frühjahr 1944) versuchte Lynen in einem Behelfslabor in Schondorf am Ammersee und, nach dem Kriegsende, als Gast im Botanischen Institut, seine Forschungen fortzusetzen. Jeder, der die damaligen Schwierigkeiten der Materialbeschaffung, des Verkehrs innerhalb einer weitgehend zerstörten Großstadt und die Vielfalt der Sorgen um das tägliche Leben kennenlernte, kann die Probleme ermessen, vor die der junge Chemiedozent gestellt war. Immerhin erhielt er, dank seiner unzweideutigen Haltung während des Dritten Reichs, sehr bald die Erlaubnis der amerikanischen Militärregierung, als Dozent für Biochemie an der Naturwissenschaftlich-Mathematischen Fakultät tätig zu sein; 1947 erfolgte seine Ernennung zum apl. Professor. Im Jahre 1949 konnte er im 5. Stock des Zoologischen Instituts in der Luisenstraße 14 einige leidlich hergestellte Laborräume beziehen. Einem Biochemiker von heute würde es allerdings schwerfallen, die dort vorhandene Einrichtung als die eines Biochemischen Laboratoriums zu erkennen.

Die Entdeckung des Kofaktors für ATP-abhängige Azetylierungsreaktionen (Coenzym A) durch F. Lipmann und D. Nachmansohn sowie die Arbeiten von E. Stadtman, A. Lehninger und F. Hunter über den Fettsäurestoffwechsel bestärkten Lynen in der Existenz einer von ihm schon 1942 postulierten „aktivierten Essigsäure". Er berichtete, daß ihm der entscheidende Gedanke über die chemische Natur dieser Verbindung bei einem nächtlichen Nachhauseweg von einer angeregten Diskussion mit seinem Schwager Theodor Wieland gekommen sei; er vermutete in der noch nicht einmal mit Sicherheit identifizierten Thiolgruppe das mechanistische Zentrum des Coenzyms A und sah die aktivierte Essigsäure als Thiolester des Coenzyms mit Essigsäure (Acetyl-S-CoA). Innerhalb kurzer Zeit gelang es ihm, diese Verbindung aus Hefezellen anzureichern und ihre chemische Natur sowie ihre enzymatische Wirksamkeit sicherzustellen. Diese Entdekkung, Anfang 1951 in einer Kurzmitteilung an die Angewandte Chemie veröffentlicht, verfehlte ihre Wirkung auf die internationale Biochemie nicht und begründete Lynens Weltruf.

Von diesem Zeitpunkt an konzentrierten sich die Forschungen seines Labors ganz auf die Ergründung Coenzym-A-abhängiger Prozesse. Mit S.

Ochoa zusammen bewies er den Mechanismus der Zitratsynthese aus Oxalazetat und Acetyl-CoA, des noch fehlenden Gliedes in dem von H.A. Krebs formulierten Zitratzyklus. Die ATP-abhängige Bildung von Acetyl-CoA aus Essigsäure und Coenzym A untersuchte er gemeinsam mit dem Labor F. Lipmanns. Die Aufklärung der bereits 1907 von F. Knoop in ihren Grundzügen erkannten β-Oxidation der Fettsäuren gelang durch die Isolierung der daran beteiligten Enzyme und Zwischenprodukte. An diesem, gegen eine starke amerikanische Konkurrenz erzielten Erfolg stand wiederum die Denkweise des Chemikers in Lynen Pate. Da es zu dieser Zeit nicht möglich war, ausreichende Mengen des Coenzyms A in der nötigen Reinheit herzustellen, versuchte Lynen es mit sehr einfach gebauten Analogen, den S-Acylderivaten des N-Acetylcysteamins. Entgegen allgemeiner Erwartung erwiesen sich diese leicht darstellbaren Modellsubstanzen als reaktionsfähige Substrate; dadurch konnte im Münchener Laboratorium der kritische Engpaß im Fortschritt der Forschungen durchbrochen werden.

Es war charakteristisch für Lynens Arbeits- und Denkweise und nicht zuletzt ein Schlüssel zu seinem Erfolg, daß er seine experimentellen Beobachtungen und Erfahrungen, negative wie positive, bewußt zu registrieren und intellektuell zu verarbeiten verstand. Hatte ihn ein Mißerfolg bei seiner Doktorarbeit bereits gelehrt, Ausdauer und Zähigkeit bei der Verfolgung schwieriger wissenschaftlicher Fragestellungen zum Leitmotiv zu machen, so zog er aus dem erfolgreichen Einsatz der N-Acetylcysteaminderivate in den Studien über die Teilreaktionen der β-Oxidation der Fettsäuren eine für ihn typische Schlußfolgerung: „Dabei erhielt ich als Wissenschaftler eine neue Lektion: Sei naiv und versuche ein Experiment, selbst wenn die Aussichten auf Erfolg gering sind. Ich habe den Eindruck, daß viele meiner biochemischen Kollegen – und das mag auch für andere Wissenschaften gelten – mehr Zeit damit verbringen, das Für und Wider eines Experiments zu diskutieren als nötig ist, um es durchzuführen. Ich glaube, daß viele Gelegenheiten für neue Entdeckungen auf diese Weise versäumt werden. Die Natur ist immer unvorhersehbar und die einzige Methode, ein biochemisches Problem aufzugreifen ist, Experimente zu machen".

Es gibt keine Patentlösung für das Angehen eines wissenschaftlichen Problems. Die Art der Bearbeitung hängt nicht nur von der jeweiligen Fragestellung und den methodischen Lösungsmöglichkeiten, sondern auch von der intellektuellen Struktur des Forschers selbst ab. Aus manchen seiner Äußerungen konnte man den Eindruck gewinnen, als ob Lynen den rein experimentellen Ansatz nach dem Prinzip "trial and error" als die ihm gemäße Methode ansah. Sicher ist richtig, daß er dem Experiment die letztlich entscheidende Funktion zuerkannte und daß er mehr als mancher andere dazu neigte, lange theoretische Überlegungen, Diskussionen und Literaturrecherchen durch den Versuchsansatz kurzzuschließen. Dennoch wird schon aus der Art und Weise, wie er zu den gezielten Versuchen über

die Natur der „aktivierten Essigsäure" gelangte, klar, daß seinen Experimenten eine beträchtliche geistige Vorarbeit voranging und daß seine Arbeitshypothesen auf dem Boden eines umfangreichen Wissens und einer besonderen Fähigkeit zur Kombinatorik standen. Wenn dieser intellektuelle Prozeß seiner Umgebung nicht immer deutlich wurde, so mag dies an seiner besonderen Fähigkeit liegen, sehr schnell aus einer Fülle von Tatsachen die entscheidenden, weiterführenden Inhalte herauszufiltern und sie mit scheinbar nicht im Zusammenhang stehenden Informationen zu kombinieren. Auch von seinen Mitarbeitern erwartete er, daß sie überlegt handelten und experimentierten; nicht unbedingt, daß sie ihn mit langatmigen Spekulationen aufhielten.

Durch seine Studien über den Abbau der Fettsäuren wurde Lynen fast zwangsläufig auch mit dem physiologisch so wichtigen Problem der Ketonkörperbildung konfrontiert. Die von anderer Seite vertretene Ansicht, daß Acetessigsäure durch Hydrolyse von Acetacetyl-CoA entstehe, konnte den Chemiker Lynen nicht befriedigen. Durch eine sehr sorgfältige Analyse dieses Vorgangs entdeckte er, daß an der Überführung von Acetacetyl-CoA in freie Acetessigsäure zwei Enzyme beteiligt sind und dieser Prozeß über β-Hydroxy-β-methyl-glutaryl-CoA als Zwischenprodukt verläuft (HMG-CoA-Zyklus).

Mit den Steroiden war Lynen bereits im Institut seines Lehrers H. Wieland bestens vertraut geworden und er hatte die Arbeiten H. Sonderhoffs miterlebt, in denen dieser ihren Aufbau aus Essigsäureeinheiten wahrscheinlich machte. Die von L. Ruzicka formulierte „Isoprenregel" und die Entdeckung der Mevalonsäure durch K. Folkers wiesen Lynen den Weg, auf dem er die Biosynthese der Terpene in Angriff nehmen wollte. Die Entdeckung des Isopentenylpyrophosphats und des Dimethylallylpyrophosphats als den biologisch wirksamen Formen des Isoprengerüsts sowie die Aufklärung des Mechanismus der Kondensationen, die zu der großen Zahl der Terpene und der Sterine führen, waren die brillanten Ergebnisse einer ungeheuer intensiven und fruchtbaren Forschungstätigkeit in der zweiten Hälfte der Fünfzigerjahre. Auch das Zytohämin, die zentrale Komponente des von O. Warburg entdeckten Atmungsferments (Zytochromoxidase) erwies sich als Isoprenabkömmling, ein Farnesylderivat des Protohämins. Es bereitete Lynen eine große Freude und Genugtuung, die Strukturaufklärung seinem verehrten Mentor zum 80. Geburtstag auf den Gabentisch legen zu können. Die Ergebnisse des Lynen-Arbeitskreises zusammen mit den Entdeckungen K. Blochs, führten in kurzer Zeit zum Verständnis des Weges, auf dem die Zellen Cholesterin aus Acetyl-CoA synthetisieren. Diese großartige wissenschaftliche Leistung wurde 1964 durch die Verleihung des Nobelpreises für Physiologie oder Medizin an Feodor Lynen und Konrad Bloch in gebührender Weise gewürdigt.

Parallel zu den Untersuchungen der Terpenbiosynthese befaßte sich Lynen mit dem Mechanismus der Karboxylierung der β-Methylcrotonsäure, in der zunächst eine Zwischenstufe auf dem Weg zum biologisch aktiven Isopren vermutet worden war. Dieser Prozeß erwies sich als ATP-abhängig und wurde durch ein biotinhaltiges Enzym katalysiert. An diesem Enzym konnte in überzeugenden Experimenten erstmals die Rolle des Vitamins Biotin bei der biologischen Karboxylierung im Detail aufgeklärt und die N-1-Karboxybiotinylgruppe als die „aktivierte Kohlensäure" identifiziert werden. Diese Versuche, die sich durch große experimentelle Eleganz auszeichneten, wurden auch durch das Glück des Tüchtigen unterstützt: Als bisher einziges bekanntes Biotinylenzym ist die Methylcrotonyl-CoA-Karboxylase in der Lage, freies Biotin zu karboxylieren. Der an diesem Enzym entdeckte Mechanismus erwies sich als allgemein gültig für die zahlreichen biotinabhängigen CO_2-Assimilationsprozesse der Zellen; er ordnete das von Fritz Kögl 1934 entdeckte Vitamin in die Reihe der Coenzymvorstufen ein.

Nachdem der Vorgang der β-Oxidation der Fettsäuren in seinen wesentlichen Zügen aufgeklärt war, wurde Lynens Interesse naturgemäß auf die Frage gelenkt, wie die Fettsäuren in der Zelle synthetisiert werden. Seine eigenen Untersuchungen und zahlreiche Beobachtungen anderer Arbeitskreise, vor allem über die andersartige subzellulare Lokalisation sowie über den ATP-, Biotin- und Bikarbonatbedarf, zwangen dazu, die Vorstellung von der physiologischen Umkehrbarkeit der β-Oxidation aufzugeben. Ähnlich wie bei der Entdeckung der aktivierten Essigsäure kam der entscheidende Gedanke im Gefolge einer Diskussion: Im Anschluß an einen Vortrag anläßlich einer Gordon Conference über Lipide im Jahre 1958, in dem über die CO_2-Abhängigkeit der Fettsäuresynthese berichtet wurde, entwickelte Lynen die Vorstellung, daß der Prozeß der Fettsäuresynthese durch eine biotinabhängige Karboxylierung von Acetyl-CoA zu Malonyl-CoA eingeleitet werden und dieses, unter Abspaltung von CO_2, die Kettenverlängerung der Fettsäuren bewerkstelligen könne. Sicherlich stand Lynens einschlägige Erfahrung mit der Karboxylierung des Methylcrotonyl-CoA bei dieser Idee Pate. Sie erwies sich, wie S. Wakil und R. Brady an tierischen Zellen und sein eigener Arbeitskreis an Hefezellen zeigen konnten, als die richtige. Nach diesem Durchbruch gelang es innerhalb weniger Jahre, den Vorgang der an einem komplizierten Multienzymkomplex ablaufenden Synthese langkettiger Fettsäuren aus Acetyl- und Malonyl-CoA in seinen Einzelheiten zu eruieren und die Architektur und den Funktionszyklus der Fettsäuresynthetase in allen Einzelheiten zu beschreiben. Die Kristallisation des Multienzymkomplexes mit einer Molmasse von 2,3 Mill. Daltons, die Entdeckung ihres Aufbaus aus zwei Arten von Untereinheiten und damit der erstmalige Nachweis eines multifunktionellen Polypeptids

sind die Höhepunkte dieser Arbeitsrichtung, die ihn bis zu seinem Tode intensiv beschäftigte.

Mit diesen Untersuchungen in engstem Zusammenhang stehen die Arbeiten über die Methylsalizylsäuresynthese durch einen Multienzymkomplex aus Penicillium patulum. Sie lieferten einen Einblick in den Vorgang der biologischen Bildung von Polyketoverbindungen und eine glänzende experimentelle Bestätigung der von A. Birch aufgestellten Polyacetatregel.

Bereits diese gedrängte Darstellung seiner wichtigsten Entdeckungen macht verständlich, daß Feodor Lynen als einer der größten und brillantesten Biochemiker seiner Zeit angesehen wird. Er gab dieser Wissenschaft entscheidende Impulse und war an ihrer spektakulären Entwicklung maßgebend beteiligt. Ca. 350 Publikationen tragen seinen Namen und zeugen von der Produktivität seines Forscherlebens. Die Bedeutung seiner Ergebnisse war unmittelbar und in ihrer vollen Tragweite erkannt worden; es fehlte deshalb auch nicht an äußeren Anerkennungen seiner Leistung, die in der Verleihung des Nobelpreises gipfelten. Schon 1953 wurde ihm das erste Ordinariat für Biochemie an einer deutschen naturwissenschaftlichen Fakultät übertragen. Berufungen an die Universitäten Bern, Zürich, Harvard und Frankfurt konnten durch die Schaffung eines Max-Planck-Instituts für Zellchemie in München abgewendet werden. Der Ehrendoktorwürde der Medizinischen Fakultät Freiburg im Jahr 1960 folgten noch sechs weitere, ferner die Verleihung der ersten Warburg-Medaille durch die Gesellschaft für Biologische Chemie (1963) und zahlreiche Ehrenmitgliedschaften bei Akademien sowie in- und ausländischen wissenschaftlichen Gesellschaften. Von zahlreichen öffentlichen Ehrungen sollen die Verleihung des Großen Verdienstkreuzes mit Stern und Schulterband des Verdienstordens der Bundesrepublik Deutschland (1965) und die Mitgliedschaft des Ordens Pour le Mérite (1971) erwähnt werden. Als Präsident der Gesellschaft Deutscher Chemiker (1971–1973), der Alexander-von-Humboldt-Stiftung (1975–1979) und als auswärtiger Vizepräsident der Deutschen Akademie der Naturforscher Leopoldina (1971–1979) stellte Lynen seine Fähigkeiten auch in den Dienst der deutschen Wissenschaft und ihrer Belange.

Es ist ein bleibendes Verdienst Lynens, nach dem Zusammenbruch, den das Dritte Reich und der Zweite Weltkrieg für die deutsche Biochemie bedeutet hatten, in München einen der ersten Kristallisationskeime für die Entwicklung einer modernen Biochemie in Deutschland geschaffen zu haben. Dank seines Ansehens als Forscher und seines integren, offenen Wesens gelang es ihm, zerrissene Bande zu den Fachkollegen im Ausland und besonders zu den zahlreichen deutschen Emigranten wieder herzustellen und neue zu knüpfen. Er erfüllte in den schwierigen Jahren des Wiederaufbaus mit großem Erfolg die Aufgabe eines Botschafters der Wissenschaft und des guten Willens.

Man wird vielleicht einmal feststellen, daß die nachhaltigste Wirkung, die von Lynens Lebensarbeit ausging, diejenige auf seine Schüler war. Es war nicht so sehr sein wissenschaftlicher Status, sondern vor allem die glückliche Paarung von wissenschaftlicher Dynamik mit menschlicher Wärme und einem ausgeprägten Sinn für Fröhlichkeit und Lebensfreude, die stets hochmotivierte junge Leute aus dem In- und Ausland um ihn versammelte. Durch die Ausstrahlungskraft seiner Persönlichkeit und durch die unerbittliche Leistungsforderung und kritische Sachlichkeit, die er selbst vorlebte und auf seine Mitarbeiter zu übertragen verstand, spornte er seine Schüler zur vollen Entfaltung ihrer Fähigkeiten an. In den 37 Jahren seiner akademischen Lehrtätigkeit arbeiteten 88 Diplomanden und Doktoranden unter seiner Leitung, daneben suchten 25 deutsche und 80 ausländische, meist amerikanische und japanische Gastprofessoren und Postdoktoranden den Weg nach München, um sich in Lynens Arbeitskreis einer starken intelektuellen Herausforderung zu stellen und gleichzeitig auch eine anregende, entspannte und an Geselligkeit reiche Atmosphäre zu erleben. In einer Festschrift zu Lynens 65. Geburtstag, in der die ehemaligen Mitarbeiter ihre Eindrücke und Erlebnisse im Münchener Laboratorium schilderten, setzten seine Schüler und Kollegen dem wissenschaftlichen Lehrmeister ein sprechendes Denkmal.

Seine Funktion als Direktor eines Max-Planck-Instituts, sein intensives Interesse an der Forschung im Labor und seine weltweite Anerkennung, die ihn während eines erheblichen Teils seiner Zeit auf Reisen sah, hinderten Lynen nicht, seiner Verantwortung als akademischer Lehrer gerecht zu werden. Seine Vorlesungen und Praktika waren von höchster Aktualität und Anschaulichkeit; er verstand es, auf seine Studenten die Faszination der biochemischen Wissenschaft zu übertragen.

Die Entwicklung der Biochemie geht mit ungebrochener Dynamik weiter; die Schwerpunkte und die Methoden der Forschung ändern sich rasch und schon zu Lynens Lebenszeiten war „seine" Biochemie der Intermediärprodukte des Stoffwechsels und der Mechanismen der Enzymreaktionen gegenüber dem Interesse an Regulationsphänomenen, der Biochemie der Zellstrukturen und der Molekularbiologie scheinbar in den Hintergrund getreten. Vielleicht werden in 50 Jahren nur noch wenige Biochemiker mit der aktivierten Essigsäure, der aktivierten Kohlensäure und dem biologisch-reaktiven Isopren automatisch den Namen Lynen verbinden. (Wieviele der heutigen jüngeren Forscher könnten wohl auf Anhieb den Entdecker des ATP nennen?) Die großen Entdeckungen der Vorfahren werden zu den Selbstverständlichkeiten der Nachkommen. Bleiben aber wird Lynens Ruhm, zu den großen Baumeistern der modernen Biochemie zu gehören, ein Vorbild an experimenteller Präzision und an Originalität des Denkens gewesen zu sein. Bleiben wird vor allem sein Stil und seine Haltung als

inspirierender Lehrer und als lebensfroher Freund seiner Schüler und Kollegen. Dieses Erbe eines großen Forschers weiterzugeben gebietet die Dankbarkeit und die Hochachtung, die wir Feodor Lynen schulden.

Aus dem Schriftenverzeichnis von F. Lynen

Lynen F (1941) Über den aeroben Phosphatbedarf der Hefe. Ein Beitrag zur Kenntnis der Pasteur'schen Reaktion. Liebigs Ann Chem 546:120–141

Lynen F, Reichert E, Rueff L (1951) Zum biologischen Abbau der Essigsäure VI. „Aktivierte Essigsäure", ihre Isolierung aus Hefe und ihre chemische Natur. Liebigs Ann Chem 574:1–32

Jones ME, Lipmann F, Hilz H, Lynen F (1953) On the enzymatic mechanism of coenzyme A acetylation with adenosine triphosphate and acetate. J Am Chem Soc 75:3285–3286

Lynen F, Ochoa S (1953) Enzymes of fatty acid metabolism. Biochim Biophys Acta 12:299–314

Lynen F (1955) Der Fettsäurecyclus. Angew Chem 67:463–470

Holzer H, Schultz G, Lynen F (1956) Bestimmung des Quotienten DPNH/DPN in lebenden Hefezellen durch Analyse stationärer Alkohol- und Acetaldehyd-Konzentrationen. Biochem Z 328:252–263

Lynen F, Decker K (1957) Das Coenzym A und seine biologischen Funktionen. Ergebn Physiol 49:327–424

Schlossmann K, Lynen F (1957) Biosynthese des Cysteins aus Serin und Schwefelwasserstoff. Biochem Z 328:591–594

Lynen F, Henning U. Bublitz C, Sörbo B, Kröplin-Rueff L (1958) Der chemische Mechanismus der Acetessigsäurebildung in der Leber. Biochem Z 330:269–295

Lynen F, Eggerer H, Henning U, Kessel I (1958) Farnesylpyrophosphat und 3-Methyl-Δ^3-butenyl-1-pyrophosphat, die biologischen Vorstufen des Squalens. Zur Biosynthese der Terpene. III. Angew Chem 70:738–742

Lynen F, Hartmann G, Netter KJ, Schuegraf A (1959) Phosphate turnover and Pasteur-Effect. Ciba foundation symposium on cell metabolism. Ciba Foundation, London, pp 256–273

Lynen F, Knappe J, Lorch E, Jütting G, Ringelmann E (1959) Die biochemische Funktion des Biotins. Angew Chem 71:481–486

Lynen F, Agranoff BW, Eggerer H, Henning U, Möslein EM (1959) γ,γ-Dimethyl-allylpyrophosphat und Geranyl-pyrophosphat, biologische Vorstufen des Squalens. Zur Biosynthese der Terpene VI. Angew Chem 71:657–663

Stadtman ER, Overath P, Eggerer H, Lynen F (1960) The role of biotin and vitamin B_{12} coenzyme in propionate metabolism. Biochem Biophys Res Commun 2:1–7

Bucher NLR, Overath P, Lynen F (1960) β-Hydroxy-β-methylglutaryl coenzyme A reductase, cleavage and condensing enzymes in relation to cholesterol formation in rat liver. Biochim Biophys Acta 40:491–501

Eggerer H, Stadtman ER, Overath P, Lynen F (1960) Zum Mechanismus der durch Cobalamin-Coenzyme katalysierten Umlagerung von Methylmalonyl-CoA in Succinyl-CoA. Biochem Z 333:1–9

Henning U, Möslein EM, Arreguin B, Lynen F (1961) Zur Bildung des Kautschuks aus Isopentenyl-pyrophosphat. Zur Biosynthese der Terpene, XI. Biochem Z 333:534–549

Knappe J, Ringelmann E, Lynen F (1961) Zur biochemischen Funktion des Biotins. III. Die chemische Konstitution des enzymatisch gebildeten Carboxy-biotins. Biochem Z 335:168–176

Grassl M, Coy U, Seyffert R, Lynen F (1963) Die chemische Konstitution des Cytohämins. Biochem Z 338:771–795

Lynen F (1965) Der Weg von der „aktivierten Essigsäure" zu den Terpenen und den Fettsäuren. Angew Chem 77:929–944, Nobel Foundation, pp 2–41

Rétey J, Coy U, Lynen F (1966) On the mechanism of the coenzyme B_{12} dependent isomerization of (R)-methylmalonyl CoA to succinyl CoA. Biochem Biophys Res Commun 22:274–277

Lynen F (1967) The role of biotin-dependent carboxylations in biosynthetic reactions. Biochem J 102:381–400

Lynen F, Hagen A, Hopper-Kessel I, Oesterhelt D, Reeves HC, Schweizer E, Willecke K, Yalpani M (1967) Multienzyme complex of fatty acid synthetase. In: Vogel HJ, Lampen JO, Bryson V (eds) Organizational biosynthesis. Academic Press, New York, pp 243–266

Gehring U, Riepertinger C, Lynen F (1968) Reinigung und Kristallisation der Thiolase, Untersuchungen zum Wirkungsmechanismus. Eur J Biochem 6:264–280

Willecke K, Ritter E, Lynen F (1969) Isolation of an acyl carrier protein component from the multienzyme complex of yeast fatty acid synthetase. Eur J Biochem 8: 503–509

Sumper M, Oesterhelt D, Riepertinger C, Lynen F (1969) Die Synthese verschiedener Carbonsäuren durch den Multienzymkomplex der Fettsäuresynthese aus Hefe und die Erklärung ihrer Bildung. Eur J Biochem 10:377–387

Back P, Hamprecht B, Lynen F (1969) Regulation of cholesterol biosynthesis in rat liver: diurnal changes of activity, influence of bile acids. Arch Biochem Biophys 133:11–21

Sumper M, Riepertinger C, Lynen F (1969) Dissociation and reconstitution of the stable multienzyme complex fatty acid synthetase from yeast. FEBS Lett 5:45–49

Oesterhelt D, Bauer H, Lynen F (1969) Crystallization of a multienzyme complex: fatty acid synthetase from yeast. Proc Natl Acad Sci USA 63:1377–1382

Kresze G-B, Oesterhelt D, Lynen F, Castorph H, Schweizer E (1976) Localization of the central and peripheral SH-groups on the same polypeptide chain of yeast fatty acid synthetase. Biochem Biophys Res Commun 69:893–899

Lynen F (1976) My expeditions into sulfur biochemistry. In: Kornberg A, Horecker BL, Cornudella L, Oro J (eds) Reflections on biochemistry. Pergamon Press, Oxford, pp 151–160

Dimroth P, Ringelmann E, Lynen F (1976) 6-Methylsalicylic acid synthetase from penicillium patulum. Some catalytic properties of the enzyme and its relation to fatty acid synthetase. Eur J Biochem 68:591–596

Wieland F, Siess EA, Renner L, Verfürth C, Lynen F (1978) Distribution of yeast fatty acid synthetase subunits: threedimensional model of the enzyme. Proc Natl Acad Sci USA 75:5792–5796

Wieland F, Renner L, Verfürth C, Lynen F (1979) Studies on the multi-enzyme complex of yeast fatty acid synthetase. Reversible dissociation and isolation of two polypeptide chains. Eur J Biochem 94:189–197

Lynen F (†) (1980) Structure and function of fatty acid synthetase. In: Nover L, Lynen F, Mothes K (eds) Cell compartmentation and metabolic channeling. VEB Fischer, Jena, Elsevier/North-Holland Biomedical, Amsterdam, pp 125–234

KARL DECKER

Biochemisches Institut
der Albert-Ludwigs-Universität
Hermann-Herder-Straße 7
D–7800 Freiburg

Rev. Physiol. Biochem. Pharmacol., Vol. 90

Control, Modulation, and Regulation of Cell Calcium

ANDRÉ B. BORLE*

Contents.

* Department of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261, USA

Abbreviations

Ca_o	extracellular ionized calcium concentration
Ca_i	intracellular (cytosolic) ionized calcium concentration
CN	cyanide
CCCP	carbonyl-cyanide,*m*-chlorophenylhydrazone
Cyclic AMP (GMP)	adenosine (guanosine) 3′-5′-monophosphate
Dibutyryl cyclic AMP	dibutyryl adenosine 3′-5′-monophosphate
DNP	dinitrophenol
EDTA	ethylenediamine tetraacetic acid
EGTA	ethyleneglycol-bis-(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid
ER	endoplasmic reticulum
FCCP	carbonyl cyanide,*p*-trifluoromethoxyphenylhydrazone
f/cs	femtomoles $cm^{-2}s^{-1}$
Hepes	*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid
IAA	iodoacetic acid
Na_o	extracellular sodium concentration
Na_i	intracellular (cystolic) sodium concentration
p/cs	picomoles $cm^{-2}s^{-1}$
SER	smooth endoplasmic reticulum
SR	sacroplasmic reticulum
Tris	tris (hydroxymethyl)aminomethane
TTX	tetrodotoxin

1 Introduction

The first encounter was inauspicious. But the serendipity of the artifact did not escape Ringer's attention. He wrote (*Ringer* 1883): "After the publication of a paper in the *Journal of Physiology*, Vol. III, no. 5, entitled 'Concerning the Influence exerted by each of the Constituents of the Blood on the Contraction of the Ventricles', I discovered that the saline solution which I had used had not been prepared with distilled water, but with pipe water supplied by the New River Water Company. As this water contains minute traces of various inorganic substances, I at once tested the action of saline solution made with distilled water and I found that I did not get the effects described in the paper referred to. It is obvious therefore that the effects I had obtained are due to some inorganic constituents of the pipe water... Calcium chloride solution added to the saline, sodium bicarbonate and potassium chloride solution, after the ventricle has lost contractility, restores good spontaneous beats which will continue for a long time... I conclude therefore that a lime salt is necessary for the maintenance of muscular contractility." Later, *Locke* (1894) and *Overton* (1904) showed that the excitation of a frog skeletal muscle by stimulation of the motor nerve was blocked if the medium calcium concentration was too high or too low, suggesting that calcium must be maintained within a narrow range for proper cell function.

The importance of calcium in maintaining membrane stability and integrity was demonstrated by *Heilbrun* (1943), who reported that *Arbacia* egg membranes disrupted by needle puncture were repaired only when calcium was present in the suspending medium. *Heilbrun* and *Wiercinski* (1947) also discovered that intracellular calcium is the trigger for muscle contraction. Since then, the list of cellular functions triggered or regulated by intracellular calcium has expanded and is becoming longer.

Since the concentration of ionized calcium in various cell compartments plays an important role in regulating many functions, intracellular calcium must in turn be controlled and regulated. Its concentration must be kept at appropriately low levels, presumably between 10^{-7} and 10^{-6} M, because higher concentrations of free calcium are known to inhibit many important physiologic processes. In addition, cell calcium must be regulated and allowed to rise and to fall in order to play its role as an intracellular signal and to trigger or modulate specific functions of the cell. There are thus two distinct aspects to cell calcium metabolism that can be represented as shown in Fig. 1: (a) the control, modulation, and regulation of cytosolic free calcium and (b) the regulation of cell functions by cytosolic free calcium. In the scheme shown in Fig. 1, a controller is a mechanism that, alone or in concert with others, maintains the intracellular free calcium

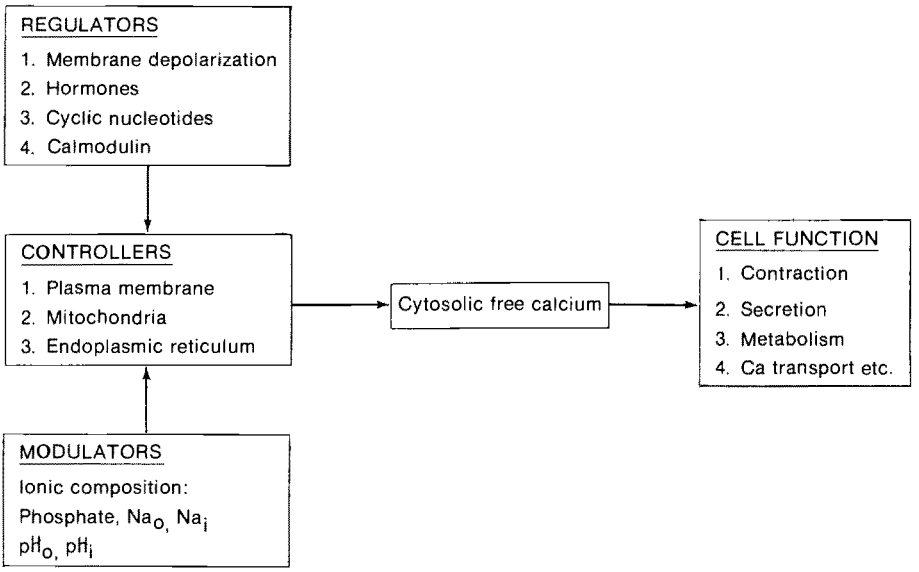


Fig. 1. Control, modulation, and regulation of cytosolic free calcium

far from its thermodynamic equilibrium; the controller determines the *set point.* A regulator, on the other hand, is any agent, electrophysiologic, endocrine or intracellular, that alters the set point, increasing or decreasing the intracellular free calcium above or below its resting or unstimulated level. Finally, a modulator is an agent that influences the level of the set point without being a part of the stimulus–signal–response system or of a negative feedback loop; it has a nonspecific influence on the level of free calcium in either the resting or the stimulated state, and may potentiate or inhibit the effects of the regulators.

This review will address itself primarily to the control, regulation, and modulation of cell calcium. Although our understanding of cellular calcium metabolism is still fragmented, we know enough to state that no single scheme can be applied to all types of cells comprising the mammalian organism. It is evident, for instance, that an erythrocyte, being devoid of a nucleus, of mitochondria, and of endoplasmic reticulum, has to rely exclusively on calcium transport across its plasma membrane to control and regulate its intracellular calcium. On the other hand, a skeletal muscle fiber that can contract and relax in the total absence of extracellular calcium must rely entirely on an intracellular redistribution of calcium between sarcoplasmic reticulum and sarcoplasm to alter its free calcium concentration. In other cells, the control of cytosolic free calcium must lie between these two extremes. But the relative importance of the various transport systems in the control and regulation of cytosolic calcium in any given cell

is still very difficult to assess. From the outset, it must be recognized that no single scheme of cellular calcium metabolism will be valid for all possible tissues.

2 Technical Considerations

Because cellular calcium metabolism is complex, no single method can provide a comprehensive picture of all its aspects. There are many methods of studying cell calcium, and each has its flaws. Obviously, our understanding of the literature must rest on a good grasp of the difficulties and the limitations of the methods used.

Chemical measurement of stable calcium poses no great problem. Atomic absorption spectrophotometry or fluorometric methods (*Borle* and *Briggs* 1968) are sufficiently sensitive, specific, and free from interference to provide reliable data. However, the measurements of total calcium in tissues or isolated cells are not good indicators of cellular calcium metabolism because 90%–95% of the cell calcium is bound to extracellular ligands (*Borle* 1968a, c, 1969a, 1975b; *Van Breemen* and *McNaughton* 1970; *Van Breemen* et al. 1977). Furthermore, measurements of stable calcium in subcellular fractions after homogenization of the tissue and ultracentrifugation may suffer from the fact that calcium redistribution among the various fractions may occur during their preparation. Recently, several quenching methods using ruthenium red and EGTA have reduced that possibility (*Reed* and *Bygrave* 1975a; *McDonald* et al. 1976b).

To eliminate the extracellular calcium bound to the cells, two methods have been used: the incubation of cells with trypsin EDTA (*Borle* 1968) and *the lanthanum method* (*Weiss* and *Goodman* 1969; *Van Breemen* and *McNaughton* 1970). The latter, used by many investigators, involves incubation of cells or tissues with ^{45}Ca; at the end of the ^{45}Ca uptake period, the tracer is displaced and washed out by lanthanum from the extracellular binding sites. The membrane-bound lanthanum is assumed to block calcium entry and the loss of ^{45}Ca from the cell. The method suffers, however, from several limitations (*Daniel* and *Janis* 1975; *Van Breemen* et al. 1977). It precludes the use of physiologic buffers containing bicarbonate and phosphate. These two ions precipitate with lanthanum, which may result in variable calcium trapping (*Van Breemen* et al. 1977), and even if amine buffers are used, there may be some leaching of phosphate from the cells. Also, since the extracellular phosphate concentration greatly influences the intracellular calcium content (*Borle* 1971a, 1972a) the data obtained by the lanthanum method will always be biased by the absence of phosphate in the incubating medium. In some cases, lanthanum does not com-

pletely inhibit calcium efflux (*Katzung* et al. 1973; *Deth* 1978; *Batra* and *Bengtsson* 1978) and may allow a 36% loss of intracellular calcium in 1 h and a 50% loss in 1–3 h (*Freeman* and *Daniel* 1973; *Van Breemen* et al. 1977). Lanthanum may even enter certain cells and mitochondria (*Hodgson* et al. 1972; *Reed* and *Bygrave* 1974c). In any case, the ^{45}Ca uptake measured by this method can only measure a pool of exchangeable calcium, and then only if isotopic steady state is reached. It does not provide any information on calcium influx, as is often claimed, unless some kind of kinetic analysis is performed.

Another method of circumventing the presence of the large extracellular calcium pool is the *kinetic analysis* of ^{45}Ca uptake or desaturation curves (*Borle* 1969a, 1972a, 1975b; *Uchikawa* and *Borle* 1978a, b; *Borle* and *Uchikawa* 1979). Since the exchange rate of ^{45}Ca with extracellular ligands is more than one order of magnitude faster than calcium transport across the plasma membrane, this extracellular pool can be kinetically determined. In addition, two distinct kinetic pools of intracellular calcium can be distinguished. However, the tracer method also has several limitations. Like most methods, it does not provide any information on a physiologically important quantity: the concentration of free calcium in any compartment. It only measures exchangeable pools. Each compartment may comprise several subpools that behave identically. The identification of the pools with anatomically distinct cellular compartments is difficult and is only supported by very indirect evidence. With three kinetic phases several possible models can be chosen, mammillary, mammillocatenary, and purely catenary. This choice is sometimes arbitrary but it can influence the absolute values of the calculated data. Finally, kinetic studies must be performed at steady state; the sequence of events leading from one steady state to another cannot always be determined.

The effects of various stimuli on cell calcium can be studied by measuring the *fractional efflux* of ^{45}Ca from prelabeled cells or tissues. While this technique can detect qualitative changes, such as gross stimulations or inhibitions of ^{45}Ca efflux, it is normally impossible to quantify the results, because the specific activity of the cells is usually unknown and decreases constantly. The interpretation derived from these experiments is often unwarranted. For instance, a sharp stimulation followed by an immediate return of fractional ^{45}Ca efflux toward baseline (spike pattern) is sometimes interpreted as a transient stimulation. This is not necessarily so because the specific activity of the compartment from which the ^{45}Ca originates may fall rapidly. Since the fractional efflux is a function of both calcium efflux and of the cell-specific activity, the fall in ^{45}Ca flux of the spike pattern may be due to a sharp drop in specific activity of the cell and not to transient nature of the stimulation of efflux. On the other hand, a progressive rise in fractional efflux produced by a stimulus and eventually

reaching a plateau does not necessarily represent a delayed or slow stimulation of calcium efflux. Such a pattern would be expected from an immediate release of calcium from an internal cell compartment slowly raising the specific activity of a cellular pool interposed between it and the external medium.

Until recently, the intracellular concentration of ionized calcium could not be measured directly. Several methods are being developed in various laboratories to measure the cellular free calcium with aequorin, with metallochromic dyes or with pCa-sensitive microelectrodes. For the moment, these techniques are still restricted to giant cells that allow micropunctures and intracellular microinjection of the dyes and they all present serious problems of calibration. Nevertheless, useful information has already been obtained.

3 Total Calcium

The total calcium of cells or tissues can be measured by atomic absorption flame photometry or by fluorometric titrations. Considering the differences in isolation techniques and in analytic methods the range of the total cell calcium as published in the literature is fairly narrow (Table 1). It is slightly but consistently higher than 1.3 mmol/kg cell water, the physiologic concentration of calcium in mammalian interstitial fluids[1]. The total calcium of liver, muscle, and nerve is about 2.2 mmol/kg wet weight; it is slightly higher in kidney, 2.4 mmol/kg wet weight. Other tissues may have higher total calcium content (Table 1), but more measurements are needed to firmly establish this point.

In tumor cells, however, it seems that the total calcium is consistently greater; the average of 5.5 mmol/kg wet weight is more than twice that of normal liver, kidney, muscle or nerve. The major fraction of the total cell calcium is extracellular, presumably bound to the glycoprotein coat called glycocalyx or to other extracellular binding sites on the lipid plasma membrane. This fraction can be displaced by lanthanum or removed with EDTA, EGTA or trypsin. In HeLa cells and in monkey kidney and pituitary cells, 90% of this extracellularly bound calcium can be removed with

1 The calcium concentration of mammalian plasma is around 2.5 mM; however, almost half is bound to plasma proteins and, therefore, nondiffusible. Consequently the concentration of calcium in the interstitial fluids bathing the cells is close to 1.3 mM. It is unfortunate that the classic reference for the preparation of suspending media to be used for the incubation of tissues in vitro (*Umbreit* et al. 1964) gives a calcium concentration of 2.5 mM. Many published experiments have been performed in buffers containing 2.5 mM calcium, which is twice the physiologic concentration.

Table 1. Total calcium of cells and tissues[a]

Cells or tissue conditions	Total calcium			Reference
	nmol/mg prot	μmol/g dry wt	mmol/kg wet wt	
Liver (rat)				
Fresh	(12)	4.1	1.8	*Van Rossum* 1970
Incubated	(10.8)	8.1	(3.6)	*Dawkins* et al. 1959
Perfused	(8.3)	(6.3)	1.25	*Dawkins* et al. 1959
Fresh slices	(10.6)	(7.9)	1.3–1.9	*Wallach* et al. 1966
Slices, incubated	(18.3)	(13.8)	2.0–3.5	*Wallach* et al. 1966
Fresh slices	(2.4)	1.7	0.34	*Judah* and *Ahmed* 1963
Slices, incubated	(5.4)	4.05	0.81	*Judah* and *Ahmed* 1963
Slices	18.0	(13.5)	3.26	*Borle* and *Studer* 1978
Slices	(11.6)	8.7	(1.74)	*Cittadini* and *Van Rossum* 1978
Perfused	(11.3)	(8.5)	1.70	*Claret-Berthon* et al. 1977
Hepatocytes	11.3	7.9	2.0	*Foden* and *Randle* 1978
Hepatocytes	15.5	11.7	2.33	*Assimacopoulos-Jeannet* et al. 1977
Mean	11.3	8.0	2.2	
Kidney				
Rabbit, slices	(14.6)	5–17	(2.2)	*Höfer* and *Kleinzeller* 1963a
Rabbit, slices	20	(15)	3.0	*Janda* 1969
Monkey, cells	17.8	(8.2)	1.63	*Borle* 1968b
Rabbit, tubules	(14.0)	10.5	(2.10)	*Rorive* and *Kleinzeller* 1972
Rat, tubules	15.2	(11.4)	1.17	*Borle* and *Studer* 1978
Rat, tubules	21.1	(15.9)	(3.2)	*Studer* 1978
Rat, slices	13.5	(10.1)	2.25	*Borle* and *Studer* 1978
Monkey, cells	20.3	(15.2)	(3.0)	*Borle* 1975
Mean	17.1	12.2	2.3	
Muscle				
Frog, skeletal	(16.1)	(11.2)	2.41	*Gibert* and *Fenn* 1957
Frog, skeletal	(16.9)	(12.7)	2.54	*Isaacson* 1969
Frog, skeletal	(13.8)	(10.4)	2.07	*Isaacson* and *Sandow* 1967
Frog, skeletal	(9.7)	(7.3)	1.45	*Cosmos* and *Harris* 1961
Guinea pig, atrium	(11.3)	(8.5)	1.69	*Winegrad* and *Shanes* 1962
Guinea pig, atrium	(20.1)	(15.1)	3.02	*Morgenstern* et al. 1972
Turtle, atrium	(18.2)	(13.2)	2.73	*Govier* and *Holland* 1964
Turtle, ventricle	(18.1)	(13.6)	2.71	*Govier* and *Holland* 1964
Human, heart	(7.6)	(5.7)	1.14	*Wester* 1965
Calf, heart	(8.8)	(6.6)	1.32	*Wester* 1965
Guinea pig, smooth	(12)	(9)	1.8	*Grosse* and *Lullmann* 1972
Rat, uterus	(15)	(11.3)	2.25	*Van Breemen* and *Daniel* 1966
Mean	13.1	10.4	2.1	
Neural Tissues				
Rat brain, slices	(10)	(7.5)	2.87	*Cooke* and *Robinson* 1971
Guinea pig cortex, slice	(16.3)	(12.3)	2.45	*Tower* 1968
Guinea pig cortex, slice	(23.4)	(17.5)	3.5)	*Stahl* and *Swanson* 1971

Table 1 (continued)

Cells or tissue conditions	Total calcium			Reference
	nmol/mg prot	μmol/g dry wt	mmol/kg wet wt	
Guinea pig cortex, slice	(14.5)	(10.9)	2.17	*Lolley* 1963
Rabbit vagus nerve	(19.1)	(14.4)	2.87	*Kalix* 1971
Crab nerve	(10.3)	(7.7)	1.54	*Bianchi* and *Lakshminarayanaiah* 1978
Neuroblasts, culture	28.7	(21.5)	3.22	*Borg* et al. 1979
Astroblasts, culture	22.2	(16.7)	3.33	*Borg* et al. 1979
Mean	18.1	13.6	2.7	
Tumors				
Ehrlich ascites, cells	35	(26)	5.0	*Levinson* 1967
Ehrlich ascites, cells	(21.3)	16	(3.2)	*Levinson* and *Blumenson* 1970
Ehrlich ascites, cells	37.1	(27.8)	(5.6)	*Cittadini* et al. 1977
HeLa cells	43.5	(25.2)	5.04	*Borle* 1968a
Hepatoma slices	(31)	23.3	(4.7)	*Van Rossum* et al. 1971
Mammary tumor, slices	(47.4)	35.6	(7.1)	*Van Rossum* et al. 1971
Glioma cells	43	(32)	(6.5)	*Lazarewicz* et al. 1977
Neuroblastoma	43	(32)	(6.5)	*Lazarewicz* et al. 1977
Mean	37.7	27.2	5.5	
Miscellaneous cells				
Rabbit pancreas, cells	14	(10.5)	(2.1)	*Renckens* et al. 1978
Rat pancreas, islets	61	(46)	9.4	*Kondo* and *Schulz* 1976a
Rat parotid, cells	23	(17.3)	3.45	*Miller* and *Nelson* 1977
Rat pituitary, cells	60	(45)	9.00	*Moriarty* 1977
Frog aorta	(26)	(19)	3.89	*Godfraind* and *Kaba* 1972
Frog skin	(16.7)	(12.5)	2.5	*Zadunaisky* and *Lande* 1972
Rat bone, cells	(112)	(84)	16.8	*Dziak* and *Brand* 1974a
Rabbit, blastocyst	(16.3)	(12.3)	2.45	*Lutwak-Mann* and *McIntosh* 1971
'Naked' cells				
HeLa cells (trypsin–EDTA)	5.74	(2.4)	0.48	*Borle* 1968a
Kidney cells (trypsin–EDTA)	3.0	(2.3)	0.45	*Borle* 1968c
Fibroblasts (trypsinized)	6.0	(4.5)	(0.90)	*Perdue* 1971
Pituitary cells (trypsin–EDTA)	3.7	(2.8)	(0.55)	*Moriarty* 1977
Rat brain (trypsin–EDTA)	3.6	(2.7)	(0.54)	*Moriarty* 1977
Mouse 3T3 cells (EGTA)	5.14	(3.8)	(0.92)	*Hazelton* and *Tupper* 1979
Mouse SV40-3T3 (EGTA)	3.03	(2.3)	(0.45)	*Hazelton* and *Tupper* 1979
Mean	4.3	3.0	0.6	

Table 1 (continued)

Cells or tissue conditions	Total calcium			Reference
	nmol/mg prot	μmol/g dry wt	mmol/kg wet wt	
Extruded axoplasm				
Squid	(0.3)	(0.3)	0.05	*Requena* et al. 1977
Squid	(0.42)	(0.32)	0.063	*Requena* et al. 1977
Mean	0.36	0.31	0.06	

[a] Chemical measurements of ^{40}Ca at physiologic extracellular calcium concentrations (1.0–1.5 mM, except for squid axon). Numbers in parentheses are recalculated assuming ratios of wet weight/dry weight/protein concentration = 100/20/15.

trypsin–EDTA (*Borle* 1968a, c; *Moriarty* 1977). In liver, EGTA or EDTA can remove 60%–70% of the total calcium (*Van Rossum* 1970; *Claret-Berthon* et al. 1977), while in mouse 3T3 cells, EGTA reduces it by 50% (*Tupper* and *Zorgniotti* 1977). Displacement of this extracellular calcium by lanthanum is the basis of the lanthanum method (*Weiss* and *Goodman* 1969; *Van Breemen* and *McNaughton* 1970). In pancreatic islets for instance lanthanum can displace 90% of total calcium of the tissue (*Hellman* et al. 1976a). The calcium concentration of 'naked' cells, stripped of their glycocalyx by trypsin or by EDTA, ranges from 0.45 mmol/kg cell water for HeLa and kidney cells (*Borle* 1968a, c) and 0.55 mmol/kg cell water for pituitary cells and brain cortex (*Moriarty* 1977) to 0.9 mmol/kg wet weight for fibroblasts (*Perdue* 1971). Axoplasm extruded from fresh squid axon contains still less calcium, about 0.05 mmol/kg wet weight (*Requena* et al. 1977).

Evidently, the chemical measurements of total tissue calcium can only provide a gross estimate of the changes in calcium content occurring in the cells. However, since cellular calcium is markedly affected by numerous factors in the extracellular milieu, total tissue calcium is a good index of the cells' viability and of the normal or steady state of the tissue.

Raising the extracellular calcium concentration above physiological levels increases total cell calcium in liver (*Wallach* et al. 1966), HeLa cells (*Borle* 1968a, 1971a, b), kidney cells (*Borle* 1970a, 1972a), squid axons (*Requena* et al. 1977, 1979), and muscle (*Gilbert* and *Fenn* 1957). Raising the extracellular concentration of phosphate also increases the total cell calcium or its ^{45}Ca uptake. This has been observed in kidney, liver, pancreas, HeLa, bone, and ascites cells, and in adipocytes (*Janda* 1969; *Van Rossum* 1970; *Borle* 1971a, b, 1972; *Dziak* and *Brand* 1974b; *Martin* et al. 1975; *Hines* and *Wenner* 1977; *Charlton* and *Wenner* 1978; *Hellman* and

Anderson 1978). There is an interaction between the calcium and the phosphate concentrations of the external milieu: the higher the phosphate concentration, the greater the gain in cell calcium with increasing extracellular calcium concentration (*Borle* 1971).

The temperature and the method of tissue preparation have important effects on tissue calcium. In Fig. 2 it is shown that incubating kidney cells at 0°C in calcium-free media rapidly depletes them of calcium. These cells may lose more than 50% of their calcium in 30 min and 95% after 2 h. Kidney slices lose 33% of their calcium (*Höfer* and *Kleinzeller* 1963a) and

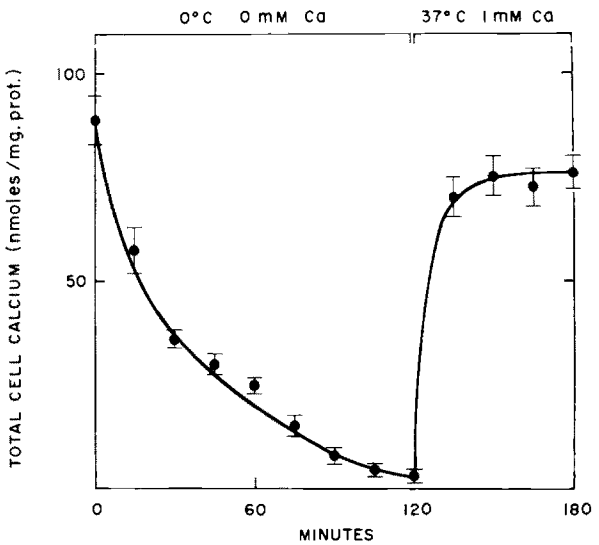


Fig. 2. Temporal relationship of the total calcium loss in calcium-free medium of 0°C and of the reaccumulation of calcium in 1 mM external calcium at 37°C. Experiments performed with cultured monkey kidney cells (LLC-MK_2) in Krebs–Henseleit bicarbonate buffer. Reproduced from *Borle* (1981), with permission from *Cell Calcium*

rat uterus 75% (*Van Breemen* et al. 1966) at 0°C in the absence of extracellular calcium. These observations may be relevant to the role of extracellular calcium and calcium influx in cell activation. Indeed, one cannot be absolutely certain that the lack of effect of a stimulus in the absence of extracellular calcium can be attributed to the lack of calcium entry into the cells, since it might be due as well to a depletion of intracellular calcium (*Borle* 1978). On the other hand, when the extracellular calcium is normal (1.3 mM) or higher, tissue calcium may increase from two- to fourfold at low temperature. This has been shown in kidney slices (*Höfer* and *Kleinzeller* 1963b), liver (*Van Rossum* 1970), hepatomas, mammary tumors (*Van Rossum* et al. 1971), and in Ehrlich ascites cells (*Cittadini* et al. 1977).

The pH of the incubating medium also affects the cell calcium: a low pH decreases while a high pH increases the total cell calcium in liver and in

kidney (*Wallach* et al. 1966; *Studer* and *Borle* 1979). The metabolic state of the cell or tissue influences the total calcium of the preparations. Prolonged incubation tends to increase the tissue calcium content in muscle, in liver, and in nerve (*Gilbert* and *Fenn* 1957; *Dawkins* et al. 1959; *Cosmos* and *Harris* 1961; *Requena* et al. 1977). This may be related to a decline in oxidative phosphorylation (*Dawkins* et al. 1959; *Wallach* et al. 1966); anaerobiosis also induces a net entry of calcium in the tissue (*Cittadini* and *Van Rossum* 1978).

In certain tissues, nerve, muscle, and pancreas, substitution of extracellular Na with choline or lithium results in a net gain of tissue calcium (*Judah* and *Ahmed* 1963; *Reuter* 1973; *Gardner* and *Hahne* 1977; *Requena* et al. 1977). Other tissues, such as kidney, do not gain calcium in Na-free media (*Borle* 1979). In the endocrine pancreas, a rise in glucose concentration of the incubating medium from 3 to 20 mM increases the tissue calcium 20%–50% (*Hellman* et al. 1976a, b; *Hellman* and *Anderson* 1978). Amine buffers, on the other hand, cause a lower total cell calcium than physiologic bicarbonate buffers, at least in kidney cells (*Borle* and *Uchikawa* 1978). Finally, in cell cultures, the total cell calcium may be different at different stages of growth (*Tupper* and *Zorgniotti* 1977). It is important to recognize that the total cell or tissue calcium is very labile and depends to a great extent on the conditions of tissue preparation and of incubation. The interpretation of many experimental results rests on the recognition of this fact.

4 Exchangeable Calcium (^{45}Ca Uptake)

Incubation of cells or tissues in the presence of a radiotracer, usually ^{45}Ca or ^{47}Ca, can give an estimate of their exchangeable calcium fraction. For a ^{45}Ca uptake value to be meaningful, the cells must be at steady state, i.e., they should not gain or lose calcium during the isotope uptake, and in theory the measurement must be made at isotopic equilibrium when the specific activity of the exchangeable fraction is maximal and equal to the specific activity of the incubating medium. In practice, it is assumed that in most tissues an incubation of 2–3 h is sufficient to reach 90% of the isotopic equilibrium, although the radioactivity of the cells may continue to increase very slowly for many hours. The exchangeable calcium fraction of various tissues slices and cells is shown in Table 2. It is severalfold higher in slices or islets than in isolated cells or in cultured cells. The average for liver, kidney, brain slices, and pancreatic islets is 17.5 ± 3.7 nmol/mg tissue protein. In freshly isolated or cultured cells there is an average of 5.0 ± 0.7 nmol exchangeable calcium/mg cell protein. Clearly, most of the dif-

Table 2. Exchangeable calcium measured by ^{45}Ca uptake at isotopic equilibrium

Tissue	Uptake time (min)	Ca_o (mM)	Exchangeable Ca (nmol/mg cell prot)	Reference
Kidney				
Kidney slices[a]	120	2.5	17[c]	*Höfer* and *Kleinzeller* 1963a
Kidney slices[a]	60	2.5	22.6[c]	*Janda* 1969
Kidney slice	120	1.3	16.5	*Borle* and *Studer* 1978
Kidney tubules	180	1.3	7.24	*Borle* and *Studer* 1978
Kidney cells	120	1.3	5.11	*Borle* 1970a
Kidney cells	120	1.3	5.01	*Borle* 1975a
Kidney cells	180	1.3	4.50	*Borle* and *Uchikawa* 1979
Liver				
Liver slices	120	1.3	18.0	*Borle* and *Studer* 1978
Liver perfused	180	2.16	8.2[c]	*Claret-Berthon* et al. 1977
Liver cells	100	1.0	2.6	*Chen* et al. 1978
Pancreas				
Pancreas islets	75	2.5	40[c]	*Hellman* et al. 1971
Pancreas islets	–	2.5	7.6	*Hellman* et al. 1976
Pancreas cells	120	1.25	3.16	*Kondo* and *Schulz* 1976
Pancreas cells	120	1.30	3.00	*Gardner* and *Hahne* 1977
Pancreas cells	90	1.50	2.50[c]	*Renkens* et al. 1978
Miscellaneous				
Brain slices	30	0.75	10.1[c]	*Stahl* and *Swanson* 1971
Aorta[b]	30	1.5	6.67[c]	*Deth* 1978
Intestinal cells	120	1.3	9.34	*Borle* 1974a
Heart cells	30	2.5	4.7[c]	*Lamb* and *McCall* 1972
Uterus[b]	180	2.5	2.0[c]	*Batra* and *Bengtsson* 1978
Bone cells	60	1.3	8.0[c]	*Dziak* and *Brand* 1974a
Crab nerve	200	1.0	4.2[c]	*Bianchi* and *Lakshminarayanaiah* 1978
3T3 cells (growth phase)	50	2.0	0.70	*Hazelton* and *Tupper* 1979
3T3 cells (quiescent)	50	2.0	3.38	*Hazelton* and *Tupper* 1979
SV40-3T3 cells (low density)	50	2.0	2.20	*Hazelton* and *Tupper* 1979
SV40-3T3 cells (high density)	50	2.0	3.08	*Hazelton* and *Tupper* 1979

a 25°C.
b La^{+++} method.
c Recalculated on a protein basis; see footnote to Table 1.

ference comes from a much larger extracellular or interstitial calcium pool in the slices. Various methods have been used to eliminate this extracellular pool after an uptake of ^{45}Ca: the cells can be washed with a medium containing lanthanum (*Weiss* and *Goodman* 1969; *Van Breemen* and

McNaughton 1970), the cells can be exposed to trypsin (*Borle* 1968a; *Moriarty* 1977) or the ^{45}Ca uptake curve can be divided into several components by kinetic analysis, an operation that can be viewed as a kinetic 'peeling' of the extracellular fraction (*Borle* 1969a; *Borle* and *Uchikawa* 1979). When the equilibrium ^{45}Ca uptake and the total calcium content of the tissues are measured simultaneously, the nonexchangeable fraction of the cell calcium can be calculated. Table 3 shows that in most tissues in

Table 3. Nonexchangeable fraction of cell calcium

Tissue	Equilibrium ^{45}Ca uptake	Total ^{40}Ca	Nonexchangeable fraction	% Exchangeable	Reference
	nmol/mg cell protein				
Liver perfused	8.2[a]	14.7[a]	6.5	56	*Claret-Berthon* et al. 1977
Liver slices	18.6	24.1	5.5	77	*Borle* (unpublished)
Kidney slices	11.6	16.5	4.9	70	*Borle* and *Studer* 1978
Kidney tubules	7.24	15.2	8.0	48	*Borle* and *Studer* 1978
Kidney cells	4.5	8.0	3.5	56	*Borle* and *Uchikawa* 1979
Kidney cells	5.1	9.8	4.7	52	*Borle* 1970a
HeLa cells	3.75	5.75	2.0	65	*Borle* 1969a
Aorta	17 [a]	26 [a]	9.0	65	*Godfraind* and *Kaba* 1972
Skeletal muscle	5.9 [a]	11.9 [a]	6.0	50	*Isaacson* and *Sandow* 1967
Uterus	11.1 [a]	14.7 [a]	3.6	76	*Van Breemen* and *Daniel* 1966
Cerebral cortex	8.9 [a]	21.3 [a]	12.4	40	*Stahl* and *Swanson* 1971
Pancreas cells	2.5	14	11.5	18	*Renckens* et al. 1978
Erythrocytes	(0.013)	9.62	(9.6)	(0.02)	*Borle* and *Studer* 1978
3T3 cells (growth phase)	0.70	5.27	4.57	13	*Hazelton* and *Tupper* 1979
3T3 cells (quiescent)	3.38	5.14	1.32	66	*Hazelton* and *Tupper* 1979
SV40-3T3 cells (low density)	2.20	2.03	–	100	*Hazelton* and *Tupper* 1979
SV40-3T3 cells (high density)	3.08	3.03	–	100	*Hazelton* and *Tupper* 1979

[a] Recalculated on a protein basis; see footnote to Table 1.

which this information is available, the nonexchangeable fraction averages 6.5 ± 0.9 nmol/mg cell protein. Since it is not significantly larger in slices than in isolated cells it is probable that this nonexchangeable fraction is mostly intracellular. The exchangeable calcium fraction expressed as a percentage of the total tissue calcium is slightly larger in slices and whole tissues than in isolated cells. On average the exchangeable cell calcium measured by ^{45}Ca uptake is about 60%. The only notable exception is

found in erythrocytes, which are known to have an extremely low permeability to calcium.

The measurement of ^{45}Ca uptake is easy and is used extensively. However, the interpretation of such data is not as simple as it appears. A ^{45}Ca uptake value obtained before the isotopic equilibrium is reached does not represent the exchangeable cell calcium or an influx of calcium, as often stated. It merely reflects the fraction of the total tissue calcium that has exchanged with the extracellular fluid calcium at that particular time. It is often claimed that an increased ^{45}Ca uptake indicates a higher rate of calcium exchange or an increased calcium transport. Conversely, a depressed ^{45}Ca uptake is sometimes interpreted as a decreased calcium influx. Although these conclusions may be correct in some cases, the fact is that, in steady-state conditions, the uptake of an isotope by a tissue is a function of two independent variables: (a) the size of the compartments with which the isotope exchanges, and (b) the rate of exchange. Consequently it is possible to observe an increased ^{45}Ca uptake when calcium transport, exchange or influx is actually depressed (*Borle* 1975a). Conversely, it is also possible to obtain a depressed ^{45}Ca uptake when calcium exchange or

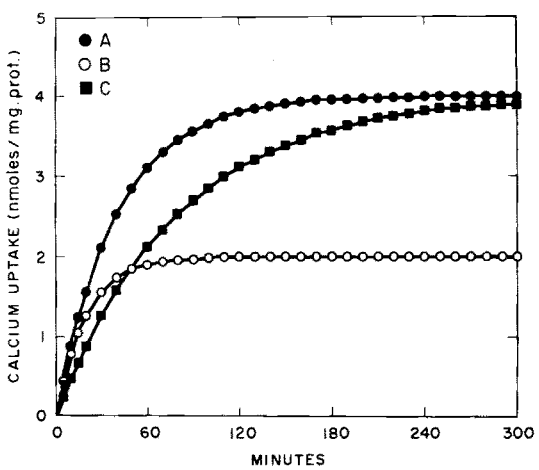


Fig. 3. Theoretical ^{45}Ca uptake curves in a two-compartment system generated by computer. Reproduced from *Borle* (1981), with permission from *Cell Calcium*

Curve A: $(dR_2/dt) = 0.1 \exp(-0.025)t$
$\rho_{12} = 100$ nmol (mg prot)$^{-1}$ min^{-1}
$S_2 = 4$ nmol /mg prot)$^{-1}$;
Curve B: $(dR_2/dt) = 0.1 \exp(-0.05)t$
$\rho_{12} = 100$ nmol /mg prot)$^{-1}$ min^{-1}
$S_2 = 2$ nmol (mg prot)$^{-1}$;
Curve C: $(dR_2/dt) = 0.05 \exp(-0.0125)t$
$\rho_{12} = 50$ nmol /mg prot)$^{-1}$ min^{-1}
$S_2 = 4$ nmol (mg prot)$^{-1}$.

transport is stimulated. Therefore, any interpretation simply based on an increase or decrease in ^{45}Ca uptake or on the visual interpretation of an uptake curve should not be accepted without reservation, unless the data are kinetically analyzed.

To illustrate this crucial point, Fig. 3 shows three theoretical uptake curves generated by computer, representing tracer exchange between two compartments (an incubation medium in which the tracer is added and one cellular compartment). In curve A the cellular exchangeable pool is 4 nmol/mg protein and the exchange rate is 100 pmol (mg prot)$^{-1}$ min^{-1}. In curve B the exchangeable pool is reduced by half, 2 nmol/mg protein, but the exchange rate is the same, 100 pmol (mg prot)$^{-1}$ min^{-1}. In curve C, on the other hand, the cellular exchangeable pool is the same as in curve A, 4 nmol/mg protein, but the exchange rate is reduced in half, 50 pmol (mg prot)$^{-1}$ min^{-1}. A comparison of curve B and curve C illustrates the difficulty of interpreting ^{45}Ca uptake curves by visual observation alone without kinetic analysis. If B were a control and C an experimental curve, one could easily conclude that since calcium uptake is increased in C compared with B, the experimental curve C reflects a stimulation of calcium transport into the cellular compartment. The fact is that calcium influx and exchange in C is only half that of curve B. The increased uptake is due exclusively to an enlarged exchangeable pool.

Another pitfall is the unrecognized nonsteady state. Very often, tissues or cells are prepared or isolated in buffers and in conditions that will lower the calcium content of the cells, for instance at 0°C and in a calcium-free medium. When the cells are placed again in a physiologic environment, in an incubating medium containing 1.3 mM calcium at 37°C, there is a net shift of calcium into the cell (Fig. 2). If a ^{45}Ca uptake experiment is performed in these conditions, one obtains spurious results which may lead to an erroneous interpretation of the data: the initial isotope uptake will be 4–5 times larger than that observed at steady state, then the radioactivity will drop as the cells unload some of the accumulated calcium. Indeed, in a ^{45}Ca uptake experiment, a drop in the cell or tissue radioactivity can only reflect a net shift of total calcium (^{40}Ca and ^{45}Ca) out of the cells. This proves that the system is not at steady state. As an example, Fig. 4 shows two uptake curves obtained with the same cell preparation: one was performed at steady state, the other when the cells were reaccumulating calcium after an incubation in a Ca-free medium at 0°C. At 20 min, calcium uptake is 5 times greater in a nonsteady state than at steady state. The drop in cellular ^{45}Ca observed in the upper curve after 20 min is the best evidence that the system is not at steady state. The cells initially depleted of calcium reaccumulate it in large excess when incubated later in a calcium-containing buffer, and they cannot be considered normal; they may require much more than 3 h to unload the calcium they accumulated. If

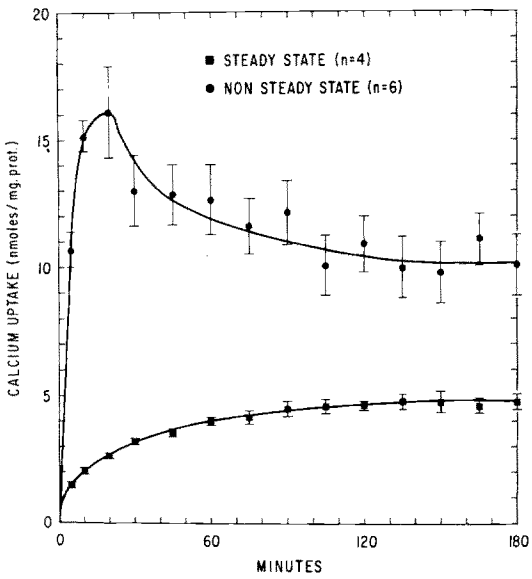


Fig. 4. ^{45}Ca uptake curves obtained at steady state and at nonsteady state. Experiments performed with cultured monkey kidney cells (LLC-MK_2) in 1 mM Ca Krebs-Henseleit bicarbonate buffer at 37°C. Nonsteady state conditions were obtained by preincubating the cells at 0°C in a calcium-free medium before the uptake incubation performed at 37°C, with 1 mM Ca and ^{45}Ca. Steady-state uptake was performed by preincubating the cells in 1 mM Ca at 37°C for 1 h before introduction of ^{45}Ca. Reproduced from *Borle* (1981), with permission from *Cell Calcium*

the 10 min uptake point was taken as representing calcium influx [15 nmol (mg prot)$^{-1}$ 10 min^{-1}] one would conclude that its magnitude is 1.5 nmol (mg prot)$^{-1}$ min^{-1}. This is 20 times greater than the calcium exchange measured between intra- and extracellular pools in steady-state conditions [0.08 nmol (mg prot)$^{-1}$ min^{-1}]. The interpretation of all published results showing these deviations from steady state is thus exceedingly difficult, if not impossible.

5 Cellular Distribution of Calcium

Cellular calcium is distributed among several extracellular and intracellular compartments. Most cells are surrounded by an extracellular coat of proteins and mucopolysaccharides called the glycocalyx, which can bind large amounts of calcium (*Chambers* 1940; *Burgos* 1960; *Brandt* 1962; *Gasic* and *Gasic* 1962; *Bennet* 1963; *Cook* et al. 1965; *Rambourg* and *Leblond* 1967; *Borle* 1968a; *Howell* and *Tyhurst* 1976). Calcium is also bound to the phospholipids and to the structural proteins of the cell plasma membrane (*Tobias* et al. 1962; *Carvalho* et al. 1963; *Koketsu* et al. 1964; *Palmer* et al. 1970; *Quinn* and *Dawson* 1972; *Duffy* and *Schwarz* 1973). As stated pre-

viously, from 50% to 90% of the cell calcium is bound extracellularly, depending on the tissue (*Borle* 1968a, c; *Van Rossum* 1970; *Claret-Berthon* et al. 1977; *Moriarty* 1977; *Tupper* and *Zorgniotti* 1977). Within the cells, four main compartments contain widely different amounts of calcium: the cytosol, the nucleus, the mitochondria, and the endoplasmic reticulum. Table 4 presents the distribution of calcium in these various cell fractions.

Table 4. Percent distribution of calcium among various cell fractions isolated by ultracentrifugation

Tissue	Nucleus and Debris	Mitochondria	Microsomes	Supernatant	Reference
Liver					
(^{40}Ca)	15.5	47.7	23.7	13.1	*Griswold* and *Pace* 1956
(^{40}Ca)	45	28.2	11.9	10.7	*Thiers* and *Vallee* 1957
(^{40}Ca)	14	48.0	20.0	17.0	*Thiers* et al. 1960
(^{45}Ca)	23	46.7	15.5	14.2	*Hohman* and *Schraer* 1966
(^{40}Ca)	23.9	30.9	26.7	11.2	*Carafoli* 1967
(^{45}Ca)	22.6	44.4	15.3	2.5	*Carafoli* 1967
Mean (liver)	24 ± 4.5	42.8 ± 4.4	18.8 ± 2.3	11.5 ± 2.0	
Other cells					
Kidney (^{40}Ca)	40	42	4	3	*Höfer* and *Kleinzeller* 1963a
Kidney (^{40}Ca)	55	30	10	5	*Borle* (unpublished)
Muscle (^{45}Ca)	26	65	7	2	*Cosmos* 1964
Ascites cells (^{45}Ca)	30.1	43.9	12.8	13.8	*Anghileri* 1972
Shell gland (^{45}Ca)	49	34	9.7	6.9	*Hohman* and *Schraer* 1966
Chorio-allantois (^{45}Ca)	31	16	3	50	*Crooks* et al. 1976
Mean (liver and other cells)	31.3 ± 4.2	43 ± 3.5	14.2 ± 2.1	9.0 ± 1.6	

In liver, the largest calcium compartment is found in mitochondria (42.8%); the nucleus and heavy cell membrane debris contain 24%, the microsomes 18.8%, and only 11.5% is left in the cytosol (supernatant). In other tissues, the calcium distribution appears to be qualitatively the same as in liver. Thus the distribution of calcium measured by cell fractionation and ultracentrifugation reveals that the mitochondria contain 3 times as much calcium as the microsomes derived from endoplasmic reticulum. About 60% of the intracellular calcium is sequestered in these two subcellular organelles while only 10% is left in the supernatant or cytosolic fraction.

If the calcium present in the 100 000 *g* supernatant after cell homogenization and ultracentrifugation (10% of total homogenate calcium) is an indication of the bound and free calcium of the cytosol it should amount to 0.220 mmol/kg wet weight, since the average cell calcium is about 2.2 nmol/kg wet tissue. This is 4 times higher than the calcium concentration of 50–60 μmol/kg wet weight measured in axoplasm extruded from the squid giant axon, which contains very few mitochondria and little endoplasmic reticulum (*Brinley* et al. 1977; *Requena* et al. 1979) (Table 1). This suggests that cell fractionation techniques overestimate this fraction or that the calcium in the squid axoplasm is unusually low. On the other hand, in kidney, liver, pancreas, muscle, and pituitary cells, the exchangeable cytosolic calcium pool measured by kinetic analysis of ^{45}Ca desaturation curves averages 0.36 mmol/kg wet tissue (Table 5), which fits in well with the value calculated from cell fractionation.

Table 5. Cytosolic and mitochondrial exchangeable calcium pools measured by kinetic analysis of ^{45}Ca desaturation curves [extracellular (Ca^{++}) = 1 mM]

Tissue	Cytosolic pool	Mitochondrial pool	Reference
	(mmol/kg wet weight)		
Kidney			
Cultured cells (LLC-MK_2)	0.27	0.38	*Borle* 1972a
Cultured cells (LLC-MK_2)	0.30	0.38	*Borle* 1972b
Cultured cells (LLC-MK_2)	0.20	0.35	*Uchikawa* and *Borle* 1978a
Cultured cells (LLC-MK_2)	0.33	0.23	*Borle* and *Uchikawa* 1979
Slices (rat)	0.57	0.50	*Uchikawa* and *Borle* 1978b
Tubules (rat)	0.56	0.38	*Studer* and *Borle* 1979
Liver			
Perfused (rat)	0.39	0.12	*Claret-Berthon* et al. 1977
Slices (rat)	0.43	0.14	*Borle* (unpublished)
Pancreas			
Cells	0.20	0.17	*Kondo* and *Schulz* 1967b
Cells	0.20	0.17	*Schulz* et al. 1977
Muscle			
Cells	0.31	0.32	*Schudt* et al. 1976
Heart	0.56	0.30	*Klaus* and *Krebs* 1974
Pituitary			
Slices	0.43	0.41	*Moriarty* 1980
Slices	0.26	0.30	*Borle* and *Zahnd* (unpublished)
Mean	0.36 ± 0.04	0.30 ± 0.03	

Table 6. Chemical measurements of ^{40}Ca in isolated mitochondria

Tissue	Mitochondrial Ca (μmol/kg wet weight)	Reference
Liver		
Rat	500–700 [a]	*Carafoli* and *Lehninger* 1971
Perfused (rat)	655	*Claret-Berthon* et al. 1977
–	400–720	*Carafoli* et al. 1977
Slices (rat)	750	*Borle* and *Studer* 1978
Rat	865	*Hughes* and *Barritt* 1978
Rat	1080	*Nicholls* 1978b
Mean	750 ± 79	
Kidney		
Cultured cells (LLC-MK_2)	280	*Borle* and *Uchikawa* 1978
Isolated tubules (rat)	640	*Borle* and *Studer* 1978
Slices (rat, Sprague Dawley)	1430	*Borle* and *Studer* 1978
Slices (rat, Holzman)	720	*Borle* and *Clark* (unpublished)
Mean	768 ± 240	
Muscle		
Heart	1600–2500	*Carafoli* et al. 1977
Myometrium	800–2600	*Carafoli* et al. 1977
Myometrium	1400	*Malmström* and *Carafoli* 1977
Mean	1750 ± 190	

[a] Recalculated, assuming 50 mg mitochondrial protein/g liver (*Carafoli* and *Crompton* 1977).

From the percentage of calcium present in the mitochondrial fraction after ultracentrifugation (43%) one can calculate that the amount of calcium in mitochondria should be around 0.95 mmol/kg wet tissue. The chemical measurements of the ^{40}Ca content of mitochondria isolated from three tissues are shown in Table 6. Kidney mitochondria contain 0.77 and liver mitochondria 0.75 mmol/kg wet tissue, both in the right order of magnitude. In muscle with 65% of the calcium in the mitochondrial fraction, the calculated value of 1.37 (65% of 2.1 mmol/kg wet weight, Tables 1 and 4) fits in well with the average chemical measurement of 1.75 mmol/kg wet tissue. From the kinetic measurements shown in Table 5, the exchangeable mitochondrial pool ranges between 0.12 for liver to an average of 0.37 mmol/kg wet tissue for kidney. This indicates that 50% or less of the mitochondrial calcium is exchangeable. The unexchangeable fraction could possibly reflect the sequestration of calcium in mitochondria as calcium phosphate precipitates (*Peachey* 1964; *Greenawalt* et al. 1964; *Weinbach* and *Von Brand* 1968; *Zadunaisky* et al. 1968; *Lehninger* 1970;

Matthews et al. 1970; *Martin* and *Matthews* 1970; *Sutfin* et al. 1971; *Ruigrok* and *Elbers* 1972; *Sayegh* et al. 1974) or as organic–inorganic complexes (*Barnard* and *Afzelius* 1972; *Bonucci* et al. 1973).

There are few data available on the calcium content of endoplasmic reticulum isolated from intact cells. The microsomal fraction obtained from the fractionation of various tissues is about 14% of the total homogenate calcium (Table 4). One can calculate that for most tissues with total calcium ranging between 2.1 and 2.5 mmol/kg wet weight the endoplasmic reticulum may contain between 0.29 and 0.35 mmol/kg wet tissue.

6 Cytosolic Ionized Calcium

The cytosolic calcium activity is the most important quantity in the metabolism of cellular calcium. It is the ionized calcium that regulates the cell functions sensitive to calcium. It is also one of the parameters that determine the thermodynamic calcium gradient between the extracellular fluids and the cytosol and between the cytosol and the mitochondrial matrix or the endoplasmic reticulum. Despite its importance, the magnitude of the cytosolic ionized calcium cannot yet be determined in mammalian cells. However, several attempts have been made in giant cells from invertebrates and estimates have been made by indirect methods in various other cells (Table 7). The best estimates have been obtained with the photoprotein aequorin or with the metallochromic indicators arsenazo III or antipyrylazo III. The advantages or disadvantages of these chemical probes with regard to selectivity, sensitivity, and possible artifacts have been reviewed elsewhere (*DiPolo* et al. 1976; *Blinks* et al. 1976; *Scarpa* et al. 1978a, b; *Blinks* 1978). In the squid axon, estimates of the axoplasmic ionized calcium made with the aid of aequorin ranges between 20 nM (*DiPolo* et al. 1976) and 350 nM (*Baker* et al. 1971). With arsenazo III, *DiPolo* et al. (1976) obtained a value of 130 nM, but assuming a free magnesium concentration of 3 mM (*Brinley* and *Scarpa* 1975) they estimate that the free calcium concentration may be as low as 50 nM. *Katz* and *Miledi* (1967) estimated the ionized calcium of the squid axon synapse from null point potential measurements: they measured the membrane potential at which no inward movement of calcium occurred. They found an $E_{Ca^{++}}$ of +130 mV with an extracellular calcium concentration of 11 mM, and calculated the intracellular ionized calcium to be 400 nM. With similar voltage clamp techniques, *Meech* and *Standen* (1975) estimated the cytosolic free calcium of snail neurons to range between 50 and 200 nM. Using a pCa microelectrode, *Christoffersen* and *Simonsen* (1977) found a calcium activity of 450 nM in snail neurons.

Table 7. Estimates of cytosolic ionized calcium in various cells

Cell	Method	Ca^{++} (μM)	Reference
Muscle fibers			
Maia squinado	Ca–EGTA, contraction threshold	0.3–1.6	*Portzehl* et al. 1964
Balanus nubilus	Ca–EGTA, contraction threshold	0.4–0.8	*Hagiwara* and *Nakajima* 1966
Maia squinado	Ca efflux, aequorin	0.05–0.12	*Caldwell* 1971
Neurons			
Squid axon	Aequorin	0.35	*Baker* et al. 1971
Squid axon	Aequorin	0.1–0.35	*Baker* 1972
Squid axon	Aequorin	0.02	*DiPolo* et al. 1976
Squid axon	Arsenazo III	0.05–0.13	*DiPolo* et al. 1976
Squid synapse	Suppression potential	0.4	*Katz* and *Miledi* 1967
Helix aspersa	Ca-EGTA, membrane resistance	0.9	*Meech* 1974
Helix aspersa	Ca-EGTA, null point potential	0.05–0.2	*Meech* and *Standen* 1975
Helix pomatia	pCa electrode	0.45	*Christoffersen* and *Simonsen* 1977
Helix pomatia	pCa electrode at 2 μM Ca_o	0.1	*Simonsen* and *Christoffersen* 1979
Helix pomatia	pCa electrode at 21 mM Ca_o	0.4	*Simonsen* and *Christoffersen* 1979
Aplysia neuron	Suppression potential	0.1	*Stinnakre* and *Tauc* 1973
Aplysia neuron	pCa electrode	0.7	*Owen* et al. 1977
Oocytes			
Lytechynus pictus	Aequorin	2.5–4.5	*Steinhardt* et al. 1977
Asterias amurensis	Aequorin	1.7	*Hamaguchi* and *Mabuchi* 1978
Asterina pectinifera	Aequorin	0.25	*Hamaguchi* and *Mabuchi* 1978

In the giant muscle fiber of the barnacle, the resting level of cytosolic free calcium has been estimated by injecting Ca–EDTA buffers and measuring the contraction threshold. Values ranging between 0.3 and 0.7 μM (*Portzehl* et al. 1964) or between 0.4 and 0.8 μM (*Hagiwara* and *Nakajima* 1966) were obtained, and these investigators estimated that the resting free calcium concentration of the sarcoplasm may be as low as 80–100 nM.

In sea urchin or starfish eggs the intracellular free calcium has also been estimated with aequorin. Values as low as 0.25 and as high as 1.7 μM were found (*Steinhardt* et al. 1977; *Hamaguchi* and *Mabuchi* 1978), and during fertilization the cytosolic ionized calcium may rise to higher levels, 2.5–4.5 μM (*Steinhardt* et al. 1977). In cultured kidney cells, calcium fluxes were measured at different intracellular free calcium concentrations, when reversible hypo-osmotic shock was used to introduce Ca–EGTA buffers

(*Borle* and *Anderson* 1976). When the fluxes observed are compared with those measured in normal conditions it can be estimated that the free calcium in kidney cells is around 0.3 μM.

From these observations one can conclude that the resting level of ionized calcium in most cells lies between 50 and 300 nM. It may even be lower (20 nM) in the axoplasm of invertebrates. The question of the absolute level of free cytosolic calcium is not academic. It will eventually establish the relative importance of the various calcium transport processes across the plasma membrane, the mitochondrial membrane or the endoplasmic reticulum membrane, and of calcium binding to specific ligands in the control and regulation of the cytosolic calcium activity. It will also determine whether the enzymatic processes, which have been reported to be stimulated or inhibited by calcium ion in cell-free systems, are actually modulated by cytosolic calcium in the intact cell. Only those enzymes whose K_i or K_a lie within a single order of magnitude of actual free calcium would be expected to be regulated by small fluctuations in cytosolic calcium activity.

7 Thermodynamic Conditions

The transmembrane potential difference (E_m) varies from cell to cell (Table 8). Excitable cells have membrane potentials ranging between –60 and –90 mV, while nonexcitable cells have lower potentials averaging about –45 mV (*Williams* 1970). Single cells and cultured cells have still lower potentials, –20 mV or less, probably because of their high cation permeability ratio P_{Na}/P_K (*Borle* and *Loveday* 1968; *Williams* 1970). Nevertheless, all cells exhibit an electrical potential difference across their plasma membrane, the inside of the cell being negative with reference to the extracellular fluids. With an average membrane potential of –60 mV and an extracellular free calcium concentration for mammalian cells of 1.3 mM, the Nernst equilibrium potential equation would predict intracellular ionized calcium in excess of 100 mM at 37°C. This is 5–7 orders of magnitude larger than the cytosolic free calcium measured or estimated in most cells. Since the intracellular free calcium is in the range of 10^{-7} M, and the extracellular calcium 1.3 mM, the Nernst equilibrium potential for Ca, E_{Ca} can be calculated to be around +126 mV [2].

This is in good agreement with the null point potential of +130 mV measured in invertebrate neurons (*Katz* and *Miledi* 1967), taking into ac-

---

2 $E_{Ca} = -(RT/zF) \ln Ca_i/Ca_o$; R = gas constant, 83 116 volt-coulomb/degree-mole; T = absolute temperature, 310° (37°C); F = Faraday, 95 520 coulomb/equivalent; z = valence (2 for calcium ion).

Table 8. Transmembrane potential of excitable and nonexcitable cells

Cell type	P.D. (mV)	Reference
Excitable cells		
Nerves		
Squid axon in vivo	−77	*Moore* and *Cole* 1960
Squid axon in vitro	−66	*Frankenhauser* and *Hodgkin* 1956
Frog myelinated nerve	−68	*Huxley* and *Stämpfli* 1951
Muscles		
Frog sartorius	−92	*Adrian* 1956
Rat skeletal muscle	−72	*Li* et al. 1957
Rabbit atrium	−82	*Vaughan-Williams* 1959
Rat myometrium (nonpregnant)	−39	*Casteels* and *Kuriyama* 1965
Rat myometrium (pregnant)	−58	*Casteels* and *Kuriyama* 1965
Nonexcitable cells		
Liver	−40 to −50	*Coraboeuf* et al. 1964; *Schanne* and *Coraboeuf* 1966; *Biederman* 1968; *Williams* 1970
Renal tubule	−70 to −75	*Giebisch* 1958, 1961; *Whittembury* and *Windhager* 1961; *Wright* 1971; *Sullivan* 1968
Adrenal cortex	−66 to −71	*Matthews* 1967
Adrenal medulla	−20 to −32	*Matthews* 1967; *Douglas* et al. 1967
Salivary duct cells	−35 to −80	*Lundberg* 1958; *Schneyer* and *Schneyer* 1965
Salivary acinar cells	−20 to −40	*Lundberg* 1958; *Schneyer* and *Schneyer* 1965
Thyroid gland	−40 to −50	*Woodbury* and *Woodbury* 1963; *Williams* 1966
Adipose tissue	−20 to −70	*Beigelman* and *Hollander* 1962; *Girardier* et al. 1968
Osteoclasts	−10 to −23	*Mears* 1971
Single cells		
Red blood cells	− 6 to −10	*Lassen* and *Sten-Knudsen* 1968; *Jay* and *Burton* 1969
Leukocytes	− 5	*Beckmann* et al. 1970
Ehrlich ascites cells	−11 to −23	*Sekiya* 1962; *Bernhardt* and *Pauly* 1967; *Aull* 1967; *Smith* et al. 1972; *Lassen* et al. 1971
Cultured cells		
Fibroblasts	−22 to −75	*Swift* and *Todaro* 1968; *Redman* 1971
Kidney cells	−12	*Redman* 1971
HeLa cells	−16 to −48	*Borle* and *Loveday* 1968; *Okada* et al. 1973
KB cells	−13	*Redman* et al. 1967
FL cells	−14	*Redman* 1971

count the slight differences in temperature and in extra- and intracellular calcium of the preparation. Evidently, calcium is not distributed passively across the cell membrane and a large chemical and electrical potential gradient exists between the exterior and interior of the cell. This gradient favors the entry of calcium into the cell and one has to postulate an active, energy-dependent transport mechanism to account for any efflux of calcium out of the cell. The driving force E, allowing the passive penetration of calcium into the cell, can be calculated from the electric field equation: $E = E_m - E_{Ca}$. E will depend on the particular transmembrane potential difference and the cytosolic calcium of each cell. It will range from a low 140 mV for nonexcitable cells with an E_m of −20 mV and a cytosolic calcium of 10^{-7} M to a high 240 mV for excitable cells with an E_m of −90 mV and a cytosolic free calcium of 10^{-8} M. The work required to maintain the cell in steady state has been calculated for skeletal muscle by *Bianchi* (1968). It was found to be only 0.6% of the total resting energy output of muscle. In rat kidney, assuming a cytosolic free calcium of $3 \cdot 10^{-7}$ M and a E_m of −70 mV (*Wright* 1971) the electric field would be 180 mV. The steady-state calcium efflux from rat kidney cells has been found to be 112 pmol (mg prot)$^{-1}$ min^{-1} or 1.0 mmol kg^{-1} h^{-1} (*Uchikawa* and *Borle* 1978a; *Studer* and *Borle* 1979). The work necessary to drive calcium transport out of kidney cells can be calculated to be 16 cal kg^{-1} [3]. This is only 0.24% of a total basal energy output ΔH based on the resting oxygen consumption of kidney of 6720 cal kg^{-1} h^{-1} (*Cohen* and *Barac-Nieto* 1973). Compared with a free energy expenditure $\Delta F'$ based on basal ATP utilization of 3600 cal kg^{-1} h^{-1} (*Cohen* and *Barac-Nieto* 1973), it would represent only 0.44%. Even if one assumes only 50% efficiency in energy utilization, active calcium transport in kidney cells would require less than 1% of the energy available in basal conditions.

In conclusion, despite uncertainties concerning the absolute levels of free calcium in most cells, calcium is not distributed passively across the plasma membrane. There is a large electrochemical gradient between the exterior and interior of the cell. An active transport mechanism must be postulated to account for the efflux of calcium out of the cells. On the other hand, calcium influx can be assumed to be passive on purely thermodynamic grounds. The energy necessary to maintain the cell in the steady state is 0.5%–1% of the total cellular energy output.

---

3 $\Delta w = (ZEF) \cdot$ (steady-state calcium efflux); z = valence, 2; electric field, $E = E_M - E_{Ca}$ [−70 mV − (+110 mV) = 0.180 V]; F = 95 520 coulomb/equivalent; 4.2 volt-coulomb/calorie; Ca efflux = 0.001 mol/kg wet weight/h.

8 Calcium Transport Across the Plasma Membrane

8.1 Magnitude of Calcium Fluxes

The published rates of calcium transport across the plasma membrane of various cells are listed in Tables 9–11. The overall magnitude of these fluxes is remarkably similar, when one takes into account the differences in tissues, in species, and in extra- or intracellular calcium concentration. In mammalian cells calcium influx ranges between 15 and 90 fmol cm^{-2} s^{-1} (f/cs), while in squid axon it varies between 60 and 150 f/cs. Since it has been shown that the calcium concentration of squid hemolymph is 7 mM, of which only about 4 mM is ionized (*Blaustein* 1974), the actual calcium influx in a normal fiber should be about 28 f/cs (*DiPolo* 1979). Thus the three- to fourfold difference in influx observed between mammalian cells and the squid axon may be due only to the high extracellular calcium concentration used in most experiments. In frog muscle calcium influx is 94 f/cs, but this value would be around 16 f/cs if corrected for

Table 9. Calcium influx in invertebrate and vertebrate cells

Tissue	Ca_o (mM)	Ca influx (f/cs)	Reference
Nerves			
Squid axon	10.4	76	*Hodgkin* and *Keynes* 1957
Squid axon	11	150	*Baker* et al. 1969
Squid axon	10	16	*Rojas* and *Taylor* 1975
Squid axon	10	64	*Requena* et al. 1977
Squid axon	10	40–100	*DiPolo* 1979
Muscles			
Barnacle	25	250	*DiPolo* 1973b
Lobster	0.1	190	*Gainer* 1968
Lobster	4.0	1740	*Gainer* 1968
Lobster	24	8550	*Gainer* 1968
Frog skeletal muscle	2.0	94	*Bianchi* and *Shanes* 1959
Cat smooth muscle	1.8	89	*Sperelakis* 1962
Mammalian cells			
Rabbit vagus nerve	2.3	25	*Kalix* 1971
Guinea pig atrium	1.25	16	*Winegrad* and *Shanes* 1962
Guinea pig atrium	2.50	29	*Winegrad* and *Shanes* 1962
Guinea pig atrium	3.75	48	*Winegrad* and *Shanes* 1962
Human HeLa	1.3	30	*Borle* 1969a
Monkey kidney	1.3	43	*Borle* 1970a
Mouse Ehrlich ascites	0.5	14	*Levinson* and *Blumenson* 1970
Rat anterior pituitary	1.0	77	*Moriarty* (pers. communication)
Mean [a]		35 ± 7.3	

[a] Omitting lobster muscle.

Table 10. Calcium efflux in invertebrate and vertebrate cells

Tissue	Ca_i (nM)	Ca efflux (f/cs)	Reference
Nerves			
Squid axon [a]	–	80–310	*Blaustein* and *Hodgkin* 1969
Squid axon	300	260	*DiPolo* 1973a
Squid axon	300	240	*DiPolo* 1974
Squid axon	100	100	*Blaustein* 1977a
Squid axon	60	20–50	*DiPolo* 1977
Squid axon	150	100	*DiPolo* 1977
Squid axon	230	320	*DiPolo* 1977
Squid axon	10–50	10–110	*Mullins* and *Brinley* 1975
Squid axon	100	22–38	*Requena* 1978
Muscles			
Crab	20	1000–2000	*Ashley* et al. 1972
Barnacle	20	400	*Ashley* et al. 1972
Barnacle	500	1000–2000	*Russell* and *Blaustein* 1974
Mammalian cells			
Monkey kidney	–	47	*Borle* 1972
Rat anterior pituitary	–	77	*Moriarty* (pers. communication)

[a] All values for calcium efflux from squid axon listed were obtained in artificial seawater with normal extracellular concentration of calcium and sodium and in the presence of ATP.

the surface area of the transverse tubular system. On the other hand, calcium influx in invertebrate muscle is much higher and this may not be due exclusively to the very high extracellular calcium used in some experiments (24 mM); even at 0.1 mM Ca_o calcium influx in lobster muscle can be as high as 190 f/cs.

The magnitude of calcium efflux in perfused squid axons and in kidney cells is the same as their calcium influx (Table 10). Indeed, since the ionized calcium of the squid axoplasm has been shown to lie between 20 and 100 nM (*DiPolo* et al. 1976), the values for calcium efflux obtained at these intracellular free calcium concentrations range between 10 and 100 f/cs. Calcium efflux from invertebrate muscle is again much higher, 400–2000 f/cs even at low Ca_i.

Several attempts have been made to measure calcium transport across mammalian cell plasma membrane by kinetic analyses of ^{45}Ca uptake or desaturation curves. The validity of these studies rests on the assumption that the initial, very fast exchange of tracer represents calcium binding to an extracellular calcium compartment and that the second, slower kinetic phase represents calcium transport across the plasma membrane (*Borle* 1969a, 1970a, 1972a, 1975b; *Uchikawa* and *Borle* 1978a, b). The values

Table 11. Calcium influx and efflux in vertebrate cells measured by kinetic analysis of ^{45}Ca movements

Cells	Ca fluxes (pmol mg^{-1} protein min^{-1})	Reference
Calcium influx		
HeLa cells	60	*Borle* 1969a
Monkey kidney cells	96	*Borle* 1970a
Monkey kidney cells	85	*Borle* 1975a
Monkey kidney cells	41	*Borle* and *Uchikawa* 1979
Dog kidney cells	118	*Borle* 1971b
Human intestinal cells	82	*Borle* 1971b
Chicken intestinal cells	144	*Borle* 1974a
Rat pancreatic cells	70	*Kondo* and *Schulz* 1976a
Mouse 3T3 cells (growth phase)	40	*Hazelton* and *Tupper* 1979
Mouse 3T3 cells (quiescent)	130	*Hazelton* and *Tupper* 1979
SV-40 3T3 (low and high density)	120	*Hazelton* and *Tupper* 1979
Mean	90 ± 11	
Calcium efflux		
Monkey kidney cells	83	*Borle* 1972a
Monkey kidney cells	53.2	*Uchikawa* and *Borle* 1978a
Monkey kidney cells	67.4	*Borle* and *Uchikawa* 1978
Monkey kidney cells	39.3	*Borle* and *Uchikawa* 1979
Rat kidney cells	135	*Studer* and *Borle* 1979
Rat kidney slices	112	*Uchikawa* and *Borle* 1978b
Chicken intestinal cells	177	*Borle* 1974
Perfused liver	187	*Claret-Berthon* et al. 1976
Pancreatic cells	81	*Kondo* and *Schulz* 1976b
Chick embryonic muscle	117	*Schudt* et al. 1976
Pituitary cells	163	*Moriarty* (pers. communication)
Mean	110 ± 15	

obtained with this method are listed in Table 11. They range between 40 and 140 pmoles (mg protein)$^{-1}$ min^{-1} for calcium influx and between 40 and 190 pmoles (mg protein)$^{-1}$ min^{-1} for calcium efflux. In most cases, the surface area of these cells has not been determined. However, in two cell types, HeLa cells and monkey kidney cells (LLC-MK_2), the surface area has been reported to be 36.7 and 33.5 cm^2/mg protein, respectively (*Borle* 1969, 1970), so that the calcium fluxes can be converted to f/cs. If one assumes that the surface area of the mammalian cells listed in Table 11 is approximately in the same order of magnitude, their calcium influx and efflux would range between 20 and 80 f/cs, which is identical with the fluxes of cells shown in Tables 9 and 10.

8.2 Mechanisms of Calcium Transport

Several mechanisms for the active transport of calcium out of the cell, across the plasma membrane, have been described: (a) an ATP-dependent calcium efflux mediated by a calcium-sensitive, magnesium-dependent ATPase (CaMgATPase) that derives its energy from the hydrolysis of ATP; (b) an efflux of calcium in exchange for extracellular sodium ions (Na–Ca exchange) that derives its energy from the sodium electrochemical gradient established across the plasma membrane by the enzyme Na-K-ATPase; (c) an efflux of calcium in exchange for an extracellular calcium ion (Ca–Ca exchange) that derives its energy from the calcium electrochemical gradient. Calcium influx, on the other hand, may be (a) a facilitated diffusion, on a uniporter or on an antiporter; or (b) a voltage-dependent influx through a calcium channel.

8.3 Calcium Efflux

8.3.1 ATP-Dependent Calcium Efflux and CaMgATPase

ATP-dependent calcium efflux and its relation to the enzyme CaMgATPase (EC 3.6, 1.3) have been extensively studied in erythrocytes. However, most other cells or tissues, kidney (*Parkinson* and *Radde* 1971; *Kinne-Saffran* and *Kinne* 1974; *Moore* et al. 1974; *Gmaj* et al. 1979), intestine (*Kurebe* 1978, 1979; *Ghijsen* and *Van Os* 1979), brain (*Ohashi* et al. 1970; *Blitz* et al. 1977; *Rahamimoff* and *Abramovitz* 1978), nerves (*DiPolo* 1976, 1977; *Baker* 1976), skeletal muscle (*Sulakhe* et al. 1973; *Casteels* et al. 1973), smooth muscle (*Thorens* 1979), fibroblasts (*Lamb* and *Lindsay* 1971), and lymphocytes (*Pau* et al. 1976), exhibit some kind of ATP-dependent calcium transport across their plasma membrane or some CaMgATPase activity. In addition, evidence for an energy-dependent calcium efflux has been offered in liver (*Wallach* 1966; *Van Rossum* 1970; *Cittadini* and *Van Rossum* 1978) and HeLa cells (*Borle* 1969b), although one group of investigators failed to detect any CaMgATPase activity in the plasma membrane of rat liver (*Chambaut* et al. 1974).

In erythrocytes, the CaMgATPase has a high affinity for calcium with a K_{Ca} of about 2 μM (Tables 12 and 13) and a V_m of 10 nmol (mg protein)$^{-1}$ min^{-1} (*Schatzmann* and *Rossi* 1971; *Scharf* and *Foder* 1978). The K_m for ATP is 40 μM but if free ATP instead of Mg-ATP is assumed to be the substrate, the K_{ATP} can be calculated to be 1–2 μM (*Schatzmann* 1977). The reaction has a Q_{+0} ranging between 3.0 and 3.5 (*Lee* and *Shin* 1969; *Schatzmann* and *Vincenzi* 1969; *Schatzmann* 1970; *Vincenzi* 1971), an energy of activation of 13–15 kcal/mol (*Lee* and *Shin* 1969; *Schatzmann* 1973; *Quist* and *Roufogalis* 1975; *Sarkadi* et al. 1977), and a

Table 12. Kinetic parameters of Ca-ATPases in different cells or tissues

Cells	High-affinity	Low-affinity		Reference
	K_{Ca} (μM)	K_{Ca} (μM)	K_{ATP} (μM)	
Erythrocytes	2.7	–	40	*Wolf* 1970
	4.1	100	–	*Schatzmann* and *Ross* 1971
	6	–	–	*Davis* and *Vincenzi* 1971
	–	30	–	*Bond* and *Green* 1971
	0.92	–	–	*Wolf* 1972
	1–4	–	–	*Schatzmann* 1973, 1975
	1.6	400	–	*Quist* and *Roufogalis* 1975
	1–4	–	–	*Scharf* 1976
	–	–	1–2	*Schatzmann* 1977
	1.8	17	–	*Gopinath* and *Vincenzi* 1977
	0.45	100	–	*Quist* and *Roufogalis* 1977
	0.9	–	–	*Hanahan* et al. 1978
	1.0	30	–	*Scharf* and *Foder* 1978
Kidney	–	1500	–	*Parkinson* and *Radde* 1971
	–	1000–2000	–	*Kinne-Saffran* and *Kinne* 1974
	<20	–	–	*Moore* et al. 1974
Intestine	–	92	–	*Kurebe* 1979
	0.5	50	–	*Ghijsen* and *Van Os* 1979
Lymphocytes	22	–	–	*Pau* et al. 1976

stoichiometry of 1 Ca/ATP according to some workers (*Schatzmann* and *Vincenzi* 1969; *Schatzmann* 1970; *Vincenzi* 1971; *Schatzmann* 1975) or 2 Ca/ATP according to others (*Quist* and *Roufogalis* 1975, 1977; *Ferreira* and *Lew* 1976; *Sarkadi* et al. 1977; *Scharf* and *Foder* 1978). The enzyme is inhibited by lanthanum, ethacrynic acid, mersalyl, chlorpromazine, and ruthenium red; it is not inhibited by ouabain, oligomycin, sodium azide, fluoride or caffeine (see refs., Table 13). Like other enzymes, CaMgATPase of erythrocytes requires the presence of phospholipids. The enzyme activity is reduced or lost after treatment with phospholipase A and C (*Cha* et al. 1971; *Quist* and *Roufogalis* 1975; *Luthra* et al. 1976a, b; *Ronner* et al. 1977). The enzyme can be reactivated by addition of phosphatidyl serine or oleate (*Ronner* et al. 1977). Phospholipase D, however, does not reduce the ATPase activity (*Cha* et al. 1971). The enzyme is presumed to be embedded in an ring of phospholipids whose fluidity influences ATPase activity (*Galo* et al. 1975; *Ronner* et al. 1977; *Strittmatter* et al. 1979).

Finally, many investigators have found that an activator protein calmodulin stimulates the CaMgATPase activity from two- to fourfold. The

Table 13. Properties of erythrocyte CaMgATPase

Parameter		Reference
High-affinity site K_{Ca}	2.0 μM	See Table 12
Low-affinity site K_{Ca}	$\sim$100 μM	See Table 12
V_m	10 nmol(mg prot)$^{-1}$ min^{-1}	*Schatzmann* and *Rossi* 1971; *Scharf* and *Foder* 1978
Affinity of ATP K_{ATP}	40 μM	*Wolf* 1970; *Schatzmann* 1975
Q_{10}	3.0–3.5	*Lee* and *Shin* 1969; *Schatzmann* and *Vincenzi* 1969; *Schatzmann* 1970; *Vincenzi* 1971
Energy of activation	13–15 kcal/mol	*Lee* and *Shin* 1969; *Schatzmann* 1973; *Quist* and *Roufogalis* 1975; *Sarkadi* 1977
Stoichiometry	a) 1 Ca/ATP	*Schatzmann* and *Vincenzi* 1969; *Schatzmann* 1970; *Vincenzi* 1971; *Schatzmann* 1975
	b) 2 Ca/ATP	*Quist* and *Roufogalis* 1975, 1977; *Ferreira* and *Lew* 1976; *Sarkadi* 1977; *Scharf* and *Foder* 1978
Inhibitors	Lanthanum, ethacrynic acid, mersalyl, chlorpromazine, ruthenium red, tetracaine, dibucaine	*Schatzmann* and *Vincenzi* 1969; *Vincenzi* 1971; *Weiner* and *Lee* 1972; *Schatzmann* 1975; *Quist* and *Roufogalis* 1975; *Hinds* et al. 1978
Not inhibited by	Ouabain, oligomycin, NaN_3, fluoride, caffeine	*Lee* and *Shin* 1969; *Schatzmann* and *Vincenzi* 1969; *Schatzmann* 1970, 1975; *Sarkadi* 1977
Depends on phospholipids		*Cha* et al. 1971; *Quist* and *Roufogalis* 1975; *Luthra* et al. 1976a, b; *Ronner* et al. 1977
Stimulated by calmodulin		*Bond* and *Clough* 1973; *Vincenzi* and *Farrance* 1977; *Jarrett* and *Penniston* 1977; *Quist* and *Roufogalis* 1977; *Gopinath* and *Vincenzi* 1977; *Hanahan* et al. 1978; *Hinds* et al. 1978; *Sarkadi* 1978

concentration of this activator in intact erythrocytes is about 8 times that necessary to saturate the enzyme (*Luthra* et al. 1976a). It is nondialyzable and it is destroyed by trypsin although it is not degraded when intact erythrocytes are exposed to trypsin (*Bond* and *Clough* 1973; *Vincenzi* and *Farrance* 1977). This suggests that the activator is located in the cell and acts by binding to the inner membrane surface (*Vincenzi* and *Farrance* 1977). It is closely related to other activator proteins such as the calcium-dependent regulator of cyclic nucleotide phosphodiesterase and skeletal muscle troponin-C (*Jarrett* and *Penniston* 1977; *Gopinath* and *Vincenzi* 1977; *Hinds* et al. 1978; *Schulman* and *Greengard* 1978). These other modulator proteins also activate the CaMgATPase activity of erythrocytes (*Gopinath* and *Vincenzi* 1977) and the ATP-dependent calcium transport (*Hinds* et al. 1978).

The ATP-dependent calcium transport and CaMgATPase of erythrocytes share many characteristics: (a) they have similar dissociation constants for calcium; (b) both require magnesium; (c) both are insensitive to ouabain; (d) both accept strontium instead of calcium; (e) both are inhibited by the same concentration of lanthanides; (f) they have the same optimal temperature; and (g) their nucleotide requirements are the same (*Cha* et al. 1971; *Weiner* and *Lee* 1972; *Porzig* 1972; *Quist* and *Roufogalis* 1977; *Scharf* and *Foder* 1978). For instance, phospholipase A and C inhibit both calcium transport and ATPase activity, while phospholipase D affects neither (*Cha* et al. 1971). Sodium and potassium stimulate a fraction of CaMgATPase in the presence of ouabain (*Schatzmann* and *Rossi* 1971; *Bond* and *Green* 1971; *Bond* and *Clough* 1973); however, calcium transport is not affected by the presence or absence of sodium or the sodium concentration ratio across the plasma membrane (*Schatzmann* 1966, 1970; *Schatzmann* and *Vincenzi* 1969; *Lee* and *Shin* 1969; *Schatzmann* and *Rossi* 1971; *Vincenzi* 1971; *Porzig* 1972). There appears to be no Na–Ca exchange in erythrocytes and the stimulation of CaATPase by sodium affects probably another enzyme that does not pertain to calcium transport (*Schatzmann* and *Rossi* 1971; *Schatzmann* 1975). Calcium transport in erythrocyte membrane vesicles has a K_{Ca} of 0.18 μM, a V_m of 0.9 nmol (mg protein)$^{-1}$ min^{-1}, and a K_{ATP} of 30–60 μM, and reconstituted vesicles require the incorporation of lipids (*Ting* et al. 1979; *Yeung* et al. 1979).

Several investigators detected a low- and a high-affinity site of the CaMgATPase for calcium (*Schatzmann* and *Rossi* 1971; *Quist* and *Roufogalis* 1975, 1977; *Gopinath* and *Vincenzi* 1977; *Scharf* and *Foder* 1978). *Scharf* and *Foder* (1978) presented evidence that the enzyme exists in two different and reversible states: (a) a resting state with low affinity for calcium (K_{Ca} = 30 μM), a Hill coefficient of 1, a low maximum velocity, and (b) an active state with high affinity for calcium (K_{Ca} = 1 μM), a Hill coefficient greater than 1, suggesting a positive cooperativity of calcium activa-

tion, and a high maximum velocity. The resting state corresponds to the enzyme free of the activator protein calmodulin, whereas the activator would be bound to the enzyme in the active state. *Scharf* and *Foder* (1978). also showed that the shift from resting to active state occurs when the enzyme is exposed to increasing calcium concentrations in the presence of the activator and the shift reaches 50% at 30 μM calcium. Conversely, the shift from active to resting state occurs at low calcium concentrations (50% shift at 0.5 μM calcium) when the activator is released from the membrane. They propose that these two states of the enzyme represent a resting and an active state of the calcium pump actively extruding calcium from the interior of the erythrocyte. A similar model has also been proposed by *Lynch* and *Cheung* (1979).

In other tissues, information concerning CaMgATPase—as related to ATP-supported calcium transport—is much more fragmentary (Table 14).

Table 14. Kinetic parameters of ATP-dependent calcium transport in cells and tissues

	K_{Ca}	V_m	Reference
	(μM)	nmol (mg prot)$^{-1}$ min^{-1}	
Erythrocytes	2–3	–	*Schatzmann* 1973
	1	–	*Ferreira* and *Lew* 1976
	10	–	*Sarkadi* 1978
	0.18	0.9	*Ting* et al. 1979; *Yeung* et al. 1979
Kidney	19.4	1.2	*Moore* et al. 1974
	0.5	1.0	*Gmaj* et al. 1979
Intestine	38	2.7	*Kurebe* 1978
Skeletal muscle	20	–	*Sulakhe* et al. 1973

Several Ca-activated ATPases described in kidney and intestine may be enzymes different from the Ca-activated Mg-dependent ATPase of erythrocytes that is characterized by its requirement for magnesium as well as for calcium. For instance *Parkinson* and *Radde* (1971) and *Kinne-Saffran* and *Kinne* (1974) described a CaATPase in kidney with a K_{Ca} greater than 1 mM that is inhibited by magnesium or depressed by high concentrations of magnesium. *Schatzmann* (1975) has suggested that such CaATPases could be identical with the protein spectrin adhering to the inner surface of some membranes and would not be related to calcium transport. Nevertheless, several investigators have described enzymes or transport processes consistent with a CaMgATPase-driven calcium pump in kidney (*Moore* et

al. 1974; *Gmaj* et al. 1979), intestine (*Kurebe* 1978; *Ghijsen* and *Van Os* 1979), brain or nervous tissues (*Ohashi* et al. 1970; *Blitz* et al. 1977; *Rahamimoff* and *Abramovitz* 1978), and lymphocytes (*Pau* et al. 1976). In brain and kidney they are not inhibited by ouabain and oligomycin (*Blitz* et al. 1977; *Gmaj* et al. 1979); in muscle and kidney they are inhibited by lanthanum and butacaine (*Sulakhe* et al. 1973; *Gmaj* et al. 1979); in kidney, muscle, and lymphocytes they have a K_{Ca} ranging between 0.5 and 20 μM (*Sulakhe* 1973; *Moore* et al. 1974; *Pau* et al. 1976; *Gmaj* et al. 1979); in intestine, delipidation decreases their activity (*Kurebe* 1979) and in synaptosomes there is a parallelism between calcium transport and the enzyme activity (*Rahamimoff* and *Abramovitz* 1977, 1978). In vesicles prepared from renal plasma membrane, sodium inhibits the ATP-dependent calcium uptake (*Moore* et al. 1974; *Gmaj* et al. 1979). *Gmaj* et al. (1979) have proposed that sodium increases the backleak of calcium from the vesicles through a Na–Ca exchange system, rather than inhibiting the ATP-dependent calcium transport directly. However, more evidence will be required to firmly establish the existence of a calcium pump dependent on a CaMgATPase similar to that of erythrocytes in most of these tissues.

8.3.2 Na_o-Activated Calcium Efflux in Squid Axons

In 1958, *Lüttgau* and *Niedergerke* (1958) reported that frog hearts depolarized in high-KCl media can be made to contract or to relax, respectively, by reducing or increasing the proportion of Na in the surrounding fluids. In 1968, *Reuter* and *Seitz* (1968) observed that calcium efflux from heart muscle decreases 30% in Ca_o-free solutions, 35% in Na_o-free solutions, and 80% in the absence of Ca and Na in the incubation medium. Moreover, the calcium content of heart muscle increases in Na_o-poor solutions and decreases again when Na is reintroduced in the extracellular fluids. *Reuter* and *Seitz* (1968) were the first to suggest a modified exchange diffusion countertransport by which calcium could be transported out of the cells in exchange for Na_o against a large electrochemical gradient without direct metabolic energy coupling.

Similar observations were made in squid axons (*Blaustein* and *Hodgkin* 1969). The giant axon of the squid has been used extensively to study Na–Ca countertransport, and several reviews on the subject have been published (*Baker* 1972, 1978; *Blaustein* 1974; *Brinley* 1976; *McNaughton* 1978; *Requena* and *Mullins* 1979). These investigations have been performed in a wide variety of experimental conditions: (a) in intact axons and in axons poisoned with CN or other metabolic inhibitors; (b) in axons into which ^{45}Ca has been injected or in internally perfused axons; (c) with very high (> 1 μM), intermediate (0.1–1.0 μM) or low intracellular free calcium concentrations (< 0.1 μM). The results obtained differ both quantita-

tively and qualitatively, depending on these conditions. The early investigations were usually done at very high Ca_i and in axons poisoned with cyanide (*Blaustein* and *Hodgkin* 1969; *Blaustein* 1974, 1976a, b; *Blaustein* et al. 1974; *Blaustein* and *Russell* 1975; *DiPolo* 1973a, *Mullins* and *Brinley* 1975). In these conditions, calcium efflux is extremely high and may exceed the physiologic rates by one to two orders of magnitude. In most conditions, however, three components of calcium efflux can be detected: (a) a Na_o-activated calcium efflux, (b) a Ca_o-activated calcium efflux, and (c) a residual calcium efflux insensitive to the presence or the absence of calcium or sodium in the extracellular fluids.

The Na_o-activated calcium efflux has several characteristics, which are summarized in Table 15. At very high Ca_i (> 1 μM) calcium efflux is very high, up to 6 p/cs, and 60%–80% is Na_o-dependent (*Blaustein* et al. 1974; *Blaustein* and *Russel* 1975; *Blaustein* 1977). The high rates observed are also due to the abnormally low concentrations of Na_i (5 mM) used in these experiments. At intermediate Ca_i (0.1–1.0 μM) and with physiologic Na_i of 60–90 mM (*Hinke* 1961; *Deffner* 1961; *Brinley* and *Mullins* 1967), calcium efflux is much lower (< 1 p/cs) and much less dependent on Na_o (*DiPolo* 1974, 1977, 1979; *Blaustein* and *Russell* 1975; *Brinley* et al. 1975; *Requena* 1978). At physiologically low Ca_i (< 0.1 μM), calcium efflux is practically insensitive to changes in Na_o (*Brinley* et al. 1975; *Mullins* and *Brinley* 1975; *DiPolo* 1976, 1977, 1979). ATP increases the sensitivity of the transport system to Na_o; it decreases the apparent activation constant K_{Na_o} from 150–300 mM (without ATP) to 40–80 mM (with ATP) (see Table 15 for references). In addition, ATP changes the shape of the curve relating the Na_o-dependent calcium efflux to the concentration of extracellular sodium: without ATP the activation curve is sigmoid with a Hill coefficient of 2–3, while in the presence of ATP it approximates a section of a rectangular hyperbola (*Blaustein* 1974, 1977; *DiPolo* 1974; *Baker* and *McNaughton* 1976a; *Baker* 1978).

Calcium efflux is also dependent on the concentration of free calcium in the axon. At low and intermediate Ca_i, calcium efflux is directly proportional to the Ca_i concentration and the slope of the curve relating efflux and Ca_i is about 1 on a double logarithmic scale (*DiPolo* 1973a; *Brinley* et al. 1975). This relation and the absolute rates are the same wether ATP is present (*DiPolo* 1973a) or absent (*Brinley* et al. 1975). Calcium efflux ranges from 5 f/cs at 5 nM Ca_i to about 1 p/cs at Ca_i concentrations close to 1 μM. At high internal calcium concentrations, the slope relating calcium efflux and Ca_i is less than 1, and suggests the initial portion of a saturation curve (*DiPolo* 1973a; *Brinley* et al. 1975). And indeed, at high Ca_i, calcium efflux appears to plateau to a maximal velocity of about 2 p/cs (*Blaustein* 1977a). The K_{Ca_i} in these conditions depends

Table 15. Na_o-dependent calcium efflux in squid axon

		Reference
Range of Ca efflux	0.005–5.0 p/cs	*DiPolo* 1973; *Blaustein* and *Russell* 1975; *Brinley* et al. 1975; *Blaustein* 1977a
Activated by Na_o		
K_{Na_o} (with ATP)	40–80 mM	*DiPolo* 1974; *Baker* and *McNaughton* 1976a; *Blaustein* 1977; *Baker* 1978; *Requena* 1978
K_{Na_o} (no ATP)	150–300 mM	*Blaustein* 1974; *DiPolo* 1974; *Baker* and *McNaughton* 1976a; *Blaustein* 1977; *Baker* 1978
Stoichiometry (no ATP)	3 Na_o/1 Ca_i	*Blaustein* 1974, 1976; *Requena* 1978
Activated by Ca_i		
At high Ca_i		
K_{Ca_i} (with ATP)	0.75 μM	*Blaustein* 1976, 1977
K_{Ca_i} (no ATP)	8 μM	*Blaustein* and *Russell* 1975; *Blaustein* 1976, 1977
At intermediate and low Ca_i		
	Linear relationship	*DiPolo* 1973; *Brinley* et al. 1975
Inhibited by Na_i		
$K_{I\ Na_i}$ (with or without ATP)	30 mM	*Blaustein* and *Russell* 1975; *Blaustein* 1977; *Requena* 1978
ATP relieves Na_i inhibition		*Requena* 1978
Activated by ATP		*Baker* and *Glitch* 1973; *DiPolo* 1974, 1976, 1977; *Mullins* and *Brinley* 1975; *Baker* and *McNaughton* 1976a; *Blaustein* 1977a; *Requena* 1978
K_{ATP_i}	0.3–0.5 mM	*DiPolo* 1974, 1976, 1977
Sensitive to the membrane PD (without ATP)		*Brinley* and *Mullins* 1974; *Blaustein* et al. 1974; *Mullins* and *Brinley* 1975; *Blaustein* and *Russell* 1975; *Baker* and *McNaughton* 1976a, b; *Blaustein* 1976
Q_{10}	2.3–3	*Luxoro* and *Yanez* 1968; *Blaustein* and *Hodgkin* 1969; *Baker* 1972
Inhibited by lanthanum		*Baker* 1972; *Brinley* et al. 1975; *Baker* and *McNaughton* 1978
Not inhibited by ouabain		*Blaustein* and *Hodgkin* 1969; *Baker* 1972; *Blaustein* 1974

on ATP. Without ATP, K_{Ca_i} is about 8 μM (*Blaustein* and *Russell* 1975; *Blaustein* 1976, 1977) while in the presence of ATP it drops to 0.75 μM (*Blaustein* 1976, 1977). These two values should be regarded with caution, however, since all rates exceeding 2 p/cs have been ignored in the calculations. Indeed, calcium efflux as high as 3–6 p/cs has been frequently reported, but discarded in the determination of the kinetic parameters (*Blaustein* et al. 1974; *Blaustein* and *Russell* 1975; *Blaustein* 1977a).

Calcium efflux is inhibited by internal sodium. At very high Ca_i, the inhibition by Na_i is not affected by ATP: the $K_{I\ Na_i}$ is 30 mM with ATP (*Blaustein* 1977) or without ATP (*Blaustein* and *Russell* 1975). *Blaustein* (1977) proposed a competitive inhibition of 2 Na_i and 1 Ca_i for a carrier site with two negative charges. However, at intermediate and low Ca_i, ATP relieves the inhibition produced by Na_i (*Requena* 1978). Furthermore, there is no significant difference between the extent of the inhibition produced by Na_i at high or low concentrations of intracellular ionized calcium (*Requena* 1978; *Brinley* et al. 1975). The fact that Ca_i does not affect $K_{I\ Na_i}$ suggests a noncompetitive interaction in which a specific site on the carrier binds exclusively internal sodium, resulting in an inhibition of calcium efflux (*Requena* 1978). Since the K of activation by Na_o is practically equal to the K of inhibition by Na_i, it appears that the postulated carrier has the same affinity for Na on either side of the membrane, and *Requena* (1978) proposed that if one assumes a single carrier for calcium translocation, Na will activate calcium transport when bound to the opposite side of the membrane, while Na inhibits calcium transport when bound to the same side.

ATP stimulates the Na_o-activated calcium efflux (*Baker* and *Glitch* 1973; *DiPolo* 1974, 1976, 1977; *Mullins* and *Brinley* 1975; *Baker* and *McNaughton* 1976a; *Blaustein* 1977; *Baker* 1978; *Requena* 1978). However, if the intracellular sodium concentration is very low, ATP has no effect on the activation produced by Na_o (*Blaustein* 1974; *Requena* 1978). As mentioned before, ATP shifts the curve relating the Na_o-activated calcium efflux to Na_o from a sigmoid shape to a simple Michaelis-Menten relationship (*DiPolo* 1974; *Baker* and *McNaughton* 1976a). The apparent activation constant of ATP, K_{ATP_i}, lies between 0.3 and 0.5 mM (*DiPolo* 1974, 1976, 1977). At physiologically low Ca_i (< 0.1 μM) more than 95% of the calcium efflux depends on ATP, while at high Ca_i only 50%–60% of the calcium efflux depends on ATP (*DiPolo* 1977). The ATP-dependent stimulation has an absolute requirement for intracellular Mg. Other high-energy phosphate compounds (UTP, GTP, CTP, UDP, ADP, AMP, cyclic AMP, and acetyl phosphate) are ineffective in supporting calcium efflux (*Baker* and *McNaughton* 1976a; *DiPolo* 1977). The compounds 2'deoxy-ATP and the hydrolyzable analog, α,β-methylene ATP are able to activate

calcium efflux, while the nonhydrolyzable analog β-methylene ATP competes with ATP for the activating site but is unable to activate calcium efflux (*DiPolo* 1977). From these data, one may conclude that ATP hydrolysis could be involved in calcium transport or at least that a phosphorylating step in the activation of calcium efflux by ATP cannot be ruled out (*Baker* and *McNaughton* 1976a; *Baker* 1976, 1978; *DiPolo* 1977).

The Na_o-activated calcium efflux is sensitive to the membrane potential difference (*Brinley* and *Mullins* 1974; *Blaustein* et al. 1974; *Mullins* and *Brinley* 1975; *Blaustein* and *Russell* 1975; *Baker* and *McNaughton* 1976a, b; *Blaustein* 1976). This could be expected in poisoned axons where the observed stoichiometry suggests an exchange of 3 Na_o for 1 Ca_i, leaving one negative charge. Na_o-activated calcium efflux should therefore be electrogenic (*Brinley* and *Mullins* 1974; *Mullins* and *Brinley* 1975; *Baker* and *McNaughton* 1976a, b). And indeed, a membrane depolarization inhibits while a hyperpolarization stimulates calcium efflux in poisoned axons. The maximum sensitivity of calcium efflux to membrane potential is in the order of an *e*-fold increase in efflux for a 25 mV increase in membrane potential (*Mullins* and *Brinley* 1975). In the presence of ATP, however, the stoichiometry is not 3/1 as indicated previously, and alterations in membrane potential have little effect on the Na_o-dependent calcium efflux (*Baker* and *McNaughton* 1976a, b; *Baker* 1976).

Finally, calcium efflux from squid axons has been shown to have a Q_{10} ranging from 2.3 to 3 (*Luxoro* and *Yanez* 1978; *Blaustein* and *Hodgkin* 1969; *Baker* 1972). It is not inhibited by ouabain (*Blaustein* and *Hodgkin* 1969; *Baker* 1972; *Blaustein* 1974) but it is inhibited by high concentrations of lanthanum (*Blaustein* and *Hodgkin* 1969; *Baker* 1972, 1976; *Brinley* 1975; *Baker* and *McNaughton* 1978).

The idea that the Na_o-dependent calcium efflux was only one aspect of a more general scheme of a Na–Ca exchange is supported by the following observations reported in squid axons: (a) there is a Na_o-dependent calcium influx since calcium influx is increased when the extracellular sodium concentration is reduced (*Baker* et al. 1969; *DiPolo* 1979); (b) calcium influx also depends on the intracellular concentration of sodium since a rise in Na_i stimulates calcium influx (*Baker* et al. 1969; *DiPolo* 1979); (c) there is a Ca_o-dependent sodium efflux since a fraction of sodium efflux is ouabain-insensitive and increases when Na_o is replaced by lithium but only in the presence of extracellular calcium (*Baker* et al. 1979). This led to the proposal already considered by *Reuter* and *Seitz* (1968), that there is a common carrier for sodium and calcium ions and that calcium can be transported out of the axon against a large electrochemical gradient by a mechanism in which external sodium exchanges for internal calcium. In such a system, sodium ions would be moving downhill and would provide the

energy needed to extrude calcium (*Baker* et al. 1969; *Blaustein* and *Hodgkin* 1979).

Several models of Na–Ca exchange have been offered. *Blaustein* (1976) proposed the model shown in Fig. 5. A carrier in the *Y* conformation is oriented with its Na-binding sites facing externally and Ca-binding site facing the axoplasm. In the *Z* form, the orientation of the sites is reversed.

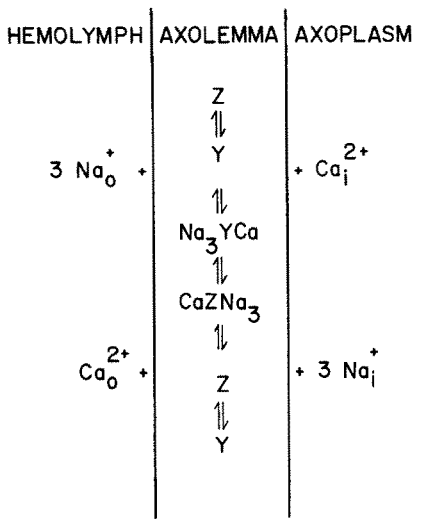


Fig. 5. Calcium–sodium exchange model proposed by Blaustein for the squid axon (*Blaustein* 1976). *Y* is the carrier oriented with its Na-binding sites facing externally and Ca-binding sites facing the axoplasm. The *Z form* represents the carrier with the sites reversed. Both unloaded and fully loaded carriers (but not partially loaded ones) may be capable of undergoing site translocation. Reprinted with permission from *Federation Proceedings* 35:2574–2578 (1976)

Both unloaded and fully loaded carriers (but not the partially loaded ones) are capable of undergoing site translocation. This model fits the 3 Na^+ for 1 Ca^{++} stoichiometry reported by Blaustein. Theoretically, if the calcium and sodium fluxes were tightly coupled, and assuming 100% efficiency the energy dissipated in moving sodium into the cell could maintain the intracellular ionized calcium at around 300 nM. The exchange would be electrogenic and sensitive to the membrane potential. ATP would increase the affinity of the carrier to Ca_i^{++}, without affecting the maximal rate of calcium efflux, and would catalyze rather than energize the transport mechanism (*Blaustein* 1976). It should be noted that the model proposed by Blaustein demands 100% efficiency for the energy exchange process and that it is based on data obtained almost exclusively from perfused poisoned axons with extremely high Ca_i and very low Na_i.

Mullins (1977) proposed another model based on more recent observations obtained at intermediate or low intracellular free calcium concentrations, normal concentrations of intracellular sodium, and with or without ATP (*DiPolo* 1974, 1976, 1977; *Requena* et al. 1977; *Brinley* et al. 1975). The model shown in Fig. 6 also provides a coupling of the electrochemical sodium gradient that energizes the efflux of calcium. The carrier is assumed

to bind and to move simultaneously 4 Na^+ inward and 1 Ca^{++} outward. The binding of 4 Na to the carrier induces a calcium binding site on the opposite side of the membrane. The high affinity of the induced site for calcium would disappear upon dissociation of Na from the carrier. Translocation requires that both sodium and calcium binding sites be fully occupied. The exchange of 4 Na^+ for 1 Ca^{++} would of course be electrogenic and sensitive to the membrane potential, as observed by several investigators (*Blaustein* 1974; *Brinley* and *Mullins* 1974; *Mullins* and *Brinley* 1975). ATP would increase the affinity of the carrier to sodium, reducing the K_{Na_o} from 140 to 30 mM, as suggested by others (*Blaustein* 1977; *Baker* and *McNaughton* 1976a). At equilibrium, the intracellular free calcium Ca_i could be brought as low as 1.5 nM. In these conditions the rate of calcium efflux would be 1/20th that of calcium influx, 1.5 vs 40 f/cs. Thus Ca_i would increase to achieve the steady-state value of 30–50 nM, at which concentration calcium efflux would be equal to influx at a rate of about 30–40 f/cs (*Mullins* 1977).

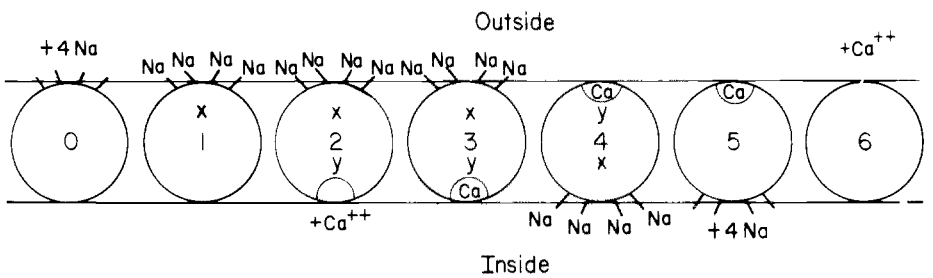


Fig. 6. Calcium–sodium exchange model proposed by Mullins for the squid axon (*Mullins* 1977), showing the sequence of Na binding to the carrier, the induction of a Ca-binding site *(2)*, the translocation *(4)*, and the dissociation of Ca *(6)*. As drawn, the scheme represents Ca efflux; on interchange of the labels 'outside' and 'inside,' it represents Ca influx. Reprinted with permission from the *Journal of General Physiology* 70:687 (1977)

In these two models the sodium electrochemical gradient provides the energy for calcium efflux, while ATP is assumed to catalzye the transport process by increasing the affinity of the carrier to sodium. However, the data are not unequivocal. Baker and his collaborators have reported that in intact, nonperfused, and unpoisoned axons calcium efflux was only reduced 25%–30% in calcium- and sodium-free seawater, whereas in a poisoned axon the residual efflux is a very much smaller percentage of the total efflux, and in absolute terms is usually smaller than the residual efflux from unpoisoned axons (*Baker* 1976; *Baker* and *McNaughton* 1976a). The residual efflux does not seem to be coupled to the movement of another ion but can be stimulated by ATP or inhibited by lanthanum and by

metabolic poisons (*Baker* and *McNaughton* 1978). Furthermore, in unpoisoned axons, alterations in membrane potential have little effect on the Na_o-dependent calcium efflux (*Baker* and *McNaughton* 1976b). The potential sensitivity of both the Na_o-dependent Ca efflux and the Ca_o-dependent Na efflux are found to be much too small to fit the 4 Na^+ to 1 Ca^{++} exchange model (*McNaughton* 1978). Similar results have been obtained in perfused axons in which the intracellular free calcium concentration is maintained at physiologic levels (< 100 nM). Even at intermediate Ca_i (≅ 230 nM), Na_o-dependent calcium efflux is only 6% of the control flux and in calcium- and sodium-free water, calcium efflux drops by only 37% (*Blaustein* and *Russell* 1975). *Mullins* and *Brinley* (1975) also reported that in poisoned axons with internal ionized calcium of 10–70 nM, the removal of sodium or of both sodium and calcium from the extracellular fluids hardly affects calcium efflux. In these axons perfused with Ca–EGTA, part of the calcium efflux could be due to leakage of the Ca–EGTA complex. However, when the values are corrected for leakage, calcium efflux is not depressed by Na-free or Na- and Ca-free external solutions, at physiologically low intracellular free calcium; in many cases, calcium efflux may even be higher, especially when lithium is substituted for Na (*Mullins* and *Brinley* 1975; *Brinley* et al. 1975). Comparable results were obtained by *DiPolo* (1977).

Furthermore, in intact axons, ATP enhances calcium efflux four- to fivefold in sodium- and calcium-free seawater (*Baker* and *McNaughton* 1976a), and in axons perfused with physiologically low free calcium solutions (60 nM) more than 95% of the calcium efflux depends on ATP (*DiPolo* 1977). ATP is able to stimulate calcium efflux only in the presence of internal magnesium and only hydrolyzable analogs of ATP can support it; nonhydrolyzable analogs cannot activate calcium efflux (*Baker* and *McNaughton* 1976a; *DiPolo* 1977).

On this basis, *Baker* et al. proposed that in intact unpoisoned axons, 50%–90% of calcium efflux can proceed in the absence of external calcium, sodium, and magnesium and may reflect an uncoupled extrusion of calcium (*Baker* 1976, 1978; *Baker* and *McNaughton* 1976a, 1978; *McNaughton* 1978). The remaining efflux is dependent on extracellular sodium. Whether ATP is metabolized is not established and there is no conclusive evidence yet that would permit us to decide whether in physiologic conditions calcium efflux from squid axons derives its energy from ATP hydrolysis or from the sodium electrochemical gradient. The choice still exists between a model where the uphill transport of calcium is fueled by ATP and activated by Na binding to the carrier or a model where calcium transport is energized by the sodium gradient and activated by ATP. It should be pointed out also that the Q_{10} of the process has been found to be high, between 2 and 3 (*Luxora* and *Yanez* 1968; *Blaustein* and *Hodgkin*

1969; *Baker* 1972; *Blaustein* and *Oborn* 1975), and that the metabolic energy necessary to maintain a very low intracellular free calcium is less than 1% of the total energy output of the cell (see Sect. 7). Finally, *DiPolo* reported the possible existence of a sodium-stimulated, ouabain-insensitive, magnesium-dependent ATPase, activated by calcium ions in the micromolar range in a highly purified membrane fraction of lobster nerves (*DiPolo* 1976, 1977).

8.3.3 Ca_o-Activated Calcium Efflux in Squid Axons

A Ca_o-dependent calcium efflux has been reported by many investigators (*Blaustein* and *Hodgkin* 1969; *DiPolo* 1974; *Blaustein* 1974, 1977a; *Blaustein* et al. 1974; *Blaustein* and *Russell* 1975; *Baker* and *McNaughton* 1976a, 1978; *Baker* 1978). In poisoned axons it represents about half the total calcium efflux (*Blaustein* and *Hodgkin* 1969; *Blaustein* and *Russell* 1975; *Baker* and *McNaughton* 1976a). In the presence of ATP, however, it is less than 10% of the total efflux (*Baker* and *McNaughton* 1978). Since calcium influx is also stimulated by raising intracellular calcium (*Blaustein* and *Russell* 1975; *DiPolo* 1979), *Blaustein* proposed that this represents a Ca–Ca exchange mediated by the same carrier as the Na_o-dependent calcium efflux, sodium and calcium competing for the same carrier site: both have the same K_{Ca_i} of 3–8 μM, both are stimulated by ATP, and both are inhibited by intracellular sodium or extracellular magnesium and strontium (*Blaustein* 1977).

But this hypothesis is open to question, because *Requena* (1978) has conclusively shown that there is no significant difference between the degree of inhibition produced by internal sodium at high or low concentrations of intracellular free calcium. This suggests a noncompetitive interaction in which a specific site on the carrier binds exclusively to internal sodium and leads to an inhibition of calcium efflux. Furthermore, *DiPolo* (1979) showed that in the absence of intracellular sodium there is no Ca_i-dependent calcium influx but there is a Ca_o-dependent calcium efflux, thus making a Ca–Ca exchange quite improbable. Finally, *Baker* and *McNaughton* (1978) showed that a large fraction of the Ca_o-dependent calcium efflux in unpoisoned axons is transient and probably does not reflect calcium transport out of the cell but rather a release of calcium from binding sites external to the plasma membrane. The transient nature of this efflux and the fact that EGTA added to calcium-free media also produces a transient rise in calcium efflux were already noticed by *Blaustein* (1977a). *Baker* and *McNaughton* (1978) conclude that three components of calcium efflux exist in squid axons: (a) an uncoupled extrusion of calcium, (b) an Na_o-activated calcium efflux, and (c) a Ca_o-activated calcium efflux. In unpoisoned axons uncoupled extrusion of calcium may predom-

inate and there is very little Ca_o-activated calcium efflux. In fully poisoned axons, uncoupled extrusion disappears and calcium efflux is activated either by external sodium or external calcium.

8.3.4 Na_o-Activated Calcium Efflux in Other Excitable Tissues

Evidence for a Na_o-activated calcium efflux is found in many other excitable tissues (Table 16). The principal observation is that calcium efflux is depressed when extracellular sodium is replaced by substituting ions such as lithium or choline. But even in the total absence of extracellular sodium and calcium, there is still a significant residual calcium efflux, which may vary from 20% of the total efflux in cardiac muscle (*Reuter* and *Seitz* 1968; *Reuter* 1974a), through 30%–60% in barnacle and in frog striated muscle (*Russell* and *Blaustein* 1974; *Caputo* and *Bolaños* 1978), to 70% in rabbit vagus nerve (*Kalix* 1971). Incubation in sodium-free media leads to an increased tissue calcium or an increased uptake of ^{45}Ca by brain tissue (*Stahl* and *Swanson* 1969, 1971, 1972; *Cooke* and *Robinson* 1971; *Swanson* et al. 1974) or by cardiac muscle (*Reuter* et al. 1973; *Reuter* 1974a; *Wendt* and *Langer* 1977). In pinched-off presynaptic nerve terminals (synaptosomes) calcium influx is stimulated by reducing Na_o and by increasing intracellular sodium (Na_i); however, in the absence of Na_i there is no stimulation of calcium influx by reducing Na_o (*Blaustein* and *Oborn* 1975). These results suggest a competitive inhibition between Na_o and Ca_o and the possible existence of a Ca–Na exchange. The relation between calcium efflux and Na_o is sigmoid and may have a stoichiometry of 3 Na^+ for 1 Ca^{++} (*Blaustein* and *Oborn* 1975; *Blaustein* et al. 1977). A stimulation of calcium influx by a reduction in Na_o or a rise in Na_i is also observed in barnacle muscle (*DiPolo* 1973b) and in cardiac muscle (*Reuter* 1970; *Glitsch* et al. 1970).

Furthermore, ouabain, which increases Na_i, has been shown to increase cellular calcium in brain tissue (*Stahl* and *Swanson* 1969, 1971) and in cardiac muscle (*Govier* and *Holland* 1964; *Reuter* 1974a; *Wood* and *Schwartz* 1978). Na_o-activated calcium efflux may be important to maintain a low cytosolic free calcium in cardiac muscle when calcium is released from intracellular stores. In this tissue, contraction is a good indication of a rise in calcium activity, and both caffeine and cyanide increase calcium efflux without inducing contraction in the presence of a normal concentration of Na_o. Without Na_o, however, calcium efflux is not increased and a contraction occurs (*Jundt* et al. 1975). In cardiac muscle contraction may be a function of $Ca_o/[Na_o]^2$ (*Lüttgau* and *Niedergerke* 1958; *Niedergerke* 1963; *Reuter* and *Seitz* 1968; *Reuter* 1970; *Reuter* et al. 1973). However, *Miller* and *Moiescu* (1976) showed that the relation does not hold true at very low Na_o concentrations when contraction is simply a

Table 16. Na_o-dependent calcium efflux reported in excitable tissues

Tissues	Reference
Neural tissues	
Squid axons	See Table 15
Crab nerve	*Baker* and *Blaustein* 1968
Rabbit vagus nerve	*Kalix* 1969, 1971
Brain slices	*Stahl* and *Swanson* 1961, 1971, 1972; *Tower* 1968; *Cooke* and *Robinson* 1971
Nerve cells	*Stallcup* 1979
Synaptosomes	*Blaustein* and *Weismann* 1970; *Swanson* et al. 1974; *Blaustein* and *Oborn* 1975; *Blaustein* and *Ector* 1976; *Blaustein* et al. 1977
Muscles	
Barnacle muscle	*Ashley* et al. 1972; *DiPolo* 1973b; *Russell* and *Blaustein* 1974, 1975; *DiPolo* and *Caputo* 1977; *Ashley* and *Lea* 1978
Skeletal muscle	*Cosmos* and *Harris* 1961; *Caputo* and *Bolanos* 1978
Cardiac muscle	*Lüttgau* and *Niedergerke* 1958; *Niedergerke* 1963; *Reuter* and *Seitz* 1968; *Glitsch* et al. 1970; *Reuter* 1970, 1974; *Kutzung* et al. 1973; *Reuter* et al. 1973; *Fundt* et al. 1975; *Miller* and *Moiescu* 1976; *Fundt* and *Reuter* 1977; *Wendt* and *Langer* 1977; *Wood* and *Schwartz* 1978
Arterial smooth muscle	*Reuter* et al. 1973; *Brading* 1973; *Raeymaekers* et al. 1973; *Van Breemen* et al. 1973, 1978; *Burton* and *Godfraind* 1974; *Droogmans* and *Casteels* 1979
Uterine muscle	*Van Breemen* et al. 1966, 1978

function of Ca_o, and they propose a competition between 4 Na_o^+ and 2 Ca_o^{++} to account for those results. There is also some evidence for a Na_o-activated calcium efflux in smooth muscle (*Brading* 1973; *Reuter* et al. 1973; *Van Breemen* et al. 1973; *Burton* and *Godfraind* 1974): in sodium-free media, tissue calcium increases, calcium influx rises while calcium efflux falls (*Reuter* et al. 1973). When intracellular sodium is raised, tissue calcium also increases. These changes are not observed, however, if the lanthanum method is used to displace extracellulary bound calcium (*Van Breemen* et al. 1973; *Burton* and *Godfraind* 1974). And indeed, *Raeymaekers* et al. (1973) showed that much, if not all, of the Na–Ca exchange could be explained by an exchange with extracellular binding sites of smooth muscle. Furthermore, in aorta smooth muscle the calcium gained by the tissue is not extruded when the sodium gradient is restored (*Van Breemen* et al. 1973). *Van Breemen* et al. (1973, 1978) conclude that in

this tissue at least the calcium gradient does not depend on the sodium gradient.

Droogmans and *Casteels* (1979) also dispute the existence of a Na–Ca exchange in the rabbit ear artery smooth muscle. Their data indicate that: (a) the contraction induced by Na_o-free solution is due to an increased calcium influx that is not mediated by a carrier mechanism exchanging internal sodium for external calcium; (b) the relaxation observed when extracellular calcium is reduced cannot be powered by the sodium gradient since it occurs in Na_o-free media; (c) reducing Na_o to zero during ^{45}Ca efflux in Ca_o-free, EGTA-containing solutions does not affect the rate of calcium efflux; (d) if calcium is present in the extracellular fluids, lowering Na_o increases calcium efflux; (e) calcium efflux is not sensitive to large changes in the membrane potential difference; and (f) the extrusion of ^{45}Ca after its release from intracellular stores by histamine or FCCP is not inhibited in tissues in which the Na gradient is reduced or eliminated by exposure to Na_o-free solutions (*Droogmans* and *Casteels* 1979). In uterine muscle, tissue calcium is not increased by ouabain or by decreasing external sodium to 10 mM, and there is no evidence that sodium competes with calcium for inward transport (*Van Breemen* et al. 1966, 1978). However, since metabolic inhibitors significantly increase uterine muscle calcium, *Van Breemen* et al. (1966) conclude that calcium efflux is probably a metabolically dependent, active transport process. *Simonsen* and *Christoffersen* (1979) report also that in *Helix pomatia* neurons there is no significant Na–Ca exchange: substitution of sodium by lithium, choline or Tris causes no change in intracellular free calcium as measured by a pCa electrode; furthermore, the rate of rise in cytosolic free calcium induced by a mitochondrial inhibitor such as azide is not affected by the external sodium concentration. Finally, it has been shown that, as in squid axon, ATP stimulates calcium efflux in sodium- and calcium-free media both in barnacle muscle (*DiPolo* and *Caputo* 1977) and in cardiac muscle (*Jundt* and *Reuter* 1977). Whether ATP increases the affinity of the postulated carrier to calcium or to Na_o or whether it provides energy to the system is unknown.

8.3.5 Na_o-Activated Calcium Efflux in Nonexcitable Tissues

Early investigations on liver had shown that slices incubated in choline chloride have a higher calcium content and that calcium extrusion is inhibited in low sodium media (*Judah* and *Ahmed* 1963, 1964; *Wallach* 1966). However, *Van Rossum* (1970) showed that net extrusion of calcium from liver is not inhibited by high intracellular sodium and is not affected by the sodium gradient. Furthermore, ouabain has no effect on liver calcium content or fluxes (*Van Rossum* 1970; *Van Rossum* et al. 1971). The uni-

directional efflux of ^{45}Ca and the net extrusion of calcium from liver cells are not prevented by ouabain or by a sodium-free medium but they are reversibly inhibited by anaerobiosis and by respiratory inhibitors, and they depend on external magnesium, suggesting that calcium extrusion is metabolically driven and independent of sodium (*Cittadini* and *Van Rossum* 1978).

Similarly, in kidney slices, early results from *Höfer* and *Kleinzeller* (1963c) indicated that the tissue calcium barely rises in sodium-free media. Later work showed that decreasing extracellular sodium from 145 mM to 100, 50 or 0 mM (with the substituting ions being either choline or TEA) does not affect at all the total slice calcium, the total exchangeable calcium or the calcium content of isolated renal mitochondria, and ouabain is also without effect (*Borle* 1979). However, the steady-state exchange of calcium across the plasma membrane is depressed in low-Na_o or in sodium-free media (*Borle* 1979); these results suggest a possible activation of the calcium carrier by sodium but do not indicate the presence of a Na–Ca exchange. In Ehrlich ascites tumor cells, there is no increase in calcium uptake if extracellular sodium is replaced by lithium, and calcium loaded cells can extrude all their accumulated calcium in sodium-free media (*Cittadini* et al. 1973, 1977). In fibroblasts, variations in extracellular or intracellular sodium do not affect the calcium fluxes and the calcium concentrations of the cells (*Lamb* and *Lindsay* 1971). In the adrenal medulla calcium efflux drops 80% on sodium- and calcium-free media, but in the presence of extracellular calcium the substitution of Na_o by lithium, choline or sucrose actually stimulates calcium efflux (*Rink* 1977; *Aguirre* et al. 1977). There is an increased ^{45}Ca uptake in Na_o-free media, but only at extracellular calcium concentration 3 times the normal (3.6 mM Ca_o) and the actual net gain of tissue calcium is very small; ouabain is without effect (*Rink* 1977). Thus, in adrenal medulla there may be a Ca–Ca exchange but the Na_o-activated calcium efflux is not dependent on the sodium gradient; it is presumably fueled by ATP (*Rink* 1977). In the pancreas, ^{45}Ca uptake is stimulated in sodium-free media (*Hellman* et al. 1971; *Case* and *Clausen* 1973; *Schulz* et al. 1977). Nevertheless, calcium efflux increases rather than decreases in the absence of extracellular sodium, even at very low extracellular calcium (*Case* and *Clausen* 1973). Similarly in the salivary gland, acetylcholine can stimulate calcium efflux to its full extent, even in sodium- and calcium-free media (*Pors Nielsen* and *Petersen* 1972). These results preclude the existence of a Na–Ca exchange mechanism in this tissue. In mammary tumor cells, ouabain has no effect on their calcium content (*Van Rossum* et al. 1971) and in platelets there is no increase in ^{45}Ca uptake if extracellular sodium is reduced from 150 to 15 mM (*Steiner* and *Tateishi* 1974). Finally, it is well documented that there is no Na–Ca

exchange in erythrocytes (*Schatzmann* 1966, 1970; *Lee* and *Shin* 1969; *Schatzmann* and *Vincenzi* 1969; *Schatzmann* and *Rossi* 1971).

In conclusion, a Na_o-dependent calcium efflux is observed in many different tissues. A broad distinction can perhaps be made between excitable (muscle and nerves) and nonexcitable tissues. In the former group a Na–Ca exchange mechanism is theoretically possible and if the stoichiometry was 4 Na_o^+ to 1 Ca_i^{++} this exchange could maintain a very low intracellular free calcium. However, such a stoichiometry has never been observed experimentally. In the squid axon the cytosolic free calcium is very low (30–50 nM) and the calcium influx and efflux are very small, in the range of 40 f/cs (*DiPolo* 1977, 1979; *Requena* et al. 1977; *Mullins* 1977; *Requena* 1978). In these conditions, calcium efflux is not depressed but sometimes increased by sodium- and calcium-free media (see above). On the other hand, when cytosolic free calcium rises by two orders of magnitude, as in muscle, a Na-Ca exchange may contribute a significant fraction of calcium efflux. In nonexcitable cells a Na_o-dependent calcium efflux is often observed, but the consensus is that it is not a Na–Ca exchange mechanism and the calcium gradient across the plasma membrane is not dependent on the sodium gradient. Calcium efflux is probably activated by extracellular sodium but energized by ATP.

8.4 Calcium Influx

8.4.1 Calcium Uptake and Metabolic Inhibitors

In all cells the thermodynamic conditions are such that calcium influx across the cell plasma membrane occurs down a steep electrochemical gradient and does not require metabolic energy. Nevertheless, in several early papers *Höfer* and *Kleinzeller* (1963a–c) and *Janda* (1969) concluded that calcium influx into kidney cells requires metabolic energy. Their conclusions were based on the facts that (a) the calcium concentration of kidney slices was higher than the incubating medium calcium concentration and (b) metabolic inhibitors such as DNP and oligomycin markedly depress the uptake of calcium or ^{45}Ca by the slices.

However, these authors ignored several facts. First, it is now clear that the measurements of cellular calcium by chemical analysis or by kinetic analysis of tracer movements do not measure the actual ionized cytosolic calcium, which is definitely not higher than the extracellular free calcium concentration. Second, in their experiments with metabolic inhibitors they were misled by their experimental design: indeed they first 'leached' tissue slices at 0°C and in Ca-free media, depleting the cells and their intracellular organelles (mitochondria and endoplasmic reticulum) of calcium.

Then they incubated these calcium-depleted slices at 25°C in a medium with a calcium concentration of 2.4 mM which is twice the physiologic concentration. In these conditions there is a net shift of calcium into the slices and the system is not at steady state since the cells, the endoplasmic reticulum, and the mitochondria reaccumulate calcium. In nonsteady state and with cells accumulating calcium the uptake of ^{40}Ca or ^{45}Ca reflects not only calcium influx across the plasma membrane but also calcium uptake by endoplasmic reticulum and mitochondria, which is metabolically dependent. Thus, when ^{45}Ca uptake is measured in a nonsteady state, when there is a net accumulation of calcium by the cells or by the tissues, the inhibition of calcium uptake observed with various metabolic inhibitors is most likely due to a depressed calcium accumulation in intracellular organelles that requires metabolic energy and not to an inhibition of calcium influx into the cell.

To illustrate this important point, Figs. 7 and 8 show the effects of metabolic inhibitors on ^{45}Ca uptake by kidney cells in steady state (no net gain of calcium) and nonsteady state (net gain of calcium). It is clear that in the former case metabolic inhibition does not depress calcium uptake, in fact isotopic equilibrium is reached more quickly, while in the latter

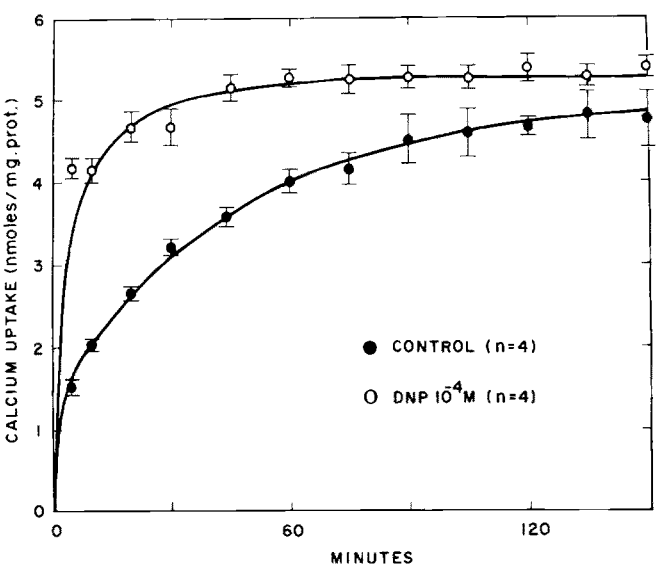


Fig. 7. Effect of a metabolic inhibitor, 10^{-4} M, DNP, on ^{45}Ca uptake in steady-state conditions. Experiments performed with cultured monkey kidney cells in conditions described in Fig. 4. Note the stimulation of calcium uptake by DNP, at steady state. Reproduced from *Borle* (1981), with permission from *Cell Calcium*

case there is a marked inhibition of uptake. Most of the time, when metabolic inhibitors (DNP, IAA) are reported to depress calcium uptake, the experimental design indicates that the cells were not at steady state (Table 17): they were preincubated at low temperature or in calcium-free media or uptake was measured in media containing unphysiologically high calcium or phosphate concentrations leading to a net shift of calcium into the cells (*Höfer* and *Kleinzeller* 1963a–c, *Janda* 1969; *Terepka* et al. 1969; *Papworth* and *Patrick* 1970; *Cittadini* et al. 1971; *Whitney* and *Sutherland* 1972; *Ewe* 1972; *Swanson* et al. 1974; *Dziak* and *Brand* 1974a, b; *Charlton* and *Wenner* 1978). However, when the cells are preincubated in physiologic media before the ^{45}Ca uptake determination, or when they are at steady state, DNP, IAA or other metabolic inhibitors do not affect calcium uptake (Table 17) (*Höfer* and *Kleinzeller* 1963a; *Levinson* 1967; *Borle* 1969a; *Levinson* and *Blumenson* 1970; *Stahl* and *Swanson* 1971; *Lamb* and *Lindsay* 1971; *Whitney* and *Sutherland* 1972; *Miller* and *Nelson* 1977). *In* some cases, metabolic inhibitors stimulate ^{45}Ca uptake (*Wallach* 1966; *Van Rossum* 1970; *Hellman* et al. 1971; *Stahl* and *Swanson* 1971; *Swanson* et al. 1974; *Borle,* unpublished). A possible explanation could be that in certain conditions these inhibitors increase the cytosolic free calcium by depressing the metabolically dependent transport of calcium into intracellular organelles (most likely mitochondria). Since a rise in cytosolic free calcium has been shown to stimulate calcium influx in several cell systems (see below), calcium uptake could thus be enhanced.

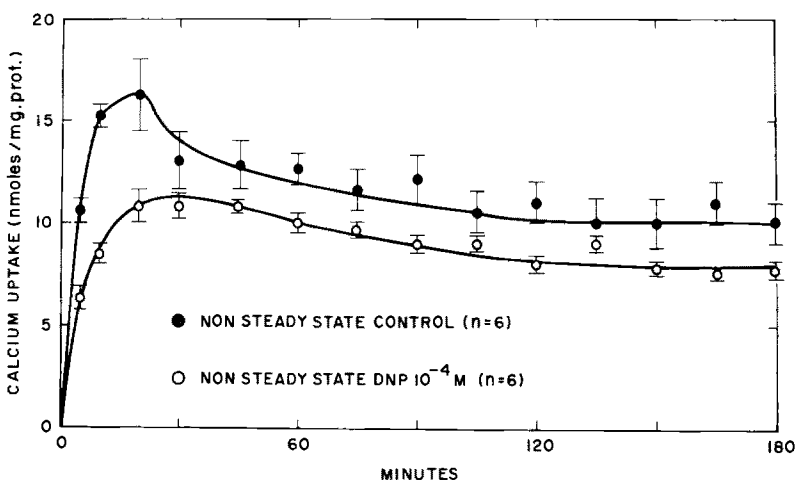


Fig. 8. Effect of a metabolic inhibitor, 10^{-4} M, DNP, on ^{45}Ca uptake in nonsteady-state conditions. Experiments performed with cultured monkey kidney cells in the conditions described in Fig. 4. Note the inhibition of calcium uptake by DNP at nonsteady state. Reproduced from *Borle* (1981), with permission from *Cell Calcium*

Table 17. Effects of metabolic inhibitors on ^{45}Ca uptake

Tissue	Inhibitor	Reference
Inhibition of ^{45}Ca uptake (nonsteady state)		
Kidney	DNP, oligomycin	*Höfer* and *Kleinzeller* 1963a, b; *Janda* 1969; *Borle*, unpublished
Ehrlich ascites cells	TTFB	*Cittadini* et al. 1971
Brain synaptosomes	DNP, antimycin A	*Swanson* et al. 1974
Lymphocytes	DNP, IAA	*Whitney* and *Sutherland* 1972
Chorioallantoic membrane	DNP, oligomycin	*Terepka* et al. 1969
Bone cells	DNP, IAA, oligomycin, antimycin A	*Dziak* and *Brandt* 1974
Intestine	DNP, IAA, CN	*Papworth* and *Patrick* 1970; *Ewe* 1972
No inhibition (steady state)		
Kidney	DNP	*Höfer* and *Kleinzeller* 1963a
Ehrlich ascites cells	DNP	*Levinson* and *Blumenson* 1970
Brain slices	DNP	*Stahl* and *Swanson* 1970
Fibroblasts	DNP, IAA	*Lamb* and *Lindsay* 1971
HeLa cells	DNP	*Borle* 1969
Parotid gland	CN	*Miller* and *Nelson* 1977
Stimulation		
Kidney cells	DNP, oligomycin, warfarin	*Borle*, unpublished
Liver slices	CN, IAA	*Wallach* et al. 1966; *Van Rossum* 1970
Pancreatic islets	DNP	*Hellman* et al. 1971
Brain slices	IAA, CN	*Stahl* and *Swanson* 1971
Brain synaptosomes	Oligomycin	*Swanson* et al. 1974

Two main mechanisms for calcium influx into cells have been postulated (a) a voltage-independent transport and (b) voltage-dependent calcium influx through 'calcium channels.'

8.4.2 Voltage-Independent Carrier-Mediated Calcium Influx

The steady-state calcium uptake or influx is a function of the extracellular calcium concentration and in nonexcitable tissues it is a saturable process, indicating that calcium transport into the cell is a carrier-mediated mechanism. Table 18 presents the K_{Ca_o} and the V_m obtained in eight different cell types. Excluding the values published by *Janda* (1969), which are 1–2 orders of magnitude larger than all others, the average K_{Ca} is about 0.5 mM and the V_m 170 pmol mg^{-1} protein min^{-1}. In squid axons, however, calcium influx is linear up to an external calcium concentration of 20 mM (*Hodgkin* and *Keynes* 1957; *Rojas* and *Taylor* 1975; *Baker* and *McNaughton* 1976a; *DiPolo* 1979). Nevertheless, the magnitude of calcium influx

Table 18. Kinetic parameters of calcium influx in different cells

Tissue	K_{Ca} (mM)	V_m [pmol (mg prot)$^{-1}$ min^{-1}]	Reference
Kidney slices	3.2	10–400	*Janda* 1969
Kidney slices	0.4	190	*Uchikawa* and *Borle*, unpublished
Kidney cells	0.37	130	*Borle* 1970
Pancreas	0.55	91	*Kondo* and *Schulz* 1976b
Bone cells	0.4 [a]	–	*Dziak* and *Brand* 1974b
Parotid gland	0.5 [a]	–	*Miller* and *Nelson* 1977
Intestine	1.00	–	*Walling* and *Rothman* 1969
Intestine	0.59	–	*Walling* and *Rothman* 1970
Intestine	1.15	190 [a]	*Papworth* and *Patrick* 1970
Fibroblasts	0.3	–	*Lamb* and *Lindsay* 1971
Synaptosomes	0.2	250 [a]	*Blaustein* and *Oborn* 1975
Synaptosomes	0.8	–	*Blaustein* 1975
Lymphocytes	0.4–1.0	–	*Whitney* and *Sutherland* 1973
Mean	0.8 ± 0.22	175 ± 23	

[a] Estimated from published data.

in mammalian cells and in squid axons is practically identical, 30–40 f/cs, when they are incubated in their respective physiologic extracellular calcium concentrations (*Requena* et al. 1977; *Requena* 1978; *DiPolo* 1979; see also Table 9). The characteristics of calcium influx are not strikingly different in excitable and nonexcitable tissues, as shown in Table 19.

In excitable tissues calcium influx is dependent on the extracellular sodium concentration. Lowering Na_o stimulates calcium influx in squid axons (*Baker* et al. 1969; *Baker* 1972; *Blaustein* 1974; *Blaustein* et al. 1974; *Blaustein* and *Russell* 1975; *DiPolo* 1979). It stimulates ^{45}Ca uptake in synaptosomes and in other neural tissues (*Baker* and *Blaustein* 1968; *Blaustein* and *Wiesman* 1970; *Stahl* and *Swanson* 1969, 1971, 1972; *Cooke* and *Robinson* 1971; *Swanson* et al. 1974; *Blaustein* and *Oborn* 1975), barnacle, cardiac and smooth muscle (*Reuter* 1970; *Glitsch* et al. 1970; *Reuter* et al. 1973; *DiPolo* 1973b; *Van Breemen* et al. 1973; *Droogmans* and *Casteels* 1979). Low Na_o also stimulates ^{45}Ca uptake in adrenal medulla (*Rink* 1977; *Aguirre* et al. 1977) and pancreas (*Case* and *Clausen* 1973; *Schulz* et al. 1977). The stimulation of calcium influx is particularly evident when Li is substituted for Na, and Li itself may promote calcium entry into the cell (*Baker* et al. 1969; *Baker* 1972; *Van Breemen* et al. 1973; *Blaustein* and *Russell* 1975; *Brinley* 1968). The relation between Na_o and calcium influx is not a simple one. Indeed, in squid axons, increasing Na_o from 0 to 100 mM activates calcium influx and only higher concentrations of Na_o inhibit it (*Baker* et al. 1969; *Baker* 1972). This has also

Table 19. Characteristics of calcium influx in excitable and nonexcitable cells

	Reference
Squid axons and other excitable tissues	
Resting influx: 30–40 f/cs	*Requena* et al. 1977; *Requena* 1978; *DiPolo* 1979
Ca_o-dependent: influx linearly related to Ca_o	*Hodgkin* and *Keynes* 1959; *Rojas* and *Taylor* 1975; *Baker* and *McNaughton* 1976a; *DiPolo* 1979
Na_o-dependent: low Na_o stimulates	
In squid axon	*Baker* et al. 1969; *Baker* 1972; *Blaustein* 1974; *Blaustein* et al. 1974; *Blaustein* and *Russell* 1975
In synaptosomes and brain tissue	*Baker* and *Blaustein* 1968; *Blaustein* and *Wiesman* 1970; *Stahl* and *Swanson* 1969, 1971, 1972; *Cooke* and *Robinson* 1971; *Swanson* et al. 1974; *Blaustein* and *Oborn* 1975
In muscle	*Reuter* 1970; *Glitsch* et al. 1970; *Reuter* et al. 1973; *DiPolo* 1973b; *Van Breemen* et al. 1973; *Droogmans* and *Casteels* 1979
Li_o-dependent: Li stimulates	*Baker* et al. 1969; *Baker* 1972; *Van Breemen* et al. 1973; *Blaustein* and *Russell* 1975; *Brinley* 1978
K_o-dependent: high K stimulates	*Hodgkin* and *Keynes* 1957; *Baker* 1972; *Blaustein* et al. 1972, 1977; *Van Breemen* et al. 1973; *Blaustein* and *Ector* 1975
pH-dependent: low pH inhibits, high pH stimulates	*Morgenstern* et al. 1972; *Van Breemen* et al. 1972, 1973; *Blaustein* and *Oborn* 1975
Na_i-dependent: high Na_i stimulates $K_{Na_i} = 50–60$ mM	*Baker* et al. 1969; *Baker* 1972; *Blaustein* et al. 1974; *Blaustein* and *Russell* 1975; *DiPolo* 1973a, 1979; *Blaustein* and *Wiesman* 1970
ATP_i-dependent: ATP stimulates $K_{ATP_i} = 0.2$ mM	*DiPolo* 1979
Ca_i-dependent: high Ca_i stimulates	*Blaustein* and *Russell* 1975; *DiPolo* 1979
Nonexcitable tissues	
Resting influx: 14–77 f/cs or 40–144 pmol (mg prot)$^{-1}$ min^{-1}	See Table 9 See Table 11
Ca_o-dependent: Michaelis-Menten kinetics $K_{Ca_o} = 0.2–1.0$ mM $V_m = 91 - 250$ f/cs	See Table 17

Table 19 (continued)

		Reference
Na_o-dependent:	possible (pancreas, adrenals)	*Hellman* et al. 1971; *Case* and *Clausen* 1973; *Schulz* et al. 1977; *Rink* 1977; *Aguirre* et al. 1977
	doubtful (kidney, cells, platelets, erythrocytes, fibroblasts)	*Höfer* and *Kleinzeller* 1963c; *Schatzmann* 1966, 1970; *Schatzmann* and *Rossi* 1971; *Lamb* and *Lindsay* 1971; *Cittadini* et al. 1973, 1977; *Steiner* and *Tateishi* 1974; *Borle* 1979
pH-dependent:	low pH inhibits high pH stimulates	*Wallach* et al. 1966; *Lamb* and *Lindsay* 1971; *Rorive* and *Kleinzeller* 1972; *Whitney* and *Sutherland* 1973; *Steiner* and *Tateishi* 1974; *Forman* et al. 1977; *Studer* and *Borle* 1979
Ca_i-dependent:	high Ca_i stimulates	*Borle* and *Anderson* 1976

been observed in kidney cells, where lowering Na_o from 140 to 0 mM decreases the steady-state exchange of calcium across the plasma membrane without increasing the calcium concentration of the tissue (*Borle* 1979).

Increasing the extracellular K concentration, which depolarizes the membrane, stimulates calcium influx in squid axons, crab nerves, synaptosomes, and smooth muscle (*Hodgkin* and *Keynes* 1957; *Baker* 1972; *Blaustein* et al. 1972, 1977; *Van Breemen* et al. 1973; *Blaustein* and *Ector* 1975). This increased calcium influx could very well occur through the calcium channels that are postulated to exist in excitable tissues (see below), although no clues are given in these published reports.

Calcium influx is also influenced by ionic composition of the intracellular phase. An increased intracellular sodium stimulates calcium influx in squid axons (*Baker* et al. 1969; *Baker* 1972; *Blaustein* et al. 1974; *Blaustein* 1974; *Blaustein* and *Russell* 1975; *DiPolo* 1979), in synaptosomes (*Blaustein* and *Wiesmann* 1970), and in barnacle muscles (*DiPolo* 1973b). *DiPolo* (1979) found that, in squid axons, the constant of activation K_{Na_i} is 50–60 mM, but the sensitivity of calcium influx to Na_i is greatest when Ca_i is elevated and is practically nil at very low Ca_i. Calcium influx is also dependent on the intracellular concentration of ATP (K_{ATP_i} = 0.2 mM) and on calcium K_{Ca_i} = 0.6 μM). *DiPolo* (1979) proposed that calcium influx into squid axon may consist of two separate components: one is dependent on Na_i, on ATP, and activated by Ca_i, and the other persisting in the absence of Na_i, Ca_i, and ATP_i. The former could be part of a Na–Ca exchange while the other would be an uncoupled passive pathway. *DiPolo* points out that under physiologic conditions (Ca_i = 60 nM, Na_i =

30 mM), calcium influx into squid axons occurs mostly through the uncoupled passive mechanism and not through a Na–Ca exchange. His conclusions are supported by *Droogmans* and *Casteels* (1979), who state that the results they obtained in smooth muscle, as already mentioned above, do not fit in with the Na–Ca exchange hypothesis but are consistent, nevertheless, with an effect of the Na gradient on the passive calcium influx. There is little direct evidence of the role of Na_i in nonexcitable tissues; however, the fact that ouabain does not increase calcium influx or ^{45}Ca uptake in liver (*Van Rossum* 1970), in kidney (*Borle* 1979), and in adrenal medulla (*Rink* 1977) suggests that the modulation of calcium fluxes by Na_o or Na_i does not represent a Na–Ca exchange mechanism in these tissues.

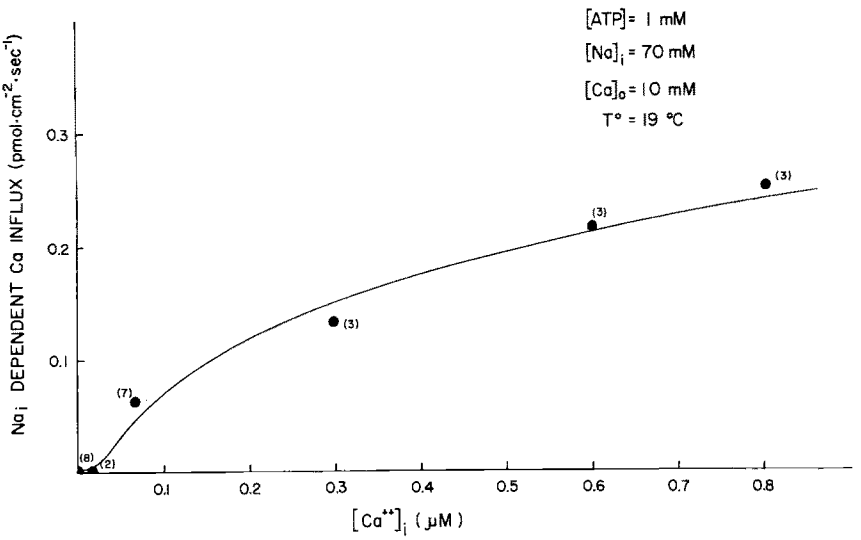


Fig. 9. Activating effect of internal calcium on the Na_i-dependent Ca influx in the squid axon. *Ordinate:* mean Na_i-dependent Ca influx in p/cs. *Abscissa:* internal ionized calcium in micromolar. All the axons were dialyzed with an internal medium containing 1 mM ATP and 70 mM Na_i. Extracellular calcium Ca_o = 10 mM; total EGTA = 0.5–1 mM. Reproduced from *DiPolo* (1979), with permission from the *Journal of General Physiology*

One of the most intriguing aspects of calcium influx is its activation by intracellular calcium (Fig. 9). A Ca_i-activated calcium influx has been reported in excitable tissues such as the squid axons (*Blaustein* and *Russell* 1975; *DiPolo* 1979) and in nonexcitable tissue such as kidney (*Borle* and *Anderson* 1976). A more indirect evidence of such an activation is the increased calcium influx observed when inhibitors of calcium uptake into mitochondria or uncouplers are present in liver (*Wallach* 1966; *Van Rossum* 1970), kidney (*Borle*, unpublished), pancreas (*Hellman* et al. 1971), and neural tissues (*Stahl* and *Swanson* 1971; *Swanson* et al. 1974), where

the cytosolic free calcium can be assumed to be elevated. *DiPolo* (1979) reported that, in squid axons dialyzed without internal sodium, the Ca_i activation of calcium is abolished, whereas Ca_o-activated calcium efflux is still present; he concludes that since there is no clear correlation between the inward and outward movements of calcium this stimulation of calcium influx does not represent a Ca–Ca exchange. *DiPolo* postulates that Ca_i activates the influx of calcium rather than exchanges with Ca_o.

Finally, the extracellular pH also markedly affects calcium influx, the ^{45}Ca uptake and, consequently, the calcium content of tissues. A low pH depresses calcium uptake and the total cell calcium while a high pH does the reverse. This effect has been observed in liver (*Wallach* et al. 1966), kidney (*Rorive* and *Kleinzeller* 1972; *Studer* and *Borle* 1979), fibroblasts (*Lamb* and *Lindsay* 1971), mast cells (*Foreman* et al. 1977), lymphocytes (*Whitney* and *Sutherland* 1973), platelets (*Steiner* and *Tateishi* 1974), cardiac muscle (*Morgenstern* et al. 1972), smooth muscle (*Van Breemen* et al. 1972, 1973), and synaptosomes (*Blaustein* and *Oborn* 1975). No direct measurement of a pH effect on calcium influx in squid axon has been published, but *Baker* and *McNaughton* (1977) reported that a low pH depresses the Ca_o-dependent Na efflux linked to calcium influx. The extracellular pH (pH_e) may not be the primary factor, but rather the shift in intracellular pH (pH_i) induced by a change in pH_e. Indeed, in kidney cells, low pH_i inhibits and high pH_i stimulates calcium influx and uptake even when the extracellular pH is maintained at the normal value of 7.4 (*Studer* and *Borle* 1979).

8.4.3 Calcium Channels and Calcium Currents

Calcium influx can take place through postulated channels in nerves and muscles of many invertebrates and vertebrates (Table 20). Several recent reviews have been published on the subject (*Hagiwara* 1973; *Reuter* 1973, 1979; *Trautwein* 1975; *Vassalle* 1979). This type of calcium influx through the plasma membrane has been called calcium current, calcium spike, late current, slow inward current, slow channel or calcium channel. It is voltage-dependent, and by definition electrogenic. This inward current is largely carried by calcium ions but the calcium channel is not specific and can also carry sodium and potassium ions (*Hagiwara* 1973; *Trautwein* 1975; *Sperelakis* and *Schneider* 1976; *Reuter* 1979; *Vassalle* 1979). However, the permeability of the calcium channel to sodium and to potassium is 100 times smaller than its permeability to calcium (*Reuter* and *Scholz* 1977a; *Reuter* 1979). The evidence supporting the idea that the slow inward current is carried by calcium ions is persuasive; it persists in the total absence of extracellular sodium and is not inhibited by tetrodotoxin (TTX) (*Reuter* 1967, 1979; *Geduldig* and *Junge* 1968; *Katz* and

Table 20. Characteristics of the slow calcium current

		Reference
Magnitude	100 fmol cm^{-2} $impulse^{-1}$	*Reuter* 1973; *Matthews* 1975
Specificity of the calcium channel	Admits Na, K, Sr, Ba	*Hagiwara* 1973; *Trautwein* 1975; *Sperelakis* and *Schneider* 1976; *Reuter* 1979; *Vassalle* 1979
	P_{Ca}/P_{Na} = 1/0.01	*Reuter* and *Schulz* 1977a; *Reuter* 1979
	P_{Ca}/P_{K} = 1/0.01	
Voltage-dependent		All authors
Ca_O-dependent	Follows Nernst or constant field equations	*Hagiwara* et al. 1964; *Reuter* 1967; *Geduldig* and *Junge* 1968; *Katz and Miledi 1969; Hagiwara* 1973; *Reuter* 1973; *Reuter* and *Scholz* 1977a
Ca_i-dependent		*Hagiwara* and *Naka* 1964; *Hagiwara* and *Nakajima* 1966; *Kostyuk* and *Krishtal* 1977b; *Brehm* and *Ecker* 1978
Na_O-independent and TTX-independent		*Reuter* 1967, 1979; *Geduldig* and *Junge* 1968; *Katz* and *Miledi* 1969; *Reuter* and *Scholz* 1973, 1977b; *Baker* and *Glitsch* 1975; *Matthews* 1975; *Sperelakis* and *Schneider* 1975; *Kostyuk* and *Krishtal* 1977a; *Vassalle* 1979
Inhibited by	Verapamil, D-600 La, Co, Mn, Mg	*Katz* and *Miledi* 1969; *Kohlhart* et al. 1972; *Hagiwara* 1973; *Reuter* 1973, 1979; *Baker* and *Glitsch* 1975; *Matthews* 1975; *Sperelakis* and *Schneider* 1976; *Newrath* et al. 1977; *Kostyuk* and *Krishtal* 1977a; *Brehm* and *Ecker* 1978; *Vasalle* 1979
Inhibited by metabolic inhibitors	IAA, CN, anoxia	*Dean* et al. 1975; *Kohlhart* and *Kübler* 1975; *Sperelakis* and *Schneider* 1976; *Kohlhart* et al. 1977
Stimulated by	cAMP, dbcAMP, catecholamines, histamine	*Reuter* 1967, 1973, 1974b, 1979; *Vassort* et al. 1969; *Brown* et al. 1975; *Sperelakis* and *Schneider* 1976; *Reuter* and *Scholz* 1977b; *Tsien* 1977
Depressed by	Acetylcholine [a]	*Giles* and *Noble* 1976; *Ten-Eick* et al. 1976

[a] In voltage-clamped myocardium.

Miledi 1969; *Reuter* and *Scholz* 1973, 1977b; *Baker* and *Glitsch* 1975; *Sperelakis* and *Schneider* 1976; *Kostyuk* and *Krishtal* 1977a; *Vassalle* 1979); it varies with extracellular calcium (*Reuter* 1967; *Geduldig* and *Junge* 1968; *Katz* and *Miledi* 1969); in sodium-free media, the overshoot sensitivity of the action potential of invertebrate neurons and muscles is 29 mV per tenfold change in extracellular calcium according to the Nernst relation (*Hagiwara* et al. 1964; *Geduldig* and *Junge* 1968; *Hagiwara* 1973; *Reuter* 1973); and in the vertebrate heart, the reversal potential of the slow inward current can be fitted by the constant field equation when Ca_o is varied (*Reuter* and *Scholz* 1977a) although the membrane does not behave as a perfect calcium electrode. The magnitude of the calcium influx through the calcium channels in the heart has been estimated to be about 100 fmol cm^{-2} $impulse^{-1}$ (*Reuter* 1973).

In invertebrate nerves and muscle the calcium inward current is sensitive to the intracellular free calcium. In mollusc neurons a Ca_i concentration of $5.8 \cdot 10^{-8}$ M completely blocks the slow inward current (*Kostyuk* and *Krishtal* 1977b). Similar effects have been reported in barnacle muscle and in *Paramecium* (*Hagiwara* and *Naka* 1964; *Hagiwara* and *Nakajima* 1966; *Brehm* and *Eckert* 1978).

The calcium inward current can be blocked by verapamil, by its methoxy derivative D-600, and by many ions: lanthanum, cobalt, manganese, magnesium (*Katz* and *Miledi* 1969; *Kohlhart* et al. 1972; *Hagiwara* 1973; *Reuter* 1973, 1979; *Baker* and *Glitsch* 1975; *Sperelakis* and *Schneider* 1976; *Nawrath* et al. 1977; *Kostyuk* and *Krishtal* 1977a; *Brehm* and *Eckert* 1978; *Vassalle* 1979). In the mammalian heart, acidosis and metabolic inhibitors also depress the inward calcium current (*Kohlhart* and *Kübler* 1975; *Sperelakis* and *Schneider* 1976; *Kohlhart* et al. 1977). The specificity of verapamil, D-600, and lanthanum for inhibiting the slow inward current is not absolute, however: they also affect the fast inward sodium current and the late outward current (*Bayer* et al. 1975; *Kass* and *Tsien* 1975).

In the heart, beta-adrenergic drugs stimulate the slow inward current, by increasing the conductance of the calcium channel (*Reuter* 1967,1973, 1974b, 1979; *Vassort* et al. 1969; *Brown* et al. 1975; *Sperelakis* and *Schneider* 1976; *Reuter* and *Scholz* 1977b). This has been interpreted as an increase in the number of functional conductance channels (*Reuter* and *Scholz* 1977b; *Reuter* 1979). The effects of catecholamines on the calcium current are probably mediated by an increase in intracellular cAMP (*Reuter* 1974b; *Brown* et al. 1975; *Sperelakis* and *Schneider* 1976; *Tsien* 1977) and indeed cAMP, db-cAMP, and agents that increase cellular cAMP, histamine, methylxantines and papaverine, also enhance the slow inward current (*Reuter* 1974b; *Sperelakis* and *Schneider* 1976; *Tsien* 1977). On the other hand, acetylcholine depresses the slow calcium current (*Giles* and

Noble 1976; *Ten-Eick* et al. 1976). *Reuter* (1979) proposed a hypothetical scheme (Fig. 10) involving cell metabolism for the regulation of the availability of calcium channels in cardiac muscle (*Kohlhart* and *Kübler* 1975; *Reuter* and *Scholz* 1977b; *Sperelakis* and *Schneider* 1976). In this model, the calcium channel consists of one filter determining its calcium selectivity and of two gating mechanisms: one would be the voltage-dependent gate and the other a phosphorylation-dependent gate. The latter would be regulated by catecholamines that would increase the number of available channels by a cAMP-dependent phosphorylation reaction, while acetylcholine would reduce their availability by dephosphorylation of the channel.

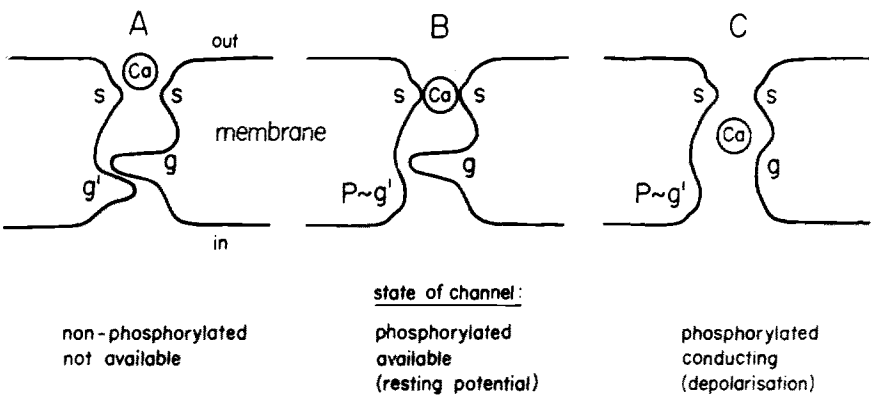


Fig. 10 A–C. Hypothetical scheme for the regulation of Ca channels in cardiac muscle proposed by *Reuter* (1979). Channel contains a filter *(s)* determining its Ca selectivity and two gates (*g* and *g'*). *g* is the voltage-dependent gate; *g'* is a phosphorylation-dependent, voltage-independent gate. Phosphorylation of *g'* may be due to a cAMP-dependent protein kinase reaction; dephosphorylation may depend on a phosphatase. **A** Without phosphorylation *g'* is closed and hence channels are not available; **B** channels are available but nonconducting when *g'* is phosphorylated ($P \sim g'$) but *g* is closed; **C** phosphorylated channels conduct when *g* opens upon depolarization of the membrane. Reproduced, with permission, from the *Annual Review of Physiology*, Vol. 41, 1979 by Annual Reviews, Inc.

Besides muscles and nerves, other cells may be electrically excitable. Secretory cells from the adrenal medulla and adrenal cortex, from the exocrine and endocrine pancreas, from salivary glands, and others can be depolarized by various agents and transmitters (*Matthews* 1967; *Matthews* and *Saffran* 1967; *Douglas* et al. 1967a, b; *Dean* and *Matthews* 1970a, b; *Matthews* and *Petersen* 1973; *Rubin* 1974). Except for the pancreatic islet cells, there is too little information to decide whether an inward calcium current through calcium channels exists in these secretory cells. But in pancreatic beta-cells the evidence is very compelling: action potentials can

be elicited by increasing the glucose concentration above 4 mM (*Dean* and *Matthews* 1970a); the spikes can be maintained even in the absence of extracellular sodium (*Dean* and *Matthews* 1970b; *Matthews* 1975; *Matthews* and *Sakamoto* 1975b); they are not blocked by TTX (*Matthews* 1975); the evoked spikes are blocked in calcium-free media, in the presence of D-600 (*Matthews* 1975; *Matthews* and *Sakamoto* 1975a) or by manganese (*Dean* and *Matthews* 1970b); a metabolic control of the calcium channel may exist, as in the myocardium, since anoxia and the metabolic inhibitor IAA block the glucose-induced action potential (*Dean* et al. 1975); the magnitude of the calcium influx calculated from the measured currents is in the same order of magnitude as in the heart, 100 fmol cm^{-2} $impulse^{-1}$ (*Matthews* 1975).

Whether calcium channels exist in nonexcitable cells is unknown. The fact that D-600 does not inhibit calcium fluxes in liver cells, kidney cells, and lymphocytes argues against such channels in these tissues (*Blackmore* et al. 1979c; *Borle* unpublished; Jan Fischer 1976, pers. communication).

9 Mitochondrial Calcium Transport

More than 40% of the total cell calcium is sequestered in mitochondria (Table 4), constituting the largest calcium compartment of the cell. In an intact cell at steady state, a cycling of calcium in and out of mitochondria occurs by two separate influx and efflux pathways. Several recent reviews have been written on this very complex problem (*Mela* 1977; *Bygrave* 1977, 1978; *Carafoli* and *Crompton* 1978b; *Lehninger* et al. 1978a). I shall present only a broad outline of the subject.

9.1 Calcium Influx into Mitochondria

9.1.1 The Driving Force

For the last 2 decades it has been recognized that the accumulation of calcium by mitochondria can be supported by ATP hydrolysis or by the oxidation of respiratory substrates (*Lehninger* 1964; *Chance* 1965; *Rasmussen* 1966) and that the uptake of calcium by mitochondria takes precedence over ADP phosphorylation (*Rossi* and *Lehninger* 1964; *Reynafarje* and *Lehninger* 1973; *Jacobus* et al. 1975). The driving force for calcium influx was first throught to involve the formation of a high-energy intermediate of oxidative phosphorylation that could energize either calcium transport or ATP synthesis. However, *Scarpa* and *Azzone* (1970) showed that, in the absence of ATP and of respiratory substrate, calcium

can be accumulated against a concentration gradient in response to a membrane potential. Following the chemiosmotic theory of *Mitchell* (1966), the current view is that an outward-directed proton pump, driven either by ATP hydrolysis or by respiration, creates a proton electrochemical potential $\Delta\bar{\mu}H$, which is composed of a membrane potential, $\Delta\psi$, and a proton concentration gradient, ΔpH: $\Delta\bar{\mu}H = \Delta\psi - 59\ \Delta pH$ (*Mitchell* and *Moyle* 1969; *Nicholls* 1974). The relative contribution of the membrane potential and of the proton gradient to $\Delta\bar{\mu}H$ depends on the presence of dissociable anions. Indeed, driven by the proton gradient, an inward transport of weak acids anions (such as acetate, phosphate, and even CO_2, which can donate a proton to the matrix) can convert most of the pH gradient into a membrane potential (*Elder* and *Lehninger* 1973a, b; *Lehninger* 1974a; *Gunter* and *Puskin* 1975). Ultimately, the potential difference across the mitochondria inner membrane is the driving force for calcium influx into the mitochondrial matrix that is electrically negative (*Scarpa* and *Azzone* 1970; *Rottenberg* and *Scarpa* 1974; *Scarpa* 1975; *Heaton* and *Nicholls* 1976; *Akerman* 1978b). Mitochondrial transmembrane potential differences ranging from −120 to −200 mV have been reported (*Mitchell* and *Moyle* 1969; *Rottenberg* 1973; *Nicholls* 1974; *Gunter* and *Puskin* 1975; *Azzone* et al. 1977). The relation among energy transduction, proton electrochemical potential (PMF = proton motive force in the figure), calcium transport, and the site of action of inhibitors is illustrated in Fig. 11, which is taken from *Bygrave* (1977).

Investigators still disagree about whether the influx of calcium driven by the membrane potential involves a net charge transfer of 1 or 2. Some find that calcium is transferred with 1 positive charge and propose the existence of a calcium–phosphate symporter rather than a Ca^{2+}/H^+ anti-

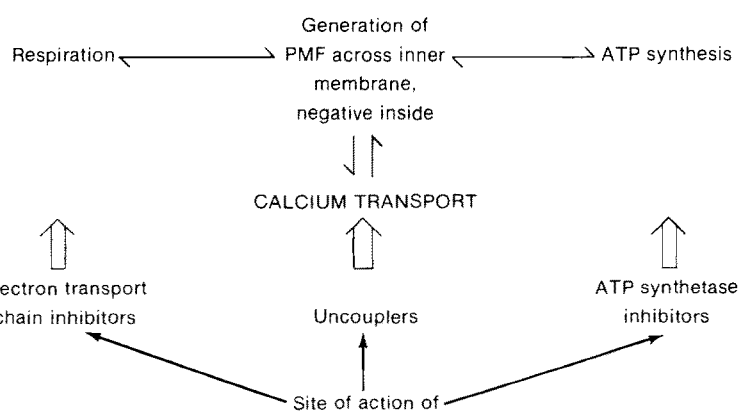


Fig. 11. Chemiosmotic model of mitochondrial energy transduction and its relation to calcium transport. *PMF* is the proton motive force ($\Delta\bar{\mu}H = \Delta\psi - 59\ \Delta pH$). Site of action of inhibitors is also shown. Adapted from *Bygrave* (1977)

porter (*Moyle* and *Mitchell* 1977a, b; *Akerman* 1978b). Others, finding a net charge transfer of 2 and a linear relation between $\Delta\psi$ and the calcium distribution ratio with a slope fitting the Nernst equation, propose the existence of a Ca^{2+} uniporter (*Rottenberg* and *Scarpa* 1974; *Scarpa* 1975; *Brand* et al. 1976; *Nicholls* 1978b; *Lehninger* et al. 1978a).

9.1.2 The Carrier

Whether symporter, antiporter or uniporter, the existence of a specific carrier for calcium transport into mitochondria seems to be well established (*Mela* 1977; *Bygrave* 1978; *Lehninger* et al. 1978a). First, it had been proposed that the high-affinity calcium-binding sites of mitochondria reflect the active site of the carrier molecule with an affinity constant K_{Ca} of 0.025 μM (*Reynafarje* and *Lehninger* 1969; *Lehninger* et al. 1978a). This has been challenged by *Reed* and *Bygrave* (1974b), who showed that the high-affinity binding is in reality the manifestation of the energy reserve of inhibited mitochondria, because the so-called bound calcium is inaccessible to EGTA chelation and thus located inside the inner membrane. In fact, the kinetic properties of the carrier are of such a kind that the measurement of the carrier specific binding properties is impossible with current techniques (*Bygrave* 1977). Lanthanum is a competitive inhibitor of calcium transport into mitochondria, presumably competing with calcium for the same site of the carrier molecule (*Mela* 1968, 1969, 1977; *Mela* and *Chance* 1969; *Reed* and *Bygrave* 1974a). From lanthanum competitive inhibition data, the number of carrier-specific binding sites has been estimated to be around 0.07 nmol/mg mitochondrial protein (*Mela* 1968, 1969, 1977; *Mela* and *Chance* 1969; *Reed* and *Bygrave* 1974a). Ruthenium red, a noncompetitive inhibitor of calcium transport into mitochondria, provides further evidence for the existence of a carrier and perhaps for its glycoprotein nature (*Moore* 1971; *Vasington* et al. 1972; *Reed* and *Bygrave* 1974a). And indeed several investigators have isolated from mitochondria a glycoprotein with a high affinity for calcium (*Sottocasa* et al. 1971, 1972; *Gomez-Puyou* et al. 1972; *Kimura* et al. 1972; *Tashmukhamedov* et al. 1972). The glycoprotein monomer has a molecular weight of 33 000, contains about 10% carbohydrate and up to 33% phospholipids, and has 2–3 high-affinity calcium binding sites per mole with a K_{Ca} of 0.1 μM (*Carafoli* 1976; *Carafoli* et al. 1978). Anti-Ca binding glycoprotein antibodies specifically inhibit calcium transport in mitochondria and the glycoprotein has been proposed to be part of a mobile carrier (*Sandri* et al. 1976; *Panfili* et al. 1976). However, it is currently postulated to be a recognition factor and not the carrier per se because (a) it is located in the inner and outer membrane and as well as in the intermembrane space, and (b) although the electrical conductance of phospho-

Table 21. Kinetic parameters of calcium uptake by mitochondria

K_{Ca} (μM)	V_m [nmol (mg prot)$^{-1}$ min^{-1}]	Hill coefficient	Substrate	[Mg] (mM)	Method [a]	Reference
Liver						
2.0	–	1.8	ATP	0	A	*Bygrave* et al. 1971a, b
2–3	–	–	Succ.	0	C	*Carafoli* and *Azzi* 1972
1.5	313	1.6	ATP	0	A	*Spencer* and *Bygrave* 1973
1.8	450	1.75	Succ.	0	A	*Spencer* and *Bygrave* 1973
4.0	–	1.7	Succ.	0	A	*Reed* and *Bygrave* 1975
1.4	–	–	β-OH but.	0	C	*Hutson* 1977
3.1	–	–	Succ.	0	C	*Hutson* 1977
4.7	–	–	Succ.	0	D	*Heaton* and *Nicholls* 1976
3–11	–	–	Succ.	0	A	*Kimura* and *Rasmussen* 1977
2.6	–	–	Succ.	0	C	*Hutson* et al. 1976
5.3	–	–	Succ.	0.25	C	*Hutson* et al. 1976
10	–	–	Succ.	0.5	C	*Hutson* et al. 1976
14.5	–	–	Succ.	0.94	C	*Hutson* et al. 1976
30	–	–	Succ.	2.0	C	*Hutson* et al. 1976
9.5	–	–	β-OH but.	1.0	C	*Hutson* 1977
17.8	–	–	β-OH but.	2.0	C	*Hutson* 1977
50	420	–	Succ.	2.0	B	*Scarpa* 1975
50–70	480	1.63	Succ.	5.0	B	*Vinogradov* and *Scarpa* 1973
20	–	–	Succ. ATP	2.0	A	*Kimura* and *Rasmussen* 1977
50–80	480	–	Succ. ATP	5.0	A	*Kimura* and *Rasmussen* 1977
Kidney						
18.7	415	1.63	Succ.	0	A	*Studer* and *Borle* 1979
Heart						
55–65	275	–	Succ.	0	B	*Sordhal* 1974
0.41	–	–	Succ.	0	C	*Jacobus* et al. 1975
5.6	426	1.9	ATP	0	A	*Noack* and *Heiner* 1977
18.5	90	1.26	Succ.	0	A	*Williams* and *Barrie* 1978

12–15	84	1.5	Succ.	1	D	*Crompton* et al. 1976a
85–105	660	2.06	Succ.	5	B	*Scarpa* and *Graziotti* 1973 (rat)
25–35	960	2.06	Succ.	5	B	*Scarpa* and *Graziotti* 1973 (frog)
60–85	360	2.06	Succ.	5	B	*Scarpa* and *Graziotti* 1973 (pigeon)
12	230	–	Succ.	5	A	*Solaro* 1972
Myometrium						
4.4	33	–	Succ.	0	A	*Malmstrom* and *Carafoli* 1977
25	780	Hyperbolic	Succ.	2	B	*Wikström* et al. 1975
Vascular smooth muscle						
17	720	–	Succ.	2	B	*Vallieres* et al. 1975
17	240	–	Succ.	2	B	*Vallieres* et al. 1975
Ascites cells						
8	1300	–	Succ.	1.0	C	*Reynafarje* and *Lehninger* 1973
Brain						
4	–	Hyperbolic	Mal.pyr.	0	D	*Nicholls* 1978

[a] Methods: A: ^{45}Ca uptake. B: Spectrophotometry with murexide or arsenazo III. C: O_2 consumption or shift in redox state of cytochrome b. D: pCa electrode or Ca conductance measurements.

lipid bilayers is increased by the glycoprotein in the presence of calcium, it does not induce a measurable efflux of calcium from these liposomes (*Carafoli* et al. 1976).

9.1.3 Kinetic Parameters

The kinetic characteristics of calcium influx into mitochondria have been studied in liver, kidney, heart, smooth muscle, and brain and they are shown in Table 21. In the absence of Mg the K_{Ca} of influx lies between 2 and 4 μM, except in heart and kidney where the range reported is much wider, 0.4–6.5 μM. The relation between the free calcium outside the mitochondria and calcium influx is not hyperbolic but sigmoidal, except in myometrium and in brain. The Hill coefficient ranges from 1.3 to 2.0 with an average of 1.68, suggesting cooperativity of 2 calcium per transport site. The maximal velocity varies considerably depending on the experimental conditions, especially on the concentration of permeable anions. V_m ranges from 100 to 1000 nmol mg^{-1} protein min^{-1}, with an average of 470 nmol mg^{-1} protein min^{-1}. Magnesium is a well-recognized inhibitor of calcium influx (Table 21) with a $K_{I_{Mg}}$ of 20 mM. And indeed, with Mg concentrations of 5 mM, the K_{Ca} of influx increases 10–20 times and ranges from 50 to 100 μM (*Vinogradov* and *Scarpa* 1973; *Scarpa* and *Graziotti* 1973; *Kimura* and *Rasmussen* 1977). However, although the total Mg in liver, kidney, and brain tissue ranges between 7 and 10 mmol/kg wet tissue, the free Mg is between 0.6 and 1.3 mmol/kg wet tissue (*Veloso* et al. 1973). In liver, the free Mg may be even less, between 0.55 and 0.93 mmol/kg wet tissue (*Veloso* et al. 1973). With these physiologic concentrations of magnesium, the K_{Ca} of influx in liver, heart and ascites cells is about 10 μM (Table 21). Other factors influence the affinity of the transport system for calcium: the temperature, the nature of the respiratory substrate, and the potassium concentration (*Hutson* et al. 1976; *Hutson* 1977; *Williams* and *Barrie* 1978). All the kinetic studies published have been conducted at 25°C or less, and *Hutson* (1977) has shown that increasing the temperature from 25°C to 35°C reduces the K_{Ca} of influx 40%. Furthermore, potassium antagonizes the Mg inhibition and the $K_{0.5}$ of calcium influx decreases 50% if the potassium concentration is raised to its physiologic value of 100 mM (*Hutson* et al. 1976; *Hutson* 1977). From the data available, one can conclude first, that the kinetic properties of calcium influx are very similar among all tissues studied; second, in physiologic conditions T = 37°C, K concentration 100 mM, Mg concentration $\approx$ 1 mM) the K_{Ca} of influx, is probably significantly less than 10 μM and close to 5 μM, and the V_m is around 400 nmol mg^{-1} protein min^{-1}.

9.1.4 Capacity of Mitochondria to Accumulate Calcium

In the absence of permeant anions, energized mitochondria can accumulate 100 nmol calcium/mg protein and this has been called 'limited' loading (*Lehninger* et al. 1967). With permeant anions such as phosphate and acetate, up to 2–3 μmol calcium/mg protein can be accumulated (*Lehninger* et al. 1967). This 'massive' loading is accompanied by mitochondrial swelling and leads to mitochondrial disruption unless ATP and Mg are present thus leading to a precipitation of calcium and phosphate in the mitochondrial matrix (*Lehninger* 1970). Although phosphate markedly increases the maximal velocity of calcium influx, it does not change the calcium affinity K_{Ca} or the Hill coefficient of the sigmoid curve relating calcium uptake and free calcium (*Spencer* and *Bygrave* 1973; *Crompton* et al. 1978). Accordingly, *Bygrave* (1977) concludes that permeant anions do not interact with the calcium carrier, but allow the dissociation of calcium from anionic sites inside the mitochondria inner membrane. According to this scheme the rate-limiting step in calcium influx is not the energy source but the dissociation of the calcium from the carrier in the internal face of the inner membrane.

The external pH or the pH gradient also affects the capacity of mitochondria to accumulate calcium. A low pH increases the K_{Ca} of influx without affecting the Hill coefficient, decreases the V_m, induces an efflux of calcium from mitochondria, and lowers the calcium content of mitochondria (*Spencer* and *Bygrave* 1973; *Reed* and *Bygrave* 1975b; *Akerman* 1978a; *Studer* and *Borle* 1979, 1980). Conversely a high external pH decreases the K_{Ca} and increases the mitochondrial calcium content and exchange rate (*Spencer* and *Bygrave* 1973; *Studer* and *Borle* 1980).

9.2 Mitochondrial Calcium Efflux and Cycling

Drahota et al. (1965) recognized that at steady state (state 4), calcium transport by mitochondria is a dynamic process in which there is a continuous efflux of accumulated calcium counterbalanced by an energized influx of calcium. Whether calcium efflux is an active or a passive process is unknown. Theoretically, since the driving force for calcium influx is the potential difference $\Delta\psi$ across the mitochondrial inner membrane it should be possible to decide whether the calcium distribution ratio between the matrix and the extramitochondrial space reflects an equilibrium governed by the Nernst relation (*Gunter* and *Puskin* 1975; *Puskin* et al. 1976). Unfortunately, too many uncertainties make such calculations difficult: (a) the $\Delta\psi$ may vary from −120 to −200 mV; (b) there is no agreement about whether calcium influx occurs on a uniporter with a charge transfer

of 2 or a symporter with a charge transfer of 1; (c) the actual distribution ratio of *free* calcium is not known. With a $\Delta\psi$ of -120 mV and assuming a symporter, the Nernst equilibrium distribution ratio of calcium should be 10^2, which is quite small. However, if one assumes a $\Delta\psi$ of -180 mV and a uniporter, the same distribution ratio should be 10^6, which is highly improbable. Consequently it is too early to predict whether calcium efflux from mitochondria represents a passive leak or an active process. At steady state the calcium distribution ratio has been shown to vary between 2000 and 7000 (*Drahota* et al. 1965; *Pozzan* et al. 1977) and since it is much greater than the Mn^{2+} distribution ratio, *Pozzan* et al. (1977) conclude that it is doubtful that these ratios depend on $\Delta\psi$. Rather, the calcium distribution between the mitochondrial matrix and the cytosol may be determined at steady state by the difference between the rates of calcium influx and efflux or by the ratio of their rate constants (*Borle* 1973a; *Pozzan* et al. 1977; *Nicholls* 1978b). At steady state, rat liver and heart mitochondria in vitro can maintain an extramitochondrial free calcium concentration of 0.3–0.8 μM (*Solaro* 1972; *Nicholls* 1978b), while myometrium mitochondria can lower it to 0.1 μM (*Batra* 1973a).

Stucki and *Ineichen* (1974) estimated that calcium efflux from liver mitochondria in state 4 amounts to 3.5 nmol mg^{-1} protein min^{-1}. Others estimated that steady-state fluxes range between 1 and 5 nmol mg^{-1} protein min^{-1} with a liver mitochondrial membrane potential difference of -200 mV (*Azzone* et al. 1977; *Pozzan* et al. 1977; *Nicholls* 1978b). In kidney mitochondria, with an external free calcium concentration buffered at 0.7 μM, and a pH of 7.4, steady-state calcium exchange (efflux = influx) across the mitochondrial inner membrane is 1.1 nmol mg^{-1} protein min^{-1} (*Studer* and *Borle* 1980).

Several physiologic factors influence calcium efflux from mitochondria. Sodium stimulates calcium efflux from mitochondria isolated from heart, cerebral cortex, adrenal cortex, parotid glands, and skeletal muscle (*Carafoli* et al. 1974; *Crompton* et al. 1976b, 1978; *Nicholls* 1978a). The relation between calcium efflux and the extramitochondrial sodium concentration is sigmoidal with a Hill coefficient of 3, suggeesting a stoichiometry of 3 Na for 1 Ca. The half-maximal activation constant K_{Na} is 8 mM and the maximum velocity of calcium release is 14 nmol mg^{-1} protein min^{-1} (*Crompton* et al. 1976b). These authors calculated a steady-state calcium efflux of 4 nmol mg^{-1} protein min^{-1} with a cytosolic sodium concentration of 6 mM, and they postulate the existence of a Na–Ca antiporter (*Crompton* et al. 1977; *Crompton* and *Heid* 1978).

Phosphoenylpyruvate also stimulates calcium efflux from heart and liver mitochondria (*Chudapongse* and *Haugaard* 1973; *Peug* et al. 1974; *Chudapongse* 1976; *Roos* et al. 1978). This effect of phosphoenolpyruvate requires phosphate and is antagonized by ATP; the release of calcium is

accompanied by an uptake of H^+ by the mitochondria (*Chudapongse* 1976).

Calcium efflux also depends upon the oxidation–reduction state of the mitochondrial pyridine nucleotides and the ATP/ADP ratio of the cytosol: a low cytosolic ATP/ADP ratio accompanied by an oxidized state of mitochondrial NAD favors calcium efflux, while a high ATP/ADP ratio with a reduced state of pyridine nucleotide would promote calcium influx (*Lehninger* et al. 1978b; *Fiskum* and *Lehninger* 1979). These authors postulate that this calcium efflux from liver mitochondria occurs through an electrically neutral $Ca^{2+}/2H^+$ antiporter.

Although it has been suggested that calcium efflux occurs through a reversal of the influx mechanism (*Pozzan* et al. 1977; *Roos* et al. 1978), a majority of investigators accept the idea of a separate efflux pathway (*Gunter* and *Puskin* 1975; *Puskin* et al. 1976; *Crompton* et al. 1976b, 1978; *Crompton* and *Heid* 1978; *Bygrave* 1978; *Nicholls* 1978a, b; *Caroni* et al. 1978; *Lehninger* et al. 1978b; *Fiskum* and *Lehninger* 1979).

In summary, calcium influx is believed to be an electrophoretic uniport process driven by the potential difference established across the mitochondrial inner membrane by a proton pump energized by respiration or ATP hydrolysis. Calcium efflux may utilize different pathways, either a Ca/Na antiporter or a Ca/H antiporter system. It is likely that mitochondrial calcium influx and efflux are independently regulated.

10 Calcium Transport by Endoplasmic Reticulum

10.1 Muscle

Calcium transport in reticulum has been studied in great detail in muscle using the reconstituted vesicles or microsomes from fragmented sarcoplasmic reticulum (SR), and many recent reviews on this subject have been published (*McLennan* and *Holland* 1975; *Martonosi* 1975; *Ebashi* 1976; *Martonosi* et al. 1978; *Tada* et al. 1978). In muscle microsomes, calcium transport is stoichiometrically coupled to ATP hydrolysis: for each ATP hydrolyzed, 2 calcium atoms are carried across the membrane in a process that is Mg-dependent. The active transport mechanism is the Mg-dependent Ca-activated ATPase that catalyzes the formation of a phosphoprotein intermediate. The enzyme phosphorylation dependence on calcium appears cooperative with a Hill coefficient greater than 2. The process is reversible because a calcium gradient across the microsomal membrane allows the synthesis of 1 mole of ATP from ADP and phosphate for each 2 calcium released from the SR vesicles.

The kinetic characteristics of calcium uptake by SR vesicles from skeletal and cardiac muscles are shown in Table 22. The K_{Ca} for both types of muscle varies between 0.1 and 12 μM with an average of about 2 μM. The maximal capacity ranges between 40 and 200 nmol/mg protein. Oxalate, however, which allows the precipitation of calcium inside the vesicles, enhances 100-fold the maximal capacity of microsomes, up to 2–10 μmol/mg protein (*Harigaya* and *Schwartz* 1969; *Worsfold* and *Peter* 1970; *Repke* and *Katz* 1972). Phosphate, on the other hand, stimulates calcium uptake by SR only very slightly and at rather unphysiologically high concentrations (*Harigaya* and *Schwartz* 1969; *Worsfold* and *Peter* 1970).

Table 22. Kinetic parameters of calcium uptake by sarcoplasmic reticulum vesicles (microsomes) of skeletal and heart muscle

K_{Ca} (μM)	V_m [μmol (mg prot)$^{-1}$ min^{-1}]	Maximal capacity [μmol (mg prot)$^{-1}$]		Reference
		Without oxalate	With oxalate	
Skeletal muscle				
0.1–1.0	1–3	0.1–0.2	8–10	*Weber* 1966
3–5	–	0.16–0.2	–	*Ogawa* 1970
12 [a]	2.8	–	–	*Worsfold* and *Peter* 1970
3 [b]	0.28	–	–	*Worsfold* and *Peter* 1970
–	–	0.08–0.16	2–4.8	*Harigaya* and *Schwartz* 1969
Cardiac muscle				
2	0.04	0.04–0.08	2–3	*Harigaya* and *Schwartz* 1969
1.2–2.3	–	no uptake	2.5	*Repke* and *Katz* 1972
1.9	1.54	–	5.6	*Solaro* and *Briggs* 1974

[a] Rat.

[b] Human.

The maximal velocity of calcium uptake by SR vesicles varies between 0.3 and 3 μmol mg^{-1} protein min^{-1} (Table 22). However, in the presence of oxalate, the relation between the extravesicular calcium and the rate of calcium uptake is often linear: velocities of 30–40 nmol mg^{-1} protein min^{-1} μM^{-1} Ca have been reported (*Repke* and *Katz* 1972).

10.2 Tissues Other than Muscle

In tissues other than muscle, calcium uptake by endoplasmic reticulum vesicles (ER microsomes) is very much slower and the capacity of the microsomes is very much less than that of sarcoplasmic reticulum (Table 23). The affinity of the transport system for calcium varies from tissue to tissue: in liver, fibroblasts, and adipocytes the K_{Ca} is about the same as in muscle, ranging from 1 to 5 μM; in kidney, brain, salivary glands, and platelets the affinity is much less and the K_{Ca} ranges from 25 to 100 μM (Tables 23 and 24). In all tissues studied, the maximal velocity is about 100 times less than in muscle, even in the presence of oxalate: it varies from 1.5 to 44 nmol mg^{-1} protein min^{-1} with an average of less than 10 nmol mg^{-1} protein min^{-1}.

Table 23. Kinetic parameters of calcium uptake by endoplasmic reticulum vesicles (microsomes) isolated from tissues other than muscle

Tissue	K_{Ca}	V_m [a]	K_{ATP}	Maximal capacity [a]	Reference
	(μM)	[nmol (mg prot)$^{-1}$ min^{-1}]	(mM)	[nmol (mg prot)$^{-1}$]	
Liver					
	4.6 [b]	11	1.8	400	*Moore* et al. 1975
	2.4 [b]	1.5	–	74	*Farber* et al. 1977
	1–2	–	0.5	–	*Bygrave* 1978
Kidney					
	25	5.4	0.3	10–30	*Moore* et al. 1974
Brain					
	50–100	–	–	65	*De Meis* et al. 1970
	67	44	–	–	*Trotta* and *De Meis* 1975
	–	–	–	78	*Otsuka* et al. 1965
	–	–	0.01	40–80	*Ohtsuki* 1969
	–	–	–	50	*Robinson* and *Lust* 1968
Fibroblasts					
	2.6 [b]	7	–	–	*Moore* and *Pastan* 1977a
	–	–	–	10	*Moore* and *Pastan* 1978
Salivary glands					
	>100	1.5	1.0	150	*Alonso* et al. 1971
Platelets					
	100	–	–	350	*Robblee* et al. 1973
Adipocytes					
	3.6 [b]	4.8	–	60	*Bruns* et al. 1976

[a] In presence of oxalate.

[b] Calculated.

Table 24. Properties of calcium uptake by microsomes (SR excluded)

Rate [nmol (mg prot)$^{-1}$ min^{-1}]			
Ca^{++} of 20–100 μM			
	Without oxalate	< 1	*Moore* et al. 1974, 1975; *Selinger* et al. 1970
	With oxalate	1–15	*Ohtsuki* 1969; *DeMeis* et al. 1970; *Selinger* et al. 1970; *Alonso* et al. 1971; *Moore* et al. 1974; *Baumrucker* and *Keenan* 1975; *Moore* and *Pastan* 1977a, 1978; *Farber* et al. 1977; *Watson* and *Siegel* 1978
Capacity [nmol (mg prot)$^{-1}$]			
	Without oxalate	0–10	*Moore* et al. 1974, 1975; *Moore* and *Pastan* 1978; *Alonso* et al. 1971
	With oxalate	10–400	*Otsuka* et al. 1965; *Robinson* and *Lust* 1968; *Ohtsuki* 1969; *DeMeis* et al. 1970; *Alonso* et al. 1971; *Robblee* et al.1973; *Moore* et al. 1974, 1975; *Moore* and *Pastan* 1977a; *Bruns* et al. 1976; *Farber* et al. 1977
K_{Ca} (μM)	Measured (total Ca)	15–100	*DeMeis* et al. 1970; *Alonso* et al. 1971; *Robblee* et al. 1973; *Moore* et al. 1974, 1975; *Moore* and *Pastan* 1977a; *Trotta* and *DeMeis* 1975; *Farber* et al. 1977
	Calculated (free Ca)	2–5	*Moore* et al. 1975; *Bruns* et al. 1976; *Moore* and *Pastan* 1977a; *Farber* et al. 1977; *Bygrave* 1978
V_m [nmol (mg prot)$^{-1}$ min^{-1}]			
	With oxalate	1–40	*Alonso* et al. 1971; *Moore* et al. 1974, 1975; *Trotta* and *DeMeis* 1971; *Bruns* et al. 1976; *Moore* and *Pastan* 1977a; *Farber* et al. 1977
	Stimulated by oxalate		*Selinger* et al. 1970; *Moore* et al. 1974, 1975; *Moore* and *Pastan* 1977a, b, 1978; *Blaustein* et al. 1978c
Inhibited by	Na, Li, sucrose, salyrgan, amytal, mersalyl, tetracaine, valinomycin, CCCP		*Robinson* and *Lust* 1968; *Selinger* et al. 1970; *Robblee* et al. 1973; *Moore* et al. 1974, 1975; *Blaustein* et al. 1978c; *Bygrave* 1978
Not inhibited by	DNP, CN, NaN_3, antimycin A, FCCP, oligomycin, ouabain, ruthenium red		*Otsuka* et al. 1965; *Ohtsuki* 1969; *Selinger* et al. 1970; *Moore* et al. 1974, 1975; *Trotta* and *DeMeis* 1975; *Farber* et al. 1977; *Ash* and *Bygrave* 1977; *Moore* and *Pastan* 1977b; *Bygrave* 1978; *Blaustein* et al. 1978c
Little or no effect of phosphate			*Otsuka* et al. 1965; *Ohtsuki* 1969; *DeMeis* et al. 1970; *Selinger* et al. 1970; *Alonso* et al. 1971

As in muscle, oxalate increases the capacity of ER vesicles to accumulate calcium, but even with oxalate the maximal capacity is very limited. In liver, kidney, brain, fibroblasts, and adipocytes the maximal capacity ranges between 10 and 80 nmol/mg protein (Tables 23 and 24.). Two re-

ports give values as high as 350–400 nmol/mg protein (*Robblee* et al. 1973; *Moore* et al. 1975). In liver and kidney there is no measurable microsomal calcium uptake in the absence of oxalate (*Moore* et al. 1974, 1975). Phosphate may stimulate calcium uptake slightly, but unphysiologically high concentrations, 40–80 mM, are needed for any significant effect (*Selinger* et al. 1970; *Alonso* et al. 1971; *Trotta* and *DeMeis* 1975). In brain microsomes, however, oxalate does not stimulate calcium uptake (*Otsuka* et al. 1965; *Ohtsuki* 1969; *Robinson* and *Lust* 1968).

Calcium uptake by ER vesicles has been shown to be inhibited in various tissues by salygran, amytal, mersalyl, tetracaine, valinomycin, CCCP, Na, Li, and sucrose (Table 24). On the other hand, it is not inhibited by DNP, CN, NaN_3, antimycin A, FCCP, oligomycin, ouabain or ruthenium red (Table 24).

There is no doubt that the cells' endoplasmic reticulum is capable of accumulating calcium by an active process requiring ATP and Mg. But, except for muscle, the capacity of ER in physiologic conditions (no oxalate, presence of phosphate, Mg $\approx$ 1 mM) appears very limited and the rate of uptake is very slow. Even with extremely high extravesicular free calcium concentrations of 20–500 μM, calcium uptake is less than 1 nmol mg^{-1} protein min^{-1} in the absence of oxalate; with oxalate it never exceeds 15–20 nmol mg^{-1} protein min^{-1} (Table 24).

11 Control of the Cytosolic Free Calcium Concentration

One or several energy requiring transport processes must intervene directly or indirectly to maintain the cytosolic concentration of ionized calcium several orders of magnitude below its thermodynamic equilibrium (cf. Sect. 7). Calcium-binding proteins, prosthetic groups with high affinity for calcium, and all the passive calcium-buffering systems, which are present in the cell (*Brink* 1954; *Nanninga* 1961a, b; *Kretsinger* 1979; *Dedman* et al. 1979), cannot determine the *level* of cytosolic calcium activity (control of the set point) since they cannot passively displace the calcium activity of the cytosol from its thermodynamic equilibrium. These passive calcium buffer systems, however, do determine the *ratio* of free calcium to bound calcium within the cell. *Mela* (1977) listed several criteria required for a cellular calcium controller to function adequately: (a) the rate of calcium transfer must be adequate for fast removal calcium from the cytosol; (b) the affinity of the transport process must be high to function at low cytosolic calcium concentration; (c) the capacity for calcium uptake and sequestration must be adequate to remove appropriate calcium loads; and (4) calcium release to the cytoplasm must be quantitatively adequate and fast enough to meet particular requirements of the cell.

Each parameter (rate, affinity, capacity) must be related to the characteristics of each particular cell: rates of calcium transfer appropriate for the slow contraction and relaxation of a smooth muscle cell may not be adequate for the fast contraction–relaxation cycle of a fast skeletal muscle fiber. The affinity for calcium of a particular controller system must also be compared to the resting level of cytosolic free calcium, which it regulates and which may vary from $5 \cdot 10^{-8}$ to 10^{-6} M, depending on the cell (cf. Table 7).

In addition, the relative role of the various transport processes as potential controllers can only be assessed when their relative mass and surface area are taken into consideration.

11.1 The Plasma Membrane as the Main Controller

In erythrocytes, the only possible controller of the intracellular free calcium concentration is the ATP-dependent calcium transport across the plasma membrane, which is catalyzed by the Mg-dependent Ca-sensitive ATPase. Erythrocytes are unique because they do not possess any other active calcium transport process and because their passive membrane permeability to calcium is much lower than that of any other cells. Calcium influx into erythrocytes is at least 2 orders of magnitude smaller than in other tissues (*Szasz* et al. 1977, 1978; *Borle* and *Studer* 1978).

Mullins (1977) has proposed on purely theoretical grounds that Na/Ca exchange across the squid axon plasma membrane could control the cytosolic free calcium and lower it below 10^{-8} M, if one assumes a stoichiometry of 4 Na for 1 Ca. However, a 4-to-1 stoichiometry has never been observed and the quantitative importance of the Na-dependent calcium efflux in unpoisoned axon is still debated (see Sect. 8.2.2).

Table 25 presents a comparison of the kinetic properties of the calcium pumps in sarcoplasmic reticulum, endoplasmic reticulum, mitochondria, and plasma membrane from different tissues. Although the affinities for calcium, K_{Ca}, are not very different, the velocity of the plasma membrane transport system is smaller than in microsomes and several orders of magnitude less than in mitochondria and sarcoplasmic reticulum, suggesting that calcium transport across the plasma membrane may be comparatively ill-suited to control the set point of the cytosolic calcium activity. This is supported by the results of *Simonsen* and *Christofferson* (1979) showing that the cytosolic free calcium of Helix neurons was hardly affected by changes in extracellular concentrations: an average ratio between changes of intracellular and extracellular pCa ($\Delta pCa_o/\Delta pCa_i$) of 7.1 was observed, suggesting that the stability of intracellular calcium activity cannot be determined by the transport capacity of the plasma membrane alone but

Table 25. Comparison of the kinetic parameters of calcium uptake (K_{Ca}, and V_m)by membranes in physiologic conditions [a]

	K_{Ca}	V_m	Hill coefficient
	(μM)	[nmol (mg prot)$^{-1}$ min^{-1}]	
Sarcoplasmic reticulum	1–12	100–3000	2
Endoplasmic reticulum	2– 5	< 1	2
Mitochondria	1–15	100–1000	2
Plasma membrane	1–20	1–3	2

[a] From Tables 13, 21, 22, 23, and 24.

rather by intracellular mechanisms. Since the mitochondrial inhibitor azide causes a rapid rise in intracellular free calcium it is likely that, in these neurons, mitochondria play an important role as a controller of cytosolic calcium. Similar results were obtained in rat kidney cells (*Uchikawa* and *Borle,* unpublished). The cytosolic exchangeable calcium pool changes little when the extracellular calcium concentration is altered between 0.7 and 2.5 mM (see Fig. 12); most of the gain or loss in cell calcium is seen in the mitochondrial calcium pool.

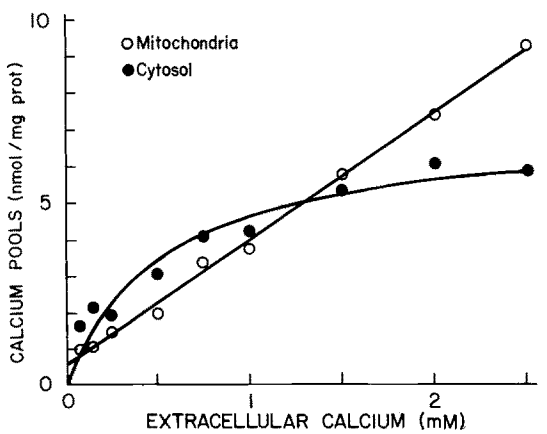


Fig. 12. Relation between the extracellular calcium concentration (Ca_O) and the size of the cytosolic and mitochondrial exchangeable calcium pools in cultured monkey kidney cells. Between a Ca_O of 0.7–2.5 mM the cytosolic pool changes relatively little compared with the loss (Ca_O < 1.2 mM) or the gain (Ca_O > 1.2 mM) in exchangeable calcium observed in the mitochondrial pool. *Uchikawa* and *Borle* (unpublished)

11.2 The Endoplasmic Reticulum as the Main Controller

In skeletal muscle, the sarcoplasmic reticulum is recognized to be the principal if not the only controller of the fiber cytosolic calcium. A detailed review of this well-known process is beyond the scope of this review. One should remember, however, that the sarcoplasmic reticulum affinity for calcium is in the same order of magnitude as the K_{Ca} of the plasma membrane, endoplasmic reticulum, and mitochondria. What distinguishes its kinetic properties is the great velocity of the transport process reflected by a V_m that may exceed 1 μmol mg^{-1} prot min^{-1} (Table 25). Thus, it is accepted that skeletal muscle sarcoplasmic reticulum can lower the cytosolic calcium activity below 10^{-8} M.

In heart muscle, the sarcoplasmic reticulum is probably not the only controller of cytosolic calcium activity. The existence of calcium channels makes this tissue more dependent on the extracellular calcium concentration and on calcium influx through the plasma membrane (see Sect. 8.4.3). In addition, many investigators have suggested that mitochondria may play a role in the control of free calcium in heart muscle (*Chance* 1965; *Patriarca* and *Carafoli* 1968; *Harigaya* and *Schwartz* 1969; *Carafoli* and *Azzi* 1972; *Carafoli* et al. 1972; *Lehninger* 1974b; *Affolter* et al. 1976; *Carafoli* and *Crompton* 1978a; *Lentz* et al. 1978; *Nayler* et al. 1979). Others doubt it because of the mitochondrial high K_{Ca} and their slow transport velocity compared with that in sarcoplasmic reticulum (*Solaro* 1972; *Scarpa* and *Graziotti* 1973; *Scarpa* 1975, 1978b; *Tsokos* et al. 1978); however, kinetic studies have often been performed at optimal conditions for calcium uptake by sarcoplasmic reticulum (presence of oxalate and high magnesium concentrations) but not for calcium uptake by mitochondria. Further physiologic conditions (i.e., presence of phosphate, absence of oxalate, free magnesium concentration of 1 mM) would undoubtedly improve the calcium transport capacity of mitochondria compared with that of sarcoplasmic reticulum. *Tsokos* et al. (1977) suggested that, although mitochondria may not play a significant role in the rapid uptake of calcium promoting relaxation of myofibrils, the possibility still remains that they participate in regulating the steady-state calcium levels and the resting tension of the myofibrils.

In uterine smooth muscle, the rate of calcium uptake is much slower in microsomes than in mitochondria; on a protein basis, the calcium capacity of microsomes is only 17% of that of mitochondria (*Batra* and *Daniel* 1971). Furthermore, while mitochondria are capable of lowering the free calcium of their incubating medium below 10^{-7} M and even down to 10^{-8} M, microsomes can only lower it slightly below 10^{-6} M (*Batra* 1973a). *Batra* (1973b, 1974) also showed that hormones and drugs that influence muscle tension of skeletal and uterine muscle, and presumably their cyto-

solic free calcium affected mostly calcium uptake or release in mitochondria and not in microsomes.

In nerve terminals, *Blaustein* et al. (1978a, b) have described a nonmitochondrial ATP-dependent sequestration mechanism that they proposed to be the smooth endoplasmic reticulum. The calcium affinity of this intracellular calcium transport appears to be greater than that of mitochondria. However, even in these nerve terminals mitochondria have a rate of calcium uptake and a capacity to sequester calcium that is one order of magnitude larger and, after loading, most of the calcium is found in mitochondria (*Blaustein* et al. 1978a, b). It is, therefore, difficult to evaluate the respective contribution of each mechanism to the control of cytosolic calcium in nerves.

In parenchymal and epithelial cells, calcium uptake by the endoplasmic reticulum has long been overlooked. Only recently, the properties of calcium transport in microsomes have been studied in liver, kidney, brain, and a few other tissues (cf. Table 23). It is increasingly evident that drugs and hormones do influence calcium transport and its sequestration by endoplasmic reitculum vesicles. Nevertheless it is still difficult to make a convincing case for the postulate that the endoplasmic reticulum is relatively more important than mitochondria in the control of cytosolic calcium. Tables 22, 24, and 25 show that even if the affinity of endoplasmic reticulum vesicles for calcium is slightly higher than that of mitochondria the velocity of transport and the capacity for calcium sequestration are lower by 1–2 orders of magnitude. *Ash* and *Bygrave* (1977) used concentrations of ruthenium red, which totally inhibit calcium uptake by mitochondria without affecting calcium transport in microsomes or plasma membrane vesicles, to assesss the potential of endoplasmic reticulum to control intracellular calcium in liver homogenate. They found that mitochondrial calcium uptake exceeds by severalfold, in terms of both initial rate and of capacity, the ruthenium red insensitive component reflecting microsomal transport. In rat liver, microsomal calcium sequestration is 1 order of magnitude smaller than mitochondrial uptake even in the presence of oxalate (*Farber* et al. 1977; *Ash* and *Bygrave* 1977; *Bygrave* and *Trauter* 1978). *Moore* et al. (1974) and *Bruns* et al. (1976) made the same observations in kidney and in adipocytes.

11.3 The Mitochondria as the Main Controller

Many investigators have proposed that except in erythrocytes and in skeletal and cardiac muscle fibers, mitochondria are the main controllers of the cytosolic calcium activity (*Lehninger* 1964; *Bygrave* 1967, 1978; *Borle* 1973; *Mela* 1977; *Carafoli* and *Crompton* 1978b). However, several others

have expressed serious doubt that mitochondria have a high enough affinity for calcium and high enough rates of calcium uptake at physiologically low cytosolic free calcium concentrations to play a significant role as controllers (*Scarpa* 1975; *Scarpa* et al. 1978b; *Brinley* 1978; *Brinley* et al. 1978).

Several lines of evidence suggest that mitochondria do play an important role in cellular calcium metabolism. First, the major part of the cell calcium is found in mitochondria (Table 4). Second, when a cell is taking up calcium (during calcium loading or in tracer experiments) the major part of the load is taken up by the mitochondria in liver (*Thiers* et al. 1960; *Farber* 1977), kidney (*Borle* 1972, 1978, 1979), muscle (*Carafoli* et al. 1969), salivary glands (*Berridge* et al. 1975), synaptosomes (*Blaustein* et al. 1978a–c), and in the squid axon (*DiPolo* et al. 1976; *Requena* et al. 1977; *Brinley* et al. 1977, 1978). Nevertheless, these observations only show that mitochondria can effectively buffer a net cellular uptake of calcium; they do not prove that mitochondria can significantly lower the cytosolic calcium or play a role as a calcium controller. On the other hand, other experiments do suggest such a role. *Rose* and *Lowenstein* (1975) showed that intracellular microinjections of calcium in the giant salivary gland cells of *Chironomus* were rapidly buffered by an energy-dependent process and did not increase the cytosolic ionized calcium monitored with aequorin. However, when cyanide or ruthenium red were injected before calcium, there was a large and diffuse increase in aequorin luminescence, indicating a large rise in cytosolic free calcium. Since ruthenium red inhibits mitochondrial calcium uptake without affecting calcium transport by microsomes or by the plasma membrane (*Ash* and *Bygrave* 1977; *Blaustein* et al. 1978c) these experiments suggest that mitochondria are the principal controllers of cytosolic free calcium in these salivary gland giant cells. In the squid axon mitochondria can accumulate great calcium loads (*Brinley* et al. 1977, 1978; *Brinley* 1978) and can rapidly release the accumulated calcium into the axoplasm if cyanide or the mitochondrial uncoupler FCCP are added (*DiPolo* et al. 1976; *Requena* et al. 1977; *Brinley* et al. 1977, 1978). More importantly, *DiPolo* et al. (1976), *Requena* et al. (1977), and *Brinley* et al. (1977) have shown that in squid axon an intracellular mechanism can rapidly lower the cytosolic free calcium to its resting levels of 30–60 nM (Figs. 13 and 14). In most of these experiments, calcium transport across the plasma membrane or into the endoplasmic reticulum is inhibited by apyrase or by a sodium-free and calcium-free external medium, leaving the substrate supported calcium uptake by mitochondria as the only explanation for the lowering of the cytosolic calcium to its resting level. *Mullins* and *Requena* (1979) also showed that after being 'primed' or initially loaded squid axon mitochondria were able to lower the cytosolic calcium below 10^{-7} M. When the calcium concen-

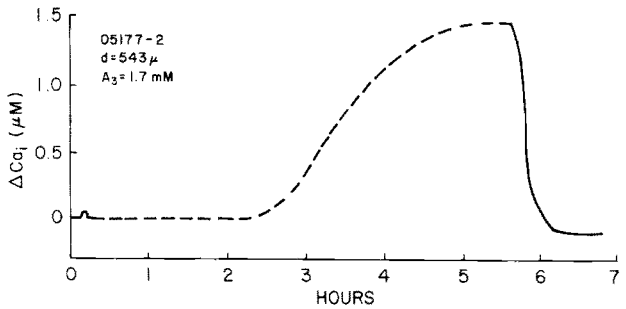


Fig. 13. Increase in cytosolic free calcium measured with arsenazo III in the squid axon, induced by cyanide and its reversal upon removal of cyanide from the solution. Fiber was initially loaded with calcium by a 10 min soak in a solution containing 10 mM Ca and 0 Na, which produced a slight rise in ionized calcium. Ionized calcium fell back to baseline after the return of the fiber to a sodium- and calcium-free solution. The fiber was maintained in 0 Ca, 0 Na solution during the entire experiment to stabilize the internal calcium content. The *dotted line* indicates the period of immersion in 2 mM CN, 0 Ca, 0 Na, artificial seawater. Approximately 2 h after the cyanide treatment there is a rise in ionized calcium to about 1.5 μM, immediately reversed back to baseline upon removal of the CN solution. *Brinley* et al. (1977). Figure reproduced with permission from the *Journal of General Physiology*

tration of the external medium is increased from 3 mM, which is the physiologic free calcium concentration of squid hemolymph (*Blaustein* 1974), to 10 mM or 112 mM and when the axon is stimulated, it appears that the mitochondria are no longer able to lower the cytosolic calcium below 0.3 –0.5 μM (*Scarpa* et al. 1978b; *Brinley* et al. 1978). In these extreme conditions, the plasma membrane calcium transport in the presence of external calcium and sodium or other intracellular buffer systems are equally unable to lower the cytosolic calcium. *Brinley* et al. (1978) calculated that squid axon mitochondria could buffer only 5% of an imposed calcium load; however, their conclusions should be regarded with caution since their own data suggest that they overestimated the loads by a factor of ten[4]. If one uses the corrected value for these calcium loads, squid axon

4 *Brinley* et al. (1977) measured the effects of stimulation at 100 impulses/s on calcium entry in squid axons bathed in 112 mM Ca seawater. They found a net entry of 0.014 pmol cm^{-2} $impulse^{-1}$ (see Fig. 5, *Brinley* et al. 1977) or 1.4 p/cs. In their calculations and in subsequent papers, however, they use the value of 14 p/cs or 140 $fmol/cm^{-2}$ imp (*Brinley* et al. 1977; *Requena* and *Brinley* 1979), 10 times their measured influx. One can calculate that for a axon of 500 μm diameter (see Table 28) the correct calcium load during stimulation would be at most 7 μmol/kg axoplasm/min and not 50 μmol/kg axoplasm/min as used by *Brinley* in this and subsequent papers (*Brinley* et al. 1977, 1978; *Scarpa* et al. 1978; *Brinley* 1978). Recently, *Requena* et al. (1979) found by direct analytical measurements that the net gain of calcium of a squid axon stimulated for 10 min at 100 impulses/s in 100 mM Ca_o was 0.25 p/cs, 40 times less than the value used by *Brinley* et al. (1977)

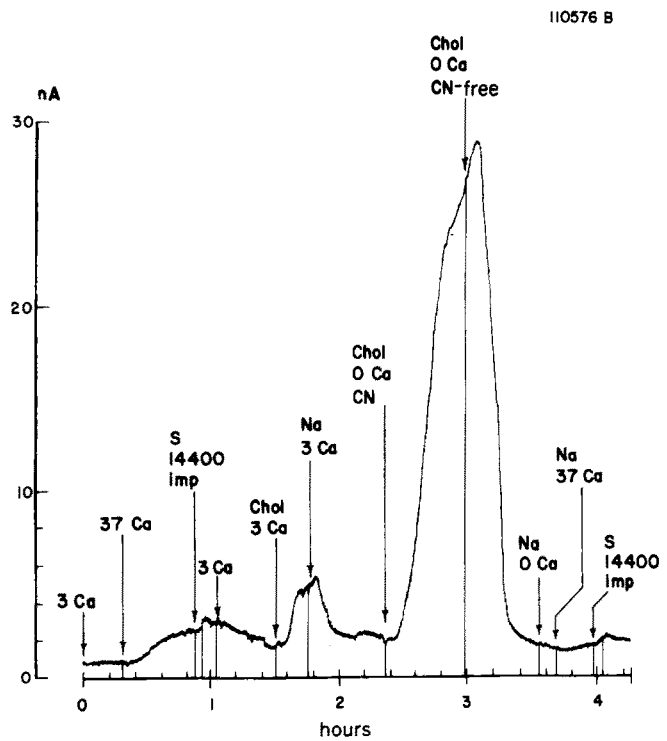


Fig. 14. Measurement of ionized calcium in the squid axon with aequorin. The axon was preinjected with apyrase and shows approximate increases in resting glow in 37 mM seawater, and recovery after its return to 3 mM Ca seawater. At about 2.2 h, when CN was applied, there was a large and virtually immediate increase in glow. The CN effect was fully reversible and the axon recovered its resting glow. *Requena* et al. 1977; reproduced with permission from the *Journal of General Physiology*

mitochondria appear to buffer 40%–50% of a calcium load, as observed in all other cell systems.

Mitochondria isolated from most tissues are capable of lowering the calcium concentration of their environment much below 10^{-6} M: mitochondria isolated from human myometrium, rat liver, and helix neurons can lower the calcium activity of their incubating media below 10^{-7} M (*Batra* 1973a; *Nicholls* 1978b; *Simonsen* and *Christoffersen* 1979). Finally, in liver, kidney, adipocytes, nerve terminals, and smooth muscle the rate of calcium uptake and the capacity of calcium sequestration of mitochondria exceed by at least one order of magnitude those of endoplasmic reticulum and of plasma membrane (*Carafoli* and *Tiozzo* 1967; *Batra* and *Daniel* 1971; *Batra* 1973a, 1974; *Moore* et al. 1974; *Bruns* et al. 1976; *Ash* and *Bygrave* 1977; *Farber* et al. 1977; *Bygrave* and *Trauter* 1978).

11.4 Theoretical Considerations

Experimental evidence suggests that, except in erythrocytes and in striated muscle, mitochondria play a major role in controlling cytosolic free calcium, but it is far from conclusive. The objections to this view are: (a) the affinity of mitochondria for calcium is simply too low ($K_{Ca} > 1$ μM); (b) the Hill coefficient close to 2 implies cooperativity; (c) calcium cycling across the inner mitochondrial membrane appears to be negligible at the physiologic cytosolic free calcium concentration ($0.5–3 \cdot 10^{-7}$ M). It is obviously true that, compared with the rate of calcium uptake by isolated mitochondria usually measured in vitro at free calcium concentrations exceeding 1 μM, calcium cycling at low calcium concentrations is very slight. However, these low rates are still faster than the calcium fluxes measured across the plasma membrane or across the endoplasmic reticulum membranes in similar physiologic conditions.

Table 25 compares the calcium affinity of various calcium-transporting systems and shows that they are all in the same order of magnitude. All have Hill coefficients close to 2. If the objections listed above were valid, one should conclude that the sarcoplasmic reticulum of skeletal muscle cannot control the free calcium of muscle fibers and cannot lower it to 10^{-8} M, the level of ionized calcium in resting muscle. What distinguishes the sarcoplasmic reticulum and the mitochondria from the other transport systems is their high maximal velocity and their capacity to sequester calcium. In spite of a K_{Ca} that is one order of magnitude larger than the cytosolic calcium activity the rates of calcium transport of sarcoplasmic reticulum and of mitochondria are so high that even at physiologically low calcium concentrations, their transport capacity or rate of cycling is significant and predominates over the other transport systems. For instance, taking an average mitochondrial K_{Ca} of 5 μM, a V_m of 400 nmol mg^{-1} (mito protein) min^{-1}, a Hill coefficient of 2, and assuming a cytosolic free calcium of 10^{-7} M, calcium cycling across the mitochondria inner membrane can be calculated (from the equation $v = V_m/(1 + (K_{Ca}/[Ca^{2+}]^2))$ to be 800 pmol mg^{-1} (mito protein) min^{-1}, one order of magnitude larger than steady-state calcium transport across the plasma membrane (Table 11).

Another important consideration is the relative mass and the relative surface area of each transport system. Obviously these will differ from cell to cell. I shall consider two cell types for which much quantitative information is available: the liver cell and the squid axon.

Liver cells contain between 700 and 2500 mitochondria per cell (*Allard* et al. 1952; *Striebich* et al. 1953; *Schneider* et al. 1953; *Lowe* et al. 1955; *Lehninger* 1964; *Weibel* et al. 1969). They occupy 18%–22% of the cell volume (*Loud* 1962; *Weibel* et al. 1969) and their protein content accounts for 33% of the cell protein concentration (*Price* et al. 1948, 1949;

Table 26. Dimensions and mass of a single liver cell

Diameter [a] (μm)	Cell	17
Volume [a] (μm^3)	Cell	4 940
	Cytoplasm	4 640
	Mitochondria	1 170
	Smooth endoplasmic reticulum (SER)	467
Surface [a] (μm^2)	Cell	1 680
	Mitochondria (inner membrane)	34 800
	SER	25 100
Surface ratio	Mitochondria/cell membrane	21
	SER/cell membrane	15
Mass (ng)	Cell wet weight (4940 μm^3)	5
	Cell protein (20% wet weight) [b]	1
	Mitochondrial protein (33% cell prot) [c]	0.33
	SER protein (10% cell prot) [d]	0.1
Surface/mass ratio $[cm^2 (mg\ prot)^{-1}]$	Mitochondria (6 m^2/ml fresh tissue) [a]	900
	SER (3.67 m^2/ml fresh tissue) [a]	1 800

[a] *Weibel* et al. 1969

[b] *Mitchell* 1966; *Foden* and *Randle* 1978

[c] *Price* et al. 1948, 1949; *Schneider* and *Hogeboom* 1951; *Hogeboom* et al. 1953; *Lowe* and *Lehninger* 1955; *Mitchell* 1966

[d] *Price* et al. 1948, 1949; *Lowe* and *Lehninger* 1955

Schneider and *Hogeboom* 1951; *Hogeboom* et al. 1953; *Lowe* and *Lehninger* 1955; *Mitchell* 1966). Table 26, based on the work of *Weibel* et al. (1969) shows that the surface of the inner mitochondrial membrane is 21 times greater than the plasma membrane surface. The specific surface of 6 m^2/ml fresh liver can be calculated to be 900 cm^2/mg mitochondrial protein. This is about twice the value of 400 cm^2/mg mitochondrial protein calculated by *Mitchell* (1966).

The protein mass of the endoplasmic reticulum of liver cells is about 10% of the total cell protein (*Price* et al. 1949; *Lowe* and *Lehninger* 1955). The surface of the smooth endoplasmic reticulum (SER) is 15 times larger than the plasma membrane surface (*Weibel* et al. 1969); its specific surface (3.67 m^2/fresh tissue) can be calculated to be 1800 cm^2/mg SER protein. Consequently the surface ratio of plasma membrane/smooth endoplasmic reticulum/mitochondria is 1/15/21. Even if the kinetic parameters of calcium transport (K_{Ca}, V_m) among the three transport systems were identical, the plasma membrane would contribute 2.7%, the endoplasmic reticulum 40%, and mitochondria 57% to the control of cytosolic calcium. But, of course, the calcium transport velocity and capacity of both the plasma

membrane and the endoplasmic reticulum are very much smaller than those of mitochondria so that their contribution is proportionately much less.

Table 27 presents a quantitative estimate of the calcium metabolism of a single liver cell based on all the available information shown in Tables 25 and 26. With a cytosolic free calcium of 10^{-7} M, calcium cycling is 6.6 times faster in mitochondria than across the plasma membrane and 800 times greater than SER calcium transport. As one would predict, the relative importance of mitochondrial calcium cycling increases with increasing cytosolic free calcium. The relation of the rates of calcium transport of mitochondria, plasma membrane, and endoplasmic reticulum as a function of the cytosolic free calcium is shown in Fig. 15.

Table 27. Calcium metabolism of a single liver cell

Calcium compartments (fmol/single cell)	
Total calcium (2.2 mmol/kg wet wt) [a]	11.0
Exchangeable cytosolic Ca (0.36 mmol/kg wet wt) [b]	1.8
Ionized cytosolic Ca (10^{-7} M) [c]	0.00046
Mitochondrial Ca (10 nmol/mg mitro protein) [d]	3.3
SER Ca (21.6 nmol/mg SER prot) [e]	2.1
Steady state calcium exchange (10^{-18} mol min^{-1} $cell^{-1}$)	
Plasma membrane (40 f/cs) [f]	40
Mitochondria [0.8 nmol (mg mito prot)$^{-1}$ min^{-1}] [g]	266
SER [3.3 pmol (mg SER prot)$^{-1}$ min^{-1}] [h]	0.33

a From Table 1.

b From Table 5.

c From Table 7.

d From Table 6 and *Carafoli* and *Lehninger* 1971.

e *Carafoli* 1967.

f From Tables 9–11.

g From Tables 21 and 25. Assumptions: Free cytosolic Ca = 10^{-7} M, K_{Ca} = 5 μM; V_m = 400 nmol (mg mito prot)$^{-1}$ min^{-1}; Hill coefficient = 2; $v = V_m/(1 + (K_{Ca}/[Ca^{2+}]^2))$.

h From Tables 23–25. Assumptions: Free cytosolic Ca = 10^{-7} M; K_{Ca} = 3 μM; V_m = 1 nmol (mg SER prot)$^{-1}$ min^{-1}; Hill coefficient 2; $v = V_m/(1 + (K_{Ca}/[Ca^{2+}]^2))$.

The giant squid axon is a cell that has been extensively studied with regard to calcium transport and metabolism. Table 28 shows the dimensions of an axon of 500 μm diameter with a plasma membrane surface area of 1 cm^2 (unit axon). Table 29 presents the calcium distribution and calcium transport in this unit axon. Several assumptions have been made here: (a) the protein mass of endoplasmic reticulum in relation to the protein mass of mitochondria is the same as in other cells; (b) the calcium content of

Table 28. Dimension of a squid axon with a membrane surface of 1 cm^2 (unit axon)

Diameter	500 μm
Length	6 cm
Surface	1 cm^2
Volume	12 μl
Wet weight (density $\approx$ 1)	12 mg
Mitochondria wet weight (10% of axoplasm) [a]	0.12 mg
Mitochondria protein (0.29% of wet weight) [a]	35 μg
Smooth ER protein (50% mito prot) [b]	17.5 μg

[a] *Brinley* et al. 1977.

[b] Assumption based on *Price* et al. 1948, 1949; *Lowe* and *Lehninger* 1955; *Carafoli* 1967.

Table 29. Calcium metabolism of a unit axon (1 cm^2)

Calcium compartments (pmol/unit axon)	
Axoplasm total calcium (50 μM) [a]	600
Ionized cytosolic Ca (50 nM) [b]	0.6
Mitochondria Ca (10 nmol/mg mito prot) [c]	84
SER Ca (20 nmol/mg SER prot) [d]	84
Steady-state calcium exchange [fmol (unit axon)$^{-1}$ sec^{-1}]	
Plasma membrane (40 f/cs) [e]	40
Mitochondria [0.2 nmol (mg mito prot)$^{-1}$ min^{-1}] [f]	120
SER [1.7 pmol (mg SER prot)$^{-1}$ min^{-1}] [g]	0.5

[a] *Requena* et al. 1977; *Brinley* 1978.

[b] *DiPolo* et al. 1976.

[c] *Carafoli* and *Lehninger* 1971

[d] *Carafoli* 1967

[e] *Mullins* 1977; *DiPolo* 1978

[f] Calculated assuming K_{Ca} = 5 μM; V_m = 400 nmol (mg mito prot)$^{-1}$ min^{-1}; Hill coefficient = 2; cytosolic free Ca^{2+} = 50 nM; $v = V_m/(1 + (K_{Ca}/[Ca^{2+}]^2))$.

[g] Calculated from *Blaustein* et al. 1978b; K_{Ca} = 0.4 μM; V_m = 275 pmol (mg SER prot)$^{-1}$ min^{-1}; Hill coefficient = 2; cytosolic free Ca^{2+} = 50 nM; $v = V_m/(1 + (K_{Ca}/[Ca^{2+}]^2))$.

axon mitochondria and their kinetic parameters are in the same order of magnitude as in other cells; (c) the kinetic parameters of calcium transport measured in microsomes from nerve terminals are applicable for the squid axon. Table 29 shows that even in the squid axon, which has far fewer mitochondria than liver cells and a lower cytosolic free calcium ($5 \cdot 10^{-8}$ M), calcium cycling in mitochondria is 4 times greater than the steady-state calcium transport across the plasmalemma. Endoplasmic reticulum calcium transport, despite a more favorable K_{Ca} of 0.4 μM, is only 1.3% of plasma-

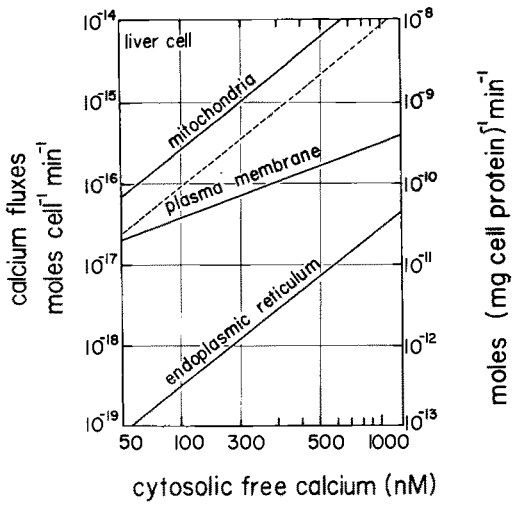


Fig. 15. Relation between the cytosolic ionized calcium and the steady-state calcium exchange across the plasmalemma, the mitochondria, and the endoplasmic reticulum membrane of a liver cell. Calculated from the parameters and equations given in Table 27. *Dotted line* shows the calculated mitochondrial calcium transport assuming a K_{Ca} of 15 μM instead of 5 μM *(solid line)*

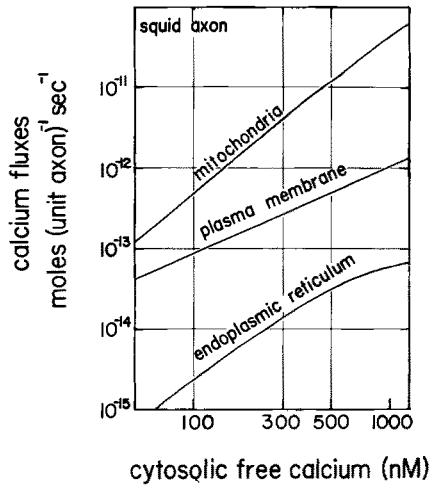


Fig. 16. Relation between the axoplasmic ionized calcium and the steady-state calcium exchange across the plasmalemma, the mitochondria, and the endoplasmic reticulum membrane of a squid axon. Calculated from the parameters and equations given in Table 29

lemmal transport and 0.5% of mitochondrial cycling. As Fig. 16 shows, at higher cytosolic free calcium concentrations, this relation remains true and mitochondrial calcium transport becomes more and more predominant.

These are, of course, theoretical considerations that cannot conclusively prove anything. They do show that mitochondrial calcium transport cannot be dismissed as unimportant controllers of cell calcium. On the contrary, they suggest that mitochondria are the best candidates for this role. However, mitochondria are not the only controllers of cell calcium and the relative importance of the three control systems, mitochondria, plasma membrane, and endoplasmic reticulum, may change under the influence of ionic or hormonal regulators. Nevertheless, when one compares the four criteria required for a cellular calcium controller with Tables 27 and 29 describing the calcium metabolism of a liver cell and of an axon, one cannot escape the conclusion that mitochondria probably play the principal role in controlling the cytosolic free calcium.

12 Modulation of the Cytosolic Free Calcium

Several ions have been shown to influence cellular calcium metabolism at the cellular and subcellular level. The effects of phosphate, sodium, and hydrogen ions are best documented. Although magnesium influences calcium uptake by endoplasmic reticulum and mitochondria, there is little information about its possible role in modulating calcium metabolism in an intact cell. Glucose, other substrates, and amine buffers have also been reported to affect cellular calcium transport but the information available is extremely fragmentary.

12.1 The Role of Phosphate

The effect of phosphate on mitochondrial calcium transport was reported early in the literature on mitochondria (*Lehninger* et al. 1963, 1967; *Lehninger* 1974a; *Brierley* et al. 1964; *Vasington* and *Greenawalt* 1964). First and foremost, inorganic phosphate increases the uptake of calcium by mitochondria (*Lehninger* 1970; *Izzard* and *Tedeschi* 1973; *Schuster* and *Olson* 1974; *Lehninger* 1974; *Reed* and *Bygrave* 1975b; *Bygrave* 1976, 1976; *Bygrave* et al. 1977; *Crompton* et al. 1978). The capacity of mitochondria to sequester calcium is also increased by phosphate (*Lehninger* 1974; *Crompton* et al. 1978). Phosphate does not stimulate the initial rate of calcium uptake and does not increase the K_{Ca} (*Reed* and *Bygrave* 1975b; *Thorne* and *Bygrave* 1975; *Bygrave* 1976; *Crompton* et al. 1978).

However, the maximal velocity of calcium transport into mitochondria (V_m) is markedly enhanced (*Thorne* and *Bygrave* 1975; *Bygrave* 1976, 1977, 1978). Since phosphate is not co-transported with calcium, it is likely that its effect occurs through its action on the mitochondrial transmembrane potential $\Delta\psi$. As reported above, the relative contribution of the membrane potential $\Delta\psi$ and of the proton gradient ΔpH to the proton electrochemical potential $\Delta\bar{\mu}H^+$ depends on the presence of dissociable anions, primarily phosphate. The inward transport of weak acid anions, driven by the proton gradient, can convert most of the pH gradient into a membrane potential by releasing a proton to the mitochondrial matrix and lowering its pH (*Lehninger* 1974; *Gunter* and *Puskin* 1975; *Reed* and *Bygrave* 1975b; *Bygrave* 1977, 1978; *Crompton* et al. 1978). The increased potential difference across the mitochondrial inner membrane could be the ultimate cause of the greater calcium uptake and sequestration. In isolated mitochondria an increased phosphate concentration produces swelling but in the presence of ATP and magnesium no mitochondrial swelling occurs and, instead, calcium and phosphate precipitate in the matrix (*Lehninger* 1970; *Crompton* et al. 1978).

Phosphate has very little effect on calcium transport or exchange in endoplasmic and sarcoplasmic reticulum (*Otsuka* et al. 1965; *Ohtsuki* 1969; *DeMeis* et al. 1970), although very high concentrations may stimulate transport slightly (*Selinger* et al. 1970; *Alonso* et al. 1971; *Trotta* and *DeMeis* 1975).

No information is available on the effect of phosphate on the different calcium transport processes across the cell plasma membrane (CaMgATPase-dependent transport, Na_o- or Ca_o-dependent calcium transport, and calcium channels).

Phosphate has a marked influence on the calcium metabolism of intact cells. A rise in extracellular phosphate concentration increases the total cell calcium and the cellular exchangeable calcium (^{45}Ca uptake) in kidney, liver, and bone cells, in pancreatic beta-cells, adipocytes, neuroblasts, astroblasts, and in HeLa and ascites cells (*Janda* 1969; *Borle* 1970b, 1971a, 1972a, 1973a, 1975a; *Van Rossum* 1970; *Dziak* and *Brand* 1974b; *Martin* et al. 1975; *Hines* and *Wenner* 1977; *Borle* and *Uchikawa* 1978; *Hellman* and *Andersson* 1978; *Charlton* and *Wenner* 1978; *Borg* et al. 1979). There is an interaction between the external calcium and phosphate concentrations (shown in Fig. 17): the higher the phosphate concentration, the greater the gain in cell calcium with increasing extracellular calcium concentrations (*Borle* 1971a). In kidney cells, the increased calcium uptake produced by phosphate is abolished by inhibitors of mitochondrial calcium transport (*Borle* 1972a) and kinetic analyses of ^{45}Ca desaturation curves suggest that most of the phosphate-induced cellular uptake of calcium is found in mitochondria (*Borle* 1972a; *Borle* and *Uchikawa* 1978).

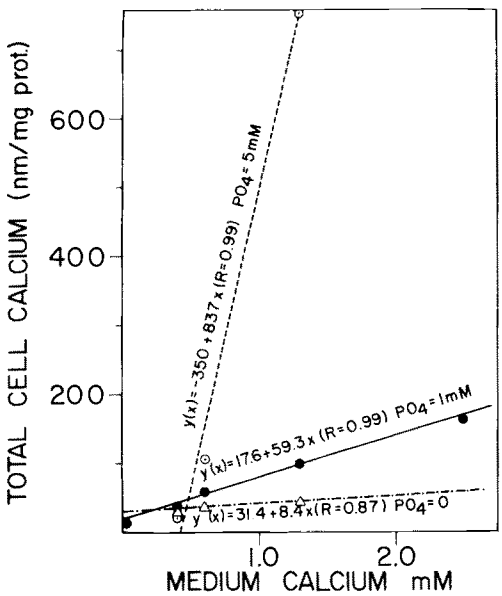


Fig. 17. Relation between the extracellular calcium concentration and the total cell calcium content at different extracellular phosphate concentrations. Data obtained with cultured monkey kidney cells incubated in Krebs-Henseleit bicarbonate buffers containing various calcium and phosphate concentrations. Adapted from *Borle* 1971a

This is supported by the finding that in Ehrlich ascites cells the uptake of calcium, which is stimulated by increasing extracellular phosphate, is sensitive to mitochondrial uncouplers (*Charlton* and *Wenner* 1978; *Hinnen* et al. 1979). The increased ^{45}Ca uptake produced by phosphate reflects an enlarged intracellular exchangeable pool and not an increased calcium influx, because at steady state the exchange of calcium across the plasma membrane is reduced by phosphate (*Borle* and *Uchikawa* 1978). In non-steady state ^{45}Ca desaturation experiments, increasing the extracellular phosphate concentration also reduces the efflux of ^{45}Ca in kidney, pancreatic beta-cells and Ehrlich cells (*Borle* 1972a; *Hellman* and *Andersson* 1978; *Charlton* and *Wenner* 1978). Conversely, lowering the phosphate concentration stimulates the tracer efflux (*Borle* 1972a).

The role of phosphate as a modulator of cellular calcium metabolism can best be seen in conjunction with hormones and other stimulators of calcium transport. Increasing extracellular phosphate reduces the stimulation produced by parathyroid hormone on cellular calcium transport in kidney cells and in HeLa cells (*Borle* 1970b; *Borle* and *Uchikawa* 1978). The glucose-stimulated ^{45}Ca and insulin release from beta-cells is inhibited by phosphate (*Hellman* and *Andersson* 1978). On the other hand, the effects of calcitonin on kidney cell calcium are enhanced by phosphate and are abolished in phosphate-free media (*Borle* 1978a). It has been proposed that the actions of parathyroid hormone on the calcium transport of kidney cells and the effects of glucose on the insulin release from beta-cells are mediated by a rise in cytosolic free calcium (*Nagata* and *Rasmussen*

1970; *Rasmussen* 1971; *Borle* 1972b, 1973a, 1974b, 1975c, 1976; *Malaisse* et al. 1978; *Kikuchi* et al. 1979). Therefore, the inhibition of these effects by phosphate suggests that increasing phosphate depresses the concentration of free calcium in the cytosol. Since other results suggest that calcitonin lowers the cytosolic free calcium (*MacManus* and *Whitfield* 1970; *Rasmussen* 1971; *Borle* 1974, 1975a, c), the enhancement of the effects of calcitonin by phosphate also supports this interpretation.

Based on the results obtained in isolated mitochondria and in intact cells, *Borle* (1973a, 1975c) has proposed that phosphate, by stimulating the uptake and sequestration of calcium into mitochondria, would shift calcium from the cytosol to the mitochondrial matrix, thereby increasing the total cellular calcium content while decreasing the cytosolic concentration of ionized calcium. Consequently, a rise in cellular phosphate induced by increasing its extracellular concentration will depress all cellular processes that are stimulated or triggered by a rise in cytosolic free calcium, but it will enhance the effects of regulators that tend to depress cytosolic calcium.

12.2 The Role of Sodium

The influence of sodium on cellular calcium metabolism varies greatly from tissue to tissue. Two broad categories can be distinguished: (a) tissues in which a Na–Ca exchange across the plasmalemma or across the mitochondrial membrane can be identified, such as muscle, nerve, and some secretory cells, and (b) tissues in which such exchange cannot be demonstrated, such as liver, kidney, adrenal medulla, fibroblasts, Ehrlich ascites cells, and erythrocytes. But, even in the latter group, sodium may influence some aspects of cellular calcium transport and distribution.

Sodium induces a release of calcium from mitochondria isolated from heart, skeletal muscle, brain, parotid gland, and adrenal cortex; but is has no effect on calcium released from mitochondria isolated from liver, kidney, lung, uterus, and ileum muscle (*Crompton* et al. 1978; *Nicholls* 1978a). The effects of sodium on mitochondrial calcium release have been best studied in heart mitochondria (*Carafoli* et al. 1974; *Carafoli* and *Crompton* 1976, 1978b; *Crompton* et al. 1976b, 1977, 1978; *Crompton* and *Heid* 1978; *Harris* 1977, 1979). The curve relating the efflux of calcium from heart mitochondria to the extramitochondrial sodium concentration is sigmoid with a Hill coefficient ranging between 2 and 3. This would suggest an exchange of 1 calcium for 2 or 3 sodium ions. The K_{Na} is 8 mM and the V_m of calcium release is 14 nmol (mg protein)$^{-1}$ min^{-1}. With an assumed cytosolic sodium concentration of 6 mM, the rate of sodium-dependent calcium efflux would be in the range of 4 nmol (mg protein)$^{-1}$

min^{-1}. Sodium may not only stimulate calcium efflux from mitochondria, but it may also inhibit calcium uptake (*Carafoli* and *Crompton* 1976; *Harris* 1979). Sodium also enhances calcium release from mitochondria isolated from nerve terminals (*Silbergeld* 1977). In liver mitochondria, sodium does not stimulate calcium efflux (*Crompton* et al. 1978) but it still influences mitochondrial calcium transport by reducing the inhibition produced by magnesium since the K_{Ca} of mitochondrial uptake may be reduced 50% (*Hutson* et al. 1976).

An effect of sodium on calcium transfer across the sarcoplasmic reticulum membrane has not been conclusive documented. Sodium inhibits calcium uptake by skeletal muscle SR when acetyl phosphate or very low concentrations of ATP are used as substrate, but no inhibition occurs at ATP concentrations higher than 10^{-4} M (*DeMeis* 1969a, b, 1971; *DeMeis* and *Hasselbach* 1971). In heart muscle, in spite of the suggestion that a rise in intracellular sodium may increase the release of calcium from the sarcoplasmic reticulum (*Busselen* and *Carmeliet* 1973), no such effect is observed in isolated SR vesicles (*Katz* et al. 1977).

The effects of sodium on calcium transport across the plasma membrane have already been reviewed above (cf. Sect. 8.3.2–5). Two issues must be clearly distinguished: (a) whether sodium influences calcium fluxes in and out of cells, which it does in a wide variety of tissues, and (b) whether these effects represent a Na–Ca exchange mechanism responsible for the uphill transport of calcium out of the cell. It is clear that in muscle and nerve a significant fraction of calcium efflux depends on a Na–Ca exchange or at least on a Na_o-activated calcium efflux, and that changes in extracellular sodium may influence the cytosolic free calcium concentration (*Blaustein* 1977b; *Roulet* et al. 1979). The active calcium transport across the cell membrane, which is catalzyed by the Ca-sensitive Mg-dependent ATPase, is not influenced by sodium (*Schatzmann* 1966, 1970; *Schatzmann* and *Vincenzi* 1969; *Schatzmann* and *Rossi* 1971, *Porzig* 1972). Sodium may affect other Ca-ATPases but these have different functions unrelated to calcium transport (*Schatzmann* and *Vincenzi* 1969; *Bond* and *Green* 1971; *Moore* et al. 1974). Nevertheless, sodium could still influence the actions of calmodulin on the CaMgATPase (*Au* 1979).

Sodium is also a modulator of calcium metabolism in intact cells. The extracellular sodium concentrations influences the total calcium content of tissues in which a Na–Ca exchange has been demonstrated. A low extracellular sodium increases tissue calcium in heart muscle (*Reuter* and *Seitz* 1968; *Reuter* 1970, 1974a; *Reuter* et al. 1973; *Wendt* and *Langer* 1977), squid axon (*Requena* et al. 1977, 1979), and brain tissue (*Stahl* and *Swanson* 1969, 1971, 1972; *Swanson* et al. 1974). Ouabain also increases tissue calcium in the myocardium (*Govier* and *Holland* 1964; *Reuter* 1974a; *Wood* and *Schwartz* 1978) and brain tissue (*Stahl* and

Swanson 1969, 1971; *Cooke* and *Robinson* 1971). In *Helix pomatia* neurons, on the other hand, changes in extracellular sodium do not affect the cytosolic free calcium and there is no evidence of a Na–Ca exchange (*Christoffersen* and *Simonsen* 1977).

In nonexcitable tissues the results are more fragmentary and sometimes conflicting. Early data show that a decrease in extracellular sodium increases the total calcium of kidney and liver slices (*Höfer* and *Kleinzeller* 1963c; *Judah* and *Ahmed* 1963). Later results show no such increases in kidney slices and Ehrlich ascites cells (*Borle* 1979; *Cittadini* et al. 1973, 1977). In fact, progressive lowering of extracellular sodium slightly lowers the total calcium mitochondrial calcium, and the exchangeable calcium pools measured by tracer kinetics (*Borle* 1979). Calcium efflux has been shown to be totally independent of extra- or intracellular sodium in kidney (*Matsushima* and *Gemba* 1979), adrenal medulla (*Rink* 1977), liver (*Cittadini* and *Van Rossum* 1978), Ehrlich ascites cells (*Cittadini* et al. 1973, 1977), and fibroblasts (*Lamb* and *Lindsay* 1971). In these tissues, ouabain has no effect on cell calcium or on calcium transport (*Lamb* and *Lindsay* 1971; *Rink* 1977; *Cittadini* and *Van Rossum* 1978; *Borle* 1979; *Matsushima* and *Gemba* 1979). In some instances calcium efflux from adrenal medulla is actually increased when extracellular sodium is substituted by sucrose or choline (*Aguirre* et al. 1977). On the other hand, in kidney slices the steady-state calcium exchange across the plasma membrane is depressed at low extracellular sodium concentrations but, since the total cell calcium and the mitochondrial calcium are also depressed in these conditions, one must conclude that both influx and efflux of calcium are inhibited (*Borle* 1979). Although there appears to be some cooperativity between sodium and calcium at the plasma membrane, there is no evidence of Na–Ca exchange in any of these tissues (*Cittadini* et al. 1977; *Rink* 1977; *Cittadini* and *Van Rossum* 1978; *Borle* 1979; *Matsushima* and *Gemba* 1979).

12.3 The Role of pH

Cellular calcium distribution and transport is markedly affected by fluctuations in pH. Even when the extracellular pH is normal, cell calcium metabolism may be altered by changes in intracellular pH (*Studer* and *Borle* 1979). Isolated subcellular organelles, mitochondria, endoplasmic reticulum vesicles, and plasma membranes are also influenced by the concentration of hydrogen ions.

In isolated mitochondria calcium binding is increased at high pH (*Scarpa* and *Azzi* 1968; *Rossi* et al. 1967). The number of low-affinity sites appears to increase when the pH is raised, while the calcium affinity of the

high-affinity site appears to decrease when the pH is lowered (*Reynafarje* and *Lehninger* 1969). The stoichiometry of calcium ions accumulated per pair of electrons passing through the respiratory chain is increased at high pH, but only in the presence of impermeant anions; the stoichiometry remains 2 in the presence of permeant anions (*Carafoli* et al. 1967). Several investigators found that pH affects the K_{Ca} of calcium uptake by mitochondria isolated from liver or from kidney: K_{Ca} is increased at low pH and decreased at high pH (*Spencer* and *Bygrave* 1973; *Reed* and *Bygrave* 1975b; *Studer* and *Borle* 1980). V_m is not affected by pH in liver mitochondria (*Spencer* and *Bygrave* 1973; *Reed* and *Bygrave* 1975b) but it is depressed at low pH in kidney mitochondria (*Studer* and *Borle* 1980); pH does not affect the Hill coefficient of calcium uptake (*Spencer* and *Bygrave* 1973; *Studer* and *Borle* 1980). Calcium efflux from liver mitochondria is fast at low pH and decreases with increasing pH (*Rossi* et al. 1966). A sudden drop in pH will also trigger a release of accumulated calcium from liver mitochondria (*Akerman* 1978a). The steady-state fluxes of calcium across the inner mitochondrial membrane or calcium cycling are increased at high pH and depressed at low pH (*Studer* and *Borle* 1980). The total calcium content of kidney mitochondria and the exchangeable mitochondrial calcium pool are both increased at high pH and decreased at low pH (*Studer* and *Borle* 1980). Finally, the capacity of isolated liver mitochondria to lower the free calcium concentration of the extramitochondrial medium is enhanced at high pH (*Nicholls* 1978b). In spite of some conflicting data (*Schraer* et al. 1973; *Hutson* 1977), these results are consistent with the idea that H^+ is a competitive inhibitor of calcium uptake by mitochondria: a high pH increases the affinity of the transport system for calcium and enhances calcium transport and its sequestration while a low pH does the reverse.

In sarcoplasmic or endoplasmic reticulum vesicles, the effects of pH are exactly the opposite of those observed in mitochondria: a high pH depresses calcium binding (*Sulakhe* et al. 1973; *Huxtable* and *Bressler* 1974; but see conflicting results of *Carvalho* and *Leo* 1967) as well as calcium uptake by the vesicles isolated from skeletal and heart muscle (*Weber* et al. 1966; *Carvalho* and *Leo* 1967; *Streter* 1969; *Nakamura* and *Schwartz* 1970, 1972; *Lacourt* 1971; *Sulakhe* et al. 1973; *Huxtable* and *Bressler* 1974; *Sorenson* and *DeMeis* 1977; *Dunnet* and *Naylor* 1979) and from kidney, liver, and nerve (*Moore* et al. 1974, 1975; *Eroglu* and *Keen* 1977). The optimal pH for calcium uptake varies between 6.0 and 7.0 with an average optimal pH of 6.6 (*Carvalho* and *Leo* 1967; *Streter* 1969; *Lacourt* 1970; *Sulakhe* et al. 1973; *Huxtable* and *Bressler* 1974; *Moore* et al. 1974; *Eroglu* and *Keen* 1977). Finally, a rise in pH will induce a release of accumulated calcium from sarcoplasmic reticulum vesicles (*Nakamura* and *Schwartz* 1970, 1972; *Dunnet* and *Nayler* 1979).

Plasma membrane vesicles are affected by pH in the same fashion as SR or ER vesicles: calcium binding is depressed (*Sulakhe* et al. 1973) and calcium uptake is inhibited by raising pH (*Sulakhe* et al. 1973; *Moore* et al. 1974). In erythrocytes, on the other hand, there is no effect of pH on the active extrusion of calcium (*Romero* and *Whittam* 1971; *Plishker* and *Gitelman* 1976).

The calcium metabolism of intact cells is also influenced by fluctuations in extra- and intracellular pH. In liver, kidney, and smooth muscle a rise in pH increases, while a low pH decreases, the total cell calcium (*Wallach* et al. 1966; *Rorive* and *Kleinzeller* 1972; *Van Breemen* et al. 1973; *Studer* and *Borle* 1979). The intracellular exchangeable calcium measured by ^{45}Ca uptake is also elevated by high pH and depressed at low pH in fibroblasts, lymphocytes, mast cells, smooth muscle, kidney, and Ehrlich ascites cells (*Lamb* and *Lindsay* 1971; *Whitney* and *Sutherland* 1973; *Morgenstern* 1972; *Foreman* et al. 1977; *Studer* and *Borle* 1979; *Hinnen* et al. 1979). In kidney cells, while acidosis depresses both total calcium and exchangeable calcium proportionately, alkalosis enhances the total calcium much more than the exchangeable calcium and most of the calcium gain is found in an unexchangeable pool, presumably in mitochondria (*Studer* and *Borle* 1979). Calcium influx and efflux across the plasma membrane of nerve terminals, squid axons, myocardial fibers, and kidney cells are also depressed at low pH (*Blaustein* and *Oborn* 1975; *Baker* and *McNaughton* 1977; *Langer* and *Pool-Wilson* 1977; *Studer* and *Borle* 1979). On the other hand, high pH does not affect calcium exchange across the plasma membrane of kidney cells (*Studer* and *Borle* 1979). *Studer* and *Borle* (1979) studied the calcium transport and distribution among intracellular pools in isolated kidney cells and found that both cytosolic and mitochondrial calcium pools are depressed in acidosis as well as calcium exchange across plasma membrane and mitochondrial membrane; in alkalosis, on the other hand, only the mitochondrial pool and transport are enhanced while the cytosolic pool and plasma membrane exchange are unaffected.

The influence of pH on the cytosolic free calcium concentrations has been studied with aequorin in the squid axon and in the barnacle muscle fiber (*Baker* and *Honerjager* 1978; *Lea* and *Ashley* 1978). Increasing the CO_2 tension decreases the intracellular pH in both tissues but has an opposite effect on the aequorin glow in each tissue. In the squid axon, an increased CO_2 decreases the aequorin glow, suggesting a lowering of the cytosolic free calcium (*Baker* and *Honerjager* 1978). In contrast, a rise in CO_2 appears to increase the glow and the free calcium of barnacle muscle (*Lea* and *Ashley* 1978). Since changes in pH have opposite effects on calcium uptake by mitochondria and by sarcoplasmic reticulum, it may not be surprising to observe different effects in different cells, depending on

the predominant controller of their cytosolic calcium. The information available is still too fragmentary to propose a coherent model for pH action in specific cells or tissues. Nevertheless, an important conclusion supported by all these studies is that the intracellular H^+ concentration is a major modulator of intracellular calcium metabolism.

13 Regulation of Cellular Calcium

Many cellular functions are mediated by intracellular calcium. Calcium is a transducing coupler between electrical or hormonal stimuli and cellular responses. Usually, the signal is a rise in the cytosolic concentration of ionized cacium, but changes in the calcium activity of other intracellular compartments, i.e., mitochondrial matrix, may also occur and regulate intramitochondrial enzyme activity. Although this general scheme is well recognized, the steps involved in the sequence of events between the stimulus and the response and the source of the calcium involved in the coupling are still controversial. Obviously, variations of the general scheme occur from tissue to tissue and with different stimuli. For many years the evidence suggesting that calcium is implicated in a stimulus–response process was rather indirect without actual measurements of cellular or subcellular calcium concentrations and fluxes. These reports relied on methods such as comparing the responses in the presence and absence of extracellular calcium and using more or less specific calcium ionophores (A23187 and X537A) or calcium channels blockers (verapamil, D-600). These experiments provided an impressive amount of useful information. Nevertheless, the interpretation of these experiments is sometimes difficult. For instance, the specificity and the mode of action of ionophores and of verapamil, and the influence of the extracellular calcium concentration on intracellular calcium stores are not well established (*Borle* and *Studer* 1978; *Borle* 1978b; *Blackmore* et al. 1979c; *Friedmann* et al. 1979). In recent years, many investigators have studied the effects of various stimuli – mostly hormonal – on several aspects of cellular calcium metabolism (Table 30). In this review I will survey the latter, omitting a wealth of elegant but indirect studies that did not report any actual measurements of cellular calcium metabolism. Two important but highly specialized topics, excitation–contraction coupling and stimulus–secretion coupling, which have been the subject of many reviews, will not be covered here.

Table 30. Hormones and other regulators affecting cell calcium metabolism

	Tissue	Reference
Peptide hormones and cyclic nucleotides		
Cyclic AMP and dibutyryl cyclic AMP	Liver	*Friedmann* and *Park* 1968; *Friedmann* and *Rasmussen* 1970; *Wallach* et al. 1971; *Friedmann* 1972; *Borle* 1973b, 1974b, 1976; *Foden* and *Randle* 1978; *Andia-Waltenbaugh* et al. 1978; *Juzu* and *Holdsworth* 1980
	Kidney	*Borle* 1972b, 1973b, 1974b, 1975b; *Borle* and *Uchikawa* 1979; *Wrenn* and *Biddulph* 1979a,b
	Heart	*Entman* et al. 1969; *Kirchberger* et al. 1972; *Meinert* et al. 1973a, b; *Tada* et al. 1974; *Borle* 1974b; *Badyshtor* and *Seredenin* 1977; *Weller* and *Laing* 1979; *Hicks* et al. 1979
	Skeletal muscle	*Cheng* and *Chen* 1975; *Bornet* et al. 1977
	Smooth muscle	*Anderson* et al. 1975; *Webb* and *Bhalla* 1976; *Thorens* and *Haeusler* 1978; *Bhalla* et al. 1979
	Uterus	*Krall* et al. 1976; *Nishikori* et al. 1977; *Nishikori* and *Maero* 1979
	Pancreas	*Brisson* et al. 1972; *Brisson* and *Malaisse* 1973; *Howell* et al. 1975; *Sehlin* 1976; *Kondo* and *Schulz* 1976; *Singh* 1979
	Salivary glands	*Prince* et al. 1972; *Prince* and *Berridge* 1973; *Kanagasuntheram* and *Randle* 1976; *Miller* and *Nelson* 1977
	Bone	*Vaes* 1968; *Klein* and *Raisz* 1971; *Herrman-Erlee* and *v.d. Meer* 1974
	Adipocytes	*Hope-Gill* et al. 1975
	Adrenals	*Matlib* and *O'Brien* 1974
	Platelets	*Käser-Glanzman* 1977
Parathyroid hormone	Kidney	*Caulfield* and *Schrag* 1964; *Cohn* et al. 1967; *Borle* 1968a–c, 1970a, b, 1972b, 1973, 1975b, c; *Nagata* and *Rasmussen* 1968, 1970; *Biddulph* and *Wrenn* 1977a, b; *Harada* et al. 1978; *Borle* and *Uchikawa* 1978; *Wrenn* and *Biddulph* 1979a, b; *Biddulph* et al. 1979
	Liver	*Wallach* et al. 1971; *Chausmer* et al. 1972
	Bone	*Vaes* 1968; *Nichols* and *Rogers* 1971; *Klein* and *Raisz* 1971; *Herrman-Erlee* and *v.d. Meer* 1974; *Dziak* and *Stern* 1975
	Chondrocytes	*Deshmukh* et al. 1977
	Frog skin	*Watlington* et al. 1968

Table 30 (continued)

	Tissue	Reference
Calcitonin	Kidney	*Borle* 1969, 1973a, 1975a, c
	Liver	*Yamaguchi* et al. 1975; *Yamaguchi* 1979
	Bone	*Harrell* et al. 1973, 1976; *Binderman* et al. 1974; *Eilam* et al. 1980
	Chondrocytes	*Deshmukh* et al. 1977
	Heart	*Hakim* 1973
Glucagon	Liver	*Friedmann* and *Park* 1968; *Friedmann* and *Rasmussen* 1970; *Yamazaki* 1975; *Keppens* et al. 1977; *Assimacopoulos-Jeannet* et al. 1977; *Hughes* and *Barritt* 1978, 1979; *Barritt* 1978; *Foden* and *Randle* 1978; *Andia-Waltenbaugh* et al. 1978; *Andia-Waltenbaugh* and *Friedmann* 1978; *Chen* et al. 1978; *Bygrave* and *Trauter* 1978; *Blackmore* et al. 1978, 1979a, b; *Friedmann* et al. 1979
	Heart	*Entman* et al. 1969; *Visscher* and *Lee* 1972
Catecholamines, α-agonists	Liver	*Friedmann* and *Park* 1968; *Assimacopoulos-Jeannet* et al. 1977; *Keppens* et al. 1977; *Foden* and *Randle* 1978; *Chen* et al. 1978 *Blackmore* et al. 1978, 1979a, b; *Chan* et al. 1979; *Parker* and *Barritt* 1979; *Babcock* et al. 1979; *Garrison* et al. 1979; *Exton* 1980
	Heart	*Entman* et al. 1969
	Smooth muscle	*Deth* and *Van Breemen* 1977
	Parotid gland	*Miller* and *Nelson* 1977
Angiotensin, vasopressin, oxytocin	Liver	*DeWulf* and *Keppens* 1976; *Keppens* et al. 1977; *Chen* et al. 1978; *Chan* et al. 1979; *Blackmore* et al. 1979b; *Garrison* et al. 1979
	Kidney	*Besley* and *Snart* 1971
	Uterus	*Akerman* and *Wikström* 1979; *Carsten* 1979
Insulin	Liver	*Dorman* et al. 1975; *Andia-Waltenbaugh* et al. 1978; *Andia-Waltenbaugh* and *Friedmann* 1978; *Blackmore* et al. 1979a; *Bömmery* and *Dargel* 1979
	Muscle	*Clausen* and *Martin* 1977; *Schudt* et al. 1976; *Grinstein* and *Erlij* 1976; *Clausen* 1977
	Adipocytes	*Hope-Gill* et al. 1975, 1976; *Kissebah* et al. 1975; *McDonald* et al. 1976a, b, 1978; *Clausen* and *Martin* 1977
	Others	*Hales* et al. 1977

Table 30 (continued)

	Tissue	Reference
Cholecystokinin, pancreozymin, and other secretagogues	Pancreas	*Matthews* et al. 1973; *Case* and *Clausen* 1973; *Heisler* and *Grondin* 1973; *Chandler* and *Williams* 1974; *Gardner* et al. 1975; *Clemente* and *Meldolesi* 1975; *Christopher* et al. 1976; *Schreurs* et al. 1976; *Deschott-Lanckman* et al. 1976; *Kondo* and *Schulz* 1976a, b; *Schulz* et al. 1977; *Gardner* and *Hahne* 1977; *Mag* et al. 1978; *Renckens* et al. 1978; *Lucas* et al. 1978; *Peterson* and *Iwatsuki* 1978; *Berridge* and *Fain* 1979; *Fain* and *Berridge* 1979; *Singh* 1979; *Schulz* and *Stolze* 1980
Thyroxine	Muscle	*Fanburg* 1968; *Suko* 1971; *Nayler* et al. 1971; *Harris* et al. 1979
Histamine	Mast cells	*Foreman* et al. 1977
Somatostatin	Pancreas	*Ishibashi* et al. 1979; *Bent-Hansen* et al. 1979
	Kidney	*Lupianez* et al. 1979
	Others	*Johansson* and *Josefsson* 1978; *Hayasaki-Kimura* and *Takahashi* 1979
Steroid hormones		
Cholecalciferol and its metabolites	Intestine	*Haussler* et al. 1970; *Kimberg* et al. 1971; *Carre* et al. 1974; *Borle* 1974a, 1975c; *Walling* et al. 1976; *Krawitt* et al. 1976; *Freedman* et al. 1977; *Baski* and *Kenny* 1978; *Fuchs* and *Peterlik* 1979; *Oswald* and *Binswanger* 1979; *Petith* et al. 1979; *Rasmussen* et al. 1979; *Feher* and *Wasserman* 1979
	Bone	*Raisz* et al. 1972; *Dziak* 1978
Glucocorticoids	Liver	*Kimura* and *Rasmussen* 1977; *Hughes* 1979; *Hughes* and *Barritt* 1979
	Intestine	*Kimberg* et al. 1971; *Krawitt* and *Stubbert* 1972; *Carre* et al. 1974; *Feher* and *Wasserman* 1979
	Bone	*Stern* 1969; *Raisz* et al. 1972; *Yasumura* 1976; *Hahn* and *Halstead* 1979
Gonadal hormones	Uterus	*Batra* and *Bengtsson* 1978; *Carsten* 1979; *Calixto* et al. 1979; *Rubanyi* and *Kovach* 1979
	Others	*Caputo* et al. 1976; *Baski* and *Kenny* 1978; *Brachet* 1978; *Navickis* et al. 1979; *Kahn* 1979
Others		
Prostaglandins	Kidney	*Biddulph* et al. 1979; *Wrenn* and *Biddulph* 1979b
	Bone	*Dziak* et al. 1979

Table 30 (continued)

	Tissue	Reference
	Liver	*Carafoli* and *Crovetti* 1973; *Malmström* and *Carafoli* 1975
	Platelets	*Rodan* and *Rodan* 1976; *Gerrard* et al. 1977, 1979
	Others	*Rasmussen* et al. 1975; *Schrier* et al. 1975; *Reed* 1977; *Greenway* and *Himms-Hagen* 1978; *Yonaga* and *Morimoto* 1979; *Kahn* and *Brachet* 1979; *Harris* et al. 1979
Calmodulin	Erythrocytes, heart, intestine, etc.	*Gopinath* and *Vincenzi* 1977; *Katz* and *Remtulla* 1978; *LePeuch* et al. 1979; *Niggli* et al. 1979; *Sudden* et al. 1979; *Vincenzi* 1979; *Wang* and *Waisman* 1979; *Ilundain* and *Naftalin* 1979
Glucose	Pancreas	*Malaisse-Lagae* and *Malaisse* 1971; *Schmidt* et al. 1976; *Hellman* et al. 1976a, b; *Kikuchi* et al. 1978; *Frankel* et al. 1978; *Hellman* and *Anderson* 1978; *Wollheim* et al. 1978; *Gylfe* et al. 1978; *Tsumura* et al. 1979; *Hersdmetz* et al. 1979, 1980; *Herschnelz* and *Malaisse* 1980

13.1 Cyclic AMP

Most peptide hormones bind to specific receptors on the membrane of their target cells, stimulate the enzyme adenylate cyclase, and increase the intracellular concentration of cyclic AMP. Several other cyclic nucleotides such as cyclic GMP are also affected and there may be complex interactions among cyclic AMP, cyclic GMP, and calcium. These interactions are beyond the scope of this review and have been discussed elsewhere (*Rasmussen* 1970; *Rasmussen* et al. 1975; *Rasmussen* and *Goodman* 1977; *Berridge* 1975, 1979).

In 1970 *Rasmussen* proposed that some of the actions of the 'second messenger' cyclic AMP could be mediated by fluctuations in cytosolic calcium activity (*Rasmussen* 1970). This assertion has never been proven directly because it is still technically impossible to measure cytosolic free calcium in small mammalian cells. Nevertheless, much evidence supports the hypothesis that cyclic AMP influences cellular calcium metabolism.

13.1.1 Effects of Cyclic AMP or Dibutyryl Cyclic AMP on Subcellular Organelles

The effects of cyclic AMP have been studied in microsomes isolated from the sarcoplasmic or endoplasmic reticulum of several different tissues. In all cases cyclic AMP stimulates microsomal calcium uptake. This has been

shown in heart (*Entman* et al. 1969; *Kirchberger* et al. 1972; *Tada* et al. 1974; *Weller* and *Laing* 1979; *Hicks* et al. 1979), in skeletal muscle (*Bornet* et al. 1977), in uterine muscle (*Krall* et al. 1976; *Nishikori* et al. 1977; *Nishikori* and *Maeno* 1979), in vascular smooth muscle (*Webb* and *Bhalla* 1976; *Thorens* and *Haeusler* 1978; *Bhalla* et al. 1979), in colon smooth muscle (*Andersson* et al. 1975), and in platelets (*Käser* and *Glanzmann* 1977). In all these studies concentrations of cyclic AMP varying between 10^{-7} and $5 \cdot 10^{-6}$ M were used, and it has been shown that concentrations greater than 10^{-5} M could inhibit microsomal calcium uptake (*Thorens* and *Haeusler* 1978). This may explain one negative report showing that 10^{-5}–10^{-4} M cyclic AMP inhibits calcium uptake in microsomes isolated from pancreatic islets (*Sehlin* 1976). In most cases the stimulation of microsomal calcium uptake by cyclic AMP requires the presence of protein kinase (*Kirchberger* et al. 1972; *Tada* et al. 1974; *Bornet* et al. 1977; *Käser* and *Glanzmann* 1977; *Thorens* and *Haeusler* 1978; *Bhalla* et al. 1979). However, two reports show that the presence of protein kinase is not necessary (*Bhalla* et al. 1979) or that the action of cyclic AMP does not require protein phosphorylation (*Weller* and *Laing* 1979). Most of these studies have been conducted with cyclic AMP, although its dibutyryl derivative is also effective (*Nishikori* et al. 1977).

The effects of cyclic AMP on mitochondrial calcium transport are more controversial. In 1973 *Borle* reported that cyclic AMP triggered a release of calcium from mitochondria isolated from liver, kidney or heart. Further studies showed that this release of mitochondrial calcium increases the concentration of ionized calcium in the extramitochondrial medium and that increasing the phosphate concentration of the medium could modulate the effects of cyclic AMP (*Borle* 1974b, 1975d). The effective range of cyclic AMP concentrations capable of releasing mitochondrial calcium was fairly narrow, from $5 \cdot 10^{-7}$ to $5 \cdot 10^{-6}$ M. These results were confirmed in mitochondria isolated from adrenal medullae (*Matlib* and *O'Brien* 1974). Furthermore, cyclic AMP inhibits calcium uptake by mitochondria isolated from liver, kidney, colon smooth muscle, uterus, and pancreatic islets (*Borle* 1974b; *Andersson* et al. 1975; *Howell* et al. 1975; *Nishikori* et al. 1977). However, a majority of investigators have been unable to reproduce these results, specifically to obtain a release of mitochondrial calcium with low concentrations of cyclic AMP (*Scarpa* et al. 196; *Borle* 1976; *Schotland* and *Mela* 1977). In spite of the importance of this problem, the physiologic implications of these results obtained in vitro in isolated mitochondria should be kept in abeyance until new and reproducible data are published. Recently two reports revived the issue by showing that cyclic AMP could induce a release of calcium from mitochondria isolated from heart and liver (*Badyshov* and *Seredenin* 1977; *Juzu* and *Holdsworth* 1980).

13.1.2 Effects of Cyclic AMP or Dibutyryl Cyclic AMP in Intact Cells

In liver, kidney, pancreas, adipocytes, barnacle muscle, bone, and salivary glands, cyclic AMP and its dibutyryl derivative stimulate the efflux of ^{45}Ca from labeled cells or tissues (*Vaes* 1968; *Friedmann* and *Park* 1968; *Friedmann* and *Rasmussen* 1970; *Wallach* et al. 1971; *Klein* and *Raisz* 1971; *Borle* 1972b, 1975b; *Prince* et al. 1972; *Prince* and *Berridge* 1973; *Herrman-Erlee* and *v.d. Meer* 1974; *Cheng* and *Chen* 1975; *Hope-Gill* et al. 1975; *Kanagasuntheram* and *Randle* 1976; *Foden* and *Randle* 1978; *Borle* and *Uchikawa* 1979; *Wrenn* and *Biddulph* 1979a, b; *Singh* 1979). More importantly, the increased efflux of ^{45}Ca induced by cyclic AMP from all these tissues occurs in the absence of extracellular calcium (*Friedmann* and *Park* 1968; *Prince* et al. 1972; *Borle* 1975b; *Cheng* and *Chen* 1975; *Kanagasuntheram* and *Randle* 1976; *Singh* 1979). This proves that cAMP mobilizes calcium from an intracellular compartment. In the presence of extracellular calcium, cyclic AMP and dibutyryl cAMP also stimulates the uptake of ^{45}Ca by kidney, heart, pancreatic beta-cells, and parotid glands (*Meinertz* et al. 1973a, b; *Miller* and *Nelson* 1977; *Borle* and *Uchikawa* 1979), indicating an increased exchangeable pool of calcium. The total calcium of kidney cells is also increased by cyclic AMP (*Borle* and *Uchikawa* 1979), although total calcium is unchanged in heart and parotid glands (*Meinertz* et al. 1973a, b; *Miller* and *Nelson* 1977). Kinetic analyses of ^{45}Ca desaturation curves obtained in cultered kidney cells also show that the two main intracellular exchangeable calcium pools, the cytosolic pool and the mitochondrial pool, are increased by cyclic AMP. In addition, cyclic AMP stimulates the steady-state calcium fluxes, i.e., the calcium exchange across the plasma membrane and across the mitochondrial membrane (*Borle* 1972b; *Borle* and *Uchikawa* 1979). In pancreatic islets dibutyryl cyclic AMP also enhances ^{45}Ca uptake while theophylline, which is assumed to increase the intracellular concentration of cyclic AMP, stimulates ^{45}Ca efflux from prelabeled cells (*Brisson* et al. 1972; *Brisson* and *Malaisse* 1973). The intracellular compartment from which calcium is mobilized by cyclic AMP in the absence of extracellular calcium is not firmly identified. It could be a pool of calcium bound to specific or nonspecific ligands in the cell cytosol or calcium stored in the endoplasmic reticulum or calcium sequestered in mitochondria. There is no evidence that cyclic AMP alters calcium binding to nonspecific binding sites. On the other hand, cyclic AMP is known to enhance calcium uptake by microsomes isolated from endoplasmic or sarcoplasmic reticulum (*Entman* et al. 1969; *Kirchberger* et al. 1972; *Tada* et al. 1974; *Andersson* et al. 1975; *Krall* et al. 1976; *Webb* and *Bhalla* 1976; *Bornet* et al. 1977; *Käser* and *Glanzmann* 1977; *Nishikori* et al. 1977; *Thorens* and *Haeusler* 1978; *Bhalla* et al. 1979; *Hicks* et al. 1979; *Nishikori* and *Maeno* 1979; *Weller* and *Laing*

1979), so that this compartment is an improbable candidate. Thus, the only likely source of calcium would be the mitochondria. And indeed, in parotid glands labeled with ^{45}Ca and incubated in calcium-free media, cyclic AMP depresses the ^{45}Ca content of mitochondria isolated at the end of the experiment compared to untreated controls (*Kanagasuntheram* and *Randle* 1976). In addition, the several, although controversial, reports showing a release of calcium from isolated mitochondria would also favor this hypothesis (*Borle* 1974b; *Andersson* et al. 1975; *Howell* et al. 1975; *Nishikori* et al. 1977).

Regardless of the intracellular source of calcium mobilized by cyclic AMP, there is an apparent conflict between the results obtained in the presence and in the absence of extracellular calcium: an increased calcium uptake in the first case and an increased efflux in the second. It is of course possible that cyclic AMP acts at two different locations: at the intracellular calcium compartment and at the cell plasma membrane. Alternatively, we have proposed that the increased ^{45}Ca uptake by the cells may be secondary to an increased cytosolic free calcium concentration induced by the release of calcium from the intracellular compartment (*Borle* 1975c; *Borle* and *Anderson* 1976; *DiPolo* 1979). If the rise in cytosolic calcium activity stimulates the passive calcium influx more than it enhances the active efflux of calcium from the cell, the increased ^{45}Ca uptake and the enlarged exchangeable calcium pools in the presence of extracellular calcium could be explained.

13.2 Parathyroid Hormone

Parathyroid hormone (PTH) regulates calcium metabolism of bone and kidney cells, although other tissues, such as liver and cartilage, respond to PTH as well. PTH increases the cellular concentration of cyclic AMP in bone (*Chase* et al. 1969; *Chase* and *Aurbach* 1970; *Peck* et al. 1973; *Rodan* and *Rodan* 1974; *Kakuta* et al. 1975; *Wong* et al. 1977) and in kidney (*Melson* et al. 1970; *Borle* 1972b; *Chabardes* et al. 1975; *Biddulph* and *Wrenn* 1977a, b; *Wrenn* and *Biddulph* 1979a, b; *Biddulph* et al. 1979). There is, however, some doubt as to whether the effects of PTH on cell calcium metabolism are mediated by cyclic AMP. Some investigators doubt it (*Butlen* and *Jard* 1972; *Agus* et al. 1973; *Kuntziger* et al. 1974; *Dziak* and *Stern* 1975), but others have shown that cyclic AMP or its dibutyryl derivative mimic the effects of PTH on calcium metabolism in bone (*Vaes* 1968; *Klein* and *Raisz* 1971; *Hermann-Erlee* and *v.d. Meer* 1974) and in kidney (*Burnatowska* et al. 1977; *Borle* and *Uchikawa* 1979; *Wrenn* and *Biddulph* 1979a, b).

In kidney cells PTH increases the uptake of ^{45}Ca (*Borle* 1968a–c, 1970b, 1975c; *Borle* and *Uchikawa* 1978). This rise in tracer uptake reflects both an increased calcium influx and a greater exchangeable pool. The effect of PTH occurs even in the absence of extracellular phosphate (*Borle* 1970b). PTH also increases the total kidney cell calcium (*Borle* 1968b, 1970b, 1975c; *Borle* and *Uchikawa* 1978). On the other hand, PTH stimulates ^{45}Ca efflux from prelabeled cells (*Borle* 1972b, 1975c; *Biddulph* and *Wrenn* 1977a, b; *Borle* and *Uchikawa* 1978; *Biddulph* et al. 1979; *Wrenn* 1979a, b). This increased ^{45}Ca efflux also occurs in the absence of extracellular calcium, indicating that PTH mobilizes calcium from an intracellular compartment. Kinetic analyses of steady-state ^{45}Ca desaturation curves show that the two exchangeable calcium compartments, cytosolic and mitochondrial, are increased by PTH and that calcium exchange across the mitochondrial membrane and the plasma membrane are both enhanced (*Borle* 1972b; *Borle* and *Uchikawa* 1978). Furthermore, the calcium content of mitochondria is increased after the administration of PTH in vivo or after an endogenous rise in circulating hormone (*Caulfield* and *Shrag* 1964; *Cohn* et al. 1967; *Borle* and *Clark* 1981). One report also shows that PTH administered to a perfused kidney stimulates calcium uptake by microsomes isolated from endoplasmic reticulum or sarcolemma (*Harada* et al. 1978). The concentration of free calcium in the cytosol has never been measured directly, but indirect evidence suggests that it is elevated by PTH (*Nagata* and *Rasmussen* 1968, 1970). In bone cells, in cultured bone rudiments or in chondrocytes PTH has similar effects: it increases ^{45}Ca uptake (*Nichols* and *Rogers* 1971; *Dziak* and *Stern* 1975; *Deshmukh* et al. 1977) but it also stimulates ^{45}Ca efflux (*Vaes* 1968; *Nichols* and *Rogers* 1971; *Klein* and *Raisz* 1971; *Hermann-Erlee* and *v.d. Meer* 1974; *Deshmukh* et al. 1977). In liver PTH increases both the total calcium and the uptake of ^{45}Ca (*Wallach* et al. 1971; *Chausmer* et al. 1972). Finally, in frog skin PTH has been reported to stimulate calcium influx (*Watlington* et al. 1968).

Phosphate appears to enhance the sequestration of calcium induced by PTH in kidney and bone (*Borle* 1970b; *Nichols* and *Rogers* 1971), while it inhibits the hormone effect on calcium efflux (*Nichols* and *Rogers* 1971; *Borle* 1973a).

The primary site of action of PTH and the sequence of events leading to these oberved changes in cellular calcium metabolism are still uncertain. It was first proposed that PTH increases the permeability of cell membranes or somehow stimulates calcium influx (*Borle* 1968b, c, 1970a, b). That would lead to an increased cytosolic calcium, an increased calcium efflux, and a sequestration of calcium in all cellular compartments. However, this interpretation cannot account for the mobilization and increased calcium efflux from the cell in the total absence of extracellular calcium.

Therefore, an alternative explanation was later advanced suggesting that, with a rise in cyclic AMP, PTH initially stimulates the release of calcium from an intracellular compartment, presumably the mitochondria, and increases the cytosolic free calcium. The rise in cytosolic free calcium secondarily enhances calcium efflux out of the cell by stimulating the 'calcium pump,' which depends on the CaMgATPase. In addition, the increased cytosolic calcium also stimulates calcium influx by the mechanism described earlier. Since the total calcium increases and all intracellular calcium compartments are larger, one can assume that the effect of cytosolic calcium on influx is greater than the effect on efflux. The net gain of cellular calcium would be sequestered in mitochondria (*Borle* 1973a, 1975c). Although this hypothesis is far from proven, a computer model of cellular calcium metabolism shows that the proposed sequence of events is possible and kinetically sound (*Borle* 1975c; *Borle* and *Anderson* 1976).

13.3 Calcitonin

The physiologic effects of calcitonin on bone, kidney, and plasma calcium are usually opposite to those of PTH. Nevertheless, like PTH, calcitonin increases the cellular concentration of cyclic AMP in bone and in kidney (*Chase* and *Aurbach* 1970; *Melson* et al. 1970; *Heershe* et al. 1974; *Rodan* and *Rodan* 1974; *Wong* et al. 1977). On a molar basis, calcitonin is about 100 times less active than PTH (*Rodan* and *Rodan* 1974) and the effects of calcitonin and PTH on cyclic AMP are additive (*Heersche* et al. 1974; *Rodan* and *Rodan* 1974). Whether the two hormones act on different cells (*Wong* et al. 1977) or on different cellular pools of cyclic AMP in the same cell or whether calcitonin increases cyclic AMP by the derepression of adenyl cyclase induced by a lower cytosolic calcium is unknown (*Rasmussen* and *Bordier* 1974; *Rasmussen* et al. 1975). The issue is beyond the scope of this review.

Calcitonin increases the total cellular calcium in kidney cells (*Borle* 1969, 1975a, c), in bone cells (*Eilam* et al. 1980), and in liver (*Yamaguchi* et al. 1975; *Yamaguchi* 1979). It also stimulates the uptake of ^{45}Ca in kidney cells (*Borle* 1969c, 1975a, c), bone cells (*Harell* et al. 1973, 1975; *Binderman* et al. 1974; *Eilam* 1980), and chondrocytes (*Deshmukh* et al. 1977). In kidney cells this increase in ^{45}Ca uptake reflects only an increased intracellular exchangeable calcium pool and not an increased influx because the steady-state calcium exchange across the plasma membrane is actually depressed by calcitonin (*Borle* 1975a). In agreement with this latter finding, ^{45}Ca efflux is depressed by calcitonin in kidney cells and in bone cells (*Borle* 1969c, 1975c; *Harell* et al. 1973; *Eilam* 1980). The increased cellular calcium appears to be sequestered in a cellular pool identified with mitochondria (*Borle* 1973a, 1975a; *Eilam* 1980).

Calcitonin stimulates calcium uptake in isolated mitochondria and in heart sarcoplasmic reticulum, but the physiologic significance of these results remains to be proven (*Hakim* 1973; *Borle* 1975a). On the other hand, calcitonin has been reported to depress the CaATPase activity in rat liver plasma membrane (*Yamaguchi* 1979). The effects of calcitonin are enhanced by phosphate and abolished by inhibitors of mitochondrial calcium uptake both in kidney and in bone (*Borle* 1975a; *Härell* et al. 1976; *Eilam* 1980), suggesting that the hormone may act by stimulating the transport or the sequestration of calcium by mitochondria (*Borle* 1973a, 1975a, c). Finally, although the effect of calcitonin on the cytosol ionized calcium has never been measured directly, indirect evidence suggests that the hormone lowers it (*MacManus* and *Whitfield* 1970; *Whitfield* et al. 1971; *Rasmussen* and *Bordier* 1974).

In order to explain the increased cellular sequestration of calcium, the depressed calcium efflux, and the assumed low cytosolic calcium activity induced by calcitonin, the following sequence of events has been proposed: calcitonin acting through an unknown intracellular messenger would primarily stimulate the uptake or the sequestration of calcium into mitochondria. This would lower the cytosolic concentration of free calcium and lead to a depression of calcium efflux out of the cell and of calcium transport in general (*Borle* 1973a, 1975c). Although this could explain most of the experimental results obtained in many different cells, this hypothesis has yet to be conclusively proven.

13.4 Glucagon

Glucagon increases cyclic AMP and also affects calcium metabolism in liver cells. But it is not firmly established whether the change in liver cell calcium is accidental or whether it is a necessary step in the sequence of events from adenylate cyclase activation to glucose formation. Calcium, for instance, activates phosphorylase b kinase, which catalyzes glycogen breakdown and suppresses glycogen synthetase (*Walsh* et al. 1968, 1970; *Soderling* and *Hickenbottom* 1970; *Exton* et al. 1972; *Exton* and *Park* 1972; *Hems* and *Whitton* 1980).

Glucagon appears to stimulate the uptake of ^{45}Ca by the liver (*Assimacopoulos-Jeannet* et al. 1977; *Keppens* et al. 1977). The total calcium, however, is unchanged (*Blackmore* et al. 1978) or slightly depressed (*Chen* et al. 1978) although high concentrations of glucagon may be required for this effect to be observed (*Blackmore* et al. 1978). The most consistent finding is the stimulation of ^{45}Ca efflux induced by the hormone (*Friedmann* and *Park* 1968; *Friedmann* and *Rasmussen* 1970; *Assimacopoulos-Jeannet* et al. 1977; *Chen* et al. 1978; *Foden* and *Randle* 1978; *Blackmore*

et al. 1978, 1979a, b; *Friedmann* et al. 1979). This stimulation of ^{45}Ca efflux from liver by glucagon occurs even in the absence of extracellular calcium (*Friedmann* and *Park* 1968; *Foden* and *Randle* 1978) or with the very low medium calcium concentration of $2 \cdot 10^{-5}$ M (*Chen* et al. 1978). The increased ^{45}Ca efflux induced by glucagon is inhibited by prior treatment with inhibitors of mitochondrial calcium uptake, FCCP, DNP or KCN (*Chen* et al. 1978), suggesting that the source of calcium mobilized by the hormone may be the mitochondria.

Glucagon, administered in vivo, also affects calcium transport in isolated organelles. The results are not always consisten, however. Some investigators found that glucagon stimulates mitochondrial calcium uptake (*Yamazaki* 1975; *Andia-Waltenbaugh* et al. 1978; *Friedmann* et al. 1979). The effect is observed when calcium uptake is supported by succinate oxidation with a concomitant H^+ ejection from mitochondria, but not when ATP is used as substrate (*Yamazaki* 1975). In other experiments, under somewhat unphysiologic conditions, glucagon also appears to increase the calcium retention time of mitochondria, an effect that is enhanced by phosphate (*Hughes* and *Barritt* 1978, 1979; *Barritt* et al. 1978). On the other hand, other investigators have reported an inhibition of mitochondrial calcium uptake induced by glucagon (*Foden* and *Randle* 1978) or even an increased efflux (*Blackmore* et al. 1979b).

Glucagon has been reported to stimulate calcium uptake by liver microsomal preparations (*Andia-Waltenbaugh* and *Friedmann* 1978) or by a ruthenium red-insensitive fraction probably derived from endoplasmic reticulum (*Bygrave* and *Trauter* 1978).

In heart muscle glucagon seems to have no effect on ^{45}Ca uptake or efflux. But since it increases tension without stimulating calcium influx it has been proposed that the hormone induces a redistribution of intracellular calcium (*Visscher* and *Lee* 1972). Another report shows an increased calcium uptake by cardiac microsomes induced by glucagon (*Entman* et al. 1969).

Although all these reports suggest that glucagon influences intracellular calcium, the significance of these findings and their relationship to the elevation in cellular cyclic AMP are still unclear.

13.5 Catecholamines

The effects of catecholamines and of other α-adrenergic agonists on cell calcium have recently been reviewed by *Exton* (1980). It is likely that their action on cell intermediary metabolism is independent of cyclic AMP and is mediated by calcium (*Garrison* et al. 1979; *Exton* 1980). Catecholamines have been reported to decrease the total liver cell calcium (*Chen* et

et al. 1978; *Blackmore* et al. 1978, 1979a; *Chan* et al. 1979) although two reports claim the opposite (*Foden* and *Randle* 1978; *Exton* 1980). Catecholamines enhance the uptake of ^{45}Ca by liver cells (*Keppens* et al. 1977; *Assimacopoulos-Jeannet* et al. 1977; *Foden* and *Randle* 1978). These hormones also stimulate the efflux of ^{45}Ca from prelabeled liver cells (*Friedmann* and *Park* 1968; *Assimacopoulos-Jeannet* et al. 1977; *Blackmore* et al. 1978, 1979b) and from other tissues, aortic smooth muscle (*Deth* and *Van Breemen* 1977) and parotid gland (*Miller* and *Nelson* 1977). The rise in ^{45}Ca efflux can be observed even in the absence of extracellular calcium, suggesting a mobilization of calcium from an intracellular pool (*Friedmann* and *Park* 1968; *Deth* and *Van Breemen* 1977). The effect is blocked by inhibitors of mitochondrial calcium uptake, which indicates that the source of intracellular calcium is the mitochondria (*Chen* et al. 1978). On the other hand, two reports record a depression of ^{45}Ca efflux induced by catecholamines (*Foden* and *Randle* 1978; *Chen* et al. 1978). The reason for these conflicting results is unclear. Catecholamines stimulate the efflux of calcium from liver mitochondria and decrease their calcium content (*Babcock* et al. 1979; *Blackmore* et al. 1979a, b; *Chan* et al. 1979). Finally, catecholamines have been reported to stimulate ^{45}Ca uptake by cardiac sarcoplasmic reticulum vesicles, an action mimicked by glucagon and cyclic AMP (*Entman* et al. 1969).

It has been proposed that catecholamines, through the action of an unknown intracellular messenger, mobilize calcium from the mitochondria and elevate the cytosolic free calcium; the rise in cytosolic calcium stimulates phosphorylase b kinase and other calcium-sensitive enzymes; at the same time, calcium efflux is increased (*Blackmore* et al. 1978, 1979b; *Garrison* et al. 1979). To explain the increased ^{45}Ca uptake induced by these hormones, one has to invoke again a stimulation of calcium influx induced by the rise in cytosolic calcium (see above).

13.6 Other Hormones

Table 30 lists several other peptide or steroid hormones that have been reported to affect cellular calcium in one way or another. Several hypotheses have been advanced for their mode of action but the available data are still too fragmentary to be reviewed in detail and to conclusively prove or disprove the proposed mechanisms.

In addition, metabolic substrates such as phosphoenolpyruvate or glucose and regulatory proteins such as calmodulin are likely candidates for the role of regulators of cell calcium metabolism. Much information may appear in the literature in the near future.

14 Conclusions

The importance of cellular calcium metabolism in cell function need not be emphasized. The range of cellular processes controlled, regulated or modulated by calcium is wide and still expanding. The best known are contraction, secretion, enzyme activation, and the regulation of metabolism, growth, motility, and ion transport. In most cases, calcium acts as a signal transducer.

Once the importance of cell calcium is recognized, it is imperative to understand the physiologic processes that control, modulate, and regulate the activity of calcium in all cellular compartments: cytosol, mitochondria, and endoplasmic (sarcoplasmic) reticulum.

The information gathered in this review appears to support the view that, with the exception of erythrocytes, the total cell calcium and its distribution among various subcellular compartments are fairly similar in all cells and in most species. The magnitude of the calcium fluxes across the plasmalemma are not very different from cell to cell and from species to species although the mechanisms may vary greatly. There is a greater difference in mitochondrial calcium transport; the number of mitochondria per cell and their affinity for calcium may vary but their high maximal velocity is about the same. The greatest difference exists between the calcium uptake by sarcoplasmic reticulum of skeletal muscle and calcium transport by endoplasmic reticulum of other cells; although their affinity for calcium may be similar, their maximal velocity differs by 2–3 orders of magnitude. This has important consequences when the relative role of each transport process (plasmalemmal, mitochondrial, SER) in the control of cytosolic free calcium is assessed. This is one of the most important problems that has yet to be conclusively settled. Other questions remain unanswered: what is the role and importance of calcium binding to various ligands of the cells, intracellular membranes, calcium binding proteins, calsequestrin, parvalbumin, and other intracellular calcium 'buffers'? Do voltage-sensitive calcium channels exist in nonexcitable cells? Is the Na–Ca exchange described in nerves and muscle operative in other cells? What is the role of calmodulin in the regulation of cellular calcium metabolism? What are the cellular targets of hormones that influence cell calcium metabolism? Besides the known cyclic nucleotides, what are the other intracellular messengers that may affect cell calcium?

One of the main obstacles to our full understanding of cellular calcium metabolism is the technical difficulty (until recently the technical impossibility) of measuring the concentration of free calcium in the cell cytosol. Cytosolic free calcium is the most important parameter of cell calcium metabolism that has yet to be measured with confidence, if at all. Once

pCa microelectrodes or techniques using ionized calcium-sensitive indicators are applied to the study of small mammalian cells, a large gap will be filled in our understanding of cellular calcium metabolism. Until then, the field still belongs to the realm of hypothesis and controversy.

References

Adrian RH (1956) The effect of internal and external potassium concentration on the membrane potential of frog muscle. J Physiol (Lond) 133:631–658

Affolter H, Chiesi M, Dabrowska R, Carafoli E (1976) Calcium regulation in heart cells. The interaction of mitochondrial and sarcoplasmic reticulum with troponin-bound calcium. Eur J Biochem 67:389–396

Aguirre J, Pinto JEB, Trifaro JM (1977) Calcium movements during the release of catecholamines from the adrenal medulla: effects of methoxyverapamil and external cations. J Physiol (Lond) 269:371–394

Agus ZS, Gardner LB, Beck LH, Goldberg M (1973) Effect of parathyroid hormone on renal reabsorption of calcium, sodium and phosphate. Am J Physiol 224:1143–1148

Akerman KEO (1978a) Effect of pH and Ca^{2+} on the retention of Ca^{2+} by rat liver mitochondria. Arch Biochem Biophys 189:256–252

Akerman KEO (1978b) Changes in membrane potential during calcium ion influx and efflux across the mitochondrial membrane. Biochim Biophys Acta 502:359–366

Akerman KEO, Wikström MKF (1979) (Ca^{2+} + Mg^{2+})-stimulated ATPase activity of rabbit myometrium plasma membrane is blocked by oxytocin. FEBS Lett 97:283–287

Allard C, Mathieu R, deLamirande G, Cantero A (1952) Mitochondrial population in mammalian cells. I. Description of a counting technique and preliminary results in rat liver in different physiological and pathological conditions. Cancer Res 12:407–412

Alonso GL, Bazerque PM, Arrigo DM, Tumilasci OR (1971) Adenosine triphosphate-dependent calcium uptake by rat submaxillary gland microsomes. J Gen Physiol 58:340–350

Andersson R, Nilsson K, Wikberg Y, Johansson S, Momhe-Lundholm E, Lundholm L (1975) Cyclic nucleotides and the contraction of smooth muscle. Adv Cyclic Nucleotide Res 5:491–518

Andia-Waltenbaugh AM, Friedmann N (1978) Hormone sensitive calcium uptake by liver microsomes. Biochem Biophys Res Commun 82:603–608

Andia-Waltenbaugh AM, Kimura S, Wood J, Divakaran P, Friedmann N (1978) Effects of glucagon, insulin and cyclic-AMP on mitochondrial calcium uptake in the liver. Life Sci 23:2437–2444

Anghileri LJ (1972) Phospholipids and calcium uptake in experimental tumors. Oncology 28:35–51

Ash GR, Bygrave FL (1977) Ruthenium red as a probe in assessing the potential of mitochondria to control intracellular calcium in liver. FEBS Lett 78:166–168

Ashley CC, Lea TJ (1978) Calcium fluxes in single muscle fibres measured with a glass scintillation probe. J Physiol (Lond) 282:307–331

Ashley CC, Caldwell PC, Lowe AG (1972) The efflux of calcium from single crab and barnacle muscle fibres. J Physiol (Lond) 223:735–755

Assimacopoulos-Jeannet FD, Blackmore PF, Exton JH (1977) Studies on α-adrenergic activation of hepatic glucose output. (Studies on role of Ca^{++}). J Biol Chem 252: 2662–2669

Au KS (1979) Observation on the protein activator of erythrocyte membrane (Ca^{2+} – Mg^{2+}) ATPase. Int J Biochem 10:637–643

Aull F (1967) Measurement of the electrical potential difference across the membrane of the Ehrlich mouse ascites cell. J Cell Physiol 69:21–32

Azzone GF, Pozzan T, Massari S, Bragadin M, Dell'Antone P (1977) H^+ site ratio and steady state distribution of divalent cations in mitochondria. FEBS Lett 78:21–24

Babcock DF, Chen J-L J, Yip BP, Lardy HA (1979) Evidence for mitochondrial localization of the hormone-responsive pool of Ca^{++} in isolated hepatocytes. J Biol Chem 254:8117–8120

Badyshtov BA, Seredenin SB (1977) Investigation of the mechanism of action of ouabain and cyclic AMP of the transport of calcium ions by rat heart mitochondria. Bull Exp Biol Med Engl Transl 83:155–158

Baker PF (1972) Transport and metabolism of calcium ions in nerve. Prog Biophys Mol Biol 24:177–223

Baker PF (1976) Regulation of intracellular Ca and Mg in squid axons. Fed Proc 35: 2589–2595

Baker PF (1978) The regulation of intracellular calcium in giant axons of Loligo and myxicola. Ann NY Acad Sci 307:250–268

Baker PF, Blaustein MP (1968) Sodium dependent uptake of calcium by crab nerve. Biochim Biophys Acta 150:167–170

Baker PF, Glitsch HG (1973) Does metabolic energy participate directly in the Na^+ dependent extension of Ca^{+2} ions from squid giant axons. J Physiol (Lond) 233: 44P–46P

Baker PF, Glitsch HG (1975) Voltage-dependent changes in the permeability of nerve membranes to calcium and other divalent ions. Philos Trans R Soc Lond (Biol) 270:389–409

Baker PF, Honerjager P (1978) Influence of carbon dioxide on the level of ionized calcium in squid axon. Nature 273:160–161

Baker PF, McNaughton PA (1976a) Kinetics and energetics of calcium efflux from intact squid giant axons. J Physiol (Lond) 259:103–144

Baker PF, McNaughton PA (1976b) The effect of membrane potential on the calcium transport systems in squid axons. J Physiol (Lond) 260:24–25P

Baker PF, McNaughton PA (1977) Selective inhibition of the Ca-dependent Na efflux from intact squid axons by a fall in intracellular pH. J Physiol (Lond) 269:78–79P

Baker PF, McNaughton PA (1978) The influence of extracellular calcium binding on the calcium efflux from squid axons. J Physiol (Lond) 276:127–150

Baker PF, Schlaepfer W (1975) Calcium uptake by axoplasm extracted from giant axons of *Loligo*. J Physiol (Lond) 249)37P–38P

Baker PF, Blaustein MP, Hodgkin AL, Steinhardt RA (1969) The influence of calcium on sodium efflux in squid axon. J Physiol (Lond) 200:431–458

Baker PF, Hodgkin AL, Ridgway EB (1971) Depolarization and calcium entry in squid giant axons. J Physiol (Lond) 218:709–755

Barnard T, Afzelius BA (1972) The matrix granules of mitochondria: a review. Subcell Biochem 1:375–389

Barritt GJ, Thorn RFW, Hughes BP (1978) Effects of hormones and $N^6O^{2'}$-dibutyruladenosine 3'5'-cyclic monophosphate, administered in vivo, on phosphate transport and metabolism in isolated rat liver mitochondria. Biochem J 172:577–585

Baski SN, Kenny AD (1978) Vitamin D metabolism in Japanese quail: gonadal hormones and dietary calcium. Am J Physiol 234:E622–E628

Batra SC (1973a) The role of mitochondrial calcium uptake in contraction and relaxation of the human myometrium. Biochim Biophys Acta 305:428–432

Batra SC (1973b) Effect of some estrogens and progesterone on calcium uptake and calcium release by myometrial mitochondria. Biochem Pharmacol 22:803–809

Batra SC (1974) The effects of drugs on calcium uptake and calcium release by mitochondria and sarcoplasmic reticulum of frog skeletal muscle. Biochem Pharmacol 23:89–101

Batra SC, Bengtsson B (1978) Effects of diethylstilboestrol and ovarian steroids on the contractile responses and calcium movements in rat uterine smooth muscle. J Physiol (Lond) 276:329–342

Batra SC, Daniel EE (1971) ATP-dependent Ca uptake by subcellular fractions of uterine smooth muscle. Comp Biochem Physiol 38A:369–385

Baumrucker CR, Keenan TW (1975) Membranes of mammary glands. X. Adenosine triphosphate dependent calcium accumulation by Golgi apparatus rich fraction from bovine mammary gland. Exp Cell Res 90:253–260

Bayer R, Kalusche D, Kaufman R, Mannhold R (1975) Inotropic and electrophysiological actions of verapamil and D-600 in mammalian myocardium. III. Effects of optical isomers on transmembrane action potentials. Arch Pharmacol 290:81–97

Beckmann A, Jenssen HL, Kalkoff W, Redman K (1970) Das bioelektrische Potential an der zytoplasmatischen Membran der Granulozyten. Experientia 26:186–187

Beigelman DM, Hollander PB (1962) Effect of insulin upon resting electrical potential of adipose tissue. Proc. Soc Exp Biol Med 110:590–595

Bennett HS (1963) Morphological aspects of extracellular polysaccharides. J Histochem Cytochem 11:14–23

Bent-Hansen L, Capito K, Hedeskov CJ (1979) The effect of calcium on somatostatin inhibition of insulin release and cyclic AMP production in mouse pancreatic islets. Biochim Biophys Acta 585:240–249

Bernhardt J, Pauly H (1967) Das Membranpotential von Ehrlich-Aszitestumorzellen. Biophysik 4:101–108

Berridge MJ (1975) The interaction of cyclic nucleotides and calcium in the control of cellular activity. Adv. Cyclic Nucleotide Res 6:1–98

Berridge MJ (1979) Relationship between calcium and the cyclic nucleotides in ion secretion. In: Binder HJ (ed) Mechanisms of intestinal secretion. Alan R Liss, New York, pp 65–82

Berridge MJ, Fain JN (1979) Inhibition of phosphatidylinositol synthesis and the inactivation of calcium entry after prolonged exposure of the blowfly salivary gland to 5-hydroxytryptamine. Biochem J 178:59–69

Berridge MJ, Oschman JC, Wall BJ (1975) Intracellular calcium reservoirs in *Calliphora* salivary glands. In: Carafoli E, Clementi F, Drabikowski W, Magreth A (eds) Calcium transport in contraction and secretion. North Holland, Amsterdam, pp 131–138

Besley GTN, Snart RN (1971) Effect of vasopressin on the uptake of calcium ions by kidney mitochondria and on the concentration of adenosine 3'-5 cyclic monophosphate in toad bladder. J Physiol (Lond) 125:60P–61P

Bhalla RC, Webb RC, Sing D, Brock T (1978) Role of cyclic AMP in rat aortic microsomal phosphorylation and calcium uptake. Am J Physiol 234:H508–H514

Bianchi CP (1968) Cell calcium. Butterworth, London

Bianchi CP, Lakshminarayanaiah N (1978) Calcium uptake and exchange in leg nerves of the crab *Libinia emarginata.* J Neurochem 30:27–33

Bianchi CP, Shanes AM (1959) Calcium influx in skeletal muscle at rest, during activity and during potassium contracture. J Gen Physiol 42:803–815

Biddulph DM, Currie MG, Wrenn RW (1979) Effects and interactions of parathyroid hormone and prostaglandins on adenosine 3'5' monophosphate concentrations in isoalted renal tubules. Endocrinology 104:1164–1171

Biddulph DM, Wrenn RW (1977a) Effect of parathyroid hormone on cyclic AMP, cyclic GMP and efflux of calcium in isolated renal tubules. J Cyclic Nucleotide Res 3:129–138

Biddulph DM, Wrenn RW (1977b) Morphology and hormonal responsiveness of renal cortical tubules in vitro. Am J Anat 150:539–558

Biedermann N (1968) Das Verhalten der Membranpotentiale von Leberzellen der Ratte während und nach Gefäßunterbindungen. Acta Biol Med Germ 21:827–833
Binderman I, Duskin D, Harell A, Sachs L, Katchalski E (1974) Formation of bone tissue in culture from isolated bone cells. J Cell Biol 61:427–439
Blackmore PF, Brumley FT, Marks JL, Exton JH (1978) Studies on α-adrenergic activation of hepatic glucose output. Relationship between α-adrenergic stimulation of calcium efflux and activation of phosphorylase in isolated rat liver parenchymal cells. J Biol Chem 253:4851–4858
Blackmore PF, Assimacopoulos-Jeannet FD, Chan TM, Exton JH (1979a) Studies on α-adrenergic activation of hepatic glucose output. Insulin inhibition of α-adrenergic and glucagon actions in normal and calcium-depleted hepatocytes. J Biol Chem 254:2828–2834
Blackmore PF, Dehaye JP, Exton JH (1979b) Studies on α-adrenergic activation of hepatic glucose output. The role of mitochondrial calcium release in α-adrenergic activation of phosphorylase in perfused rat liver. J Biol Chem 254:6945–6950
Blackmore PF, El-Refai MF, Exton JH (1979c) Alpha-adrenergic blockage and inhibiton of A23187 mediated Ca^{2+} uptake by the calcium antagonist verpamil in rat liver cells. Mol Pharmacol 15:598–606
Blaustein MP (1974) The interrelationship between sodium and calcium fluxes across cell membranes. Rev Physiol Biochem Pharmacol 70:33–82
Blaustein MP (1975) Effects of potassium veratridine and scorpion venom on calcium accumulation and transmitter release by nerve terminals in vitro. J Physiol (Lond) 247:617–655
Blaustein MP (1976) The ins and outs of calcium transport in squid axons: internal and external ion activation of calcium efflux. Fed Proc 35:2574–2578
Blaustein MP (1977a) Effects of internal and external cations and of ATP on sodium-calcium and calcium-calcium exchange in squid axons. Biophys J 20:79–111
Blaustein MP (1977b) Sodium ions, calcium ions, blood pressure regulation and hypertension: a reassessment and a hypothesis. Am J Physiol 232:C165–C173
Blaustein MP, Ector AC (1975) Barbiturate inhibition of calcium uptake by depolarized nerve terminals in vitro. Mol Pharmacol 11:369–378
Blaustein MP, Ector AC (1976) Carrier-mediated sodium dependent and calcium dependent calcium efflux from pinched-off presynaptic nerve terminals (synaptosomes) in vitro. Biochim Biophys Acta 419:295–308
Blaustein MP, Hodgkin AL (1969) The effect of cyanide on the efflux of calcium from squid axons. J Physiol (Lond) 200:497–527
Blaustein MP, Oborn CJ (1975) The influence of sodium on calcium fluxes in pinched-off nerve terminals in vitro. J Physiol (Lond) 247:657–686
Blaustein MP, Russell JM (1975) Sodium-calcium exchange and calcium-calcium exchange in internally dialyzed squid giant axons. J Membr Biol 22:285–312
Blaustein MP, Wiesmann WP (1970) Effect of sodium ions on calcium movements in isolated synaptic terminals. Proc Natl. Acad Sci USA 66:664–671
Blaustein MP, Johnson EM, Needleman P (1972) Calcium-dependent norepinephrine release from presynaptic nerve endings in vitro. Proc Natl Acad Sci USA 69:2237–2240
Blaustein MP, Russell JM, DeWeer P (1974) Calcium efflux from internally dialyzed squid axons: the influence of external and internal cations. J Supramol Struct 2: 558–581
Blaustein MP, Kendrick NC, Fried RC, Ratzlaff RW (1977) Calcium metabolism of the mammalian presynaptic nerve terminal: lessons from the synaptosome. In: Cowan WM, Ferrendelli JA (eds) Approaches to the cell biology of neurones. Society for Neuroscience Symposia, Society for Neuroscience, Bethesda, pp 172–194
Blaustein MP, Ratzlaff RW, Kendrick NC, Schweitzer ES (1978a) Calcium buffering in presynaptic nerve terminals. I. Evidence for involvement of a nonmitochondrial ATP-dependent sequestration mechanism. J Gen Physiol 72:15–41

Blaustein MP, Ratzlaff RW, Schweitzer ES (1978b) Calcium buffering in presynaptic nerve terminals. II. Kinetic properties of the nonmitochondrial Ca sequestration mechanism. J Gen Physiol 72:43–66

Blaustein MP, Ratzlaff RW, Kendrick NK (1978c) The regulation of intracellular calcium in presynaptic nerve terminals. Ann NY Acad Sci 307:195–211

Blinks JK (1978) Measurement of calcium ion concentration with phosphoproteins. Ann NY Acad Sci 307:71–85

Blinks JR, Pendergast FG, Allen DG (1976) Phosphoproteins on biological calcium indicators. Pharmacol Rev 28:1–93

Blitz AL, Fine RE, Toselli PA (1977) Evidence that coated vesicles isolated from brain are calcium-sequestering organelles resembling sarcoplasmic reticulum. J Cell Biol 75:135–147

Bond GH, Clough DL (1973) A soluble protein activator of (Mg^{2+} + Ca^{2+})-dependent ATPase in human red cell membranes. Biochim Biophys Acta 323:592–599

Bond GH, Green JW (1971) Effects of monovalent cations on the (Mg^{2+} + Ca^{2+})-dependent ATPase of the red cell membrane. Biochim Biophys Acta 241:393–398

Bonucci E, Derenzini M, Marinozzi V (1973) The organic-inorganic relationship in calcified mitochondria. J Cell Biol 59:185–211

Borg J, Mark J, Mandel P (1979) Effects of amino acids on calcium uptake by glial and neuroblastoma cells. J Neurobiol 10:31–40

Borle AB (1968a) Calcium metabolism in HeLa cells and the effects of parathyroid hormone. J Cell Biol 36:567–582

Borle AB (1968b) Effects of purified parathyroid hormone on the calcium metabolism of monkey kidney cells. Endocrinology 83:1316–1322

Borle AB (1968c) Calcium transport in cell culture and the effects of parathyroid hormone. In: Talmage RV, Belanger LF, Clark I (eds) Parathyroid hormone and thyrocalcitonin (calcitonin). Excerpta Medica, New York, pp 258–272

Borle AB (1969a) Kinetic analyses of calcium movements in HeLa cell cultures. I. Calcium influx. J Gen Physiol 53:43–56

Borle AB (1969b) Kinetic analyses of calcium movements in HeLa cell cultures. II. Calcium efflux. J Gen Physiol 53:57–69

Borle AB (1969c) Effects of thyrocalcitonin on calcium transport in kidney cells. Endocrinology 85:194–199

Borle AB (1970a) Kinetic analyses of calcium movements in cell cultures. III. Effect of calcium and parathyroid hormone in kidney cells. J Gen Physiol 55:163–186

Borle AB (1970b) Kinetic analyses of calcium movements in cell cultures. IV. Effects of phosphate and parathyroid hormone in kidney cells. Endocrinology 86:1389–1393

Borle AB (1971a) Effets du phosphate sur les mouvements du calcium en cultures cellulaires. In: Hioco DJ (ed) Phosphate et metabolisme phosphocalcique. Expansion Scientifique, Paris, pp 29–43

Borle AB (1971b) Calcium transport in kidney cells and its regulation. In: Nichols G Jr, Wasserman RH (eds) Cellular mechanisms for calcium transfer and homeostasis. Academic Press, New York, pp 151–174

Borle AB (1972a) Kinetic analyses of calcium movements in cell cultures. V. Intracellular calcium distribution in kidney cells. J Membr Biol 10:45–66

Borle AB (1972b) Parathyroid hormone and cell calcium. In: Talmage RV, Munson PL (eds) Calcium, parathyroid hormone and the calcitonins. Excerpta Medica, Amsterdam, pp 484–491

Borle AB (1973a) Calcium metabolism at the cellular level. Fed Proc 32:1944–1950

Borle AB (1973b) Cyclic AMP regulation of calcium efflux from liver, kidney and heart mitochondria. J Int Res Commun 1:9

Borle AB (1974a) Kinetic studies of calcium movements in intestinal cells: effect of vitamin D deficiency and treatment J. Membr Biol 16:207–220

Borle AB (1974b) Cyclic AMP stimulation of calcium efflux from kidney, liver and heart mitochondria. J Membr Biol 16:221–236
Borle AB (1975a) Regulation of cellular calcium metabolism and calcium transport by calcitonin. J Membr Biol 21:125–146
Borle AB (1975b) Methods for assessing hormone effects on calcium fluxes in vitro. In: Hardman JG, O'Malley BW (eds) Methods in enzymology, hormone action. Part D: Isolated cells, tissues and organ systems. Academic Press, New York, v 39, pp 513–573
Borle AB (1975c) Regulation of the mitochondrial control of cellular calcium homeostasis and calcium transport by phosphate, parathyroid hormone, calcitononin, vitamin D and cyclic AMP. In: Talmage RV, Owen M, Parsons JA (eds) Calcium regulating hormones. Excerpta Media, Amsterdam, pp 217–228
Borle AB (1975d) Modulation of mitochondrial control of cytoplasmic and calcium activity. In: Carafoli E, Clementi F, Drabikowski W, Magreth A (eds) Calcium transport in contraction and secretion. North Holland, New York, p 77
Borle AB (1976) On the problem of the release of mitochondrial calcium by cyclic AMP. J Membr Biol 29:205–208
Borle AB (1978) On the difficulty of assessing the role of extracellular calcium in cell function. Ann NY Acad Sci 307:431–432
Borle AB (1979) Na-Ca exchange and renal cell Ca homeostasis. Fed Proc 38:1392
Borle AB (1981) Pittfalls of the ^{45}Ca method. Cell Calcium (in press)
Borle AB, Anderson JH (1976) A cybernetic view of cell calcium metabolism. In: Duncan CJ (ed) Calcium in biological systems. Soc Exp Biol Symp, pp 141–160
Borle AB, Briggs FN (1968) Microdetermination of calcium in biological material by automatic fluorometric titration. Anal Chem 40:339–344
Borle AB, Clark I (1981) Effects of phosphate induced hyperparathyroidism and parathyroidectomy on rat kidney calcium in vivo. Am J Physiol (in press)
Borle AB, Loveday J (1968) Effects of temperature, potassium and calcium on the electrical potential difference in HeLa cells. Cancer Res 28:2401–2405
Borle AB, Studer R (1978) Effects of calcium ionophores on the transport and distribution of calcium in isolated cells and in liver and kidney slices. J Membr Biol 38: 51–72
Borle AB, Uchikawa T (1978) Effects of parathyroid hormone on the distribution and transport of calcium in cultured kidney cells. Endocrinology 102:1725–1732
Borle AB, Uchikawa T (1979) Effects of adenosine 3',5'-monophosphate dibutyryl adenosine 3',5'-monophosphate, aminophylline and imidazole on renal cellular calcium metabolism. Endocrinology 104:122–129
Bornet EP, Entman ML, Van Winkle WB, Schwartz A, Lehotay DC, Levey, GS (1977) Cyclic AMP modulation of calcium accumulation by sarcoplasmic reticulum from fast skeletal musle. Biochim Biophys Acta 468:188–193
Brachet J (1978) The hormonal induction of maturation in amphibian oocytes. Med Biol 56:304–309
Brading AF (1973) Ion distribution and ion movements in smooth muscle. Philos Trans R Soc Lond (Biol) 265:35–46
Brand MD, Chen CH, Lehninger AL (1976) Stoichiometry of the ejection during respiration-dependent accumulation of Ca^{2+} by rat liver mitochondria. J Biol Chem 251:968–974
Brandt PW (1962) A consideration of the extraneous coats of the plasma membrane. Circulation 26:1075–1091
Brehm P, Eckert R (1978) Calcium entry leads to inactivation of calcium channel in paramecium. Science 202:1203–1206
Brierly GP, Murer E, Bachmann E (1964) Studies on ion transport. III. The accumulation of calcium and inorganic phosphate by heart mitochondria. Arch Biochem Biophys 105:89–102
Brink F (1954) The role of calcium ions in neural processes. Pharmacol Rev 6:243–298

Brinley FJ Jr (1968) Sodium and potassium fluxes in isolated barnacle muscle fibers. J Gen Physiol 51:445–477
Brinley FJ Jr (1976) Calcium and magnesium metabolism in cephalopod axons. Fed Proc 35:2572–2595
Brinley FJ Jr (1978) Calcium buffering in squid axons. Ann Rev. Biophys Bioeng 7: 363–392
Brinley FJ Jr, Mullins LJ (1967) Sodium extrusion by internally dialyzed squid axons. J Gen Physiol 50:2303–2331
Brinley FJ Jr, Mullins LJ (1974) Effects of membrane potential on sodium and potassium fluxes in squid axons. Ann NY Acad Sci 242:406–433
Brinley FJ Jr, Scarpa A (1975) Ionized magnesium concentration in axoplasm of dialyzed squid axons. FEBS Lett 50:82–85
Brinley FJ Jr, Spangler SG, Mullins LJ (1975) Calcium and EDTA fluxes in dialyzed squid axons. J Gen Physiol 66:223–250
Brinley FJ Jr, Tiffert T, Scarpa A (1978) Mitochondria and other calcium buffers of squid axon studied in situ. J Gen Physiol 72:101–127
Brinley FJ Jr, Tiffert T, Scarpa A, Mullins LJ (1977) Intracellular buffering capacity in isolated squid axons. J Gen Physiol 70:355–384
Brisson GR, Malaisse WJ (1973) The stimulus-secretion coupling of glucose induced insulin release. XI. Effects of theophylline and epinephrine on ^{45}Ca efflux from perfused islets. Metabolism 22:455–465
Brisson GR, Malaisse-Lagae F, Malaisse WJ (1972) The stimulus secretion coupling of glucose-induced insulin release. VII. A proposed site of action for adenosine 3',5'-cyclic monophosphate. J Clin Invest 51:232–241
Brömme HJ, Dargel R (1979) Effect of in vivo and in vitro application of glucagon, insulin and epinephrine on Ca^{++}-transport properties of liver mitochondria. Acta Biologica et Medica Germanica 38:1365–1977
Brown HF, McNaughton PA, Noble D, Noble SJ (1975) Adrenergic control of pacemaker currents. Philos Trans R Soc Lond (Biol) 270:527–537
Bruns DE, McDonald JM, Jarett L (1976) Energy-dependent calcium transport in endoplasmic reticulum of adipocytes. J Biol Chem 251:7191–7197
Burgos MH (1960) The role of amorphous cellular coatings in active transport. Anat Rec 137:171
Burnatowska MA, Harris CA, Sutton RAL, Dirks JH (1977) Effect of PTH and cAMP on renal handling of calcium, magnesium and phosphate in the hamster. Am J Physiol 233:F514–F518
Burton J, Godfraind T (1974) Sodium-calcium sites in smooth muscle and their accessibility to lanthanum. J Physiol (Lond) 241:287–298
Busselen P, Carmeliet E (1973) Protagonistic effects of Na and Ca on tension development in cardiac muscle at low extracellular Na concentrations. Nature New Biol 243:57–59
Butlen D, Jard S (1972) Renal handling of 3',5'-cyclic AMP in the rat. Pfluegers Arch 331:172–190
Bygrave FL (1967) The ionic environment and metabolic control. Nature 214:667–671
Bygrave FL (1976) Mitochondrial calcium transport and the regulation of metabolism by calcium in tumor cells. In: Criss WE, Ono T, Sabine JR (eds) Control mechanisms in cancer. Raven Press, New York, pp 411–423
Bygrave FL (1977) Mitochondrial calcium transport. Curr Top Bioenergetics 6:259–318
Bygrave FL (1978) Mitochondria and the control of intracellular calcium. Biol Rev 53: 43–79
Bygrave FL, Ramachandran C, Smith RL (1977) On the mechanism by which inorganic phosphate stimulates mitochondrial calcium transport. FEBS Lett 83:155–158

Bygrave FL, Reed KC, Spencer T (1971a) Cooperative interactions in energy-dependent accumulation of Ca^{2+} by isolated rat liver mitochondria. Nature New Biol 230:89

Bygrave FL, Reed KC, Spencer T (1971b) Sigmoidal kinetics associated with Ca^{2+} uptake and related ATPase in rat liver mitochondria. In: Quagliariello E, Papa S, Rossi CS (eds) Energy transduction in respiration and photosynthesis. Adriatica Editrice, Bari, pp 981–988

Bygrave FL, Trauter CJ (1978) The subcellular location, maturation and response to increased plasma glucagon of ruthenium red-insensitive calcium-ion transport in rat liver. Biochem J 174:1021–1030

Caldwell PC (1971) Calcium movement in muscle. In: Podolsky RJ (ed) Contractility of muscle cells and related processes. Prentice Hall, Englewood Cliff, pp 105–114

Calixto JB, Aucélio JG, Turkiewicz A (1979) Relationship between modulation by estradiol, progesterone and calcium upon the pharmacological reactivity of uteri of dogs. Res Commun Chem Pathol Pharmacol 25:447–460

Caputo C, Bolanos P (1978) Effect of external sodium and calcium on calcium efflux in frog striated muscle. J Membr Biol 41:1–14

Caputo CB, Meadows D, Raisz LG (1976) Failure of estrogens and androgens to inhibit bone resorption in tissue culture. Endocrinology 98:1065–1068

Carafoli E (1967) In vivo effect of uncoupling agents on the incorporation of calcium and strontium into mitochondria and other subcellular fractions of rat liver. J Gen Physiol 50:1849–1864

Carafoli E (1976) Mitochondrial calcium transport and calcium binding proteins. In: Packer L, Gomez-Puyou A (eds) Mitochondria, bioenergetics, biogenesis and membrane structure. Academic Press, New York, pp 47–60

Carafoli E, Azzi A (1972) The affinity of mitochondria for calcium. Experientia 27: 906–908

Carafoli E, Crompton M (1976) Calcium ions and mitochondria. Soc Exp Biol Symp 30:89–115

Carafoli E, Crompton M (1978a) The regulation of intracellular calcium by mitochondria. Ann NY Acad Sci 307:269–284

Carafoli E, Crompton M (1978b) The regulation of intracellular calcium. Curr Top Membr Transp 10:151–216

Carafoli E, Crompton M, Malmström K, Siegel E, Salzmann M, Chiesi M, Affolter H (1977) Mitochondrial calcium transport and the intracellular calcium homeostasis. In: Semenza G, Carafoli E (eds) Biochemistry of membrane transport. FEBS Symposium No. 42, Springer, Berlin Heidelberg New York, pp 535–551

Carafoli E, Crovetti F (1973) Interactions between prostaglandin E_1 and calcium at the level of the mitochondrial membrane. Arch Biochem Biophys 154:40–46

Carafoli E, Gamble RL, Rossi CS, Lehninger AL (1967) Super-stoichiometric ratios between ion movements and electron transport in rat liver mitochondria. J Biol Chem 242:1199–1204

Carafoli E, Lehninger AL (1971) A survey of the interaction of calcium ions with mitochondria from different tissues and species. Biochem J 122:681–690

Carafoli E, Malmström K, Sigel E, Crompton M (1976) The regulation of intracellular calcium. Clin Endocrinology 5:Suppl 49s–59s

Carafoli E, Patriarca P, Rossi CS (1961) A comparative study of the role of mitochondria and the sarcoplasmic reticulum in the uptake and release of Ca^{++} by the rat diaphragm. J Cell Physiol 74:17–30

Carafoli E, Schwarzmann K, Roos I, Crompton M (1978) Protein in mitochondrial calcium transport. In: Blauer G, Sund H (eds) Transport by proteins. de Gruyter, Berlin, pp 171–186

Carafoli E, Tiozzo R (1967) Time course of the distribution of in vivo administered ^{89}Sr in rat liver subcellular fraction. Experientia 23:1017–1020

Carafoli E, Tiozzo R, Lugli G, Crovetti F, Kratzing C (1974) The release of calcium from heart mitochondria by sodium. J Mol Cell Cardiol 6:361–371

Carafoli E, Tiozzo R, Rossi CS, Lugli G (1972) Mitochondrial Ca^{2+} uptake and heart relaxation. In: Bolis L, Keynes RD, Wilbrandt W (eds) Role of membranes in secretory processes. North Holland, Amsterdam, pp 175–181

Caroni P, Schwerzmann K, Carafoli E (1978) Separate pathways for Ca^{2+} uptake and release in liver mitochondria. FEBS Lett 96:339–342

Carré M, Ayigbedé O, Miravet L, Rasmussen H (1974) The effect of prednisolone upon the metabolism and action of 25-hydroxy- and 1,25-dihydroxyvitamin D_3. Proc Natl Acad Sci USA 71:2996–3000

Carsten GE (1979) Calcium accumulation by human uterine microsomal preparations – effects of progesterone and oxytocin. Am J Obstet Gynecol 133:598–601

Carvalho AP, Leo B (1967) Effects of ATP on the interaction of Ca^{++}, Mg^{++} and K^+ with fragmented sarcoplasmic reticulum isolated from rabbit skeletal muscle. J Gen Physiol 50:1327–1352

Carvalho AP, Sanui H, Pace N (1963) Calcium and magnesium binding properties of cell membrane materials. J Cell Physiol 62:311–317

Case RM, Clausen T (1973) The relationship between calcium exchange and enzyme secretion in the isolated rat pancreas. J Physiol (Lond) 235:75–102

Casteels R, Goffin J, Raeymaekers L, Wuytack F (1973) Calcium pumping in smooth muscle cells of *Taenia coli*. J Physiol (Lond) 231:19P

Casteels R, Kuriyama H (1965) Membrane potential and ionic current in pregnant and nonpregnant rat myometrium. J Physiol (Lond) 177:263–287

Caulfield JB, Schrag PE (1964) Electron microscopic study of renal calcification. Am J Pathol 44:365–381

Cha YN, Shin BC, Lee KS (1971) Active uptake of Ca^{++} and Ca^{++}-activated Mg^{++} ATPase in red cell membrane fragments. J Gen Physiol 57:202–215

Chabardès DM, Imbert M, Clique A, Montégut M, Morel F (1975) PTH sensitive adenyl cyclase activity in different segments of the rabbit nephron. Pfluegers Arch 354:229–239

Chambaut AM, Leray-Pecker F, Feldmann G, Hanoune J (1974) Calcium binding properties and ATPase activities of rat plasma membranes. J Gen Physiol 64:104–126

Chambers R (1940) The relation of the extraneous coats to the organization and permeability of cell membranes. Cold Spring Harbor Symp Quant Biol 8:144–153

Chan TM, Blackmore PF, Steiner KE, Exton JH (1979) Effects of adrenalectomy on hormone action on hepatic glucose metabolism. Reciprocal change in α- and β-adrenergic activation of hepatic glycogen phosphorylase and calcium mobilization in adrenalectomized rats. J Biol Chem 254(7):2428–2433

Chance B (1965) The energy-linked reaction of calcium with mitochondria. J Biol Chem 240:2729–2748

Chandler DE, Williams JA (1979) Pancreatic acinar cells: effects of lanthanum ions on amylase release and calcium ion fluxes. J Physiol (Lond) 243:831–846

Charlton RR, Wenner CE (1978) Calcium ion transport by intact Ehrlich ascites tumor cells. Role of respiratory substrates, P_i and temperature. Biochem J 170:537–544

Chase LR, Aurbach GD (1970) The effect of parathyroid hormone on the concentration of adenosine 3',5'-monophosphate in skeletal tissue in vitro. J Biol Chem 245: 1520–1526

Chase LR, Fedak SA, Aurbach GD (1969) Activation of skeletal adenyl cyclase by parathyroid hormone in vitro. Endocrinology 84:761–768

Chausmer AB, Sehrman BS, Wallach S (1972) The effect of parathyroid hormone on hepatic cell transport of calcium. Endocrinology 90:663–672

Chen JLJ, Babcock DF, Lardy HA (1978) Norepinephrine, vasopressin, glucagon and A23187 induce efflux of caclium from an exchangeable pool in isolated rat hepatocytes. Proc Natl Acad Sci USA 75:2234–2238

Cheng SC, Chen SS (1976) Stimulation by cyclic nucleotides of calcium efflux in barnacle muscle fibers. Life Sci 16:1711

Christoffersen GRJ, Simonsen L (1972) Ca^{2+} sensitive microelectrode – intracellular steady state measurement in nerve cell. Acta Physiol Scand 101:492–494

Christophe JP, Frandsen EK, Conlon TP, Krishna G, Gardner JD (1976) Action of cholecystokinin, cholinergic agents, and A-23187 on accumulation of guanosine 3′-5′-monophosphate in dispersed guinea pig pancreatic acinar cells. J Biol Chem 251:4640–4645

Chudapongse P (1976) Further studies on the effect of phosphoenol pyruvate on respiration dependent calcium transport by rat heart mitochondria. Biochim Biophys Acta 423:196–202

Chudapongse P, Haugaard N (1973) The effect of phosphoenol pyruvate on calcium transport by mitochondria. Biochim Biophys Acta 307:599–606

Cittadini A, Bossi D, Rosi G, Wolf F, Terranova T (1977) Calcium metabolism in Ehrlich ascites tumour cells. Biochim Biophys Acta 469:345–349

Cittadini A, Scarpa A, Chance B (1971) Kinetic evidence for Ca^{2+} uptake by intact Ehrlich ascites tumor cells. FEBS Lett 18:98–102

Cittadini A, Scarpa A, Chance B (1973) Calcium transport in intact Ehrlich ascites tumor cells. Biochim Biophys Acta 291:246–259

Cittadini A, Van Rossum GDV (1978) Properties of the calcium-extruding mechanism of liver cells. J Physiol (Lond) 281:29–43

Claret-Berthon B, Claret M, Mazet JL (1977) Fluxes and distribution of calcium in rat liver cells: kinetic analysis and identification of pools. J Physiol (Lond) 272:529–552

Clausen T (1977) Calcium, glucose transport and insulin action. In: Semenza G, Carafoli E (eds) Biochemistry of membrane transport. Springer, Berlin Heidelberg New York, pp 481–499

Clausen T, Martin BR (1977) The effect of insulin on the washout of [^{45}Ca] calcium from the adipocytes and soleus muscle of the rat. Biochem J 164:251–255

Clemente F, Meldolesi J (1975) Calcium and pancreatic secretonin – dynamics of subcellular pools in resting and stimulated acinar cells. Br J Pharmacol 55:369–379

Cohen JJ, Barac-Nieto M (1973) Renal metabolism of substrates in relation to renal function. In: Orloff J, Berliner RW, Geiger SR (eds) Renal physiology. American Physiological Society, Washington (Handbook of physiology, Sec 8, pp 909–1001)

Cohn DV, Bawdon R, Eller G (1967) The effect of parathyroid hormone in vivo on the accumulation of calcium and phosphate by kidney and kidney mitochondrial function. J Biol Chem 242:1253–1258

Cook GMW, Laico MT, Eylar EH (1965) Biosynthesis of glycoproteins of the Ehrlich ascites carcinoma cell membranes. Proc Natl. Acad Sci USA 54:247–252

Cooke WJ, Robinson JP (1971) Factors influencing calcium movements in rat brain slices. Am J Physiol 221:218–225

Coraboeuf E, Beigelman PM, Breton D (1964) Potentiels électriques du foie de rat in situ. Effets de l'asphyxie. CR Acad Sci (D) (Paris) 25q:2300–2302

Cosmos E (1964) Intracellular distribution of calcium in developing breast muscle of normal and dystrophic chicks. J Cell Biol 23:241–252

Cosmos E, Harris EJ (1961) In vitro studies of the gain and exchange of calcium in frog skeletal muscle. J Gen Physiol 44:1121–1130

Crompton M, Heid I (1978) The cycling of calcium, sodium and protons across the inner membrane of cardiac mitochondria. Eur J Biochem 91:599–608

Crompton M, Sigel E, Salzmann M, Carafoli E (1967a) Kinetic study of the energy-linked influx of Ca^{2+} into heart mitochondria. Eur J Biochem 69:429–434

Crompton M, Capano M, Carafoli E (1976b) The sodium induced efflux of calcium from heart mitochondria. A possible mechanism for the regulation of mitochondrial calcium. Eur J Biochem 69:453–462

Crompton M, Künzi M, Carafoli E (1977) The calcium-induced and sodium-induced effluxes of calcium from heart mitochondria. Evidence for a sodium calcium carrier. Eur J Biochem 79:549–558

Crompton M, Hediger M, Carafoli E (1978a) The effect of inorganic phosphate on calcium influx into rat heart mitochondria. Biochem Biophys Res Commun 80:540–546

Crompton M, Moser R, Lüdi H, Carafoli E (1978b) The interrelations between the transport of sodium and calcium in mitochondria of various mammalian tissues. Eur J Biochem 82:25–31

Crooks JR, Kyriakides CPM, Simkiss K (1976) Routes of calcium movements across the chick chorioallantois. Q J Exp Physiol 61:265–274

Daniel EE, Janis RA (1975) Calcium regulation in the uterus. Pharmacol Ther (B) 1: 695–729

Davis PW, Vincenzi FF (1971) Ca-ATPase activation and NaK-ATPase inhibition as a function of calcium concentration in human red cell membranes. Life Sci 10:401–406

Dawkins MJR, Judah JD, Rees KR (1959) Factors influencing the survival of liver cells during autolysis. J Path Bact 77:257–275

Dean PM, Matthews EK (1970a) Glucose induced electrical activity in pancreatic islet cells. J Physiol (Lond) 210:255–264

Dean PM, Matthews EK (1970b) Electrical activity in pancreatic islet cells: effect of ions. J Physiol (Lond) 210:265–275

Dean PM, Matthews EK, Sakamoto Y (1975) Pancreatic islet cells: effects of monosaccharides, glycolytic intermediates and metabolic inhibitors on membrane potential and electrical activity. J Physiol (Lond) 246:459–478

Dedman JR, Brinkley BR, Means AR (1979) Regulation of microfilaments and microtubules by calcium and cyclic AMP. Adv Cyclic Nucl Res 11:131–174

Deffner GGJ (1961) The dialysable free organic constitutents of squid blood: a comparison with nerve axoplasm. Biochim Biophys Acta 47:378–388

DeMeis L (1969a) Activation Ca^{2+} uptake by acetylphosphate in muscle microsomes. Biochim Biophys Acta 172:343–344

DeMeis L (1969b) Ca^{2+} uptake and acetylphosphate of skeletal muscle microsomes. Inhibition by Na^+, K^+, Li^+ and adenosine triphosphate. J Biol Chem 244:3733–3739

DeMeis L (1971) Allosteric inhibition by alkali ions of the Ca^{2+} uptake and adenosine triphosphatase activity of skeletal muscle microsomes. J Biol Chem 246:4764–4773

DeMeis L, Hasselbach W (1971) Acetylphosphate as substrate for Ca^{2+} uptake in skeletal muscle microsomes. Inhibition by alkali ions. J Biol Chem 246:4759–4763

DeMeis L, Rubin-Attschul BM, Tillack TW (1970) Comparative data of Ca^{2+} transport in brain and skeletal muscle microsomes. J Biol Chem 245:1883–1889

Deschodt-Lanckman M, Robberecht P, De Neef P, Lammens M, Christophe J (1976) In vitro action of bombesin and bombesin-like peptides on amylase secretion, calcium efflux, and adenylate cyclase activity in the rat pancreas. J Clin Invest 58:891–898

Deshmukh K, Kline WG, Sawyer BD (1977) Effect of calcitonin and parathyroid hormone on the metabolism of chondrocytes in culture. Biochim Biophys Acta 499: 28–35

Deth RC (1978) Effect of lanthanum and reduced temperature on ^{45}Ca efflux from rabbit aorta. Am J Physiol 234:C139–C145

Deth R, Van Breemen C (1977) Agonist induced release of intracellular Ca^{2+} in the rabbit aorta. J Membr Biol 30:363–380

DeWulf H, Keppens S (1976) Is calcium 2nd messenger in liver for cyclic AMP independent glycogenolytic hormones? Arch Int Physiol Biochim 84:159–160

DiPolo R (1973a) Calcium efflux from internally dialyzed squid giant axons. J Gen Physiol 62:575–589

DiPolo R (1973b) Sodium dependent calcium influx in dialyzed barnacle muscle fiber. Biochim Biophys Acta 298:279–283

DiPolo R (1974) Effect of ATP on the calcium efflux in dialzyed squid giant axon. J Gen Physiol 64:503–517
DiPolo R (1976) The influence of nucleotides on calcium fluxes. Fed Proc 35:2579–2582
DiPolo R (1977) Characterization of the ATP-dependent calcium efflux in dialyzed squid giant axons. J Gen Physiol 69:795–813
DiPolo R (1979) Calcium influx in internally dialyzed squid giant axons. J Gen Physiol 73:91–113
DiPolo R, Caputo C (1977) The effect of ATP on calcium efflux in dialyzed barnacle muscle fibres. Biochim Biophys Acta 470:389–394
DiPolo R, Requena J, Brinley FJ Jr, Mullins LJ, Scarpa A, Tiffert T (1976) Ionized calcium concentration in squid axons. J Gen Physiol 67:433–467
Dorman DM, Barritt GJ, Bygrave FF (1975) Stimulation of hepatic mitochondrial calcium transport by elevated plasma insulin concentration. Biochem J 150:389–395
Douglas WW, Kanno T, Sampson SR (1967a) Effects of acetylcholine and other medullary secretagogues and antagonists on the membrane potential of adrenal chromaffin cells: an analysis employing technique of tissue culture. J Physiol (Lond) 188: 107–120
Douglas WW, Kanno T, Sampson SR (1976b) Influence of the ionic environment on the membrane potential of adrenal chromaffin cells and on the deploarizing effect of acetylcholine. J Physiol (Lond) 191:107–121
Drahota Z, Carafoli E, Rossi CS, Gamble RL, Lehninger AL (1965) The steady state maintenance of accumulated Ca^{++} in rat liver mitochondria. J Biol Chem 240:2712–2720
Droogmans G, Casteels R (1979) Sodium and calcium interactions in vascular smooth muscle cells of the rabbit ear artery. J Gen Physiol 74:57–70
Duffy MJ, Schwarz V (1973) Calcium binding to erythrocyte membrane. Biochim Biophys Acta 330:294–301
Dunnett J, Nayler WG (1979) Effect of pH on calcium accumulation and release by isolated fragments of cardiac and skeletal muscle sarcoplasmic reticulum. Arch Biochem Biophys 198:434–438
Dziak R (1978) Effects of vitamin D_3 metabolites on bone cell calcium transport. Calcif Tissue Res 26:65–70
Dziak R, Brand JS (1974a) Calcium transport in isolated bone cells. I. Bone cell isolation procedure. J Cell Physiol 84:75–74
Dziak R, Brand JS (1974b) Calcium transport in isolated bone cells. II. Calcium transport studies. J Cell Physiol 84:85–96
Dziak R, Hausmann E, Chang YW (1979) Effects of lipopolysaccharides and prostaglandins on rat bone cell calcium and cyclic AMP. Arch Oral Biol 24:347–354
Dziak R, Stern PH (1975) Calcium transport in isolated bone cells. III. Effects of parathyroid hormone and cyclic 3′5′ AMP. Endocrinology 97:1281–1287
Ebashi S (1976) Excitation-contraction coupling. Ann Rev Physiol 38:293–313
Eilam Y, Szydel N, Harell A (1980) Effect of calcitonin on transport and intracellular distribution of exchangeable Ca^{2+} in primary culture of bone cells. Mol Cell Endocrinol 18:215–225
Elder JA, Lehninger AL (1973a) Respiration dependent transport of carbon dioxide into rat liver mitochondria. Biochemistry 12:976–982
Elder JA, Lehninger AL (1973b) Energy linked uptake of Ca^{2+} supported by carbon dioxide: inhibition by diamox. In: Azzone GF, Ernster L, Papa S, Quagliariello E, Siliprandi N (eds) Mechanisms in bioenergetics. Academic Press, New York, pp 513–526
Entman ML, Levey GS, Epstein SE (1969) Mechanism of action of epinephrine and glucagon on the canine heart. Evidence for increase in sarcotubular calcium stores mediated by cyclic 3′5′ AMP. Circ Res 25:429–438
Eroglu L, Keen P (1977) Active uptake of ^{45}Ca by a microsomal fraction prepared from rat dorsal roots. J Neurochem 29:905–909

Ewe K (1972) Calcium transport in rat small intestine in vitro and in vivo. Arch Pharmacol 273:352–365

Exton JH (1980) Mechanisms involved in α-adrenergic phenomena: role of calcium ions in actions of catecholamines in liver and other tissues. Am J Physiol 238:E3–E12

Exton JH, Park CR (1972) Interaction of insulin and glucagon in the control of liver metabolism. In: Greep RO, Astwood EB (eds) Endocrinology, endocrine pancreas. American Physiological Society, Washington, D.C. (Handbook of physiology, vol I/7, pp 437–455)

Exton JH, Robinson GA, Sutherland EW (1972) Glucagon and cyclic AMP. In: Endocrinology, endocrine pancreas. American Physiological Society, Washington, D.C. (Handbook of physiology, vol I/7, pp 425–436)

Fain JN, Berridge MJ (1979) Relationship between hormonal activation of phosphatidylinositol hydrolysis, fluid secretion and calcium flux in the blowfly salivary gland. Biochem J 178:45–58

Fanburg BL (1968) Calcium transport by skeletal muscle sarcoplasmic reticulum in the hypothyroid rat. J Clin Invest 47:2499–2506

Farber JL, El-Mofty SK, Schanne FAX, Aleo JJ Jr, Serroni A (1977) Intracellular calcium homeostasis in galactosamine-intoxicated rat liver cells. Active sequestration of calcium by microsomes and mitochondria. Arch Biochem Biophys 178:617–624

Feher JJ, Wasserman RH (1979) Intestinal calcium-binding protein and calcium absorption in cortisol-treated chicks: effects of vitamin D_3 and 1,25-dihydroxyvitamin D_3. Endocrinology 104:547–551

Ferreira HG, Lew VL (1976) Use of ionophore A23187 to measure cytoplasmic Ca buffering and activation of the Ca pump by internal Ca. Nature 259:47–49

Fiskum G, Lehninger AL (1979) Regulated release of calcium from respiring mitochondria by $Ca^{2+}/2H^+$ antiport. J Biol Chem 254, 6236–6239

Foden S, Randle PJ (1978) Calcium metabolism in rat hepatocytes. Biochem J 170: 615–625

Foreman JC, Hallett MB, Mongar JL (1977) The relationship between histamine secretion and ^{45}calcium uptake by mast cells. J Physiol (Lond) 271:193–214

Frankel BJ, Imagawa WT, O'Connor MDL, Lundquist I, Kromhout JA, Fanska RE (1978) Glucose stimulated ^{45}calcium efflux from isolated rat pancreatic islets. J Clin Invest 62:525–531

Frankenhauser B, Hodgkin AL (1956) The after effects of impulses in the giant nerve fibres of *Loligo*. J Physiol (Lond) 131:341–376

Freedman RA, Weiser MM, Isselbacher KJ (1977) Calcium translocation by Golgi and lateral-basal membrane vesicles from rat intestine: decrease in vit-D-deficient rats. Proc Natl Acad Sci USA 74:3612–3616

Freeman DJ, Daniel EE (1973) Calcium movements in vascular smooth muscle and its detection using lanthanum as a tool. Can J Physiol Pharmacol 51:900–913

Friedmann N (1972) Effects of glucagon and cyclic AMP on ion fluxes in the perfused liver. Biochim Biophys Acta 274:214–225

Friedmann N, Divakaran P, Kirkland J, Kimura S, Wood J (1979) Effects of the calcium ionophore A23187 on liver metabolism. J Pharmacol Exp Ther 211:127–132

Friedmann N, Park CR (1968) Early effect of 3′5′ AMP on the fluxes of Ca and potassium in the perfused liver of normal and adrenalectomized rats. Proc Natl Acad Sci USA 61:504–508

Friedmann N, Rasmussen N (1970) Relationship between Ca^{2+} movement and gluconeogenesis in the perfused liver. Biochim Biophys Acta 222:41–52

Fuchs R, Peterlik M (1979) Vitamin D-induced transepithelial phosphate and calcium transport by chick jejunum: effect of microfilamentous and microtubular inhibitors. FEBS Lett 100:357–359

Gainer H (1968) The role of calcium in excitation-contraction coupling. J Gen Physiol 52:88–110

Galo MG, Bloj B, Farias RN (1975) Kinetic changes of erythrocyte (Mg^{2+} + Ca^{2+})-adenosine triphosphatase of rats fed different fat-supplemented diets. J Biol Chem 250:6204–6207

Gardner JD, Conlon TP, Klaeveman HL, Adams TO, Ondetti MA (1975) Action of cholecystokinin and cholinergic agents on calcium transport in isolated pancreatic acinar cells. J Clin Invest 56:366–375

Gardner JD, Hahne WF (1977) Calcium transport in dispersed acinar cells from rat pancreas. Biochim Biophys Acta 471:466–476

Garrison JC, Borland MK, Florio VA, Twible DA (1979) The role of calcium ions as a mediator of the effects of angiotensin II. Catecholamines and vasopressin on the phosphorylation and activity of enzymes in isolated hepatocytes. J Biol Chem 254: 7147–7156

Gasic G, Gasic T (1962) Removal and regeneration of the cell coating in tumour cells. Nature 196:170

Geduldig D, Junge D (1968) Sodium and calcium components of action potentials in the *Aplysia* giant neurone. J Physiol (Lond) 199:347–365

Gerrard JM, Townsend D, Stoddard S, Witkop CJ, White JG (1977) The influence of prostaglandin G_2 on platelet ultrastructure and platelet secretion. Am J Pathol 86(1):99–115

Gerrard JM, Kindom SE, Peterson DA, Peller J, Krautz KE, White J (1979) Lysophosphatidic acid's influence on platelets aggregation and intracellular calcium flux. Am J Pathol 96:423–438

Ghijsen WEJM, Van Os CH (1979) Ca-stimulated ATPase in brush border and basolateral membranes of rat duodenum with high affinity sites for Ca ions. Nature 279: 802–803

Giebisch G (1958) Electrical potential measurements on single nephrons of *Necturus*. J Cell Comp Physiol 51:221–239

Giebisch G (1961) Measurements of electrical potential differences on single nephrons of the perfused *Necturus* kidney. J Gen Physiol 44:659–678

Gilbert DL, Fenn WD (1957) Calcium equilibrium in muscle. J Gen Physiol 40:393–408

Giles W, Noble SJ (1976) Changes in membrane currents in bullfrog atrium produced by acetylcholine. J Physiol (Lond) 261:103–123

Girardier L, Seydoux J, Clausen T (1968) Membrane potential of brown adipose tissue. A suggested mechanism for the regulation of thermogenesis. J Gen Physiol 52: 925–940

Glitsch HG, Reuter H, Scholz H (1970) The effect of the internal sodium concentration on calcium fluxes in isolated guinea-pig auricles. J Physiol (Lond) 209:25–43

Gmaj P, Murer H, Kinne R (1979) Calcium ion transport across plasma membranes isolated from rat kidney cortex. Biochem J 178:549–557

Godfraind T, Kaba A (1972) The role of calcium in the action of drugs on vascular smooth muscle. Arch Int Pharm Therap 196 (Suppl):35–49

Gomez-Puyou A, Tuena de Gomez-Puyou M, Becker G, Lehninger AL (1972) An insoluble Ca^{2+}-binding factor from rat liver mitochondria. Biochem Biophys Res Commun 47:814–819

Gopinath RM, Vincenzi FF (1977) Phosphodiesterase protein activator mimics red blood cell cytoplasmic activator of (Ca^{2+} – Mg^{2+}) ATPase. Biochem Biophys Res Commun 77:1203–1209

Govier WC, Holland WC (1964) Effect of ouabain on tissue calcium and calcium exchange in pacemaker of turtle heart. Am J Physiol 207:195–198

Greenawalt JW, Rossi CS, Lehninger AL (1964) Effect of active accumulation of calcium and phosphate ions on the structure of rat liver mitochondria. J Cell Biol 23: 21–38

Greenway DC, Himms-Hagen J (1978) Increased calcium uptake by muscle mitochondria of cold acclimated rats. Am J Physiol 234:C7–C13

Grinstein S, Erlij D (1976) Action of insulin and cell calcium: effect of ionophore A23187. J Membr Biol 29:313–328
Griswold RL, Pace N (1956) The intracellular distribution of metal ions in rat liver. Exptl Cell Res 11:362–367
Grosse D, Lüllman H (1972) Tissue Ca content of intestinal smooth muscles and the Ca^{++} concentration of the incubation medium. Experientia 28:412–413
Guerrero-Munoz F, Cerreta KW, Guerrero ML, Way EL (1979a) Effect of morphine on synaptosomal Ca^{++} uptake. J Pharmacol Exper Therap 209:132–135
Guerrero-Munoz F, De Lourdes Guerrero M, Way EL (1979b) Effect of morphine on calcium uptake by lysed synaptosomes. J Pharmacol Exp Ther 211:370–374
Guerrero-Munoz F, DeLourdes Guerrero M, Way EL, Li CH (1979c) Effect of β-endorphin on calcium uptake in the brain. Science 206:89–90
Gunter TE, Puskin JS (1975) The use of electron paramagnetic resonance in studies of free and bound divalent cation: the measurement of membrane potential in mitochondria. Ann NY Acad Sci 264:112–122
Gylfe E, Buitrago A, Berggren PO, Hammarström K, Hellman B (1978) Glucose inhibition of ^{45}Ca efflux from pancreatic islets. Am J Physiol 235:E191–E196
Hagiwara S (1973) Ca spike. Adv Biophys 4:71–102
Hagiwara S, Chichibu S, Naka K (1964) The effects of various ions on resting and spike potentials of barnacles. J Gen Physiol 48:163–179
Hagiwara S, Naka K (1964) The initiation of spike potential in barnacle muscle fibers under low intracellular Ca^{++}. J Gen Physiol 48:141–162
Hagiwara S, Nakajima S (1966) Effects of the intracellular Ca ion concentration upon excitability of the muscle fiber membrane of a barnacle. J Gen Physiol 49:807–818
Hahn TJ, Halstead LR (1979) Cortisol enhancement of PTH-stimulated cyclic AMP accumulation in cultured fetal rat long bone rudiments. Calcif Tissue Int 29:173–175
Hakim A (1973) Effect of human calcitonin on the sarcoplasmic reticulum of the human heart. Naturwissenschaften 60:53
Hales CN, Campbell AK, Luzio JP, Siddle K (1977) Calcium as a mediator of hormone action. Biochem Soc Trans 5:866–871
Hamaguchi Y, Mabuchi I (1978) Measurement of intracellular free calcium concentration in the starfish egg by means of the microinjection of aequorin. Cell Struct Func 3:259–264
Hanahan DJ, Taverna RD, Flynn DD, Echolm JE (1978) The interaction of Ca^{2+}/Mg^{2+} ATPase activator protein and Ca^{2+} with human erythrocyte membranes. Biochem Biophys Res Commun 84:1009–1017
Harada E, Laychock SG, Rubin RP (1978) Parathyroid hormone induced stimulation of calcium uptake by renal microsomes. Biochem Biophys Res Commun 84:396–402
Harell A, Binderman I, Gues M (1976) Tissue culture of bone cells: mineral transport, calcification and hormonal effects. Isr J Med Sci 12:115–123
Harell A, Binderman I, Rodan GA (1973) The effect of calcium concentration on calcium uptake by bone cells treated with thyrocalcitonin (TCT) hormone. Endocrinology 92:550–555
Harigaya S, Schwartz A (1969) Rate of calcium binding and uptake in normal animal and failing human cardiac muscle. Membrane vesicles (relaxing system) and mitochondria. Circ Res 25:781–794
Harris EJ (1977) The uptake and release of calcium by heart mitochondria. Biochem J 168:447–456
Harris EJ (1979) Modulation of Ca^{2+} efflux from heart mitochondria. Biochem J 178: 673–680
Harris EJ, Al-Shaikhaly M, Baum H (1979) Stimulation of mitochondrial calcium ion efflux by thiolspecific reagents and by thyroxine. The relationship to adenosine diphosphate retention and to mitochondrial permeability. Biochem J 182:455–464

Harris RH, Ramwell PW, Gilmer PJ (1979) Cellular mechanisms of prostaglandin action. Ann Rev Physiol 41:653–658

Haussler MR, Nagode LA, Rasmussen H (1970) Induction of intestinal brush border alkaline phosphatase by vitamin D and identity with Ca-ATPase. Nature 228:1199–1201

Hayasaki-Kumura N, Takahashi K (1979) Studies on action of somatostatin on growth hormone release in relation to calcium and cyclic AMP. Proc Soc Exp Biol Med 161:312–318

Hazelton BJ, Tupper JT (1979) Calcium transport and exchange in mouse 3T3 and SU40-3T3 cells. J Cell Biol 81:538–542

Heaton GM, Nicholls DG (1976) The calcium conductance of the inner membrane of rat liver mitochondria and the determination of the calcium electrochemical gradient. Biochem J 156:635–646

Heersche JNM, Marcus R, Aurbach GD (1974) Calcitonin and the formation of 3',5'-AMP in bone and kidney. Endocrinology 94:241–247

Heilbrunn L (1943) An outline of general physiology. Saunders, Philadelphia

Heilbrunn LV, Wiercinski FJ (1947) The action of various cations on muscle protoplasm. J Cell Physiol 29:15–32

Heisler S, Grondin G (1973) Effect of lanthanum of ^{45}Ca flux and secretion of protein from rat exocrine pancreas. Life Sci 13:783–794

Hellman B, Anderson T (1978) Calcium and pancreatic β-cell function. IV. Evidence that glucose and phosphate stimulate Ca^{45} incorporation into different intracellular pools (BBA 28582). Biochim Biophys Acta 541:483–491

Hellman B, Sehlin J, Täljedal IB (1971) Calcium uptake by pancreatic β-cells as measured with the aid of ^{45}Ca and mannitol-^{3}H. Am J Physiol 221:1795–1801

Hellman B, Sehlin J, Täljedal IB (1976a) Effects of glucose on $^{45}Ca^{++}$ uptake by pancreatic islets as studied with the lanthanum method. J Physiol (Lond) 254:639–656

Hellman B, Sehlin J, Täljedal IB (1976b) Calcium and secretion: distinction between two pools of glucose-sensitive calcium in pancreatic islets. Science 194:1421–1423

Hems DA, Whitton PD (1980) Control of hepatic glycogenolysis. Physiol Rev 60:1–50

Herchuelz A, Couturier E, Malaisse WJ (1980) Regulation of calcium fluxes in pancreatic islets: glucose-induced calcium-calcium exchange. Am J Physiol 238:E96–E103

Herchuelz A, Delcroix C, Malaisse WJ (1979) Regulation of calcium fluxes in pancreatic islets. Quantification of calcium movements. Biochem Med 22:156–164

Herchuelz A, Malaisse WJ (1980) Regulation of calcium fluxes in pancreatic islets: two calcium movements' dissociated response to glucose. Am J Physiol 238:E87–E95

Herrman-Erlee MPM, v.d. Meer JM (1974) The effect of dibutyryl cyclic AMP, aminophylline, propranolol on PTE-induced bone resorption in vitro. Endocrinology 94: 424–434

Hicks MJ, Shigekawa M, Katz AM (1979) Mechanism by which cyclic adenosine 3',5' monophosphate-dependent protein kinase stimulates calcium transport in cardiac sarcoplasmic reticulum. Circ Res 44:384–391

Hinds TR, Larsen FL, Vincenzi FF (1978) Plasma membrane Ca^{2+} transport: stimulation by soluble proteins. Biochem Biophys Res Commun 81:455–461

Hines RN, Wenner CE (1977) The role of Pi in glycolytic inhibition of calcium ion uptake by ELD ascites tumor cells. Biochim Biophys Acta 465:391–399

Hinke JAM (1961) The measurement of sodium and potassium activities in the squid axon by means of cation selective glass microelectrodes. J Physiol (Lond) 156: 314–335

Hinnen R, Miyamoto H, Racker E (1979) Ca^{2+} translocation in Ehrlich ascites tumor cells. J Membr Biol 49:309–324

Hodgkin AL, Keynes RD (1957) Movements of labelled calcium in squid giant axons. J. Physiol (Lond) 138:253–281
Hodgson BJ, Kidwai AM, Daniel EE (1972) Uptake of lanthanum by smooth muscle. Can J Physiol Pharmacol 50:730–733
Höfer M, Kleinzeller A (1963a) Calcium transport in slices of rabbit kidney cortex: the uptake and distribution of calcium. Physiol Bohemoslov 12:405–416
Höfer M, Kleinzeller A (1963b) Calcium transport in slices of rabbit kidney cortex: the steady state compartmentalization of Ca and rate constants of ^{45}Ca efflux. Physiol Bohemoslov 12:417–424
Höfer M, Kleinzeller A (1963c) Calcium transport in slices of rabbit kidney cortex: the loss of calcium from Ca-enriched slices. Physiol Bohemoslov 12:425–434
Hogeboom GH, Schneider WC, Striebich MJ (1953) Localization and integration of cellular function. Cancer Res 13:617–632
Hohman W, Schraer H (1966) The intracellular distribution of calcium in the mucosa of the avian shell gland. J Cell Biol 30:317–331
Hope-Gill H, Kissebah A, Tulloch B, Clarke P, Uydelingum N, Fraser TR (1975) The effects of insulin on adipocyte calcium fluxes and the interaction with the effects of dibutyryl cyclic AMP and adrenaline. Horm Metab Res 7:195–196
Hope-Gill HF, Kissebah AH, Clarke P, Vydelingum N, Tulloch B, Fraser TR (1976) Effects of insulin and procaine hydrochloride on glycogen synthetase activation and adipocyte calcium flux – evidence for a role of calcium in insulin activation of glycogen synthetase. Horm Metab Res 8:184–190
Howell SL, Mantague W, Tyhurst M (1975) Calcium distribution in islets of Langerhans: a study of calcium concentrations and calcium accumulation in 3 cell organelles. J Cell Sci 19:395–409
Howell SL, Tyhurst M (1976) ^{45}Calcium localization in islets of Langerhans, a study by electron-microscopy autoradiography. J Cell Sci 21:415–422
Hughes BP, Barritt GJ (1978) Effects of glucagon and N_6,O_2 dibutyryladenosine 3′:5′-cyclic monophosphate on calcium transport in isolated rat liver mitochondria. Biochem J 176:295–304
Hughes BP, Barritt GJ (1979) Interaction between glucocorticoids and glucagon in the hormonal modification of calcium retention by isolated rat liver mitochondria. Biochem J 180:291–295
Hutson SM (1977) Steady state kinetics of energy-dependent Ca^{2+} uptake in rat liver mitochondria. J Biol Chem 252:4539–4545
Hutson SM, Pfeiffer DR, Lardy HA (1976) Effect of cations and anions on the steady state kinetics of energy-dependent Ca^{2+} transport in rat liver mitochondria. J Biol Chem 251:5251–5258
Huxley AF, Stämpfli R (1951) Direct determination of membrane resting potential and action potential in single myelinated nerve fibers. J Physiol (Lond) 112:476–495
Huxtable R, Bressler R (1974) The effect of deuterium ion concentration on the properties of sarcoplasmic reticulum. J Membr Biol 17:189–197
Ilundain A, Naftalin RJ (1979) Role of Ca^{2+}-dependent regulator protein in intestinal secretion. Nature 279:446–448
Isaacson A (1969) Caffein-induced contracture and related calcium movements of muscle in hypertonic media. Experientia 25:1263–1265
Isaacson A, Sandow A (1967) Quinine and caffeine effects on ^{45}Ca movements in frog sartorius muscle. J Gen Physiol 50:2109–2128
Ishibashi F, Sato T, Onari K, Kawate R (1979) Effect of somatostatin on glucose-induced Ca^{45} uptake in the pancreatic islets. Endocrinol Jpn 26:585–590
Izzard S, Tedeschi H (1973) Characterization of orthophosphate induced active cation transport in isolated liver mitochondria. Arch Biochim Biophys 154:527–539
Jacobus WE, Tiozzo R, Lugli G, Lehninger AL, Carafoli E (1975) Aspects of energy-linked Ca^{2+} accumulation by rat-heart mitochondria. J Biol Chem 250:7863–7870

Janda S (1969) Mechanism of calcium transport in kidney cortex slices. Physiol Bohemoslov 18:413–423

Jarrett HW, Penniston JT (1977) Partial purification of the (Ca^{+2} + Mg^{+2}) ATPase activator from human erythrocytes: its similarity to the activator of 3′:5′ cyclic nucleotide phosphodiesterase. Biochem Biophys Res Commun 77:1210–1216

Jay AWL, Burton AC (1969) Direct measurement of potential difference across the human red blood cell membrane. Biophys J 9:115–121

Johansson P, Josefsson J (1978) Evidence for a dual effect of intracellular calcium ion on pinocytosis. Acta Physiol Scand 102:71A–72A

Judah JD, Ahmed K (1963) Role of phosphoproteins in ion transport: interactions of sodium with calcium and potassium in liver slices. Biochim Biophys Acta 71:34–44

Judah JD, Ahmed K (1964) The biochemistry of sodium transport. Biol Rev 39:160–193

Jundt H, Porzig H, Reuter H, Stucki JW (1975) The effect of substances releasing intracellular calcium ions on sodium-dependent calcium efflux from guinea pig auricles. J Physiol (Lond) 246:229–253

Jundt H, Reuter H (1977) Is sodium-activated calcium efflux from mammalian cardiac muscle dependent on metabolic energy. J Physiol (Lond) 266:78P–79P

Juzu HA, Holdsworth ES (1980) New evidence for the role of cyclic AMP in the release of mitochondrial calcium. J Membr Biol 52:185–189

Kakuta S, Suda T, Sasaki S, Kimura N, Nagata N (1975) Effects of parathyroid hormone on the accumulation of cyclic AMP in bone of vitamin D deficient rats. Endocrinology 97:1288–1293

Kalix P (1969) Na-activated calcium efflux in rabbit vagus nerve fibres. Experientia 25:1293–1294

Kalix P (1971) Uptake and release of calcium in rabbit vagus nerve. Pfluegers Arch 326:1–14

Kanagasuntheram P, Randle P (1976) Calcium metabolism and amylase release in rat parotid acinar cells. Biochem J 160:547–564

Käser-Glanzmann R, Jakabova M, George JN, Lüscher EF (1977) Stimulation of calcium uptake in platelet membrane vesicles by adenosine 3′5′-cyclic monophosphate and protein kinase. Biochim Biophys Acta 466:429–440

Kass RS, Tsien RW (1975) Multiple effects of calcium antagonists on plateau currents in cardiac Purkinje fibers. J Gen Physiol 66:169–192

Katz AM, Repke DL, Fudyma G, Shigekawa M (1977) Control of calcium efflux from sarcoplasmic reticulum vesicles by external calcium. J Biol Chem 252:4210–4214

Katz B, Miledi R (1967) A study of synaptic transmission in the absence of nerve impulses. J Physiol (Lond) 192:407–436

Katz B, Miledi R (1969) Tetrodotoxin-resistant electric activity in presynaptic terminals. J Physiol (Lond) 203:459–487

Katz S, Remtulla MA (1978) Phosphodiesterase protein activator stimulates calcium transport in cardiac microsomal preparations enriched in sarcoplasmic reticulum. Biochem Biophys Res Commun 83:1373–1379

Katzung BG, Reuter H, Portzig H (1973) Lanthanum inhibits Ca inward current but not Na-Ca exchange in cardiac muscle. Experientia 29:1073–1075

Keppens S, Vendenheede JR, DeWulf H (1977) On the role of calcium as second messenger in liver for the hormonally induced activation of glycogen phosphorylase. Biochim Biophys Acta 496:448–457

Khan AR (1979) Effects of diethylstilbestrol on single fibers of frog skeletal muscle. Acta Physiol Scand 106:69–74

Kahn A, Brachet E (1979) The permeability coefficient of albumin of the isolated rat mesentery – a modification by some mediators of inflammation, cyclic AMP and calcium. Biochim Biophys Acta 588:219–231

Kikuchi M, Wollheim CB, Cuendet GS, Renold AE, Sharp WG (1978) Studies on the dual effects of glucose on ^{45}Ca efflux from isolated rat islets. Endocrinology 102: 1339–1349

Kikuchi M, Wollheim CB, Seigel EG, Renold AE, Sharp GWG (1979) Biphasic insulin release in rat islets of Langerhans and the role of intracellular Ca^{++} stores. Endocrinology 105:1013–1019

Kimberg DV, Baerg RD, Gershon E, Graudusius RT (1971) Effect of cortisone treatment on the active transport of calcium by the small intestine. J Clin Invest 50: 1309–1321

Kimura S, Rasmussen H (1977) Adrenal glucorticoids, adenine nucleotide translocation, and mitochondrial calcium accumulation. J Biol Chem 252:1217–1225

Kimura T, Chu JW, Mukai R, Ishizuka I, Ymamkawa T (1972) Some properties of a glycoprotein isolated from adrenal cortex mitochondria. Biochem Biophys Res Commun 49:1678–1683

Kinne-Saffran E, Kinne R (1974) Localization of a calcium-stimulated ATPase in the basal-lateral plasma membranes of the proximal tubule of rat kidney cortex. J Membr Biol 17:263–274

Kirchberger MA, Tada M, Repke DL, Katz AM (1972) Cyclic adenosine 3′5′-monophosphate-dependent protein kinase stimulation of calcium uptake by canine cardiac microsomes. J Mol Cell Cardiol 4:673–680

Kissebah AH, Clarke P, Vydelingum N, Hope-Gill H, Tulloch B, Fraser TR (1975) The role of calcium in insulin action. III. Calcium distribution in fat cells; its kinetics and the effects of adrenaline, insulin and procaine-HCl. Eur J Clin Invest 5:339–349

Klaus W, Krebs R (1974) Analysis of the calcium compartments in guinea pig hearts under control conditions and under the influence of ouabain. Arch Pharmacol 283: 277–292

Klein CD, Raisz LG (1971) Role of adenosine-3′,5′-monophosphate in the hormonal regulation of bone resorption: studies with cultured fetal bone. Endocrinology 89: 818–826

Kohlhardt M, Bauer B, Krause H, Fleckenstein A (1972) Differentiation of the transmembrane Na and Ca channels in mammalian cardiac fibres by the use of specific inhibitors. Pflügers Arch 335:309–322

Kohlhart M, Kübler M (1975) The influence of metabolic inhibitors upon the transmembrane slow inward current in the mammalian ventricular myocardium. Arch Pharmacol 290:265–274

Kohlhart M, Mnich Z, Maier G (1977) Alteration of the excitation process of the sinoatrial pacemaker cell in the presence of anoxia and metabolic inhibitors. J Mol Cell Cardiol 9:477–488

Koketsu K, Kitamura R, Tanaka R (1964) Binding of calcium ions to cell membrane isolated from bullfrog skeletal muscle. Am J Physiol 207:509–512

Kondo S, Schulz I (1976a) Calcium ion uptake in isolated pancreas cells induced by secretagogues. Biochim Biophys Acta 419:76–92

Kondo S, Schulz I (1976b) Ca^{++} fluxes in isolated cells of rat pancreas. Effect of secretagogues and different Ca^{++} concentrations. J Membr Biol 29:185–203

Kostyuk PG, Krishtal OA (1977a) Separation of sodium and calcium currents in the somatic membrane of mollusc neurones. J Physiol (Lond) 270:545–568

Kostyuk PG, Krishtal OA (1977b) Effects of calcium and calcium-chelating agents on the inward and outward current in the membrane of mollusc neurones. J Physiol (Lond) 270:569–580

Krall JF, Swensen JL, Korenman SG (1976) Hormonal control of uterine contraction characterization of cyclic AMP-dependent membrane properties in the myometrium. Biochim Biophys Acta 448:578–588

Krawitt EL, Kunin AS, Bettejayne FB (1976) Intestinal mitochondrial calcium uptake during adaptation to dietary calcium restriction. Calcif Tissue Res 21:129–133

Krawitt EL, Stubbert PR (1972) The role of intestinal transport proteins in cortisone-mediated suppression of Ca^{2+} absorption. Biochim Biophys Acta 274:179–188

Kretsinger RH (1979) The informational role of calcium in the cytosol. Adv Cyclic Nucl Res 11:1–26

Kuntzinger H, Amiel C, Roinel N, Morel F (1974) Effects of parathyroidectomy and cyclic AMP on renal transport of phosphate, calcium, and magnesium. Am J Physiol 227:905–911

Kurebe M (1978) Interaction of dibucaine and calcium ion on a calcium pump reconstituted from defined components of intestinal brush border. Mol Pharmacol 14: 138–144

Kurebe M (1979) Effect of lipid on the access of ATP and calcium to the delipidated Ca^{++}-ATPase of intestinal brush border membrane. Life Sci 24:275–282

Lacourt A (1971) Action post mortem du pH et de la temperature sur le captage de calcium et l'activité ATPasique du réticulum sarcoplasmique fragmenté du muscle bovin. Ann Biol Anim Bioch Biophys 11:681–694

Lamb JF, Lindsay R (1971) Effect of Na, metabolic inhibitors and ATP on Ca movements in L cells. J Physiol (Lond) 218:691–708

Lamb JF, McCall D (1972) Effect of prolonged ouabain treatment on Na, K, Cl and Ca concentration and fluxes in cultured human cells. J Physiol (Lond) 225(3):599–617

Langer G, Poole-Wilson P (1977) The effect of acidosis and maganese on calcium exchange in the myocardium of the rabbit. J Physiol (Lond) 265:20

Lassen UV, Nielsen AMT, Pape L, Simonsen LO (1971) The membrane potential of Ehrlich ascites tumor cells – microelectrode measurements and their critical evaluation. J Membr Biol 6:269–288

Lassen UV, Sten-Knudsen O (1968) Direct measurements of membrane potential and membrane resistance of human red cells. J Physiol (Lond) 195:681–696

Lazarewicz JW, Kanje M, Sellström A, Hamberger A (1977) Calcium fluxes in cultures and bulk isolated neuronal and glial cells. J Neurochem 29:495–502

Lea TJ, Ashley CC (1978) Increase in free Ca^{2+} in muscle after exposure to CO_2. Nature 275:236–238

Lee KS, Shin BC (1969) Studies on the active transport of Ca^{++} in human red cells. J Gen Physiol 54:713–729

Lehninger AL (1964) The mitochondrion. Benjamin, New York

Lehninger AL (1970) Mitochondria and calcium ion transport. Biochem J 119:129–138

Lehninger AL (1974a) Role of phosphate and other proton-donating anions in respiration coupled transport of calcium by mitochondria. Proc Natl Acad Sci USA 71: 1520–1524

Lehninger AL (1974b) Ca^{2+} transport by mitochondria and its possible role in the cardiac contraction-relaxation cycle. Circ Res 35(Suppl III):83–90

Lehninger AL, Rossi CS, Greenwalt JW (1963) Respiration-dependent accumulation of inorganic phosphate and Ca^{++} by rat liver mitochondria. Biochem Biophys Res Commun 10:444–448

Lehninger AL, Carafoli E, Rossi CS (1967) Energy-linked ion movements in mitochondrial systems. Adv Enzymol 29:259–320

Lehninger AL, Reynafarje B, Vercesi A, Tew WP (1978a) Transport and accumulation of calcium mitochondria. Ann NY Acad Sci 307:160–176

Lehninger AL, Vercesi A, Bababunmi ES (1978b) Regulation of Ca^{2+} release from mitochondria by the oxidation-reduction state of pyridine nucleotides. Proc Natl Acad Sci USA 75:1690–1694

Lentz RW, Harrison CE Jr, Dewey JD, Barnhorst DA, Danielson GK, Pluth JR (1978) Functional evaluation of cardiac sarcoplasmic reticulum and mitochondria in human pathologic states. J Mol Cell Cardiol 10:3–30

LePeuch CJ, Haiech J, Demaille JG (1979) Concerted regulation of cardiac sarcoplasmic reticulum calcium transport by cyclic adenosine monophosphate-dependent and calcium-calmodulin-dependent phosphorylation. Biochemistry 18:5150–5157

Levinson C (1967) Calcium exchange in Ehrlich mouse ascites tumor cells. Biochim Biophys Acta 135:921–926
Levinson C, Blumenson LE (1970) Calcium transport and distribution in Ehrlich mouse ascites tumor cells. J Cell Physiol 75:231–240
Li CL, Shy GM, Wells J (1957) Some properties of mammalian skeletal muscle fibres with particular reference to fibrillation potentials. J Physiol (Lond) 135:522–535
Locke F (1894) Notiz über den Einfluß physiologischer Kochsalzlösung auf die elektrische Erregbarkeit von Muskel und Nerv. Zentralbl für Physiologie 8:166
Lolley RN (1963) The calcium content of isolated cerebral tissues and their steady state exchange of calcium. J Neurochem 10:665–676
Loud AV (1962) A method for the quantitative estimation of cytoplasmic structures. J Cell Biol 15:481–487
Lowe CU, Lehninger AL (1955) Oxidation and phosphorylation in liver mitochondria lacking "polymerized" ribonuclear acid. J Biophys Biochem Cytol 1:89–92
Lowe CU, MacKinney D, Sarkaria D (1955) Effect of cortisone on rat liver mitochondria. J Biophys Biochem Cytol 1:237–244
Lucas M, Schmid G, Kromao R, Löffler G (1978) Calcium metabolism and enzyme secretion in guinea pig pancreas. Uptake, storage and release of calcium in whole cells and mitochondrial and microsomal fractions. Eur J Biochem 85:609–619
Lundberg A (1958) Electrophysiology of salivary glands. Physiol Rev 38:21–40
Lupianez JA, Dileepan KN, Wagle SR (1979) Interrelationship of somatostatin, insulin and calcium in the control of gluconeogenesis in kidney cortex slices. Biochem Biophys Res Commun 90:1153–1158
Luthra MG, Hildebrandt GR, Hanahan DJ (1976a) Studies on an activator of the (Ca^{++} + Mg^{++})-ATPase of human erythrocyte membranes. Biochim Biophys Acta 419:164–179
Luthra MG, Hildenbrandt GR, Kim HD, Hanahan DJ (1976b) Observations on the (Ca^{++} + Mg^{++})ATPase activator found in various mammalian erythrocytes. Biochim Biophys Acta 419:180–186
Lüttgau HC, Niedergerke R (1958) The antagonism between Ca and Na ions on the frog's heart. J Physiol (Lond) 143:486–505
Lutwak-Mann C, McIntosh JEA (1971) Calcium content and uptake of ^{45}Ca in rabbit blastocysts and their environment. J Reprod Fertil 27:471–475
Luxoro M, Yanez E (1968) Permeability of the giant axon of *Dosidicus gigas* to calcium ions. J Gen Physiol 51:1115–1225
Lynch TJ, Cheung WY (1979) Human erythrocyte Ca^{2+}-Mg^{2+}-ATPase: mechanisms of stimulation by Ca^{2+}. Arch Biochem Biophys 194:165–170
MacManus JP, Whitfield JF (1970) Inhibition by thyrocalcitonin of the mitogenic action of parathyroid hormone and cyclic adenosine 3′,5′-monophosphate on rat thymocytes. Endocrinology 86:934–939
Malaisse WJ, Herchuelz A, Devis G, Somers G, Boschero AC, Hutton JC, Kawazu S, Sener A (1978) Regulation of calcium fluxes and their regulatory roles in pancreatic islets. Ann NY Acad Sci 307:562–581
Malaisse-Lagae F, Malaisse WJ (1971) Stimulus-secretion coupling of glucose-induced insulin release. III. Uptake of ^{45}calcium by isolated islets of Langerhans. Endocrinology 88:72–80
Malmström K, Carafoli E (1977) The interaction of Ca^{2+} with mitochondria from human myometrium. Arch Biochem Biophys 182:657–666
Martin BR, Clausen T, Gliemann J (1975) Relationships between the exchange of Ca^{++} and PO_4^{-2} in isolated fat cells. Biochem J 152:121–129
Martin JH, Matthew JL (1970) Mitochondrial granules in chondrocytes osteoblasts and osteocytes. An ultrastructural and microincineration study. Clin Orthop 68:273–278
Martonosi AN (1975) The mechanism of Ca^{2+} transport in sarcoplasmic reticulum. In: Carafoli E, Clementi F, Drabikowski W, Magreth A (eds) Calcium transport in contraction and secretion. North Holland, Amsterdam, pp 313–327

Martonosi AN, Chyn TL, Schibeci A (1978) The calcium transport of sarcoplasmic reticulum. Ann NY Acad Sci 307:148–156
Matlib A, O'Brien JP (1974) Adenosine 3':5'-cyclic monophosphate stimulation of calcium efflux. Biochem Soc Trans 2:997–1000
Matsushima Y, Gemba M (1979) Divalent cation transport in kidney slices. I. Properties of calcium transport in slices of rat kidney cortex and the effects of diuretics. Jpn J Pharmacol 29:367–374
Matthews EK (1967) Membrane potential measurement in cells of the adrenal gland. J Physiol (Lond) 189:139–148
Matthews EK (1975) Calcium and stimulus-secretion coupling in pancreatic islet cells. In: Carafoli E, Clementi F, Drabikowski W, Magreth A (eds) Calcium transport in contraction and secretion. North Holland, Amsterdam, pp 203–210
Matthews EK, Petersen OH (1973) Pancreas acinar cells: ionic dependence of the membrane potential and acetylcholine-induced depolarization. J Physiol (Lond) 231:283–295
Matthews EK, Saffran M (1967) Steroid production and membrane potential measurement in cells of the adrenal cortex. J Physiol (Lond) 189:149–161
Matthews EK, Sakamoto Y (1975a) Electrical characteristics of pancreatic islet cells. J Physiol (Lond) 246:421–437
Matthews EK, Sakamoto Y (1975b) Pancreatic islet cells: electrogenic and electrodiffusional control of membrane potential. J Physiol (Lond) 246:439–457
Matthews EK, Petersen OH, Williams JA (1973) Pancreatic acinar cells: acetylcholine-induced membrane depolarization, calcium efflux and amylase release. J Physiol (Lond) 234:689–701
Matthews JL, Martin JH, Sampson HW, Kunin AS, Roan JH (1970) Mitochondrial granules in the normal and rachitic rat epiphysis. Calcif Tissue Res 5:91–99
May RJ, Conlon TP, Erspamer V, Gardner JD (1978) Actions of peptides isolated from amphibian skin on pancreatic acinar cells. Am J Physiol 235:E112–E118
McDonald JM, Bruns DE, Jarett L (1976a) Ability of insulin to increase calcium binding by adipocyte plasma membranes. Proc Natl. Acad Sci USA 73:1542–1546
McDonald JM, Bruns DE, Jarett L (1976b) The ability of insulin to alter the stable calcium pools of isolated adipocyte subcellular fractions. Biochem Biophys Res Commun 71:114–121
McDonald JM, Bruns DE, Jarett L (1978) Ability of insulin to increase calcium uptake by adipocyte endoplasmic reticulum. J Biol Chem 253:3504–3508
McLennan DH, Holland PC (1975) Calcium transport in sarcoplasmic reticulum. Ann Rev Biophys Bioengin 4:377:404
McNaughton PA (1978) Calcium transport in excitable membranes. In: Morad M, Tabatabai M, Smith S (eds) Biophysical aspects of cardiac muscle. Academic Press, New York, pp 107–128
Mears DC (1971) Effects of parathyroid hormone and thyrocalcitonin on the membrane potential of osteoblasts. Endocrinology 88:1021–1028
Meech RW (1974) The sensitivity of *Helix aspersa* neurones to injected calcium ions. J Physiol (Lond) 237:259–277
Meech RW, Standen NB (1975) Potassium activation in *Helix aspersa* neurones under voltage clamp: a component mediated by calcium influx. J Physiol (Lond) 249: 211–239
Meinertz T, Nawrath H, Scholz H (1973a) Dibutyryl cyclic AMP and adrenaline increase contractile force and ^{45}Ca uptake in mammalian cardiac muscle. Naunyn Schmiedebergs Arch Pharmacol 277:107–112
Meinertz T, Nawrath H, Scholz H (1973b) Stimulatory effects of DB-c-AMP and adrenaline on myocardial contraction and ^{45}Ca exchange. Experiments at reduced calcium concentration and low frequencies of stimulation. Naunyn Schmiedebergs Arch Pharmacol 279:327–338
Mela L (1968) Interaction of La^{+3} and local anesthetic drugs with mitochondrial Ca^{+2} and Mn^{+2} uptake. Arch Biochem Biophys 123:286–293

Mela L (1969) Inhibition and activation of calcium transport in mitochondria. Effect of lanthanides and local anesthetic drugs. Biochemistry 8:2481–2486
Mela L (1977) Mechanism and physiological significance of calcium transport across mammalian mitochondrial membranes. In: Bronner F, Kleinzeller A (eds) Current topics in membranes and transport, vol 9. Academic Press, New York, pp 321–366
Mela L, Chance B (1969) Calcium carrier and the "high affinity calcium binding site" in mitochondria. Biochem Biophys Res Commun 35:556–559
Melson GL, Chase LR, Aurbach GD (1970) Parathyroid hormone sensitive adenyl cyclase in isolated renal tubules. Endocrinology 86:511–518
Miller BE, Nelson DL (1977) Calcium fluxes in isolated acinar cells from rat parotid. Effect of adrenergic and cholinergic stimulation. J Biol Chem 252:3629–3635
Miller DJ, Moiescu DG (1976) The effects of very low external calcium and sodium concentration on cardiac contractile strength and calcium-sodium antagonism. J Physiol (Lond) 259:283–308
Mitchell P (1966) Metabolic flow in the mitochondrial multiphase system: an appraisal of the chemi-osmotic theory of oxydative phosphorylation. In: Tager JM, Papa S, Quagliariello E, Slater EC (eds) Regulation of metabolic processes in mitochondria. Elsevier, Amsterdam, pp 65–85
Mitchell P, Moyle J (1969) Estimation of membrane potential and pH difference across the cristae membrane of rat liver mitochondria. Eur J Biochem 7:471–484
Moore CL (1971) Specific inhibition of mitochondrial Ca^{++} transport by ruthenium red. Biochem Biophys Res Commun 42:298–305
Moore JW, Cole KS (1960) Resting and action potentials of squid giant axon in vivo. J Gen Physiol 43:961–970
Moore L, Chen T, Knapp HR Jr, Landon EJ (1975) Energy dependent calcium sequestration activity in rat liver microsomes. J Biol Chem 250:4562–4568
Moore L, Fitzpatrick DF, Chen TS, Landon EJ (1974) Calcium pump activity of the renal plasma membrane and renal microsomes. Biochim Biophys Acta 345:405–418
Moore L, Pastan J (1977a) Regulation of intracellular calcium in chick embryo fibroblast: calcium uptake by the microsomal fraction. J Cell Physiol 91:289–296
Moore L, Pastan I (1977b) Energy dependent calcium uptake in cultured mouse fibroblast microsomes. Regulation of the uptake system by cell density. J Biol Chem 252:6304–6309
Moore L, Pastan I (1978) Energy dependent calcium uptake by fibroblast microsomes. Ann NY Acad Sci 307:177–193
Morgenstern M, Noack E, Köhler E (1972) The effects of isoprenaline and tyramine on the ^{45}calcium uptake, the total calcium content and the contraction force of isolated guinea pig atria in dependence on different extracellular hydrogen ion concentrations. Arch Pharmacol 274:125–137
Moriarty CM (1977) Involvement of intracellular calcium in hormone secretion from rat pituitary cells. Mol Cell Endocrinol 6:349–361
Moriarty CM (1980) Kinetic analysis of calcium distribution in rat anterior pituitary slices. Am J Physiol 238:E167–E173
Moyle J, Mitchell P (1977a) Electric charge stoichiometry of calcium translocation in rat liver mitochondria. FEBS Lett 73:131–136
Moyle J, Mitchell P (1977b) The lanthanide-sensitive calcium phosphate porter of rat liver mitochondria. FEBS Lett 77:136–140
Mullins LJ (1976) Steady-state calcium fluxes: membrane versus mitochondrial control of ionized calcium in axoplasm. Fed Proc 35:2583–2588
Mullins LJ (1977) A mechanism for Na/Ca transport. J Gen Physiol 70:681–695
Mullins LJ, Brinley FJ Jr (1975) Sensitivity of calcium efflux from squid axons to changes in membrane potential. J Gen Physiol 65:135–152
Mullins LJ, Requena J (1979) Calcium measurement in the periphery of an axon. J Gen Physiol 74:393–413

Murad F, Brewer HB Jr, Vaughan M (1970) Effect of thyrocalcitonin on adenosine 3′,5′-cyclic phosphate formation by rat kidney and bone. Proc Natl Acad Sci 65: 446–453

Nagata N, Rasmussen H (1968) Parathyroid hormone and renal cell metabolism. Biochemistry 7:3728–3733

Nagata N, Rasmussen H (1970) Parathyroid hormone, 3′-5′ AMP, Ca^{++} and renal gluconeogenesis. Proc Natl Acad Sci USA 65:368–374

Nakamura Y, Schwartz A (1970) Possible control of intracellular calcium metabolism by $[H^+]$: sarcoplasmic reticulum of skeletal and cardiac muscle. Biochem Biophys Res Commun 41:830–836

Nakamura Y, Schwartz A (1972) The influence of hydrogen ion concentration on calcium binding and release by skeletal muscle sarcoplasmic reticulum. J Gen Physiol 59:22–32

Nanninga LB (1961a) The association constant of the complexes of adenosine triphosphate with magensium, calcium, strontium and barium ions. Biochim Biophys Acta 54:330–338

Nanninga LB (1961b) Calculation of free magnesium, calcium and potassium in muscle. Biochim Biophys Acta 54:338–344

Navickis RJ, Dial OK, Katzenellenbogen BS, Nalbandov AV (1979) Effect of gonadal hormones on calcium-binding protein in chick duodenum. Am J Physiol 237: E409–E417

Nawrath H, TenEick RE, McDonald TF, Trautwein W (1977) On the mechanism underlying the action of D-600 on slow inward current and tension in mammalian myocardium. Circ Res 40:408–414

Nayler WG, Merrillees NCR, Chipperfield D, Kurtz JB (1971) Influence of hyperthyroidism on the uptake and binding of calcium by cardiac microsomal fractions and on mitochondrial structures. Cardiovasc Res 5:469–482

Nayler WG, Poole-Wilson PA, Williams A (1979) Hypoxia and calcium. J Mol Cell Cardiol 11:683–706

Nicholls DG (1974) The influence of respiration and ATP hydrolysis on the proton-electrochemical gradient across the inner membrane of rat liver mitochondria as determined by ion distribution. Eur J Biochem 50:305–315

Nicholls DG (1978a) Calcium transport and proton electron chemical potential gradient in mitochondria from guinea pig cerebral cortex and rat heart. Biochem J 170: 511–522

Nicholls DG (1978b) The regulation of extramitochondrial free calcium ion concentration by rat liver mitochondria. Biochem J 176:463–474

Nichols G Jr, Rogers P (1971) Mechanism for the transfer of calcium into and out of the skeleton. Pediatrics 47:211–228

Niedergerke R (1963) Movements of Ca in beating ventricles of the frog heart. J Physiol (Lond) 167:551–580

Niggli V, Ronner P, Carafoli E, Penniston JT (1979) Effect of calmodulin on the (Ca^{2+} + Mg^{2+}) ATPase partially purified from erythrocyte membranes. Arch Biochem Biophys 198:124–13-

Nishikori K, Maeno H (1979) Close relationship between adenosine 3′,5′-monophosphate-dependent endogenous phosphorylation of a specific protein and stimulation of calcium uptake in rat uterine microsomes. J Biol Chem 254:6099–6106

Nishikori K, Takenaka T, Maeno H (1977) Stimulation of microsomal calcium uptake and protein phosphorylation by adenosine cyclic 3′5′-monophosphate in rat uterus. Mol Pharmacol 13:671–678

Noack EA, Heinen EM (1977) A kinetic study of calcium transport by heart mitochondria. Eur J Biochem 79:245–250

Ogawa Y (1970) Some properties of fragmented frog sarcoplasmic reticulum with particular reference to its response to caffeine. J Biochem (Tokyo) 67:667–683

Ohashi T, Uchida S, Nagai K, Yoshida H (1970) Studies on phosphate hydrolyzing activities in the synaptic membrane. J Biochem (Tokyo) 67:635–641

Ohtsuki I (1969) ATP-dependent Ca uptake of brain microsomes. J Biochem (Tokyo) 66:645–650

Okada Y, Ogawa M, Aoki N, Izutsu K (1973) The effect of K^+ on the membrane potential in HeLa cells. Biochim Biophys Acta 291:116–126

Oswald N, Binswanger U (1979) Proximal and distal intestinal calcium transport in vitro as influenced by low calcium diet, uremia, parathyroidectomy and 1,25-dihydroxycholecalciferol treatment in rats. Res Exp Med 175(1):19–30

Otsuka M Ohtsuki I, Ebashi S (1965) ATP dependent Ca binding of brain microsome. J Biochem (Tokyo) 58:188–190

Overton E (1904) Beiträge zur allgemeinen Muskel- und Nervenphysiologie. Pfluegers Arch 105:176–290

Owen JD, Brown HM, Pemberton JP (1977) Neurophysiological applications of a calcium selective microelectrode. Anal Chim Acta 90:241–244

Palek J, Curby WA, Lionetti FJ (1971) Relation of Ca^{++}-activated ATPase to Ca^{++} linked shrinkage of human red cell ghosts. Am J Physiol 220:1028–1032

Palmer RF, Posey VA (1970) Calcium and adenosine triphosphate binding to renal membranes. J Gen Physiol 55:89–103

Panfili E, Sandri G, Sottocasa GL, Lunazzi G, Liut G, Graziosi G (1976) Specific inhibition of mitochondrial Ca^{2+} transport by antibodies directed to the Ca^{2+}-binding glycoprotein. Nature 264:185–186

Papworth DG, Patrick G (1970) The kinetics of influx of calcium and strontium into rat intestine in vitro. J Physiol (Lond) 210:999–1020

Parker JC, Barritt GJ (1979) Effects of epinephrine on the subcellular distribution of exchangeable calcium in isolated rat heptaocytes. Proc Aust Biochem Soc 12:52

Parkinson DK, Radde IC (1971) Properties of a Ca^{2+} and Mg^{2+}-activated ATP hydrolyzing enzyme in rat kidney cortex. Biochim Biophys Acta 242:238–246

Patriarca P, Carafoli E (1968) Study of the intracellular transport of calcium in rat heart. J Cell Physiol 72:29–38

Pau B, Dornand J, Mani JC (1976) Etude critique de l'activité ATPase activée par Ca^{2+} ou Mg^{2+} des membranes plasmiques lymphocytaires. Biochimie 58:593–599

Peachy LD (1964) Electron microscopic observations on the accumulation of divalent cations in intramitochondrial granules. J Cell Biol 20:95–108

Peck WA, Carpenter K, Messinger K, DeBra D (1973) Cyclic 3′5′ adenosine monophosphate in isolated bone cells: response to low concentrations of parathyorid hormone. Endocrinology 92:692–697

Perdue JF (1971) The isolation and characterization of plasma membranes from cultured cells. III. The adenosine triphosphate-dependent accumulation of Ca^{2+} by chick embryo fibroblasts. J Biol Chem 246:6750–6759

Petersen OH, Iwatsuki N (1978) The role of calcium in pancreatic acinar cell stimulus-secretion coupling: an electrophysiological approach. Ann NY Acad Sci 307:599–617

Petith MM, Wilson HD, Schedl HP (1979) Vitamin D dependence of in vivo calcium transport and mucosal calcium binding protein in rat large intestine. Gastroenterology 76:99–104

Peug CF, Price DW, Bhuvaneswaran C, Wadkins CL (1974) Factors that influence phosphoenolpyruvate-induced calcium efflux from rat liver mitochondria. Biochem Biophys Res Commun 56:134–141

Plishker G, Gitelman H (1976) Calcium transport in intact human erythrocytes. J Gen Physiol 68:29–41

Pors-Nielsen S, Petersen OH (1972) Transport of calcium in the perfused submandibular gland of the cat. J Physiol (Lond) 223:685–697

Portzehl H, Caldwell PC, Rüegg JC (1964) The dependence of contraction and relaxation of muscle fibres from the crab *Maia squinado* on the internal concentrations of free calcium ions. Biochim Biophys Acta 79:581–591

Porzig H (1972) ATP-independent calcium net movements in human red cell ghosts. J Membr Biol 8:237–258

Pozzan T, Bragadin M, Azzone GF (1977) Disequilibrium between steady-state Ca^{2+} accumulation ratio and membrane potential in mitochondria. Pathway and role of calcium efflux. Biochemistry 16:5618–5625

Price JM, Miller EC, Miller JA (1948) The intracellular distribution of protein, nucleic acids, riboflavin and protein bound aminoazo dye in the liver of rats fed p-dimethylaminoazobenzene. J Biol Chem 173:345–353

Price JM, Miller JA, Miller EC, Weber GM (1949) Studies on the intracellular composition of liver and liver tumor from rats fed 4-dimethylaminoazobenzene. Cancer Res 9:96–102

Prince WT, Berridge MJ (1973) The role of calcium in the action of 5-hydroxytryptamine and cyclic AMP on salivary glands. J Exp Biol 58:367–384

Prince WT, Berridge MJ, Rasmussen H (1972) Role of calcium and adenosine 3′:5′-cyclic monophosphate in controlling fly salivary gland secretion. Proc Natl Acad Sci USA 69:553–557

Puskin JS, Gunter TE, Gunter KK, Russell PR (1976) Evidence for more than one Ca^{2+} transport mechanism in mitochondria. Biochemistry 15:3834–3842

Quinn PJ, Dawson RMC (1972) The pH dependence of calcium absorption onto anionic phospholipid monolayers. Chem Phys Lipids 8:1–9

Quist EE, Roufogalis BD (1975) Calcium transport in human erythrocytes starvation and reconstitution of high and low affinity (Mg + Ca) ATPase activities in membranes prepared at low ionic strength. Arch Biochem Biophys 168:240–251

Quist EE, Roufogalis BD (1977) Association of (Ca + Mg)-ATPase activity with ATP-dependent Ca uptake in vesicles prepared from human erythrocytes. J Supramol Struct 6:375–381

Raeymaekers L, Wuytack F, Casteels R (1973) Na-Ca exchange in smooth muscle cells of *Taenia coli*. Arch Int Pharmacodyn 204:196–197

Rahamimoff H, Abramovitz E (1978) Calcium transport and ATPase activity of synaptosomal vesicles from rat brain. FEBS Lett 92:163–167

Raisz LG, Trummel CL, Wener JA, Simmons H (1972) Effect of glucocorticoids on bone resorption in tissue culture. Endocrinology 90:961–967

Rambourg A, Leblond CP (1967) Electron microscope observations on the carbohydrate-rich cell coat present at the surface of cells in the rat. J Cell Biol 32:27–53

Rasmussen H (1966) Mitochondrial ion transport: mechanism and physiological significance. Fed Proc 25:903–911

Rasmussen H (1970) Cell communication, calcium ion, and cyclic adenosine monophosphate. Science 170:404–412

Rasmussen H (1971) Ionic and hormonal control of calcium homeostasis. Am J Med 50:567–588

Rasmussen H, Bordier P (1974) The physiological and cellular basis of metabolic bone disease. Williams & Wilkins, Baltimore

Rasmussen H, Goodman DP (1977) Relationships between calcium and cyclic nucleotides in cell activation. Physiol Rev 57:421–509

Rasmussen H, Jensen P, Lake W, Friedmann N, Goodman DBP (1975a) Cyclic nucleotides and cellular calcium metabolism. Adv Cyclic Nucleotide Res 5:375–394

Rasmussen H, Lake W, Allen JE (1975b) The effect of catecholamines and prostaglandins upon human and rat erythrocytes. Biochim Biophys Acta 411:63–73

Redmann K (1971) Messungen elektrischer Membranpotentiale kultivierter Einzelzellen mittels Mikroelektroden. Acta Biol Med Germ 27:55–68

Redmann K, Stolte C, Lüders D (1967) Membranpotential-Messungen an KB-Zellkulturen. Naturwissenschaften 54:255

Reed KC, Bygrave FL (1974a) The inhibition of mitochondrial calcium transport by lanthanides and ruthenium red. Biochem J 140:143–155

Reed K, Bygrave F (1974b) A reevaluation of energy-independent calcium ion binding by rat liver mitochondria. Biochem J 142:535–566
Reed KC, Bygrave FL (1974c) Accumulation of lanthanum by rat liver mitochondria. Biochem J 138:239–252
Reed KC, Bygrave LF (1975a) Methodology for in vitro studies of Ca^{2+} transport. Anal Biochem 67:44–54
Reed KC, Bygrave FL (1975b) A kinetic study of mitochondrial calcium transport. Eur. J Biochem 55:497–504
Reed PW (1977) Calcium ionophore activity of prostaglandin endoperoxides and stabilized analogs of prostaglandin H-2. Fed Proc 36:673
Renckens BAM, Schrijen JJ, Swarts HGP, De Pont JJHHM, Bonting SL (1978) Role of calcium in exocrine pancreatic secretion. IV. Calcium movements in isolated acinar cells of rabbit pancreas. Biochim Biophys Acta 544:338–350
Repke DI, Katz AM (1972) Calcium binding and calcium uptake by cardiac microsomes: a kinetic analysis. J Mol Cell Cardiol 4:401–416
Requena J (1978) Calcium efflux from squid axons under constant sodium electrochemical gradient. J Gen Physiol 72:443–470
Requena J, DiPolo R, Brinley FJ, Mullins LJ (1977) The control of ionized calcium in squid axon. J Gen Physiol 70:329–353
Requena J, Mullins LJ (1979) Calcium movements in nerve fibers. Q Rev Biophys 12: 371–460
Requena J, Mullins LJ, Brinley FJ Jr (1979) Calcium content and net fluxes in squid giant axons. J Gen Physiol 73:327–342
Reuter H (1967) The dependence of slow inward current in Purkinje fibres on the extracellular calcium concentration. J Physiol (Lond) 192:479–492
Reuter H (1970) Calcium transport in cardiac muscle. In: Bolis L (ed) Permeability and function of biological membranes. North Holland, Amsterdam, pp 342–347
Reuter H (1973) Divalent cations as charge carriers in excitable membranes. Prog Biophys Mol Biol 26:1–43
Reuter H (1974a) Exchange of calcium ions in the mammalian myocardium: mechanisms and physiological significance. Circ Res 34:599–605
Reuter H (1974b) Localization of β-adrenergic receptors and effects of noradrenaline and cyclic nucleotides on action potentials, ionic currents and tension in mammalian cardiac muscle. J Physiol (Lond) 242:429–451
Reuter H (1979) Properties of two inward membrane currents in the heart. Annu Rev Physiol 41:413–424
Reuter H, Blaustein MP, Haeusler G (1973) Na-Ca exchange and tension development in arterial smooth muscle. Philos Trans R Soc Lond (Biol) 265:87–94
Reuter H, Scholz H (1977a) A study of the ion selectivity and the kinetic properties of the calcium dependent slow inward current in mammalian cardiac muscle. J Physiol (Lond) 264:17–47
Reuter H, Scholz H (1977b) The regulation of the calcium conductance of cardiac muscle by adrenaline. J Physiol (Lond) 264:49–62
Reuter H, Seitz N (1968) The dependence of calcium efflux from cardiac muscle on temperature and external ion composition. J Physiol (Lond) 195:451–470
Reynafarje B, Lehninger AL (1969) High affinity and low affinity binding of Ca^{++} by rat liver mitochondria. J Biol Chem 244:584–593
Reynafarje BL, Lehninger AL (1973) Ca^{2+} transport by mitochondria from L-1210 mouse ascites tumor cells. Proc Natl Acad Sci USA 70:1744–1748
Ringer S (1883) A further contribution regarding the influence of the different constituents of the blood on the contraction of the heart. J Physiol (Lond) 4:29–49
Rink TJ (1977) The influence of sodium on calcium movement and catecholamine release in thin slices of bovine adrenal medulla. J Physiol (Lond) 266:297–325
Robblee LS, Shepro D, Belamarich FA (1973) Calcium uptake and associated ATPase activity of isolated platelets membranes. J Gen Physiol 61:462–481

Robinson JD, Lust WD (1968) Adenosine triphosphate-dependent calcium accumulation by brain microsomes. Arch Biochem Biophys 125:286–294
Rodan GA, Feinstein MB (1976) Interrelationships between Ca^{++} and adenylate cyclase in the control of platelet secretion and aggregation. Proc Natl Acad Sci USA 73(6):1829–1833
Rodan S, Rodan G (1974) The effect of parathyroid hormone and thyrocalcitonin on the accumulation of cyclic adenosine 3′,5′-monophosphate in freshly isolated bone cells. J Biol Chem 249:3068–3074
Rodesch F, Bogaert C, Dumont JE (1976) Compartmentalization and movements of calcium in the thyroid. Mol Cell Endocrinol 5:303–313
Rojas E, Taylor RE (1975) Simultaneous measurements of magnesium, calcium and sodium influxes in perfused giant axons under membrane potential control J Physiol (Lond) 252:1–27
Romero P, Whittam R (1971) The control by internal calcium of membrane permeability to sodium and potassium. J Physiol (Lond) 214:481–507
Ronner P, Gazzotti P, Carafoli E (1977) A lipid requirement for the (Ca^{2+} + Mg^{2+}) activated ATPase of erythrocyte membranes. Arch Biochem Biophys 179:578–583
Roos I, Crompton M, Carafoli E (1978) The effect of phosphoenolpyruvate on the retention of calcium by liver mitochondria. FEBS Lett 94:418–421
Rorive G, Kleinzeller A (1972) The effect of ATP and Ca^{2+} on the cell volume in isolated kidney tubules. Biochim Biophys Acta 274:226–239
Rose B, Lowenstein WR (1975) Calcium ion distribution in cytoplasm visualized by aequorin. Diffusion in the cytosol is restricted due to energized sequestring. Science 190:1204–1206
Ross DH, Cardenas HL (1979) Nerve cell calcium as a messenger for opiate and endorphin action. In: Loh HH, Ross PH (eds) Neurochemical mechanisms of opiates and endorphins. Raven Press, New York. (Advances in biochemical psychopharmacology, vol 20, pp 301–338)
Rossi C, Azzi A, Azzone GF (1967) Ion transport in liver mitochondria. I Metabolism-independent Ca^{++} binding and H^+ release. J Biol Chem 242:951–957
Rossi CS, Bielawski J, Carafoli E, Lehninger AL (1966) The relationship of the Ca^{++} efflux rate to the "super-stoichiometry" of respiration-linked Ca^{++} accumulation by mitochondria. Biochem Biophys Res Commun 22:206–210
Rossi CS, Lehninger A (1974) Stoichiometry of respiratory stimulation, accumulation of Ca^{++} and phosphate, and oxidative phosphorylation in rat liver mitochondria. J Biol Chem 239:3971–3980
Rossi JPFC, Garrahan PJ, Rega AF (1978) Reversal of the calcium pump in human red cells. J Membr Biol 44:37–46
Rottenberg H (1973) The mechanism of energy-dependent ion transport in mitochondria. J Membr Biol 11:117–137
Rottenberg H, Scarpa A (1974) Calcium uptake and membrane potential in mitochondria. Biochemistry 13:4811–4817
Roulet MJ, Mongo KG, Vassort G, Ventura-Clapier R (1979) The dependence of twitch relaxation and sodium ions and on internal Ca^{2+} stores in voltage clamped frog atrial fibres. Eur J Physiol 379:259–268
Rubanyi G, Kovach AGB (1979) Effect of ovarian steroid hormones on superficial activator calcium in the rabbit uterus. Acta Physiol Acad Sci Hung 53:71–80
Rubin RP (1974) Calcium and the secretory process. Plenum, New York
Ruigrok JC, Elbers PF (1972) The effects of calcium acetate on mitochondria in the perfused rat liver. I. Accumulation of Ca^{++} and concomitant swelling. Cytobiologie 5:51–64
Russell JM, Blaustein MP (1974) Calcium efflux from barnacle muscle fibres: depen- on external cations. J Gen Physiol 63:144–167
Russell JM, Blaustein MP (1975) Calcium fluxes in internally dialyzed giant barnacle muscle fibres. J Membr Biol 23:157–179

Sandri G, Panfili E, Sottocasa GL (1976) The calcium-binding glycoprotein and mitochondrial calcium movements. Biochem Biophys Res Commun 68:1272–1279
Sarkadi B, MacIntyre JD, Gardos G (1978) Kinetics of active calcium transport in inside-out red cell membrane vesicles. FEBS Lett 89:78–82
Sarkadi B, Szasz I, Gerlocozy A, Gardos G (1977) Transport parameters and stoichiometry of active calcium ion extrusion in intact human red cells. Biochim Biophys Acta 464:93–107
Sayegh FS, Davis RW, Solomon GC (1974) Mitochondrial role in cellular mineralization. J Dent Res 53:581–587
Scarpa A (1975) Kinetic and energy-coupling of Ca^{++} transport in mitochondria. In: Carafoli E, Clementi F, Drabikowski W, Magreth A (eds) Calcium transport in contraction and secretion. North Holland, Amsterdam, pp 65–76
Scarpa A, Azzi A (1968) Cation binding to submitochondrial particles. Biochim Biophys Acta 150:473–481
Scarpa A, Azzone G (1970) The mechanism of ion translocation in mitochondria. Eur J Biochem 12:328–335
Scarpa A, Graziotti P (1973) Mechanisms for intracellular calcium regulation in heart. J Gen Physiol 62:756–772
Scarpa A, Malmström K, Chiesi M, Carafoli E (1976) On the problem of the release of mitochondrial calcium by cyclic AMP. J Membr Biol 29:205
Scarpa A, Brinley FJ, Dubyak G (1978a) Antipyrylazo, III, a "middle range" Ca^{2+} metallochromic indicator. Biochemistry 17:1378–1386
Scarpa A, Brinley FJ, Tiffert T, Dubyak GR (1978b) Metallochromic indicators of ionized calcium. Annal NY Acad Sci 307:86–112
Schanne O, Coraboeuf E (1966) Potential and resistance measurements of rat liver cells in situ. Nature 210:1390–1391
Scharf O (1976) Ca^{+2} activation of membrane-bound (Ca^{+2} + Mg^{+2})-dependent ATPase from human erythrocytes prepared in the presence or absence of Ca^{+2}. Biochim Biophys Acta 443:206–218
Scharf O, Foder B (1978) Reversible shift between two states of Ca-ATPase in human erythrocytes mediated by Ca^{+2} and á membrane bound activator. Biochim Biophys Acta 509:67–77
Schatzmann HJ (1966) ATP dependent Ca^{2+}-extrusion from human red cells. Experientia 22:364–365
Schatzmann HJ (1970) Transmembrane calcium movements in released human red cells. In: Cuthbert AW (ed) Calcium and cellular function. Macmillan, London, pp 85–95
Schatzmann HJ (1973) Dependence on calcium concentration and stoichiometry of the calcium pump in human red cells. J Physiol (Lond) 235:551–569
Schatzmann HJ (1975) Active calcium transport and Ca^{2+}-activated ATPase in human red cells. In: Bronner F, Kleinzeller A (eds) Current topics in membranes and transport, vol 6, pp 125–168
Schatzmann HJ (1977) Role of magnesium in the (Ca^{+2} + Mg^{+2})-stimulated membrane ATPase of human red blood cells. J Membr Biol 35:149–158
Schatzmann HJ, Rossi GL (1971) (Ca^{2+} + Mg^{2+})-activated membrane ATPases in human red cells and their possible relations to cation transport. Biochim Biophys Acta 241:379–392
Schatzmann HJ, Vincenzi FJ (1969) Calcium movements across the membrane of human red cells. J Physiol (Lond) 201:369–395
Schinger Z, Naim E, Lasser M (1970) ATP-dependent calcium uptake by microsomal preparation from rat parotid and submaxillary glands. Biochim Biophys Acta 203: 326–334
Schneider WC, Hogeboom GH (1951) Cytochemical studies of mammalian tissues: the isolation of cell components by differential centrifugation: a review. Cancer Res 11:1–22

Schneider WC,Hogeboom GH, Shelton E, Striebich MJ (1953) Enzymatic and chemical studies on the liver and liver mitochondria of rats fed 2-methyl-or 3'-methyl-4-dimethylaminoazobenzene. Cancer Res 13:285–288

Schneyer LH, Schneyer CA (1965) Membrane potentials of salivary gland cells of rat. Am J Physiol 209:1304–1310

Schotland J, Mela L (1977) Role of cyclic nucleotides in the regulation of mitochondrial calcium uptake and efflux kinetics. Biochem Biophys Res Commun 75:920–924

Schraer R, Elder JA, Schraer H (1973) Aspects of mitochondrial function in calcium movement and calcification. Fed Proc 32:1938–1943

Schreurs VVAM, Swarts HGP, De Pont JJHHM, Bontin SL (1976) Role of calcium in exocrine pancreatic secretion. II. Comparison of the effects of carbachol and the ionophore A-23187 on enzyme secretion and calcium movements in rabbit pancreas. Biochim Biophys Acta 419:320–330

Schrier SL, Bensch KG, Johnson M, Junga I (1975) Energized endocytes in human erythrocyte ghosts. J Clin Invest 56:8–22

Schudt C, Gaertner U, Pette D (1976) Insulin action on glucose transport and calcium fluxes in developing muscles cells in vitro. Eur J Biochem 68:103–111

Schulman H, Greengard P (1978) Ca^{2+}-dependent protein phosphorylation system in membranes from various tissues and its activation by "calcium-dependent regulator". Proc Natl Acad Sci USA 75:5432–5436

Schulz I, Kondo S, Sachs G, Milutinović S (1977) The role of Ca^{++} in pancreatic enzyme secretion. In: Bonfils S, Fromageot P, Rosselin G (eds) Hormonal receptors in digestive tract physiology. North Holland, Amsterdam, pp 275–288

Schulz I, Stolze HH (1980) The exocrine pancreas: the role of secretagogues, cyclic nucleotides, and calcium in enzyme secretion. Annu Rev Physiol 42:127–156

Schuster SM, Olson MS (1974) Studies of the energy-dependent uptake of divalent metal ions by beef heart mitochondria. J Biol Chem 249:7151–7158

Sehlin J (1976) Calcium uptake by subcellular fractions of pancreatic islets: effects of nucleotides and theophylline. Biochem J 156:63–69

Sekiya T (1962) Studies on the membrane potential of Ehrlich ascites tumor cell. Gann 53:41–57

Selinger Z, Naim E, Lasser M (1970) ATP-dependent calcium uptake by microsomal preparation from rat parotid and submaxillary glands. Biochim Biophys Acta 203: 326–334

Silbergeld EK (1977) Na^+ regulates release of Ca^{++} sequestered in synaptosomal mitochondria. Biochem Biophys Res Commun 77:464–469

Simonsen L, Christoffersen GRJ (1979) Intracellular Ca^{2+} activity in *Helix* neurons: effects of extracellular Ca^{2+}, H^+, Na^+ and N_3^-. Comp Biochem Physiol (A) 63:615–618

Singh M (1979) Calcium and cyclic nucleotide interaction in secretion of amylase from rat pancreas in vitro. J Physiol (Lond) 296:159–176

Smith TC, Mikiten TM, Levinson C (1972) The effect of multivalent cations on the membrane potential of the Ehrlich ascites tumor cell. J Cell Physiol 79:117–126

Soderling TR, Hickenbottom JP (1970) Inactivation of glycogen synthetase and activation of phosphorylase b kinase by the same cyclic 3'-5'-AMP dependent kinase. Fed Proc 29:601

Solaro RJ (1972) Cardiac contractility: a problem in calcium conservation. Doctoral Thesis, Univ Pittsburgh School of Medicine

Solaro RJ, Briggs FN (1974) Estimating the function capabilities of sarcoplasmic reticulum in cardiac muscle – calcium binding. Circ Res 34:531–540

Sordahl LA (1974) Effect of magnesium, ruthenium red and the antibiotic ionophore A23187 on initial rates of calcium uptake by heart mitochondria. Arch Biochem Biophys 167:104–115

Sorensen MM, DeMeis L (1977) Effects of anions, pH and magnesium on calcium accumulation and release by sarcoplasmic reticulum. Biochim Biophys Acta 465: 210–223

Sottocasa GL, Sandri G, Panfili E, de Bernard B (1971) A glycoprotein located in the membrane space of rat liver mitochondria. FEBS Lett 17:100–105

Sottocasa GL, Sandri G, Panfili E, de Bernard B, Gazzotti P, Vasington FD, Carafoli E (1972) Isolation of a soluble Ca^{2+} binding glycoprotein from ox liver mitochondria. Biochem Biophys Res Commun 47:808–813

Spencer T, Bygrave FL (1973) The role of mitochondria in modifying the cellular ionic environment: studies of the kinetic accumulation of calcium by rat liver mitochondria. Bioenergetics 4:347–362

Sperelakis N (1962) Ca^{45} and Sr^{89} movements with contraction of depolarized smooth muscle. Am J Physiol 203:860–866

Sperelakis N, Schneider JA (1976) A metabolic control mechanism for calcium ion influx that may protect the ventricular myocardial cell. Am J Cardiol 37:1079–1085

Stahl WL, Swanson PD (1969) Uptake of calcium by subcellular fractions isolated from ouabain treated cerebral tissues. J Neurochem 16:1553–1563

Stahl WL, Swanson PD (1971) Movements of calcium and other cations in isolated cerebral tissues. J Neurochem 18:415–427

Stahl WL, Swanson PD (1972) Calcium movements in brain slices in low sodium or calcium media. J Neurochem 19:2395–2407

Stallcup WB (1979) Sodium and calcium fluxes in a clonal nerve cell line. J Physiol (Lond) 286:525–540

Steiner M, Tateishi T (1974) Distribution and transport of calcium in human platelets. Biochim Biophys Acta 367:232–246

Steinhardt R, Zucker R, Schatten G (1977) Intracellular calcium release at fertilization in the sea urchin egg. Dev Biol 58:185–196

Stern PH (1969) Inhibition by steroids of parathyroid hormone-induced ^{45}Ca release from embryonic rat bone in vitro. J Pharmacol Exp Ther 168:211–217

Stinnakre J, Tauc L (1973) Calcium influx in active *Aplysia* neurones detected by injected aequorin. Nature (New Biol) 242:113–115

Streter FA (1969) Temperature, pH and seasonal dependence of Ca-uptake and ATPase activity of white and red muscle microsomes. Arch Biochem Biophys 134:25–33

Striebich MJ, Shelton E, Schneider WC (1953) Quantitative morphological studies on the livers and liver homogenates of rats fed 2-methyl- or 3'methyl-4-dimethylaminoazobenzene. Cancer Res 13:279–284

Strittmatter WJ, Hirata F, Axelrod J (1979) Increased Ca^{2+}-ATPase activity associated with methylation of phospholipids in human erythrocytes. Biochem Biophys Res Commun 88:147–153

Struyvenberg A, Morrison R, Relman A (1968) Acid-base balance of separated canine renal tubule cells. Am J Physiol 214:1155–1162

Stucki JW, Ineichen EA (1974) Energy dissipation by calcium recycling and the efficiency of calcium transport in rat liver mitochondria. Eur J Biochem 48:365–375

Studer RK (1978) The influence of hydrogen ion on calcium metabolism of renal cell. Ph.D. Thesis, University of Pittsburgh

Studer RK, Borle AB (1979) Effect of pH on the calcium metabolism of isolated rat kidney cells. J Membr Biol 48:325–341

Studer RK, Borle AB (1980) The effect of hydrogen ion on the kinetics of calcium transport by rat kidney mitochondria. Arch Biochem Biophys 203:707–718

Sugden MC, Christie MR, Ashcroft SJH (1979) Presence and possible role of calcium-dependent regulator (calmodulin) in rat islets of Langerhans. FEBS Lett 105:95–100

Suko J (1971) Alterations of Ca^{2+} uptake and Ca^{2+}-activated ATPase of cardiac sarcoplasmic reticulum in hyper- and hypothyroidism. Biochim Biophys Acta 252:324–327

Sulakhe PV, Drummond GI, Ng DC (1973) Calcium binding by skeletal muscle sarcolemma. J Biol Chem 248:4150–4157
Sullivan WJ (1968) Electrical potential differences across distal renal tubules of *Amphiuma*. Am J Physiol 214:1096–1103
Sutfin LV, Holtrop ME, Oglivie RE (1971) Microanalysis of individual mitochondrial granules with diameters less than 1000 angstroms. Science 174:947–949
Swanson PD, Anderson L, Stahl WL (1974) Uptake of calcium ions by synaptosomes from rat brain. Biochim Biophys Acta 356:174–183
Swift MR, Todaro GJ (1968) Membrane potential of human fibroblast strains in culture. J Cell Physiol 71:61–64
Szasz I, Sarkodi B, Gardos G (1977) Mechanism of Ca^{2+}-dependent selective rapid K^+-transport induced by propranolol in red cells. J Membr Biol 35:75–93
Szasz I, Sakardi B, Gardos G (1978) Mechanisms for passive calcium transport in human red cells. Acta Biochim Biophys Acta Sci Hung 13:239–242
Tada M, Kirchberger MA, Repke DI, Katz AM (1974) The stimulation of calcium transport in cardiac sarcoplasmic reticulum by adenosine 3′5′-monophosphate-dependent protein kinase. J Biol Chem 249:6174–6180
Tada M, Yamamoto T, Tonomura Y (1978) Molecular mechanism of active calcium transport by sarcoplasmic reticulum. Physiol Rev 58:1–79
Tashmukhamedov BA, Gagelgans AI, Mamatkulov K, Makhmodova EM (1972) Inhibition of Ca^{2+} transport in mitochondria by selective blockade of membrane mucopolysaccharides by hexamine cobaltichloride. FEBS Lett 28:239–242
Ten Eick R, Nawrath H, McDonald TF, Trautwein W (1976) On the mechanism of the negative inotropic effect of acetylcholine. Pfluegers Arch 361:207–213
Terepka AF, Stewart ME, Merkel U (1969) Transport functions of the chick chorioallantoic membrane. II. Active calcium transport in vitro. Exp Cell Res 58:107–117
Thiers RE, Villee BL (1957) Distribution of metals in subcellular fractions of rat liver. J Biol Chem 226:911–920
Thiers RE, Reynolds ES, Villee BL (1960) The effect of carbon tetrachloride poisoning on subcellular metal distribution in rat liver. J Biol Chem 235:2130–2133
Thorens S (1979) Ca^{2+}-ATPase and Ca uptake without requirement for Mg^{2+} in membrane fractions of vascular smooth muscle. FEBS Lett 98:177–180
Thorens S, Haeusler G (1978) Effects of adenosine 3′:5′-monophosphate and guanosine 3′:5′-monophosphate on calcium uptake and phosphorylation in membrane fractions of vascular smooth muscle. Biochim Biophys Acta 512:415–428
Thorne RFW, Bygrave FL (1975) Kinetic evidence for calcium-ion and phosphate-ion transport systems in mitochondria from Ehrlich ascites tumour cells. FEBS Lett 56: 185–188
Ting A, Lee JW, Vidaver GA (1979) Calcium transport by pigeon erythrocyte membrane vesicles. Biochim Biophys Acta 555:239–248
Tobias JM, Agin DP, Pawlowski R (1962) The excitable system in the cell surface. Circulation 26:1145–1150
Tower DB (1968) Ouabain and the distribution of calcium and magnesium in cerebral tissues in vitro. Exp Brain Res 6:273–283
Trautwein W (1975) Membrane currents in cardiac muscle fibers. Physiol Rev 53:793–835
Trotta EE, DeMeis L (1975) ATP-dependent calcium accumulation in brain microsomes. Enhancement by phosphate and oxalate. Biochim Biophys Acta 394:239–247
Tsien RW (1977) Cyclic AMP and contractile activity in heart. Adv Cyclic Nucl Res 8: 364–420
Tsokos J, Sans R, Bloom S (1977) Ca^{2+} uptake by hyperpermeable mouse heart cells: effects of inhibitors of mitochondrial function. Life Sci 20:1913–1922
Tsumura Y, Kagawa S, Yoshika K, Koboyashi K, Matsuoka A (1979) Insulin release and metabolism of calcium, adenine and adenine 3′-5′ cyclic monophosphate. Endocrinol Jpn 26:359–370

Tupper JT, Zorgniotti F (1977) Calcium content and distribution as a function of growth and transformation in the mouse 3T3 cell. J Cell Biol 75:12–22
Uchikawa T, Borle AB (1978a) Kinetic analysis of calcium desaturation curves from isolated kidney cells. Am J Physiol 234:R29–R33
Uchikawa T, Borle AB (1978b) Studies of calcium-45 desaturation from kidney slices in flow-through chambers. Am J Physiol 234:R34–R38
Umbreit W, Burris RH, Stauffer JF (1964) Manometric techniques, 4th ed. Burgess, Minneapolis, pp 132
Vaes (1968) Parathyroid hormone-like action of N^6-2′-0-dibutyryladenosine-3′,5′ (cyclic) monophosphate on bone explant in tissue culture. Nature 219:939–940
Vallieres J, Scarpa A, Somlyo AP (1975) Subcellular fractions of smooth muscle. Isolation, substrate utilization and calcium transport by main pulmonary artery and mesenteric vein mitochondria. Arch Biochem Biophys 120:659–669
Van Breemen C, Aaronson P, Loutzenhiser R (1978) Sodium calcium interactions in mammalian smooth muscle. Pharmacol Rev 30:167–208
Van Breemen C, Daniel EE, Van Breemen D (1966) Calcium distribution and exchange in the rat uterus. J Gen Physiol 49:1265–1297
Van Breemen C, Farinas BR, Casteels R, Gerba B, Wuytack F, Deth R (1973) Factors controlling cytoplasmic Ca^{2+} concentration. Philos Trans R Soc Lond (Biol) 265: 57–71
Van Breemen C, Farinas BR, Gerba P, McNaughton ED (1972) Excitation-contraction coupling in rabbit aorta studied by the lanthanum method for measuring cellular calcium influx. Circ Res 30:44–54
Van Breemen C, McNaughton E (1970) The separation of cell membrane calcium transport from extracellular calcium exchange in vascular smooth muscle. Biochem Biophys Res Commun 39:567–574
Van Breemen C, Hwang O, Siegel B (1977) The lanthanum method. In: Casteels R, Goodfraind T, Rüegg JC (eds) Excitation-contraction coupling in smooth muscle. Elsevier/North Holland, Amsterdam, pp 243–252
Van Rossum GDV (1970) Net movements of calcium and magnesium in slices of rat liver. J Gen Physiol 55:18–32
Van Rossum GVD, Gosalvez M, Galeotti T, Morris HP (1971) Net movements of monovalent and bivalent cations and their relation to energy metabolism in slices of hepatoma 3924A and of a mammary tumour. Biochim Biophys Acta 245:263–276
Vasington FD, Gazzotti P, Tiozzo R, Carafoli E (1972) The effect of ruthenium red on Ca^{2+} transport and respiration in rat liver mitochondria. Biochim Biophys Acta 256: 43–54
Vasington FD, Greenawalt JW (1964) Ca^{++} and Pi uptake by non-phosphorylating mitochondrial preparations. Biochem Biophys Res Commun 15:133–138
Vassalle M (1979) Electrogenesis of the plateau and pacemaker potential. Annu Rev Physiol 41:425–440
Vassort G, Rougier O, Garnier D, Sanviat MP, Coraboeuf E, Gardouil YM (1969) Effects of adrenalin on membrane inward currents during the cardiac action potential. Pfluegers Arch 309:70–81
Vaughan-Williams EM (1959) The effect of changes in extracellular potassium concentration on the intracellular potentials of isolated rabbit atria. J Physiol (Lond) 146: 411–427
Veloso D, Guynn RW, Oskarsson M, Veech I (1973) The concentration of free and bound magnesium in rat tissues. J Biol Chem 248:4811–4819
Vincenzi FF (1971) A calcium pump in red cell membranes. In: Nichols G Jr, Wasserman RH (eds) Cellular mechanisms for calcium transfer and homeostasis. Academic Press, New York, pp 135
Vincenzi FF (1979) Calmodulin in the regulation of intracellular calcium. Proc West Pharmacol Soc 22:289–294

Vincenzi FF, Farrance ML (1977) Interaction between cytoplasmic (Ca^{2+} – Mg^{2+}) ATPase activator and erythrocyte membrane. J Supramol Struc 7:301–306
Vinogradov A, Scarpa A (1973) The initial velocities of calcium uptake by rat liver mitochondria. J Biol Chem 248:5527–5531
Visscher MB, Lee YCP (1972) Calcium ions and the cardiotonic action of glucagon. Proc Natl Acad Sci 69:463–465
Wallach S, Chausmer AB, Sherman BS (1971) Hormonal effects on calcium transport in liver. Clin Orthop 78:40–46
Wallach S, Reizenstein DL, Bellavia JV (1966) The cellular transport of calcium in rat liver. J Gen Physiol 49:743–762
Walling MW, Kimberg DV, Wasserman RH, Feinberg RR (1976) Duodenal active transport of calcium and phosphate in vitamin D-deficient rats; effects of nephrectomy, *cestrum diurnum* and 1α-25-dihydroxyvitamin D_3. Endocrinology 98:1130–1134
Walling MW, Rothman SS (1969) Phosphate independent, carrier-mediated active transport of calcium by rat intestine. Am J Physiol 217:1144–1148
Walling MW, Rothman SS (1970) Apparent increase in carrier affinity for intestinal calcium transport following dietary calcium restriction. J Biol Chem 245:5007–5011
Walsh DA, Krebs EG, Reimann EM, Brostrom MA, Corbin JD, Hickenbottom JP, Soderling TR, Perkins JP (1970) The receptor protein for cyclic AMP in the control of glycogenolysis. In: Greengard P, Costs E (eds) Role of cyclic AMP in cell function. Raven Press, New York, pp 265–285
Walsh DA, Perkins JP, Krebs EG (1968) An adenosine 3′,5′-monophosphate-dependent protein kinase from rabbit skeletal muscle. J Biol Chem 243:3763–3774
Wang JH, Waisman DM (1979) Calmodulin and its role in the second-messenger system. Curr Top Cell Regul 15:47–107
Watlington CO, Burke PK Estep HL (1968) Calcium flux in isolated frog skin; the effect of parathyroid substance. Proc Soc Exp Biol Med 128:853–856
Watson EL, Siegel IA (1978) Factors affecting calcium accumulation and release in canine submandibular salivary microsomes. Arch Oral Biol 23:323–328
Webb RC, Bhalla RC (1976) Calcium sequestration by subcellular fractions isolated from vascular smooth muscle: effect of cyclic nucleotides and prostaglandins. J Mol Cell Cardiol 8:145–147
Weber AM (1966) Energized calcium transport and relaxing factor. Curr Top Bioenerg 1:203–254
Weber A, Herz R, Reiss I (1966) Study of the kinetics of calcium transport by isolated fragmented sarcoplasmic reticulum. Biochem Z 345:329–369
Weibel ER, Stäubli W, Gnägi HR, Hess FA (1969) Correlated morphometric and biochemical studies on the liver cell. I. Morphometric model, stereologic methods and normal morphometric data for rat liver. J Cell Biol 42:68–91
Weinbach EC, Von Brand T (1965) The isolation and composition of dense granules from Ca^{++}-loaded mitochondria. Biochem Biophys Res Commun 19:133–137
Weiner ML, Lee KS (1972) Active calcium ion uptake by inside-out and right-side-out vesicles of red blood cell membranes. J Gen Physiol 59:462–475
Weiss GB, Goodman FR (1969) Effects of lanthanum on contraction, calcium distribution and ^{45}Ca movements in intestinal smooth muscle. J Pharmacol Exp Ther 169:46–55
Weller M, Laing W (1979) The effect of cyclic nucleotides and protein phosphorylation on calcium permeability and binding in the sarcoplasmic reticulum. Biochim Biophys Acta 551:406–419
Wendt IR, Langer GA (1977) The sodium-calcium relationship in mammalian myocardium: effect of sodium deficient perfusion on calcium fluxes. J Mol Cell Cardiol 9:551–564
Wester PO (1965) Concentration of 17 elements in subcellular fractions of beef heart tissue determined by neutron activation. Biochim Biophys Acta 109:268–283

Whitfield JF, MacManus JP, Gilland DJ (1971) Inhibition by thyrocalcitonin (calcitonin) of the cyclic AMP-mediated stimulation of thymocyte proliferation by epinephrine. Horm Metab Res 3:348–351

Whitney RB, Sutherland RM (1972) Enhanced uptake of calcium by transforming lymphocytes. Cell Immunol 5:137–147

Whitney RB, Sutherland RM (1973) Characteristics of calcium accumulation by lymphocytes and alterations in the process induced by phytohemagglutinin. J Cell Physiol 82:9–20

Whittembury G, Windhager E (1961) Electrical potential difference measurements in perfused single proximal tubule of *Necturus* kidney. J Gen Physiol 44:679–687

Wikström M, Ahonen P, Luukkainen T (1975) The role of mitochondria in uterine contraction. FEBS Lett 56:120–123

Williams AJ, Barrie SE (1978) Temperature effects on the kinetics of calcium transport by cardiac mitochondria. Biochem Biophys Res Commun 84:89–94

Williams JA (1966) Effect of external K^+ concentration on transmembrane potentials of rabbit thyroid cells. Am J Physiol 211:1171–1174

Williams JA (1970) Origin of transmembrane potentials in non-excitable cells. J Theor Biol 28:287–296

Winegrad S, Shanes AM (1962) Calcium flux and contractility in guinea pig atria. J Gen Physiol 45:371–394

Wolf HU (1970) Purification of the Ca^{2+} dependent ATPase of human erythrocyte membranes. Biochim Biophys Acta 219:521–524

Wolf HU (1972) Studies on a Ca^{2+} dependent ATPase of human erythrocyte membranes. Effects of Ca^{2+} and H^+. Biochim Biophys Acta 266:361–375

Wollheim CB, Kikuchi M, Renold AE (1978) The roles of intracellular and extracellular Ca^{++} in glucose-stimulated biphasic insulin release by rat islets. J Clin Invest 62: 451–458

Wong GL, Luben RA, Cohn DV (1977) 1,25-dihydroxycholecalciferol and parathormone: effects on isolated osteoclast-like and osteoblast-like cells. Science 197:663–665

Wood JM, Schwartz A (1978) Effects of ouabain on calcium-45 flux in guinea pig cardiac tissue. J Mol Cell Cardiol 10:137–144

Woodbury DM, Woodbury JW (1963) Correlation of micro-electrode potential recordings with histology of rat and guinea pig thyroid glands. J Physiol (Lond) 169: 553–567

Worsfold W, Peter JB (1970) Kinetics of calcium transport by fragmented sarcoplasmic reticulum. J Biol Chem 245:5545–5552

Wrenn RW, Biddulph DM (1979a) Parathyroid hormone-induced calcium efflux from isolated renal cortical tubules – evidence for cyclic AMP mediation. Mol Cell Endocrinol 15:29–40

Wrenn RW, Biddulph DM (1979b) Correlation between cyclic AMP levels and calcium efflux in isolated renal cortical tubules. J Cyclic Nucleotide Res 5:239–250

Wright FS (1971) Increasing magnitude of electrical potential along the renal tubule. Am J Physiol 220:624–638

Yamaguchi M, Takei Y, Yamamoto T (1975) Effect of thyrocalcitonin on calcium concentration in liver of intact and thyroparathyroidectomized rats. Endocrinology 96:1004–1008

Yamaguchi M (1979) Effect of calcitonin on Ca-ATPase activity of plasma membrane in liver of rats. Endocrinol Jpn 26:605–609

Yamazaki RK (1975) Glucagon stimulation of mitochondrial respiration. J Biol Chem 250:7924–7930

Yasamura S (1976) Effect of adrenal steroids on bone resorption in rat. Am J Physiol 230:90–93

Yeung WK, Weisman G, Vidaver GA (1979) active Ca^{2+} transport by vesicle reconstituted from Triton X-100-solubilized pigeon erythrocyte membrane. Biochim Biophys Acta 555:249–288

Yonaga T, Morimoto S (1979) A calcitonin-like action of prostaglandin E-1. Prostaglandins 17:801–820

Zadunaisky JA, Lande MA (1972) Calcium content and exchange in amphibian skin and its isolated epithelium. Am J Physiol 222:1309–1315

Zadunaisky JA, Gennaro JF Jr, Bashirelahi N, Hilton M (1968) Intracellular redistribution of sodium and calcium during stimulation of sodium transport in epithelial cells. J Gen Physiol 51:290–302

Rev. Physiol. Biochem. Pharmacol., Vol. 90

Calcium Transport in the Kidney

ZALMAN S. AGUS, STANLEY GOLDFARB, and
ALAN WASSERSTEIN *

Contents

1 Introduction

Initial studies of calcium transport utilizing clearance techniques emphasized the striking similarity between the urinary excretion of sodium and that of calcium and the effects of a variety of maneuvers on both (*Walser* 1961). Early results from micropuncture studies also suggested that calcium was reabsorbed throughout the nephron in a pattern very similar to that of sodium transport (*Lassiter* et al. 1963; *Duarte* and *Watson* 1967). Since then studies utilizing more sophisticated techniques combining papillary tip, superficial proximal, and distal micropuncture and the recent application of the techniques of isolated tubular perfusion have markedly refined our concepts of renal calcium transport. These studies have indicat-

* Renal-Electrolyte Section, Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA

ed that calcium transport occurs in most segments of the nephron in a manner similar to that of sodium but can be dissociated from sodium transport in several segments beyond the proximal convoluted tubule. The principal factors influencing urinary calcium excretion that may be important in calcium homeostasis include parathyroid hormone, phosphate balance, acid–base balance, and the state of the extracellular fluid volume. With the possible exception of extracellular fluid volume contraction, all these factors affect urinary calcium excretion by altering transport in the distal portions of the nephron; thus the terminal nephron appears to be responsible for the regulation of urinary calcium excretion. In this review, we will first consider the sites of calcium transport within the nephron and then discuss those factors that alter transport with emphasis on recent advances.

2 Sites of Transport

In normal man, the filtered load of calcium is slightly greater than 500 mEq/day. As calcium excretion is normally 5–10 mEq/day during an average calcium intake of less than 50 mEq/day, the fraction of filtered calcium excreted is less than 1%. Approximately 50%–55% of the filtered load is reabsorbed in the proximal convoluted tubule, 20%–30% in the pars recta and loop of Henle, 10%–15% in the distal convoluted tubule, and 2%–8% in the terminal portions of the nephron.

2.1 Glomerular Filtration

Calcium in plasma exists both in the free ionic form, bound to protein, and complexed with various ions such as phosphate and citrate. It has generally been assumed that calcium concentration in glomerular ultrafiltrate can be estimated with laboratory methods of ultrafiltration across artificial membranes. Micropuncture studies with direct measurements of fluid from surface glomeruli have verified that about 60% of the total plasma calcium is ultrafiltered across the glomerulus. Thus *Harris* et al. (1974) found an ultrafiltration ratio of 0.63 ± 0.02. *Le Grimellec* et al. (1975) found similar values but with a marked scatter that they attributed to glomerular heterogeneity.

2.2 Proximal Convoluted Tubule

A number of micropuncture studies have revealed ratios of tubular fluid to ultrafilterable calcium consistenly greater than 1 (recently summarized by *Suki* 1979). This is consistent with a passive transport mechanism that could be dependent upon sodium and water reabsorption and many investigators have documented a parallelism between sodium and water transport in the proximal tubule. *Ullrich* et al. (1976), however, provided evidence for active calcium transport in addition to passive diffusion. Using tubular microperfusion in combination with simultaneous perfusion of the peritubular capillaries, they demonstrated an active component of approximately 20% of the total reabsorptive rate. Removal of sodium from the perfusion solution or inhibition of sodium transport with ouabain also inhibited the active component of calcium transport. Thus both passive diffusion and active transport of calcium appear to be dependent upon sodium transport. The mechanism for the linkage of active transport with sodium transport is unclear. One possibility is via an ATPase system. Another possibility is a sodium–calcium exchange system across the basolateral plasma membrane, a phenomenon described in a variety of tissues (*Taylor* and *Windhager* 1979). According to this hypothesis, intracellular calcium activity is regulated by calcium extrusion in exchange for sodium diffusing into the cell down a concentration gradient. Inhibition of the sodium pump and removal of peritubular sodium would reduce both the gradient for sodium entry into the cell across the basolateral membrane and calcium extrusion. The resultant increase in cytosolic calcium activity would serve to inhibit calcium transport across the luminal membrane. Recent studies in vesicles isolated from rat renal cortex have in fact demonstrated both an ATP-driven calcium pump and a Na–Ca countertransport system in the basolateral plasma membrane but not in brush border membranes (*Gmaj* et al. 1979).

The original interdependence between the handling of sodium and calcium by the kidney, therefore, seems to be characteristic of the proximal tubule, and with a few exceptions, multiple studies have been unable to dissociate the transport of these ions at this site. Some exceptions to this rule include calcium infusion (*Edwards* et al. 1976; *Le Grimellec* et al. 1974) and sulfate infusion (*Le Chene* et al. 1975) and possibly acute parathyroidectomy (*Kuntziger* et al. 1974). The ratio of calcium concentration in tubular fluid to that in ultrafiltrate has been observed to increase with infusion of calcium and sulfate, probably because of an increase in the complexed fraction that may not be available for reabsorption. The changes in calcium concentration observed with acute parathyroidectomy have not been uniformly observed in other laboratories, and current data suggest that the important effect of parathyroid hormone (PTH) on urinary

calcium excretion is due to an effect in distal portions of the nephron. As a general rule, therefore, it is still reasonable to consider that, under conventional conditions, sodium and calcium transport in the proximal convoluted tubule are parallel.

2.3 Pars Recta and Descending Limb of Henle's Loop

Studies in the rat (*Jamison* et al. 1974) and *Psammomys* (*De Rouffignac* et al. 1973) have suggested that the transport of sodium and of calcium may not be parallel between the end of the proximal convoluted tubule and the ascending limb. Both groups of investigators found that the ratio of tubular fluid calcium to ultrafilterable calcium concentrations (TF/UF calcium) at the papillary tip was significantly lower than the ratio of tubular fluid sodium to plasma sodium concentrations (TF/P sodium) in contrast to the accessible proximal convoluted tubule, where the reverse is found. These data thus suggested a dissociation between calcium and sodium reabsorption in either the pars recta or the thin descending limb. *Rocha* et al. (1977), however, found no evidence for calcium reabsorption in the latter segment with isolated perfusion. When an osmotic gradient was imposed to stimulate in vivo conditions of osmotic water extraction, the rise in concentration of the volume marker was associated with a parallel rise in ^{45}Ca concentration, indicating the absence of calcium efflux despite water loss. More recently, *Rouse* et al. (1980) have provided direct evidence that the pars recta of superficial nephrons is a site of significant net calcium efflux in excess of water reabsorption. As efflux occurred against an electrochemical gradient, they suggested that this transport was active. It was not inhibited by ouabain but was dependent upon cellular metabolism, as transport was inhibited by cooling to room temperature. The transport process was not saturated and the transport rate increased with increasing load. The exact nature of this process, its physiological role, and the factors that regulate it remain to be determined. Whether or not this transport process also occurs in the pars recta of juxtamedullary nephrons remains to be evaluated.

2.4 Ascending Limb of Henle's Loop

As in the thin descending limb, there is no evidence of net calcium transport in the thin ascending limb (*Rocha* et al. 1977). In contrast, net calcium reabsorption occurs in the isolated perfused thick ascending limb as originally demonstrated by *Rocha* et al. (1977) and confirmed in several laboratories (*Shareghi* and *Stoner* 1978; *Imai* 1978; *Bordeau* and *Burg*

1979; *Suki* et al. 1977). The mechanism of calcium transport in this segment is controversial, however. Earlier micropuncture studies revealed that furosemide inhibited calcium reabsorption in the loop of Henle, suggesting a relationship between sodium and calcium transport. While it seems that passive forces could account for net reabsorption because of the lumen-positive potential difference that occurs in this segment, data have suggested that other factors must play a role. Thus, in two studies, alteration of the potential difference (PD) by ouabain or furosemide was not associated with parallel changes in calcium transport (*Rocha* et al. 1977; *Imai* 1978) and the flux ratio was higher than that predicted by simple diffusion. In fact, *Imai* (1978) was able to demonstrate significant efflux despite reversal of the PD. *Bordeau* and *Burg* (1979) also found a flux ratio greater than that predicted by the positive potential, but were able to show that there was no net flux when the PD was zero. *Shareghi* and *Stoner* (1978) found that the observed PD more than adequately accounted for the calcium flux. It should be pointed out that deviations of flux ratios from values predicted by 'passive' forces is not necessarily an index of active transport. Thus a variety of types of flux coupling (single file diffusion, exchange diffusion, solvent drag) as well as heterogeneity in the membrane can alter the flux ratio. These explanations, however, would not account for the persistence of transport with marked alterations in the potential differences. It is not clear why these discrepancies exist. All investigators, however, agree that at least part of calcium transport in this segment may be accounted for by passive forces. Some of the discrepancies may relate to differences in experimental techniques. It is also possible that heterogeneity of function between cortical and medullary segments may account for some of these differences. Preliminary data seem to suggest that the medullary segment exhibits passive transport characteristics while persistent transport despite reduction of the PD is characteristic of the cortical segment (*Suki* et al. 1977).

2.5 Distal Convoluted Tubule

The first micropuncture studies suggested that 10%–11% of the filtered load of calcium was reabsorbed in the distal tubule (*Lassiter* et al. 1963). More recent experiments utilizing measurements of distal tubule length have confirmed these data, revealing reabsorption of 9% of the filtered load or 89% of the delivered load during free-flow micropuncture (*Costanzo* and *Windhager* 1978b). The reabsorptive rate during in vivo microperfusion increased linearly with increasing load and there was virtually no detectable 'backleak' of calcium into the tubule lumen despite the imposition of large concentration gradients (*Costanzo* and *Windhager* 1978).

These data suggest the presence of active, load-dependent calcium transport in the distal convoluted tubule. Similar evidence for calcium transport in this segment, and possibly later segments as well, has been obtained in ^{45}Ca microinjection experiments (*Greger* et al. 1978). Under normal conditions, calcium transport in the distal tubule seems to be parallel to sodium transport, as demonstrated by most in vivo micropuncture studies. As discussed subsequently, in vivo transport can be dissociated with thiazides (*Costanzo* and *Windhager* 1978b). In vitro, *Shareghi* and *Stoner* (1978) were able to inhibit the PD and reduce sodium reabsorption with furosemide and amiloride, but these agents did not alter calcium transport. As will be discussed below, the distal convoluted tubule may consist of several morphologically distinct segments. Studies have not been performed to evaluate transport specifically in the bright segment of the distal tubule, and thus the homogeneity of the distal convoluted tubule with respect to calcium transport remains unclear.

2.6 Collecting Duct System

Comparison of the amount of calcium remaining at the end of the protion of the distal tubule accessible to micropuncture with the final urine concentration at the same time have been utilized to estimate calcium transport in the terminal portions of the nephron. These estimates have ranged from 3% to 10% of the filtered load (*Agus* et al. 1977; *Lassiter* et al. 1963). Additional evidence for transport beyond the late distal tubule, namely in the cortical collecting tubule, has been found with tracer microinjection studies (*Greger* et al. 1978). Recent studies utilizing in vitro microperfusion, however, have suggested heterogeneity of function in the collecting duct system with regard to calcium transport. Thus the early cortical collecting duct, which contains granular epithelium similar to that of the late distal tubule, transports calcium and responds to PTH but there is no detectable transport in the later light segment (*Sharegi* and *Stoner* 1978). As the nephrons of the mid-cortex and the deep cortex are characterized by longer granular segments (connecting tubule, arcade), these data have particular relevance to the interpretation of earlier micropuncture studies. It now seems likely that the calcium transport previously attributed to the terminal nephron as a whole may in fact be restricted to the granular segment. Careful study of the medullary collecting duct system is required to complete our understanding of calcium transport beyond the distal tubule.

3 Factors Influencing Calcium Transport

Many factors alter tubular calcium transport and thereby urinary calcium excretion. These factors can be grouped into those that primarily alter calcium transport and those that primarily alter sodium excretion and secondarily affect calcium excretion by virtue of the transport relationship between these ions in certain segments of the nephron, principally the proximal convoluted tubule and the loop of Henle (possibly only the medullary segment of the thick ascending limb – Sects. 2.2 and 2.4). Thus, acute increase in glomerular filtration rate produce only slight changes in calcium and sodium excretion (*Massry* and *Kleeman* 1972), presumably because of the maintenance of glomerulotubular balance, a function primarily of the proximal tubule. Infusion of renal vasodilators, saline infusion (*Agus* et al. 1977), acetazolamide (*Beck* and *Goldberg* 1973), parathyroid hormone (*Agus* et al. 1973), dibutyryl cyclic AMP (*Agus* et al. 1973), and mild hypercalcemia (*Edwards* et al. 1974), all of which inhibit proximal tubular sodium reabsorption, are associated with parallel changes in proximal tubular calcium reabsorption. Whether or not these events are associated with significant changes in urinary excretion depends upon their effects on sodium and calcium transport in more distal portions of the nephron where the handling of these ions may be dissociable. Thus extracellular fluid volume expansion produces natriuresis and calciuria associated with inhibition of transport beyond the late distal tubule, while parathyroid hormone reduces urinary calcium excretion despite comparable inhibition of proximal tubular reabsorption. Similarly, vasodilators increase urinary excretion of sodium and calcium, while acetazolamide produces a mild natriuresis and little change in calcium excretion (*Massry* and *Kleeman* 1972). While dissociation of tubular transport of sodium and calcium can be demonstrated in in vitro perfusion of the pars recta and the cortical thick ascending limb (see 2.4), the physiologic significance of these events has not been demonstrated. Thus inhibition of sodium transport in the loop of Henle with furosemide produces comparable changes in both TF/P sodium and calcium in the early distal tubule (*Edwards* et al. 1973). Factors that appear to selectively alter calcium transport and urinary excretion seem to exert this action in the distal convoluted tubule and early portions of the collecting duct system. These include changes in acid–base balance, phosphate balance, parathyroid hormone, and thiazides. One possible exception to this rule is the administration of calcitonin, as will be discussed below.

3.1 Parathyroid Hormone

Parathyroid hormone (PTH) stimulates the net tubular reabsorption of calcium, presumably via the production of cyclic AMP. The intrarenal sites of PTH-sensitive adenylate cyclase activity have been elegantly detailed in the last few years by the application of enzymatic analysis to anatomic subsections of isolated, collagenase-treated rabbit tubules (*Chabardes* et al. 1975, 1980; *Imbert* et al. 1975; *Morel* et al. 1976). These studies identified the proximal convoluted tubule, pars recta, cortical thick ascending limb, and granular segments of the distal nephron as the major sites of activity. The 'bright' and 'light' segments of the distal convoluted tubule were found to possess little activity, while the apparently granular epithelium of the arcade or connecting tubule segment of deeper cortical collecting tubules, as well as of late segments of the distal convoluted tubule, was highly responsive to PTH. It is also worth noting that differences in adenylate cyclase sensitivity to PTH were found in the various segments. Thus much higher concentrations of PTH were required for stimulation of adenylate cyclase in the cortical thick ascending limb than in proximal or distal tubules, a difference that may have physiologic significance, as will be discussed below.

The localization of the effects of PTH upon renal tubular calcium transport correlates very well with the sites of adenylate cyclase activity. Initial stop-flow studies suggested that the site of the hypocalciuric effect of PTH was in the distal nephron (*Widrow* and *Levinsky* 1962). Micropuncture studies in the dog subsequently indicated that acute administration of parathyroid hormone inhibited proximal tubular calcium reabsorption, also suggesting a distal site for the hypocalciuric effect (*Agus* et al. 1973). Subsequent studies on the rat suggested that PTH enhanced calcium reabsorption in the terminal portions of the nephron beyond the late distal puncture site (*Agus* et al. 1977). More recently, direct evidence has been obtained for an effect within the distal convoluted tubule with in vivo and in vitro studies. *Costanzo* and *Windhager* (1978a) found a twofold increase in distal tubule calcium transport with intravenous PTH infusion or intratubular cyclic AMP perfusion. As discussed above, however, the distal nephron is extremely heterogeneous as regards PTH-sensitive adenylate cyclase activity. *Shareghi* and *Stoner* (1978) demonstrated PTH-sensitive calcium transport in distal convoluted tubules containing granular-type epithelium and in cortical collecting tubules containing granular epithelium. PTH-sensitive calcium transport has also been recently found in cortical thick ascending limb segments. Absorption is stimulated, however, by high (*Bordeau* and *Burg* 1979; *Shareghi* and *Agus* 1979) but not by low levels of PTH in vitro (*Shareghi* and *Stoner* 1978), a finding that is compatible with the reduced sensitivity of this segment demonstrated by

histochemical techniques (*Chabardes* et al. 1975). Thus all the data taken together suggest that it is the granular segment that is the distal site of calcium transport previously demonstrated by other techniques.

3.2 Other Hormones

Remarkably inconsistent effects of calcitonin on urinary calcium excretion have been reported in a number of studies. The data suggest that if there is an effect it is principally to decrease calcium excretion, but in many studies it has not been possible to distinguish the relative roles of changes in filtered load and direct tubular effects. Recent micropuncture studies in young, parathyroidectomized rats revealed that calcitonin reduced calcium excretion, in association with increased reabsorption in the loop of Henle. The enhanced reabsorption, however, was dependent upon hypocalcemia, and when this was prevented by calcium infusion there were no changes in calcium transport (*Quamme* 1980). This observation may explain the variable results in the literature, as the serum calcium response to calcitonin is also very variable. Calcitonin-sensitive adenylate cyclase activity has been described in the medullary thick ascending limb (*Chabardes* et al. 1976). Preliminary studies in perfused rabbit tubules are consistent with this observation and reveal enhanced calcium transport in the medullary portion of the thick ascending limb (*Suki* and *Rouse* 1980). There are very marked species differences, however, in calcitonin-sensitive adenylate cyclase activity (*Chabardes* et al. 1980), and more definitive studies are needed to assess both the effects and physiologic importance of calcitonin.

Enhanced tubular calcium reabsorption has been shown in thyroparathyroidectomized dogs and rats with acute administration of vitamin D, 25-hydroxy-cholecalciferol and 1,25-dihydroxycholecalciferol (*Puschett* et al. 1972a, b). Chronic administration of physiologic doses of $1,25(OH)_2D$ to TPTX rats, however, has no effect upon intrinsic renal calcium handling as assessed during calcium infusion, despite demonstrable alterations in intestinal calcium calcium absorption (*Hugi* et al. 1979). Thus it does not seem likely that $1,25(OH)_2D$ plays an important role in the homeostatic regulation of urinary calcium excretion.

Chronic administration of growth hormone, thyroid, hormone, and glucocorticoids is associated with increased urinary calcium excretion. The mechanisms for these alterations include increased $1,25(OH)_2D$ production and/or enhanced bone resorption but there is little evidence for a direct renal tubular effect of these hormones.

The acute administration of mineralocorticoids does not alter calcium excretion. Chronic administration, however, is associated with a progres-

sive increase in urinary calcium excretion. The calciuria can be prevented by restriction of sodium intake implying that extracellular fluid (ECF) volume expansion is the mediator of the reduced tubular reabsorption of calcium (*Suki* et al. 1968; *Massry* et al. 1968; *Rastegar* et al. 1972).

Insulin infusion and oraal glucose ingestion are associated with an increase in calcium excretion that cannot be accounted for by alterations in renal hemodynamics and appear to be related to changes in tubular calcium reabsorption (*Lennon* and *Piering* 1970; *De Fronzo* et al. 1975). The tubular site of action is unclear; although inhibition of proximal tubular reabsorption has been demonstrated with both insulin and glucose administration in the dog, fractional calcium excretion did not increase in those studies (*De Fronzo* et al. 1976).

3.3 Acid–Base Alterations

A number of studies utilizing clearance techniques have shown that metabolic acidosis increases and metabolic alkalosis decreases urinary calcium excretion. These effects occur in the absence of parathyroid hormone and appear to be the result of direct tubular effects. Micropuncture studies suggest a dual effect in the nephron, depending upon the duration of the acid–base disturbance. Acute metabolic acidosis in the rat inhibited proximal tubular sodium and calcium transport (*Dubb* et al. 1977) but did not alter the final urine excretion or dissociate sodium and calcium. Chronic ammonium chloride loading in the dog produced a dissociation of such a kind that, at any level of sodium excretion, acidotic dogs exhibited a significantly higher level of calcium excretion than normal dogs. Micropuncture studies revealed that the site of inhibition was not in the proximal tubule but was apparent in the distal tubule and beyond (*Sutton* et al. 1979). These changes were reversed with bicarbonate correction of the acidosis in both intact and parathyroidectomized dogs.

3.4 Diuretics

The effect of a natriuretic agent upon calcium excretion is dependent upon its principal site of action and the relationship between sodium and calcium transport at that site. Thus acetazolamide inhibits proximal reabsorption but the augmented delivery is reabsorbed distally and there is little change in urinary calcium excretion. Osmotic diuretics that seem to inhibit sodium reabsorption in the loop of Henle tend to produce parallel changes in urinary excretion of sodium and calcium (*Wesson* 1962), presumably reflecting the reabsorptive parallelism of these ions in the loop.

Loop-active diuretics such as ethacrynic acid and furosemide produce a marked calciuria that may be proportionately greater than the corresponding natriuresis. Micropuncture studies suggest that this dissociation is not apparent prior to the cortical portion of the distal tubule and reflects calcium-independent sodium reabsorption at more distal sites in the nephron (*Edwards* et al. 1973).

Acute administration of benzothiadiazides produces natriuresis and variable changes in calcium excretion. Chronic administration invariably reduces urinary calcium excretion. Part of the hypocalciuria has been attributed to ECF volume contraction and enhanced proximal tubular reabsorption of sodium and calcium. Thus the hypocalciuria can be markedly attenuated if sodium losses are replaced concurrent with drug administration (*Brickman* et al. 1972). Since the reduction in calcium excretion is less marked in patients with hypoparathyroidism it has also been suggested that thiazides potentiate the effects of parathyroid hormone in the distal nephron to enhance calcium reabsorption (*Brickman* et al. 1972). More recently, evidence has been obtained for a direct tubular effect of thiazides (*Costanzo* and *Windhager* 1978b). In microperfusion studies of the distal convoluted tubule, these investigators were able to demonstrate enhancement of absolute calcium reabsorption with increasing load compared with controls, despite simultaneous inhibition of sodium transport. The observation that thiazides were effective when present only in luminal perfusion fluid is consistent with a site of action on the luminal side. As the distal convoluted tubule is a heterogeneous morphological structure, however, the exact cellular site of action cannot be determined from these studies.

3.5 Phosphate Depletion

Phosphate depletion produces a rapid and marked increase in urinary calcium excretion (*Coburn* and *Massry* 1970). Several mechanisms have been proposed to account for this phenomenon. Dissolution of bone and enhanced intestinal absorption may tend to raise serum calcium. The combination of increased filtered load of calcium and reduced levels of PTH could combine to account for calciuria. Several laboratories, however, have shown that the hypercalciuria cannot be totally corrected with either intravenous (*Coburn* and *Massry* 1970) or chronically PTH administration (*Grabie* et al. 1978). Alternatively, phosphate depletion could directly alter tubular calcium transport, as suggested by *Coburn* and *Massry* (1970). Recent studies have corroborated the presence of a tubular defect. Micropuncture studies in the dog demonstrated inhibition of proximal tubular calcium and sodium reabsorption (*Goldfarb* et al. 1977) Acute infusion with phosphate, however, to parathyroidectomized, phosphate-depleted

dogs reduced calcium excretion to normal levels, but the proximal tubular defect was unchanged, suggesting a more distal site of action of phosphate. Subsequent studies in the rat revealed that the site of the defect in calcium transport was beyond the late distal tubule puncture site and that this was the site where phosphate infusion acted to reduce calcium excretion (*Lau* et al. 1979). Thus the evidence currently strongly favors the presence of intrinsic renal tubular defects as the cause of hypercalciuria in phosphate depletion. While the proximal tubule is the site of reduced transport of sodium and calcium, it is the distal nephron, specifically that portion of the nephron beyond the accessible portions of the late distal tubule, that is responsible for the changes in the final urine.

3.6 Effect of Filtered Load

In contrast to increases in filtered load produced by a rise in glomerular filtration rate (GFR), calcium excretion increases markedly with calcium infusion. Recent studies in TPTX rats (*Hugi* et al. 1979) indicate that in the absence of PTH urinary calcium excretion is a linear function of filtered load with 35%–50% of the increases in filtered calcium appearing in the urine. With PTH infusion, the threshold is increased but the slope remains the same. In intact animals, a steeper slope is observed as PTH secretion falls, and both intact and TPTX animals reach a similar urinary calcium excretion at a plasma calcium of 12–13 mg%. Micropuncture studies during mild hypercalcemia reveal little change in TF/UF calcium in the proximal tubule and suggest an important role for the distal nephron. With more marked hypercalcemia, TF/UF calcium increases but this may reflect the presence of filterable but nonreabsorbable calcium complexes rather than saturation of proximal tubular reabsorption (*Edwards* et al. 1974).

4 Summary

Calcium is reabsorbed in most segments of the mammalian nephron in a pattern generally similar to that of sodium reabsorption. Of the filtered load, 50%–60% is reabsorbed in the proximal convoluted tubule, where there appears to be a strong link between sodium and calcium transport. There is now evidence for active calcium reabsorption in the pars recta but there is virtually no transport in the thin descending or ascending limb of Henle's loop. A significant fraction of the filtered load of calcium is transported in the thick ascending limb but the mechanism of transport remains controversial and the possibility of heterogeneity between cortical and

medullary segments is being actively investigated. There is active calcium transport in the distal tubule and the granular segment of the collecting duct with reabsorption of up to 10% of the filtered load.

The terminal nephron, i.e., the late distal tubule and early collecting duct, which are morphologically characterized as the granular segment, may be the major site for the final regulation of urinary calcium excretion. Current data suggest that these segments are the major site of action of parathyroid hormone and are responsible for the alterations in calcium excretion observed with metabolic acidosis, volume expansion, and chronic phosphate depletion.

References

Agus ZS, Gardner LB, Beck LH, Goldberg M (1973) Effects of parathyroid hormone on renal tubular reabsorption of calcium, sodium and phosphate. Am J Physiol 224:1143–1154

Agus ZS, Chiu PJS, Goldberg M (1977) Regulation of urinary calcium excretion in the rat. Am J Physiol 232:F545–F549

Beck LH, Goldberg M (1973) Effects of acetazolamide and parathyroidectomy on renal transport of sodium, calcium and phosphate. Am J Physiol 224:1136

Bordeau JE, Burg M (1979) Voltage dependence of calcium transport in the thick ascending limb of Henle's loop. Am J Physiol 236:F357–F364

Brickman AS, Massry SG, Coburn JW (1972) Changes in serum and urinary calcium during treatment with hydrochlorothiazide: studies on mechanisms. J Clin Invest 51:945–954

Chabardes D, Imbert M, Clique A, Montegut M, Morel F (1975) PTH-sensitive adenyl cyclase in different segments of the rabbit nephron. Pfluegers Arch 354:229–239

Chabardes D, Gagnan-Brunette M, Imbert-Teboul M, Gontcharevskaia O, Montegut M, Clique A, Morel F (1980) Adenylate cyclase responsiveness to hormones in various portions of the human nephron. J Clin Invest 65:439–448

Coburn JW, Massry SG (1970) Changes in serum and urinary calcium during phosphate depletion: Studies on mechanisms. J Clin Invest 49:1073

Costanzo LS, Windhager EE (1978a) Effect of parathyroid hormone and cyclic AMP on calcium and sodium transport in the distal tubule. Kidney Int 14:638a

Costanzo LS, Windhager EE (1978b) Calcium and sodium transport by the distal convoluted tubule of the rat. Am J Physiol 235:F492–F506

De Fronzo RA, Cooke CR, Andres R, Faloona GR, Davis PJ (1975) The effect of insulin on renal handling of sodium, potassium, calcium and phosphate in man. J Clin Invest 55:845–855

De Fronzo RA, Goldberg M, Agus ZS (1976) The effects of glucose and insulin on renal electrolyte transport. J Clin Invest 58:83–90

De Rouffignac C, Morel F, Moss N, Roinel N (1973) Micropuncture study of water and electrolyte movements along the loop of Henle in Psammomys with special reference to magnesium, calcium, and phosphorus. Pfluegers Arch 344:309–326

Duarte CG, Watson JF (1967) Calcium reabsorption in proximal tubule of the dog nephron. Am J Physiol 212:355–356

Dubb J, Goldberg M, Agus ZS (1977) Tubular effects of acute metabolic acidosis in the rat. J Lab Clin Med 90:318–323

Edwards BR, Baer PG, Sutton RAL, Dirks JH (1973) Micropuncture study of diuretic effects on sodium and calcium reabsorption in the dog nephron. J Clin Invest 52: 2418–2427

Edwards BR, Sutton RAL, Dirks HJ (1974) Effect of calcium infusion on renal tubular reabsorption in the dog. Am J Physiol 227:13–18

Goldfarb S, Westby GR, Goldberg M, Agus ZS (1977) Renal tubular effects of chronic phosphate depletion. J Clin Invest 59:770

Gmaj P, Murer H, Kinne R (1979) Calcium ion transport across plasma membranes isolated from rat kidney cortex. Biochem J 178:549–557

Grabie M, Lau YK, Agus ZS, Goldberg M, Goldfarb S (1978) Role of parathyroid hormone in the hypercalciuria of chronic phosphate depletion. Miner Electrolyte Metab 1:279

Greger RF, Lang F, Oberleithner H (1978) Distal site of calcium reabsorption in the rat nephron. Pfluegers Arch 374:153–157

Harris CA, Baer PG, Chirito E, Dirks HJ (1974) Composition of mammalian glomerular filtrate. Am J Physiol 227:972–976

Hugi K, Bonjour J-R, Fleisch H (1979) Renal handling of calcium: influences of parathyroid hormone and 1,25-dihydroxyvitamin D. Am J Physiol 236:F349–F356

Imai M (1978) Calcium transport across the rabbit thick ascending limb of Henle's loop perfused in vitro. Pfluegers Arch 374:255–263

Imbert M, Chabardes D, Montegut M, Clique A, Morel F (1975) Adenylate cyclase along the rabbit nephron as measured in single isolated segments. Pfluegers Arch 354:213–228

Jamison RL, Frey N, Lacy FB (1974) Calcium reabsorption in the thin loop of Henle. Am J Physiol 227:745–751

Kuntziger H, Amiel C, Roinel N, Morel F (1974) Effects of parathyroidectomy and cyclic AMP on renal transport of phosphate, calcium and magnesium. Am J Physiol 227:905–911

Lassiter WE, Gottschalk CW, Mylle M (1963) Micropuncture study of renal tubular reabsorption of calcium in normal rodents. Am J Physiol 204:771–775

Lau YK, Agus ZS, Goldberg M, Goldfarb S (1979) Renal tubular sites of altered calcium transport in phosphate depleted rats. J Clin Invest 64:1681

Le Chene C, Abraham E, Warner R (1975) Effect of sulfate loading on ionic distribution along the rat nephron. Clin Res 23:432A

Le Grimellec C, Roinel N, Morel F (1974) Simultaneous Mg, Ca, P, K and Cl analysis in rat tubular fluid. III. During acute Ca plasma loading. Pfluegers Arch 346:171–188

Le Grimellec C, Poujeol P, De Rouffignac C (1975) ^{3}H-inulin and electrolyte concentrantions in Bowman's capsule in rat kidney. Pfluegers Arch 354:117–131

Lennon EJ, Piering WF (1970) A comparison of the effects of glucose ingestion and NH_4Cl acidosis on urinary calcium and magnesium excretion in man. J Clin Invest 49:1458–1465

Massry SG, Kleeman CR (1972) Calcium and magnesium excretion during acute rise in glomerular filtration rate. J Lab Clin Med 80:654–664

Massry SG, Coburn JW, Chapman LW, Kleeman CR (1968) The effect of long term desoxycorticosterone acetate administration on the renal excretion of calcium and magnesium. J Lab Clin Med 7:212–219

Morel F, Chabardes D, Imbert M (1976) Functional segmentation of the rabbit distal tubule by microdetermination of hormone dependent adenylate cyclase activity. Kidney Int 9:264–277

Puschett JB, Moranz J, Kurnick WD (1972a) Evidence for a direct action of cholecalciferol and 25-hydroxycholecalciferol on the renal transport of phosphate, sodium and calcium. J Clin Invest 51:373–385

Puschett JB, Fernandez PD, Boyle IT, Gray RW, Omdahl JL, De Luca HF (1972b) The actue renal tubular effects of 1,25 dihydroxycholecalciferol. Proc Soc Exp Biol Med 141:379–390

Quamme GA (1980) Effect of calcitonin on calcium and magnesium transport in rat nephron. Am J Physiol 238:E573–578
Rastegar A, Agus ZS, Connor TB, Goldberg M (1972) The renal handling of calcium and phosphate during mineralocorticoid escape in man. Kidney Int 2:279–286
Rocha A, Magaldi JB, Kokko JP (1977) Calcium and phosphate transport in isolated segments of rabbit Henle's loop. J Clin Invest 59:975–983
Rouse D, Ng RCK, Suki WN (1980) Calcium transport in the pars recta and thin descending limb of Henle of the rabbit, perfused in vitro. J Clin Invest 65:37–42
Shareghi GR, Agus ZS (1979) Magnesium transport in the rabbit cortical thick ascending limb. Clin Res 27:430A
Shareghi GR, Stoner LC (1978) Calcium transport across segments of the rabbit distal nephron in vitro. Am J Physiol 4:F367–375
Suki WN (1979) Calcium transport in the nephron. Am J Physiol 237:F1–F6
Suki WN, Rouse D (1980) Heterogeneity of calcium transport in the medullary and cortical thick ascending limb of Henle: Effect of calcitonin. Clin Res 28:536A
Suki WN, Schwettmann RS, Rector FC Jr, Seldin DW (1968) Effect of chronic mineralocorticoid administration on calcium excretion in the rat. Am J Physiol 215: 71–74
Suki WN, Rouse D, Kokko JP (1979) Calcium transport in the thick ascending limb of Henle. Kidney Int 12:461a
Sutton RAL, Wong NLM, Dirks JH (1979) Effects of metabolic acidosis and alkalosis on sodium and calcium transport in the dog kidney. Kidney Int 15:520–533
Taylor A, Windhager EE (1979) Possible role of cytosolic calcium and Na-Ca exchange in regulation of transepithelial sodium transport. Am J Physiol 236:F505–F512
Ullrich KJ, Rumrich G, Kloss S (1976) Active Ca^{2+} reabsorption in the proximal tubule of the rat kidney. Pfluegers Arch 364:223–228
Walser M (1961) Calcium clearance as a function of sodium clearance in the dog. Am J Physiol 200:1099–1104
Wesson LG Jr (1962) Magnesium, calcium and phosphate excretion during osmotic diuresis in the dog. J Lab Clin Med 60:422–432
Widrow SH, Levinsky NG (1962) The effect of parathyroid extract on renal tubular calcium reabsorption in the dog. J Clin Invest 41:2151–2159

Rev. Physiol. Biochem. Pharmacol., Vol. 90

Central Chemosensitivity: A Respiratory Drive

MARIANNE E. SCHLAEFKE *

Dedicated to Professor Dr. Dr. h.c. Hans H. Loeschcke on the occasion of his 68th birthday

Contents

* Arbeitsgruppe Physiologie der Regulation, Institut für Physiologie, Ruhr-Universität Bochum, D–4630 Bochum/GFR

1 Introduction

1.1 Central Chemosensitivity as Seen in Two Earlier Articles by Winterstein and Wyss

Two previous articles on the respiratory control system of mammals in this review series form the basis for the following one, which deals with central respiratory chemosensitivity: One emphasizes the humoral homeostatic part (*Winterstein* 1958), the other the neural reflexogenic part (*Wyss* 1964). In „Die chemische Steuerung der Atmung" *Winterstein* discussed the four forms of the "reaction theory" founded on the idea that lung ventilation is governed by the H^+ ion concentration of the body fluids. Lung ventilation should keep the pH constant by releasing more or less CO_2. *Winterstein* believed that H^+ ions were also responsible for the respiratory drive produced by hypoxia. The mechanism he thought likely to exist was a possible influence of H^+ ions on the cholinergic system of the peripheral chemoreceptors. In addition *Winterstein* at the same time speculated that the site of H^+ ion sensitivity behind the blood-brain barrier was located within the respiratory centers. *Winterstein*'s concept of chemical regulation fundamentally influenced the research on acid-base homeostasis as controlled by the respiratory system, up to the present time.

Wyss (1964) in „Die nervöse Steuerung der Atmung" described the neural mechanism of peripheral and central reflexogenic nature. He dealt with the rhythmogenesis of breathing and stated the basic role of the vagal reflexes for the autonomy of respiration, which, however, as was shown later, in the absence of any chemosensitive input into the rhythm generator, are unable to contribute to the generation of respiration (*See* 1973). *Wyss*, in his contribution, did not discuss the chemical reflexes in detail but evaluated their component as incorporated within the "vegetative basic structure", the chemical reflexes thus being part of the basic conditions for the autonomy of bulbar activity. *Wyss* questioned the idea of the respiratory center being sensitive to CO_2 or H^+ ions and, referring to the early experiments of *Loeschcke* and co-workers (*Loeschcke* and *Koepchen* 1958a–c; *Loeschcke* et al. 1958), he concluded that a special chemosensitive apparatus existed in the brain outside the respiratory centers, this theory being later accepted by *Winterstein* (1960).

This article is concerned with the central chemosensitive mechanism, its location, its morphological correlate, some basic functions, and some clinical aspects. The main aim is to describe the present opinions regarding central H^+ ion sensitivity as a respiratory afferent system.

Any references from the older literature may be obtained from the two quoted reviews. Previous reviews on central chemosensitivity have been published by *Loeschcke* (1974a) and *Loeschcke* and *Schlaefke* (1976). For

further reviews on the control of respiration and some special aspects see *Berger* et al. (1977a–c), *Cohen* (1979), *Cunningham* (1973), *Duffin* (1971), *Guz* (1975), *Karczewski* (1974), *Heinemann* and *Goldring* (1974), *Loeschcke* (1969, 1971, 1972, 1973a–c, 1977), and some recent symposia edited by *Paintal* and *Gill-Kumar* (1977), *v. Euler* and *Lagercrantz* (1979), *Fitzgerald* et al. (1978), *Duron* (1976), *Karczewski* and *Widdicombe* (1973), *Koepchen* et al. (1980), *Leusen* (1972), *Loeschcke* (1976), *Paintal* (1976), *Neil* (1973), *Umbach* and *Koepchen* (1974), *Siesjö* and *Sørensen* (1971), and the "Warsaw Symposium" on "CO_2 and Breathing" (1976).

1.2 Is Chemosensitivity a General Characteristic of the Respiratory Centers?

Based on the essential discoveries in respiratory physiology by *Pflüger* (1868), *Haldane* and *Priestley* (1905), and *Fredericq* (1901), *Winterstein* (1910, 1911, 1923) attributed the extent of lung ventilation to the H^+ ion concentration within the respiratory centers. The H^+ ion concentration would depend upon the metabolism, the pH of the surrounding fluids, and upon the permeability of components determining the pH. *Gesell* (1923, 1925) also saw the important contribution of the acidity within the fluid surrounding the respiratory centers to the regulation of respiration. In 1908 *Robertson* had already reported experiments in which he could elicit respiratory responses by injecting acids into the respiratory center of the frog. In mammals, as is quoted later, accounts concerning stimulation of the respiratory center with drugs by different workers are rather controversial. Regarding this vagueness in the field of central chemosensitivity, *Gesell* (1939) praised *Heymans* and his co-workers for their discovery of the carotid and aortic chemoreceptors (*Heymans* and *Heymans* 1927; *Heymans* and *Bouckaert* 1930, 1939; *Heymans* et al. 1931) as it gave "a new outlook on respiration for which physiology is deeply indebted ... No one had actually demonstrated that either excess of carbon dioxide or lack of oxygen, restricted to the medulla, is capable of augmenting pulmonary ventilation". This statement held for the effect of hypoxia, as well as for CO_2 when its action was restricted to the medullary respiratory centers.

Following *Heymans* and *Bouckaert* (1939), *Heymans* (1951), *Hesser* (1949), and *Gray* (1950) there still remains an insufficient number of scientists who have studied chemical control of ventilation after peripheral chemoreceptor denervation. Depending on the species, the experimental conditions and purpose, it was more or less clearly shown that CO_2 or H^+ ions still influence respiration without peripheral chemoreceptors (*Adolph* et al. 1961; *Anitschkow* 1936; *Banus* et al. 1944; *Belville* et al. 1959;

Berger et al. 1971, 1973; *Berndt* et al. 1972a–c; *Bisgard* and *Vogel* 1971; *Borison* and *McCarthy* 1973; *Bouverot* 1976; *Bouverot* and *Bureau* 1975; *Bouverot* et al. 1961, 1963, 1965; *Dejours* 1962; *v. Euler* and *Liljestrand* 1940; *Florez* and *Borison* 1971; *Gautier* 1976a, b; *Guz* et al. 1966; *Heeringa* 1979; *Jones* and *Purves* 1970; *Katsaros* 1965a, b; *Kiwull* et al. 1972, 1976; *Kiwull-Schöne* and *Kiwull* 1979; *Kiwull-Schöne* et al. 1976; *Loeschcke* 1960; *Loeschcke* et al. 1958, 1979; *Mitchell* et al. 1964; *Neil* and *O'Regan* 1969; *Perkins* 1968; *Rosenstein* et al. 1974; *Rutherford* et al. 1967; *Schlaefke* and *Loeschcke* 1967; *Schlaefke* et al. 1970; *Schmidt* and *Comroe* 1940; *Sörensen* and *Mines* 1970; *Wiemer* et al. 1964; *Winterstein* 1950; *Winterstein* and *Gökhan* 1953).

The chemosensitivity of the respiratory center was questioned by investigators who had observed the selective decrease of the respiratory response to CO_2 and not to hypoxia under anesthesia (*Åström* 1952; *Benzinger* et al. 1938; *Dripps* and *Dumke* 1943; *Dripps* and *Severinghaus* 1955; *Florez* and *Borison* 1969; *Hesser* 1949; *Marshall* and *Rosenfeld* 1936; *Schmidt* and *Comroe* 1940) or by sleep (*Phillipson* 1977, 1978a, b; *Phillipson* et al. 1978; *Reed* and *Kellogg* 1958, 1960). Selective loss or lowering of CO_2 sensitivity has also been observed in patients with chronic lung emphysema (*Alexander* et al. 1955; *Comroe* et al. 1950; *Prime* and *Westlake* 1954; *Scott* 1920), or with local lesions (*Richter* et al. 1957), or in cases of primary alveolar hypoventilation (*Fruhmann* et al. 1961; *Chiesa* et al. 1970).

After *Pitts* (1940), *Pitts* et al. (1939a–c) and *Stella* (1938a, b) had detected the topographical location for where the respiratory rhythm should be generated and afferent input should be coordinated, *Comroe* (1943) and *Liljestrand* (1953, 1958) compared effects produced by bicarbonate injections or electrical stimulation within the respiratory centers or in the medulla oblongata respectively, and obtained diverging results. *Comroe* observed coincidence of the chemically and electrically-elicited effects, while *Liljestrand* discovered some incongruity.

Von Euler and *Söderberg* (1952a, b) recorded the first chemically-evoked action potentials from the "completely denervated" rhombencephalon in response to carbon dioxide. In addition they measured slow potential shifts in the medulla oblongata as a response to alterations in blood chemistry. The authors were also able to show that the neural response to CO_2 was diminished by anesthesia without impediment of other peripheral respiratory reflexes. They drew the following conclusions: (1) The bulbar chemosensitive structures could not be involved in the respiratory reflex and coordinating pathways, but should be located as a special apparatus within the respiratory centers. (2) Differing from the ideas of *Gesell* (1940) and *Pitts* (1946) who believed that CO_2 would modify a basic discharge activity of neurons due to metabolic gradients from the dendrites to the

axon hillock, *von Euler* and *Söderberg* (1952a, b) proposed a mechanism of impulse generation by CO_2.

Another approach was made by various authors applying substances which act on the peripheral chemoreceptors, to the floor of the fourth ventricle, or by suboccipital injections, by perfusing the ventricular system, or by injection into the cisterna pontis. The observed respiratory responses to nicotine, lobeline, cyanide, cocaine, acetylcholine, acids, or cooling were equivocal (*Comroe* 1943; *Gesell* et al. 1943; *Loeschcke* and *Koepchen* 1958a–c; *Mitchell* et al. 1960; *Wiemer* 1959; *Winterstein* and *Gökhan* 1953; *Winterstein* and *Wiemer* 1959).

A new attempt to investigate central chemosensitivity was begun by *Leusen* (1954a, b). Acid solutions perfused through the ventricular spaces produced an increase in ventilation. *Leusen* showed that the relationship between H_2CO_3 and $BHCO_3^-$ was decisive for the effect on respiration, but he could not prove that the true stimulus was provided by the H^+ ions. *Leusen* believed like *Winterstein* (1955), *Gesell* (1940), and *Comroe* (1943), that the respiratory centers underlying the fourth ventricle would respond to the changes of acidity directly.

Considering *Winterstein*'s concept regarding the predominant role of H^+ ions on one hand and the equivocal findings on the questioned central site of chemical action, *Loeschcke* started his work on central chemosensitivity and gave his first report in 1957, a paper which was later published, together with *Koepchen* and *Gertz* (1958), and which may be regarded as the first one dealing with medullary surface pH sensitivity.

2 The Structure

2.1 The Localization

The most important step in the investigation of central chemosensitivity was taken by *Loeschcke* et al. (1958) isolating the stimulus, to show whether it is CO_2 or pH. Three types of techniques were used in the series, from which decisive proposals could be made:

1) A catheter was advanced 2 mm through the roof to the third ventricle.

2) The catheter was pushed forward to the third ventricle, so that the injected solutions could run back along the catheter and reach the bulbar base through the foramina Luschkae.

3) The cerebellum was removed by suction and test solutions were applied to the floor of the fourth ventricle by pledgets.

The authors used $BHCO_3^-/H_2CO_3$ buffers equilibrated with CO_2 in air, in which $BHCO_3^-$ and NaCl were exchanged equivalently. All solutions had the same K^+ and Ca^{++} ion concentration and were isotonic. These were the

results: An acid shift of pH at constant PCO_2 of the perfusate was followed by an increase in tidal volume, and an alkaline shift by a decrease in tidal volume. An increase of PCO_2 at constant pH was accompanied by a gentle diminution of tidal volume. The respiratory response could only be seen when the solutions reached the lateral recessus. Application of acid to the floor of the fourth ventricle produced no effect. With these results *Winterstein*'s concept was proved for the first time, as the results showed that H^+ ions, and not specifically CO_2, influence the sensitive structure. In addition, the findings contradicted the idea of a general chemosensitivity of the respiratory centers. From the results of the experiments of *Jacobs* (1920a, b) and the theory of *Winterstein* (1910, 1911, 1923, 1955), and of *Gesell* (1923, 1925, 1940), *Loeschcke* and his co-workers considered that an increase in CO_2 at constant pH which did not stimulate breathing, indicated a very superficial location for the sensory structure. Deeper-lying sensor elements should have been reached by the easily penetrating CO_2 with the consequence of hyperpnea. This however, was not observed. The authors suspected free nerve endings within the lateral recessus and within the chorioid plexus of the fourth ventricle, as had been described by *Clark* (1934), to be possible candidates for a H^+ ion sensitive structure.

In two further experimental studies by *Loeschcke* and *Koepchen* (1958a, c), procaine was applied using *Leusen*'s method (1954a), by which the whole ventricular system of the cerebrum was reached, or by perfusing only the fourth and third ventricle as described before. Procaine (2%) was also applied locally to the floor of the fourth ventricle without effect, but a decrease in respiration and blood pressure was observed following the perfusion technique as well as the local application on the lateral recessus. Apnea of the chemodenervated cat could be interrupted by electrical stimulation of the inspiratory center. From these results the authors found support for their assumption that the centers, when deprived of peripheral chemoreceptors under resting conditions, are dependent upon H^+ ion sensitive afferent impulses. They further concluded that the vasomotor center may also receive afferent impulses, and that both afferent systems may be superficially located within the region of the lateral recessus and the medullary base.

With these results *Loeschcke* joined *Mitchell*'s group, which had also worked on chemically-induced effects on respiration obtained from the floor of the fourth ventricle in dogs (*Mitchell* et al. 1960). In 1963 together they were able to confirm that the increase in respiration due to H^+ ions and PCO_2, nicotine and acetylcholine was obtained from the subarachnoid space on the ventrolateral surface of the medulla, whereas no effects or depressive ones were observed when the substances were applied to the fourth ventricle. Procaine, lobeline, NaCN, and cold CSF in the subarachnoid space on the lateral surface, depressed ventilation (*Mitchell* et al.

1963b) and in another paper (*Mitchell* 1965). Unfortunately the two drawings of the medulla oblongata published in the two papers differ from each other and from the topographical anatomy of the cat. The following description was given: The chemosensitive area is bordered rostrally by the pons, extending 6–7 mm caudal, bounded laterally by the roots of the 7th–11th cranial nerves and medially by the pyramidal tract (*Mitchell* et al. 1963b). The authors attributed the superficial location of the sensitive structure to the fast respiratory responses following local application of the chemical substances. In addition it was shown that apnea occurred with 1% procaine in the perfusate, and, using a staining method for procaine (*Grodinski* et al. 1933), it was shown not to penetrate deeper than 350 μm below the surface, after a contact time of 5 min.

Schwanghart et al. (1974) applied procaine to the ventral medullary surface and studied inspiratory and expiratory discharge as well as reticular neuronal discharge. Inspiratory discharge was abolished within a very short time, expiratory discharge became tonic, and reticular neurons were either activated or inhibited. Histochemical tests revealed that procaine had not penetrated deeper than 30–50 μm, which again provides evidence for a very superficial location of the structure mediating afferent respiratory drive.

The most intensive research conducted on central chemosensitivity during the past 20 years was done by the group led by *Loeschcke*. His coworker *Berndt* tested a geometric localization (*Berndt* et al. 1972a–d). The authors experimentally established stationary gradients of the extracellular pH between the ventral surface and deeper parts of the medulla. They showed by calculation that ventilatory responses were correlated best with extracellular pH changes occurring at 250–300 μm below the ventral medullary surface. *Berndt* et al. (1970) also showed that the respiratory center could still be activated by electrical stimulation when the ventral surface was superfused with either procaine or solutions of high potassium concentration, although K^+ ions may rapidly diffuse through the extracellular spaces of the medulla in contrast to procaine.

Trouth et al (1973a) by systematic electrical stimulation with a unipolar electrode elicited respiratory responses from the surface, and from different points at 0.1–0.2 mm up to a depth of 1 mm and from thereon at millimeter intervals up to a depth of 6 mm below the surface. The authors observed the strongest effects from the surface medial of the hypoglossal root, still obtainable at 0.2 mm but not at about 0.6–0.8 mm below the surface. Respiratory effects could again be evoked from 2 mm below the surface and deeper. From both studies the authors, using the two different experimental approaches, saw in their results further confirmation of the hypothesis that the sensitive system was located within the ventral medullary surface layer and therefore could not be identical with the respiratory reflex and coordinating centers.

Three further studies were performed in order to obtain a more precise localization of the medullary surface area with respiratory effects, aiming at providing information for subsequent tissue withdrawals for morphological investigations (*Loeschcke* et al. 1970; *Schlaefke* and *Loeschcke* 1967; *Schlaefke* et al. 1970). The outcome was that not one but three areas on the ventral medullary surface were found to be involved in central chemosensitivity (Fig. 1). The first area was localized by cold block, cooling with

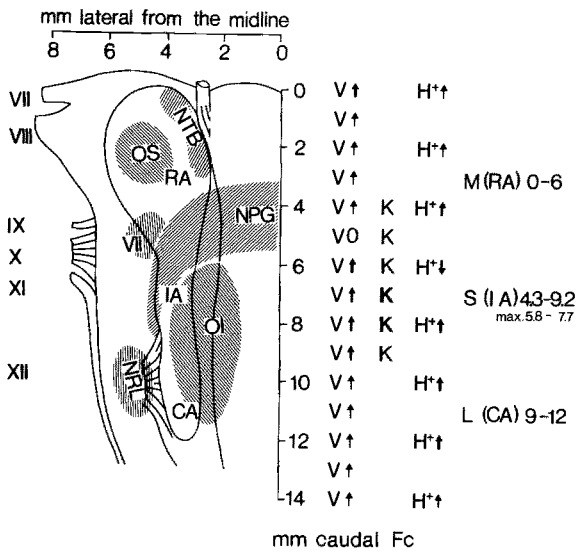


Fig. 1. *Left side:* Scheme of ventral surface of the medulla oblongata of the cat. VI–XII cranial nerve roots. *O,* Foramen caecum *(Fc).* Projected on the surface: *NTB,* nucleus trapezoid body; *OS,* superior olive; *NPG,* paragigantocellular nucleus; *OI,* inferior olive; *NRL,* lateral reticular nucleus; *VII,* VIIth nerve nucleus. *Right side:* Strongest respiratory responses were elicited from 3 mm lateral of the midline and at points in different caudal distances from the foramen caecum. *V* for electrical stimulation; *K* for cold block; H^+ for local superfusion with artificial cerebrospinal fluid of varied pH. *Arrows upward,* increase; *arrows downward,* decrease of tidal volume. *0,* almost no change. Maximal responses to electrical stimulation: 0 and 11 mm caudal of Fc. *K fat:* largest effect of cold block. The rostrocaudal extension from *M, S,* and *L* areas is given

a thermode 1 mm in diameter to 8°–10°C or 281–283 K, respectively. The characteristic result was apnea, when the corresponding contralateral area was coagulated, and sinus nerves and vagi were cut (*Schlaefke* and *Loeschcke* 1967). Later, in different papers, the extension of this area varied. For this reason it was reinvestigated under the same conditions, the rostrocaudal boundary referring to the foramen caecum (*Schlaefke* et al. 1970, Fig. 6 there): The maximal effect, which was rapid apnea following the onset of cold block, was obtained from an area 5.8–7.7 mm caudal of

the foramen caecum (the border between pons and medulla, Fig. 1). Apnea, after a longer latency, was observed up to 4.3 and 9.2 mm caudal of the foramen caecum. The lateral boundary was 2.9–3.8 mm lateral from the midline. The area was at first named „Kälte-empfindliches Feld", later it was called area S and finally intermediate area (IA). In two parallel studies electrical and chemical stimulation of the ventral medullary surface was performed with the aim of comparing respiratory effects (*Loeschcke* et al. 1970; *Schlaefke* et al. 1970). The conditions of the two series varied, however. The electrical stimulation was done in intact cats; the chemical stimulation, namely superfusing 1 mm^2 areas with artificial CSF of varying pH while keeping the surrounding area neutral, was performed in cats in which the contralateral intermediate area had been coagulated, and sinus nerves and vagi had been cut.

For electrical stimulation a core electrode with 0.6 mm inner and 1.6 mm outer diameter was used. The voltage used was 2/3 of that delivered to a point of maximal respiratory response medial of the hypoglossal root, for the individual cat. Electrical stimulation produced increases in tidal volume within areas 3 mm lateral to the midline, from 0–4 mm caudal, and from 6–14 mm caudal of the foramen caecum with maxima at 0 and 11 mm caudal of the reference point. Between these limits there appeared to be a steep trough with its minimum at 5 mm caudal from the foramen caecum, showing either no increase, or only small increases in tidal volume. However, higher voltages at this point caused apneusis. Local chemical stimulation elicited only small responses regarding tidal volume in areas from 0–4 mm and from 8–12 mm caudal from the foramen caecum. A trough with significant inhibition of tidal volume by acid buffer was found at 6 mm from the foramen caecum. The trough obtained by both types of stimuli projected to the intermediate area, where cold block maximally depressed ventilation. The profiles of respiratory responses over various points of stimulation on the ventral medullary surface led to a working hypothesis which suggested that the sensory sites within the rostral area M (*Mitchell* et al. 1963a), and the caudal area with positive respiratory responses, which was called area L (*Loeschcke* et al. 1970) might converge within the intermediate area S (*Schlaefke* and *Loeschcke* 1967), from where the "bundled" information should be linked with the respiratory centers.

Central chemosensitivity of respiration could be located also for the rat in an area corresponding to the area which has been described by *Mitchell* and co-workers. *Mitchell*'s as well as *Loeschcke*'s results were obtained from the cat, and the effects were seen in principle in dogs as well (*Loeschcke* et al. 1958a; *Mitchell* et al. 1963a). The evidence for central chemosensitive areas on the ventral medullary surface of the rat's brain has been provided by *Hori* et al. (1970), *Fukuda* and *Honda* (1975), *Fukuda* and

Loeschcke (1977), and by *Fukuda* et al. (1978, 1979). *Fukuda* and coworkers observed H^+ ion sensitive neurons in brain slices of the rat in an area corresponding to the location of the intermediate area of the cat. However, nobody so far has shown that central chemosensitivity is represented by three different areas in the rat also. Since in rabbits bilateral cold block of an area corresponding to the intermediate area of the cat causes apnea, it is likely that the central chemosensitive apparatus may also be comparable to that of the cat (*Katzorke* and *Schlaefke*, unpublished data). Nothing is known so far about any H^+ ion sensitive characteristics of the ventral brainstem surface of man.

However, recent histopathological findings in cases of sudden infant death syndrome give some hints that similarities to the cat's chemosensitive apparatus may exist (*Schlaefke* et al. 1980a, b).

2.2 The Search for the Receptor – Without Success

The search for the responsible correlate of an H^+ ion sensitive receptor initiated several histological studies. A first effort by *Loeschcke* (1965), who studied the superficial tissue of the lateral recessus of the fourth ventricle and the adjacent region around the tuberculum acusticum, led to the description of superficial nerve cells surrounded by fine axons. In addition he saw cell clusters of glial nature interwoven with a network of fine axons. *Fleischhauer* and *Petrovický* (1968) within the superficial layer of the lateral recessus described the "nucleus z", neuronal elements surrounded by subependymal glia fibers. So far no one has studied the physiology of these neuronal or glial elements, which is not surprising, since in the meantime the site of interest shifted to the ventral surface.

Petrovický (1968) was the first to compare the results of systematic histological studies on the marginal layer of the ventral brain stem with physiological data. He described, within a field corresponding to the intermediate area (*Schlaefke* and *Loeschcke* 1967) a thin and spongy layer of glia fibers with two types of nerve cells; one, small, round, and pale, with diffusely scattered Nissl substance; and the other medium-sized and multipolar, with dense clusters of Nissl substance. Both types form a small nucleus (2 mm long, 1 mm wide, 1 mm deep), known as the lateral reticular nucleus Olszewski (*Petrovický* 1968). Rostrally, the nucelus turns away from the surface into the depth and contacts the paragigantocellular nucleus (NPG; Figs. 1, 2). Rostrally, within the superficial part, one finds the small pale type of cells; caudally, the larger, dark type. Some cells of the dark type accompany the hypoglossal fibers up to the region of the nucleus interfascicularis hypoglossi.

By systematic electrical stimulation of the medulla, and electrical marking, *Trouth* et al. (1973b) identified 7–26 characteristic cells. These he described to be 40–70 μm in diameter and situated at the site of maximal respiratory responses within the caudal area, at the level of the middle of the hypoglossal root fan. They are arranged as single cells or in groups of 2–8 between the ventral surface and the ventral side of the inferior olive. Their distance from the ventral surface varies from 50–450 μm, but mostly they are found between 150 and 200 μm. A small number has been observed between 450 and 800 μm below the surface. They are easily distinguished by their long multipolar protoplasmic processes. The authors also observed some superficial and some deeper lying nerve cells, hardly distinguishable from reticular neurons, within the rostral area. The authors also missed the grouping here which is characteristic for the caudal area.

Schlaefke (1972), *Kille* and *Schlaefke* (1974), *Schlaefke* et al. (1974) and *Loeschcke* and *Schlaefke* (1976), systematically mapped neurons within the ventral medullary surface layer, extending from the surface to 300 μm depth, up to 12 mm caudal from the foramen caecum and 2–4 mm lateral to the midline. The material was obtained after fixation of the medulla in glutaraldehyde and embedding it in Epon or paraffin from which transverse sections of 2 μm or 8 μm, respectively, as well as horizontal sections, were taken and stained either by silver impregnation or methylene blue. The upper part of Fig. 2 shows the surface projection of the cells found in the different distances from the surface layer (lower part). The different symbols correspond to the different levels below the surface at which cells can be seen. Some neurons of the superficial layer form part of the nucleus reticularis lateralis (NRL, lateral to and underneath the caudal area), of the nucleus paragigantocellularis (NPG, underneath the most superficial cell layer in the intermediate area, and underneath the rostral area), and of the nucleus trapezoidalis (NTP, underneath the rostral area) (*Berman* 1968; *Taber* 1961). The cells first described by *Petrovický* (1968), and those described by *Trouth* et al. (1973b), are indicated by the author's initial. The superficial nerve cells within the intermediate area which have been described by *Petrovický* in 1968 have recently been rediscovered by *Shahar* and *Edery* (1976) and by *Peskov* et al. (1980). The latter found the multipolar neurons distributed within the intermediate area between 9 and 380 μm below the surface. The authors also observed that the cell clusters are located near venules and that they branch out in the immediate vicinity of the microvessels.

The blood supply to the medulla has recently been studied by *Cragg* et al. (1977), who injected indian ink into the vertebral arteries. They found the highest density of small arterioles and venules close to the hypoglossal root and on the ventrolateral surface, while the highest density of small

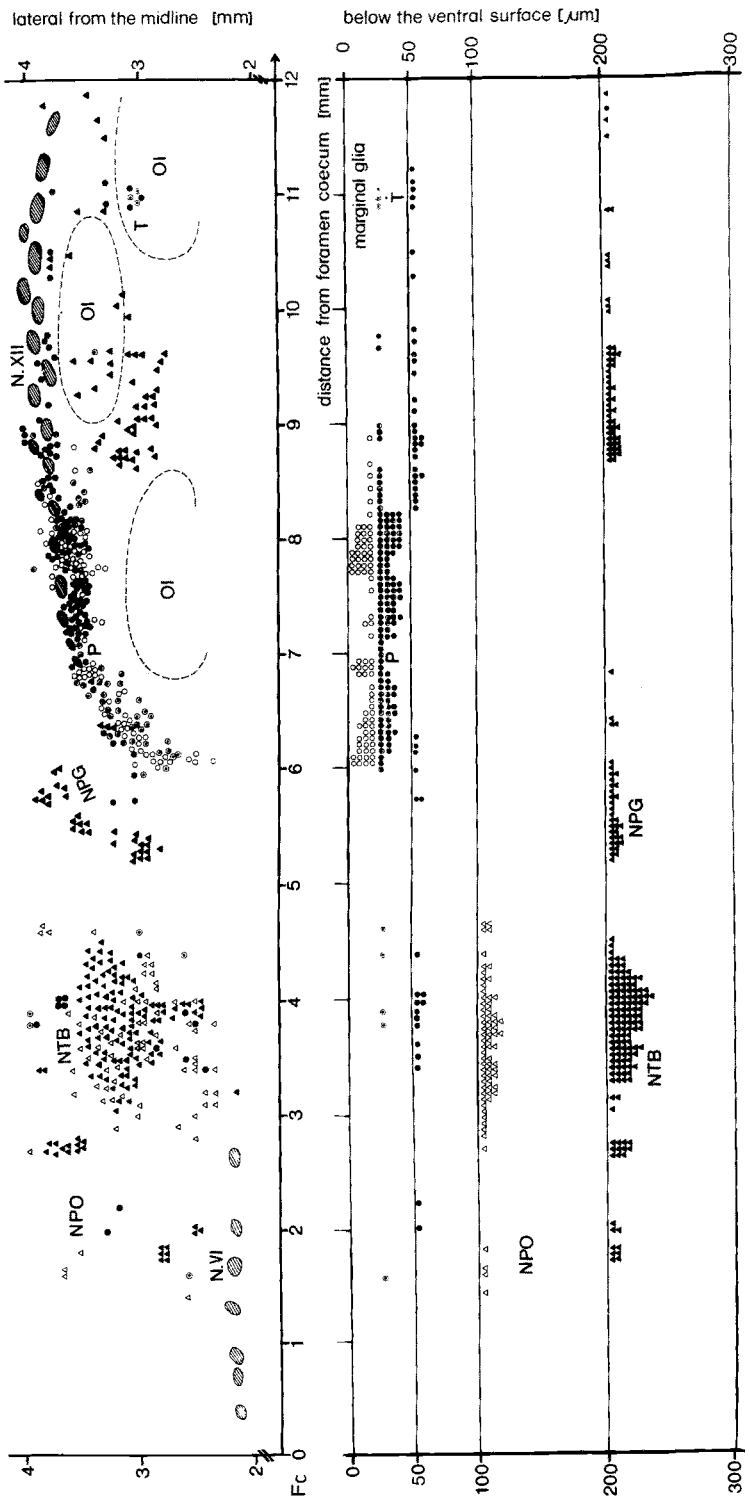


Fig. 2. Number of nerve cells found within different distances from the surface (between 0 and 50, 50 and 100, 100 and 200, and 200 and 300 µm below the surface) are indicated by various symbols (according to the layer below th surface) in the lower part. The same symbols are used for the projection of the cells on the surface in the upper part. *NPO*, preolivary nucleus. *T, Trouth* et al. (1973a, b); *P, Petrovický* (1968). Marginal glia around the hypoglossal root ca. 23 µm in the thickness. H^+ sensitive neurons were recorded from the *NTB* or the rostral area by *Cakar* and *Terzioglu* (1976), *Pokorski* (1976); from the *NPG* or the intermediate area by *Schlaefke* et al. (1975, 1979), *Schlaefke* (1976); from the *NRL* or the caudal area by *Shimada* et al. (1969); *Prill* et al. (1975), *Prill* (1977)

arterioles was found in a zone corresponding to the intermediate area. Fig. 3 shows a plasticized vessel preparation from the medullary base performed by *Kille* (1969), who used Plastogen G for the injection into the vertebral arteries. A very dense coil of vessels project onto the intermediate area. The cold block effect, by which this area is characterized, is only obtained if the thermode is placed between or caudal from the vessels, but never when actually on the vessels, as far as can be controlled using the operation microscope. For a discussion of the argument that the blood supply of deeper medullary structures from the ventral side of the medulla may mimic the ventral superficial location of central chemosensitivity see Sects. 2.5 and 3.4 (*Cragg* et al. 1977; *Lipscomb* and *Boyarski* 1972).

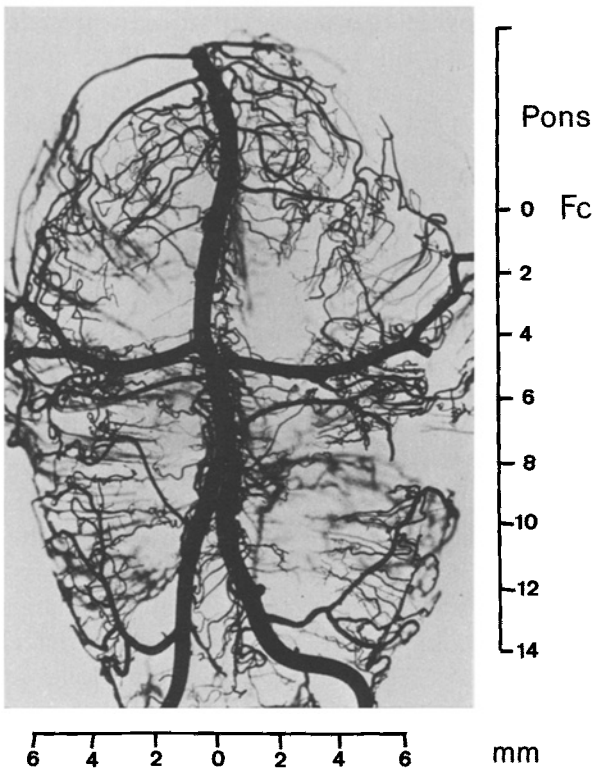


Fig. 3. Medullary base vessels filled via the aorta with Plastogen G (*Kille* 1969). Compare projections of areas M, S, L (Fig. 1). No or opposite respiratory effects elicited by chemical or electrical stimulation were obtained between 5 and 6 mm caudal from the Foramen caecum, an area with a dense coil of vessels. The thermode had to be placed caudally or between the vessels, not on the vessels. Compare *Cragg* et al. (1977)

2.3 The Ultrastructure

Electron micrograph studies (*Ullah* 1973; *Brettschneider* et al. 1974; *Dermietzel* et al. 1974, 1975; *Leibstein* et al. 1975; *Leibstein* 1979; *Luber* 1976; *Shahar* and *Edery* 1976) were combined with scanning techniques, freeze-etching methods, and the local application of horseradish peroxidase and lanthanhydroxide (*Dermietzel* 1976; *Dermietzel* et al. 1977, 1978; *Leibstein* 1979). The following description was given: The most superficial glia fibers run parallel to the surface and become vertical within the area medial and rostromedial of the hypoglossal root. Fingerlike invaginations of the brain surface with an entrance diameter of 0.2–5 μm extend to 10 μm below the surface and show a labyrinthine formation. The glia fibers with microvillilike processes, border the invaginations which are completely covered by the basal membrane. Wide intercellular spaces between the glia fibers contain unmyelinated axons and perikarya of nerve cells. The authors call this configuration the basal membrane labyrinth (*Dermietzel* 1976), in accordance with *Leonhardt* (1967, 1970) who found similar structures on the surface of the rabbit brain ventricles. The invaginations can be clearly distinguished from perivascular spaces of pial blood vessels which enter the marginal glia. Rostromedial of the hypoglossal root a dense population of perikarya as well as dendritic and axonal profiles were described, with a maximum concentration 1 mm rostral from the rostral end of the hypoglossal roots, which projects onto the intermediate area. Cells rich in mitochondria and surrounded by numerous large synaptic boutons have been described for the intermediate area by *Shahar* and *Edery* (1976). According to *Dermietzel* the number of superficially sectioned axons and dendritic profiles increases from the caudal to the rostral part of the ventral medullary surface. In the intermediate (*Shahar* and *Edery* 1976; *Peskow* and *Piatin* 1980) and rostral (*Dermietzel* 1976) area, cell groups are in close contact with capillaries and with the surrounding glia spongiosa. In the rostral area (*Dermietzel* 1976) the maximally enlarged spaces resemble lakes of intercellular cerebral fluid and are typical for this area. The dendrites of the cells form a large number of synaptic connections. The radially arranged axons form a rosette-like grouping around a central dendrite, the latter being characterized by aggregates of mitochondria. *Dermietzel* (1976) suggests that, according to *Gray*'s categorization (1959), the different synapses observed on the dendrites belong to the type I as well as type II, thus probably providing for an inhibitory as well as an excitatory action on the dendrites.

Dermietzel (1976) also described wide spaces surrounding the capillaries of the chemosensitive zones. Horseradish peroxidase (HRP) administered in the subarachnoid region, accumulated in the perivascular space (Fig. 4). Freeze-etch studies revealed a high pinocytotic activity of the capillary

endothelium in contrast to typical cerebral capillaries. In general, *Dermietzel* (1976) finds morphological evidence for a free exchange between the cerebrospinal and the interstitial cerebral compartment, a feature which was required for those ions determining the pH (*Katzmann* et al. 1968; *Pappenheimer* 1967).

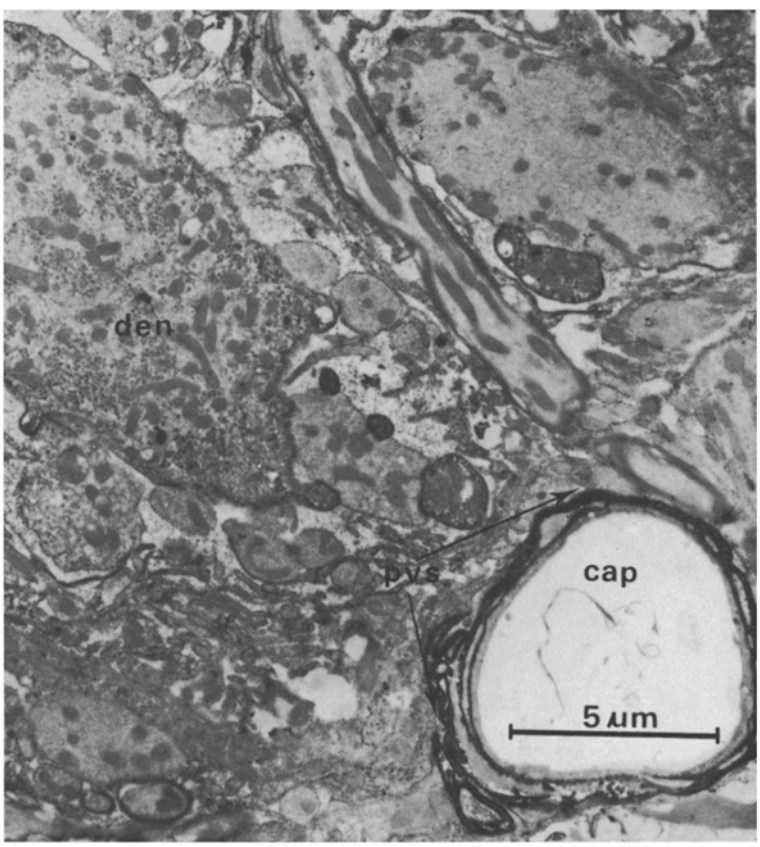


Fig. 4. Electron micrograph from M area. *pvs*, perivascular space filled with subarachnoidally applied horseradish peroxidase. *den*, dendrite, axondendritic synapses. By courtesy of *Rolf Dermietzel*

2.4 Histochemical Tracing of Neurons

By labelling neuronal elements situated within the ventral medullary surface layer with topically applied horseradish peroxidase (HRP, 2%) and incubating for 4 hours *Dermietzel* et al. (1977, 1978) were able to reconstruct the three-dimensional formation of a neuronal complex underneath

the ventral surface, which probably was identical with the nucleus paragigantocellularis (NPG). By shortening the exposure time to 30 min the authors were able to differentiate two classes of neurons. The first group is represented by small-sized cells with a high peroxidase acitivity. Electron microscopical investigations showed that these cells and their dendritic processes show a diffuse HRP labelling of their cytoplasma. The second group of large-sized neurons revealed a less intensive labelling. They showed a vesicular incorporation and storage of the HRP. In addition, within the intermediate area numerous superficially located processes could be observed by HRP labelling, as well as neurons 200 μm below the surface (Fig. 5a).

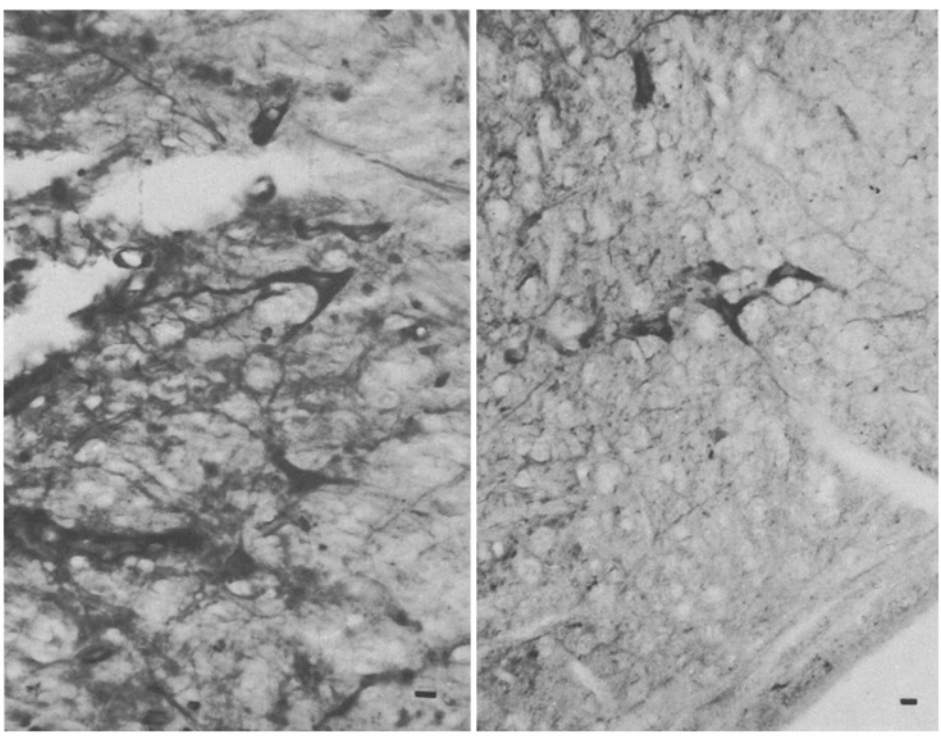


Fig. 5. a Nerve cell 250 μm below the intermediate area, labelled by HRP applied on the ventral medullary surface. *Bar* indicates 10 μm. **b** Acetylcholine esterase in neurons 200 μm below the intermediate area. By courtesy of *Willenberg, Leibstein* and *Dermietzel*

Errington and *Dashwood* (1979) used a 20% solution of HRP and an incubation time of 0.5–5 hours. The HRP was applied bilaterally 8.5–11.5 mm caudal of the Horsley-Clark zero which corresponds to the location of the intermediate area. The authors found numerous labelled cells

and fibers immediately below the surface. 40–400 μm below the surface multipolar neurons could be identified, some of their processes extending toward the ventral surface. In addition fibers were labelled passing dorsally from the ventral surface and originating from two groups of labelled cell bodies. One group was part of the nucleus tractus solitarii, the other group, which was less intensively stained, was situated within the dorsal nucleus of the vagus. Cell bodies were also labelled within the nucleus cochlearis.

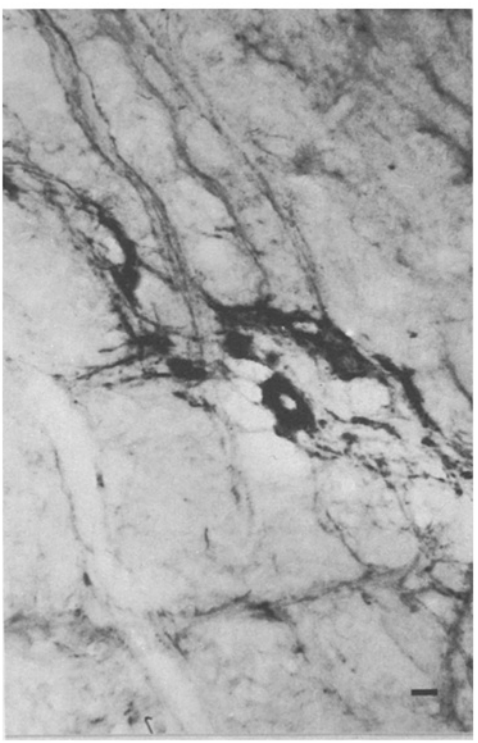


Fig. 6. Acetylcholine esterase in neurons 400 μm below the rostral area. *Bar* indicates 10 μm. By courtesy of *Willenberg, Leibstein* and *Dermietzel*

Some recent data of *Dermietzel* and co-workers (unpublished) were obtained by the histochemical proof of specific acetylcholine esterase within all three areas (Figs. 5b, 6, 7).

2.5 Neurophysiological Tracing

The caudal area: Shimada, Trouth and *Loeschcke* (1969) were the first to succeed in showing that neurons a little beneath the ventral surface of the caudal area (Figs. 1, 2) were able to respond to pH changes induced by superfusing the ventral surface with artificial cerebrospinal fluid of varied

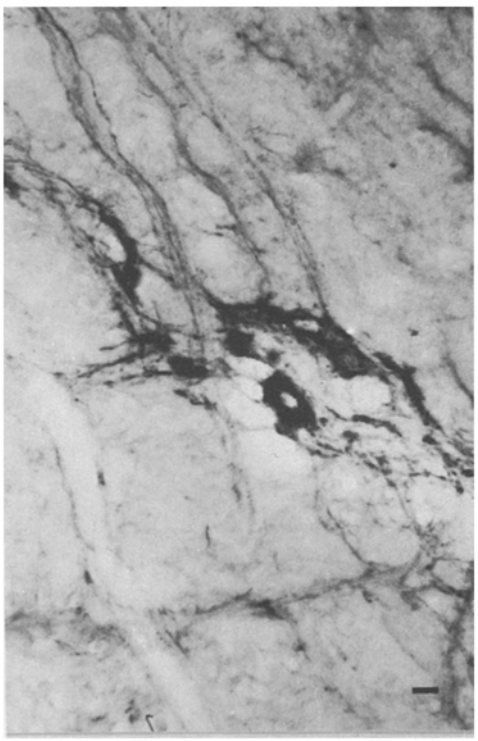


Fig. 7. Acetylcholine esterase in neurons 800 μm below the caudal area. *Bar* indicates 10 μm. By courtesy of *Willenberg, Leibstein* and *Dermietzel*

pH at constant PCO_2. At a CSF pH of 7.8 they measured an impulse frequency of less than 1 per sec. Within 3–4 min after the perfusate was changed to pH 7.0 the authors observed a rapid and thereafter a slower increase of discharge frequency up to an average of 20 per sec. The time course of the unit's response corresponded to that of the phrenic nerve discharge. The authors regarded the discharge pattern of the pH sensitive unit as similar to that of the peripheral chemoreceptors as recorded by *Biscoe* and *Taylor* (1963) and *Eyzaguirre* and *Koyano* (1965). *Prill* (*Prill* et al. 1975; *Prill* 1977) recorded from 47 neurons located immediately below the surface of the caudal area, at the level of the middle of the hypoglossal root, 3.0–4.5 mm lateral to the midline. 16 of these neurons responded to changes in pH induced by intravenous injections of either 0.1 n HCl or 1 M $NaHCO_3$.

Prill distinguished between three types of chemosensitive neurons, fast-responding with a frequency response similar to that which was found by *Shimada* et al. (1969), and regularly firing neurons with a higher frequency. The third type responded in a paradoxical way, showing an increase with bicarbonate and a decrease of discharge activity with HCl. Since *Prill* did not measure pH locally he left it open, whether the paradoxical effect was truly paradoxical or whether it was the consequence of a para-

doxical behaviour of the cerebral extracellular pH following metabolic disturbances in the blood as long as peripheral chemoreceptors are intact (*Loeschcke* and *Sugioka* 1969; *Loeschcke* and *Ahmad* 1980). There were two other remarkable observations on neurons underlying the caudal area. The first was the capability of the neurons to respond in an opposite manner to changes in pH and also to respond to passive movement of the contralateral foreleg. The second was a strong increase in the discharge frequency following HCl injection together with a change of the interspike interval histogram from a unimodal to a trimodal one, which has been described as characteristic for reticular neurons by *Segundo* and *Perkel* (1969) and by *Segundo* et al. (1967). This same neuron turned out to be located 1750 μm below the surface, namely within the lateral reticular nucleus. This is within a depth which is not thought to be the location of the chemosensitive structure (*Trouth* et al. 1973a, b). *Prill*'s study demonstrated that the consideration of the change in frequency following changes in pH, as well as the discharge pattern of a neuron responding to pH changes, may be of help in deciding whether or not a neuron may belong to a specific H^+ ion sensitive apparatus. At this time however, nothing definite can be said regarding the specific sensor element lying within the surface layer. There is evidence, however, that neuronal activity can be increased by H^+ ions within the structure of the caudal area.

The rostral area: Cakar and *Terzioglu* (1976) located neurons which responded to breathing of CO_2 as well as to injections of CO_2 equilibrated Ringer-Locke solutions into the vertebral artery, within 400–1400 μm below the surface and most of them between 600–1000 μm below the surface of the caudal and of the rostral area. The corresponding nuclei were thought to be the nucleus preolivaris and the nucleus trapezoid body (Fig. 2). The authors are in doubt about the hypothetical superficial location of the central chemosensitive apparatus; however, they did not publish any further details regarding frequency characteristics, and also the location of the neurons was given only approximately.

Pokorski (*Pokorski* et al. 1975; *Pokorski* 1976) recorded from neurons located within the first 1 mm layer below the rostral area, most being between 100 and 500 μm beneath the surface (Fig. 2). He found 12 neurons out of 44 to be chemosensitive with an irregular tonic firing pattern, similar to that described for the neurons within the caudal area by *Shimada* et al. (1969) and by *Prill* (1977). *Pokorski* found that neurons with such a firing pattern developed a grouped discharge upon HCl injection. He also saw inversely reacting neurons. He could show, however, by comparison of interspike interval histograms, that in both types of neurons, in those which increased their mean discharge frequency with HCl and those which decreased it, there was an increased number of shorter interspike intervals

after HCl, which would indicate an acceleration in spike generation. The author also described neurons which responded to "touch" of the contralateral limb, which was observed within the caudal area as well. Such neurons within the rostral area showed a tendency for increased activity following intravenous injections of either HCl or $NaHCO_3$. Since local pH was not measured, interpretations can only be speculative. For example *Pokorski* (1976) attributed very short "latencies" of chemosensitive neurons responding to intravenous injections of either HCl or $NaHCO_3$ within 5 sec, to some unknown peripheral chemoreceptors, different from those of the carotid and aortic bodies. However, very rapid changes in pH in the brain extracellular fluid have been measured during intravenous injections of the above mentioned solutions (*Ahmad* 1976), which were accompanied by changes in neuronal discharge (*Schlaefke* and *See* 1980; *Schlaefke* et al. 1979). One also has to consider the possibility that stimulation of "unspecific" nature by the injections, e.g. pain, may increase the synaptic input into the central chemosensitive apparatus (*Schlaefke* et al. 1969; *Spode* 1980). For further discussion of this concept see Sects. 3.3, 3.6 and Figs. 10 and 11, pp. 199, 201).

The intermediate area: Chemical stimulation by either the ventral surface superfusion technique or intravenous injections of HCl or $NaHCO_3$ caused a response of neuronal discharge also underneath the intermediate area (*Schlaefke* 1976a; *Schlaefke* et al. 1974, 1975a, b). So the neurons behave according to the concept of *Lambertsen* et al. (1961), responding to changes in acid-base parameters in the blood as well as in the CSF. As in the caudal area non-chemosensitive neurons responding to "touch" of a contralateral limb were observed. Unlike the studies on the rostral and the caudal area, no "paradoxical" responses to pH changes were recorded. In more recent experiments the authors measured the pH on the ventral medullary surface simultaneously (which, with the method of a floating flat surface pH electrode gives a pH reading between CSF and ECF pH) (*Schlaefke* 1967b; *Schlaefke* et al. 1977a, b, 1978, 1979, *Schlaefke* and *See* 1978, 1979, 1980; *See* et al. 1977, 1978; *See* and *Schlaefke* 1978, 1980). In these experiments it could be shown in a few cases that intravenous injections of HCl may cause an increase in pH on the ventral medullary surface, which was accompanied by a decrease in the firing frequency of single units. Using the same technique, further recordings revealed that only slight shifts of pH to the acid side were necessary in order to obtain a remarkable increase in the firing frequency of units. The alkaline or slight acid shifts of the surface pH following HCl injections are in accordance with observations of *Robin* et al. (1958), *Loeschcke* and *Sugioka* (1969), *Fencl* et al. (1966, 1971), *Pappenheimer* (1967), and *Mitchell* et al. (1965), who found opposite or only small pH changes in

the cerebrospinal fluid following metabolic disturbances. This is also expressed in a mathematical study of *Middendorf* and *Loeschcke,* demonstrating that in the transient phase of nonrespiratory acidosis the peripheral chemoreceptors mediate the fast respiratory feed back (*Middendorf* and *Loeschcke* 1976a, b, 1978) and may be the cause of the alakline shift in the extracellular pH of the brain. In connection herewith and with the true pH change following HCl injections, the "paradoxical" reaction of neurons recorded by *Pokorski* (1976) whithin the rostral and by *Prill* (1977) within the caudal area must be regarded with caution, because the pH was not measured at the same time. There are however, criteria which would not reject the possibility of paradoxical pH responses within the chemosensitive zones (see Sect. 3.2, 33.).

Neurons of the intermediate area, the discharge frequency of which increased following a fall in the surface pH and decreased following a rise in pH, have been picked up between the surface and 1600 μm below, mostly at 200–300 μm below the surface. Of a total of 92 units tested for their reaction to pH changes, 51 behaved in the described manner (Table 1).

Table 1. Number of units recorded from the ventral layer underneath the caudal (L), intermediate (S), and rostral (M) areas in the cat. Percentage is given in addition of H^+ ion modulated neurons from the totally recorded number of neurons in the corresponding field, as well as for the non-responsive neurons in relation to the total number. The data refer to *Pokorski* (1976), *Prill* (1977) *and Schlaefke* (1976), *Schlaefke* et al. (1975, 1978).

	Caudal area (L)	Intermediate area (S)	Rostral area (M)
Total no. of units	49	92	44
H^+ -responding	23 (46.93%)	51 (55.43%)	9 (20.45%)
Nonresponsive	26 (12 touch) (53.06%)	41 (14 touch) (44.56%)	35 (12 touch) 79.54%)

The location was determined either by iontophoretic application of procion yellow through the recording electrode, or by histological verification of the position of the electrode tip. Figs. 8 and 9 give examples of cells located with both techniques. Both neurons have been identified as "chemosensitive" by superfusion of the ventral surface with artificial cerebrospinal fluid of varied pH according to the solutions used by *Loeschcke* et al. (1958). The figures also represent the two types of neurons which have been described by *Petrovický* (1968) and by *Dermietzel* et al. (1978), namely the larger one (Fig. 8) which belongs to the ventral part of the NPG, its topographical location being 8.24 mm caudal to the foramen caecum,

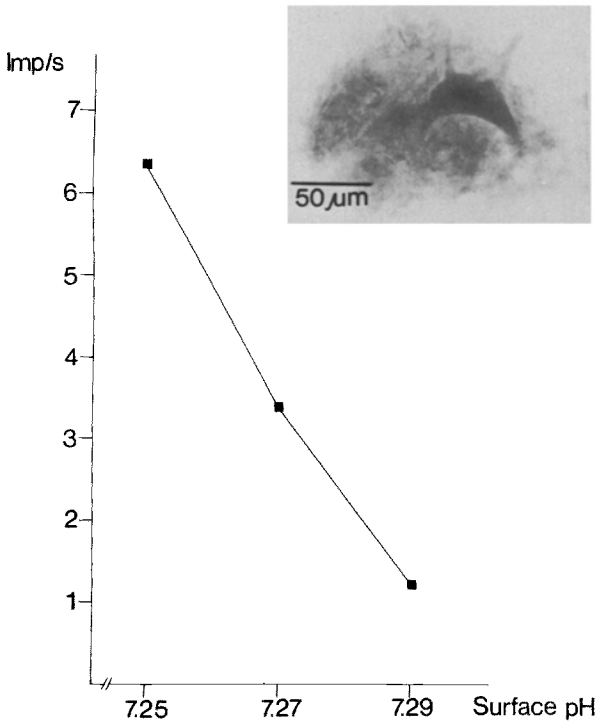
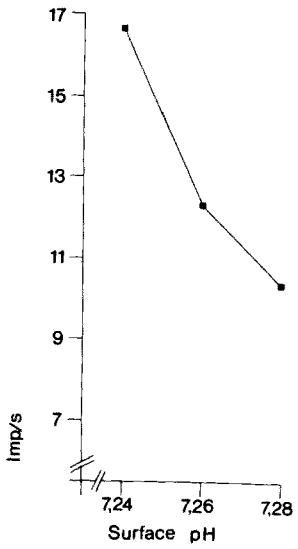


Fig. 8. Procion yellow stained neuron within the ventral pole of the NPG, 1600 µm below the intermediate area. Impulse frequency response per second to changes of pH on the surface, mean values of steady states. Compare Fig. 10

2.80 mm lateral to the midline, 1600 µm below the surface. The group of the small-sized type (Fig. 9) was found at the tip of the electrode 200 µm below the surface and is typical for the intermediate area (*Schlaefke* et al. 1979a). The coordinates were 6.7 mm caudal to the foramen caecum and 3.6 mm lateral to the midline (Figs. 1, 2). The mean values for impulse frequency per second refer to steady state conditions. The units recorded from the intermediate area could be classified as fiber recordings and recordings from close to the soma, using standard criteria given by *Porter* (1963), *Nakayama* and *Baumgarten* (1964), *Bishop* (1964) and *Hukuhara* jr. (personal communication). It turned out that neurons the discharge frequency of which followed the pH variations were picked up close to the soma, whereas the nonchemosensitive units were from fiber recordings. Regarding the latencies between variations of the pH, measured on the ventral medullary surface, and the neuronal responses, in the cases of superfusion, the neuronal discharge changed simultaneously, with the pH electrode measurements having a response time of about one second. In some cases the chemosensitive neuron responded faster than the pH elec-



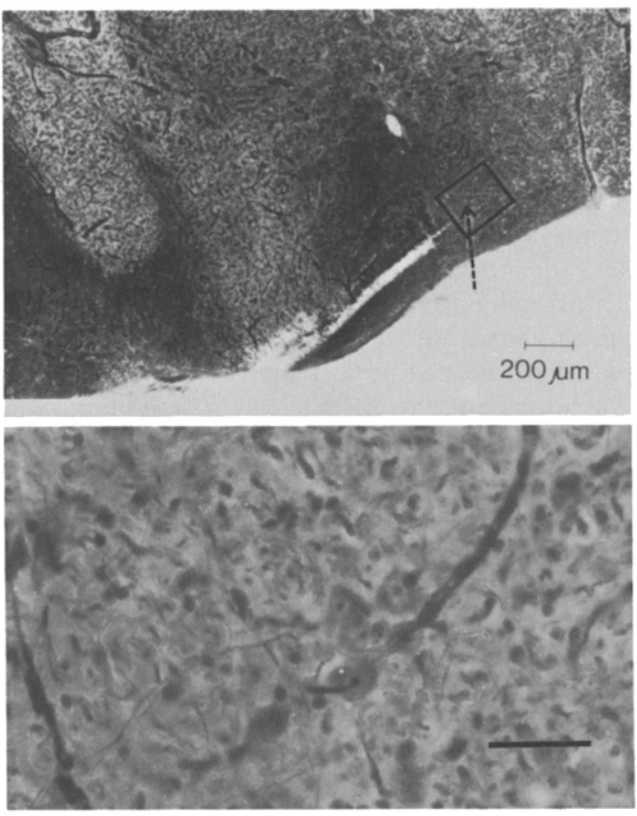


Fig. 9. *Top:* Mean values of impulse frequency per second during steady state of various pH measured on the ventral medullary surface. The single unit was recorded as indicated by the elctrode channel 200 µm below the surface of the intermediate area *(middle)*. Typical in this region small-sized cells as here at the tip of the electrode *(bottom)*. 50 µm indicated by the bar

trode, especially when pH was varied by intravenous injections. This observation however, may be due to methodical conditions and must not necessarily indicate any physiological significance. Latencies obtained by indirect criteria will be presented in connection with the discussion of the mechanism (see Sect. 3.3).

pH sensitive neurons could be recorded also from brain slices of the rat and the cat (*Fukuda* and *Honda* 1975; *Fukuda* and *Loeschcke* 1977; *Fukuda* et al. 1978). From the rat ventral medullary surface within an area corresponding to the intermediate area of the cat, namely from the rostromedial part of the hypoglossal root to the trapezoid body (compare Fig. 1), *Fukuda* and *Loeschcke* (1977) recorded spontaneous unit activity. The authors compared this spontaneous unit activity with spontaneous activity recorded from slices taken from the floor of the fourth ventricle. The recording site from the ventral slice was 20–100 μm below the ventral surface, the one from the dorsal slice between 20 and 200 μm below the dorsal surface. The firing pattern was either random or regular. The mean frequency of firing in the ventral slice was 4.7 impulses per second and in the dorsal slice 13.0 impulses per second when the pH of the bathing solution was 7.4. In the tissue slice from the ventral side, the authors found 31 neurons out of a total of 56 neurons which increased their discharge frequency when the pH was lowered, however, such a correlation between pH and neuronal discharge was limited to a range of pH 7.6–7.2. Beyond this range, the response to pH was reversed, which means that a further shift to the acid side caused an inhibition. Within the given range the excited neurons increased their firing frequency by 0.99 impulses per second per 0.1 pH reduction. 23 neurons inhibited by pH reduction fired with 5.5 impulses per second at a pH of 7.4 and their rate was decreased by 1.22 impulses per second per 0.1 pH reduction. Two neurons were found which did not respond to pH changes. The units located within the dorsal slice fired with a higher frequency and reacted inversely to pH changes in 57% of the cases. Only 4 neurons out of 30 were excited by H^+ ions.

Fukuda et al. (1978) found some evidence that glia cells may be involved in the central chemosensitve mechanism. Again they compared the reaction of structures from ventral with those from dorsal slices. The authors measured a depolarization of silent cells induced by acid solutions, without significant changes in membrane resistance, in 44% of the cells from ventral slices. In the dorsal tissue they found 34% hyperpolarized under the same condition, whereas only a few were slightly depolarized. From both experimental series the authors concluded that the density of neurons and silent cells exhibiting an excitatory reaction is especially high within the ventral medullary surface layer.

Lipscomb and *Boyarski* (1972) were unable to record from neurons within the ventral medullary surface layer responding to changes in pH on

the surface. They postulated the nonexistence of any acid-base dependent activity in ventral medullary structures. Their interpretation was recently supported by a study by *Cragg* et al. (1977). They concluded that due to the blood supply of the whole medulla, including the respiratory neurons of the solitary tract and the nucleus retroambigualis, stemming from the ventral side, the respiratory responses to chemical stimuli from the surface would be the consequence of direct action on deeper structures. The difficulty in avoiding experimental errors due to changes in blood supply has been overcome by *Fukuda*'s tissue slice techniques. Furthermore the slices did not contain the ventral respiratory nuclei, thus ruling out an effect on or from the respiratory "centers".

3 The Mechanism

3.1 Respiratory Effects of Ions, Drugs, and Putative Transmitters

Berndt et al. (1969) investigated the dependence of the respiratory response to pH changes on different concentrations of Ca^{++} and Mg^{++} ions. Since the same respiratory effects were obtained when Ca^{++} ions were partly replaced by Mg^{++} ions, the authors believed more in an unspecific neuronal response to these ions, than in an effect from a cholinergic synaptic system. The latter would require a counteraction between the two types of ions if their action was comparable to that at the neuromuscular junction (*Katz* and *Miledi* 1963; *Miledi* and *Slater* 1966; *Elmqvist* and *Feldman* 1965; *Hubbard* et al. 1968).

The effect of increasing respiration by local application of acetylcholine, which had been observed by *Mitchell* et al. (1963a, b) was recently further elaborated by *Dev* and *Loeschcke* (1979a, b) and *Loeschcke* (1978, 1979, 1980). Local application of 10^{-4} g · ml^{-1} acetylcholine on the rostral and caudal chemosensitive field either by superfusion according to the method used by *Schlaefke* et al. (1970), or by using plexiglass rings as described by *Guertzenstein* (1973), caused an increase in ventilation, mainly due to an increase in tidal volume. When atropine at a concentration of 10^{-3} g · ml^{-1} was applied 10 min before the acetylcholine was given, the hyperventilatory effect was diminished or abolished. CO_2 response curves were flattened and shifted to higher CO_2 values when atropine was allowed to act on the rostral and caudal chemosensitive fields. 10^{-5} g · ml^{-1} nicotine affected respiration like acetylcholine and was inhibited by intravenously injected hexamethonium (10^{-3} g · ml^{-1}). Physostigmine 10^{-4} g · ml^{-1} in the same areas enhanced resting ventilation but did not affect the slope of the respiratory response curve to CO_2 inhalation. The authors also observed that the hyperventilation caused by nico-

tine was remarkably inhibited by bilateral application of 10^{-2} g · ml^{-1} procaine on the intermediate area. This was considered to be further support for the hypothetical convergence of chemosensitive elements from the rostral and caudal field within the intermediate area (*Loeschcke* et al. 1970). Since there was a similarity between the site from which H^+ ions elicited positive respiratory responses and the site from which acetylcholine acted, including the inhibition by atropine, and since a clear connection between atropine and the respiratory response to CO_2 could be found, the authors concluded that the H^+ ion sensitive reactions were dependent upon a cholinergic transmission in the surface layer.

3.2 Neuronal Effects of Ions, Drugs, and Transmitter Substances

The excitatory effect of acetylcholine and its inhibition by atropine was also shown for ventral medullary neurons in spontaneously breathing or artificially ventilated cats, when the substances were applied locally to the ventral medullary surface by superfusion (*Schlaefke* et al. 1977, 1978; *Schlaefke* and *See* 1978, 1980). *Folgering* et al. (1979) gave a report on the phrenic nerve activation by intravenous application, or by injection into the vertebral artery of an acetylcholine releasing drug (4-aminopyridine, 4-AP), and suggested its possible effect on central chemosensitive structures. This was examined by *See* et al. (1978) by local application of this substances to the ventral medullary surface. Indeed the authors observed a strong increase in ventral neuronal and phrenic nerve activity which was inhibited by atropine. However, it was still not possible to show that the effect of either acetylcholine or 4-AP acted by excitation of the H^+ ion sensitive mechanism alone. The authors observed that neurons, which did not respond to CO_2 or H^+ ions but which are probably part of a descending pathway related to the sympathetic system, were excited also (*Schlaefke* and *See* 1978, 1980a, b; *Schlaefke* et al. 1977; *See* et al. 1978).

In addition, reticular neurons, characterized by a change of their firing pattern from an irregular one to a grouped one when excited, did respond to both substances. Since acetylcholine seems to act also in the nucleus retroambigualis in a strong inhibitory way, cholinergic mechanisms may be involved in various systems within the ventral portion of the medulla oblongata. As a result local superfusion of the medullary surface can only serve as a method for pioneer studies and should be followed by more precise application techniques (*Jordan* and *Spyer* 1979; *Kirsten* et al. 1978). The involvement of a cholinergic mechanism in the role played by the ventral medullary surface for the cardiovascular system will be discussed (see Sect. 3.5). The critical argùments regarding the experimental difficulties encountered when working on the warmblooded animal, with the

intention of analyzing basic mechanisms situated within a small piece of the brain, were again overcome by *Fukuda* et al. (1978). *Fukuda* et al. incubated 400–600 μm thick slices taken from the ventral medullary surface of the rat, and picked up action potentials, observing them for periods of 60–360 min. Neurons were studied between the rostral part of the hypoglossal root and the trapezoid body within 20–100 μm from the ventral surface. The authors found that H^+ ion sensitive neurons, responding with excitation, were also always excited by acetylcholine. Cholinergic blocking agents like atropine, hexamethonium and mecamylamine depressed the H^+ ion elicited excitation of neurons. Eserine acted synergistically with H^+ ions and strengthened the effect of pH, and also the excitatory effect of acetylcholine was prolonged and augmented by H^+ ions. In most cases effects of noradrenaline and serotonine were opposite to those elicited by H^+ ions. There was another surprising observation made regarding silent cells, which to a high percentage had been depolarized by H^+ ions in slices from the ventral surface in contrast to those from the floor of the fourth ventricle. Silent cells were depolarized by acetylcholine and lost their ability to depolarize as a response to an increase in the H^+ ion concentration when, following the method described by *Del Castillo* and *Engbeak* (1954), *Hubbard* et al. (1968), *Hutter* and *Kostial* (1954), and *Richard* and *Sercombe* (1970), the transmitter release in the slice was depressed by a simultaneous reduction in the Ca^{++} ion concentration and an increase in the Mg^{++} ion concentration. The authors concluded at first, that on the base of previous investigations of glia cells (*Kelly* and *Van Essen* 1974; *Krnjević* and *Schwartz* 1967; *Ranson* and *Goldring* 1973; *Somjen* 1975), the potential shifts of silent cells were recorded from glia cells.

In contrast to the findings of *Carpenter* et al. (1974) who did not find any effect of CO_2 on the cortical neuroglia, *Fukuda* et al. (1978) directed the attention to the existence of glia cells which depolarize in the presence of H^+ ions within the dorsal side of the medulla, although there is a strikingly high density of such a cell type within the ventral surface layer. The absence of any significant change in the membrane resistance during slow depolarization by H^+ ions led the authors to reject the possibility that the silent cells may constitute the true receptor correlate or deliver the receptor potential. They suggested therefore that the general characteristics of the neuroglia as had been described by *Krnjević* (1974), *Kuffler* and *Nichols* (1966, 1976), *Grossman* and *Hampton* (1968), *Karahashi* and *Goldring* (1966), and *Ransom* and *Goldring* (1973b), namely its protective role against disturbances of the environment of the neurons by ions and transmitters, may be involved specifically in the central chemosensitive mechanism, on behalf of the dense distribution of the cell type which can be depolarized by acetylcholine and H^+ ions in the ventral surface layer. Since low Ca^{++} and high Mg^{++} ion concentrations had depressed not only

the depolarizing effect of H^+ ions on silent cells but also the H^+ ion induced stimulation of neuronal activity, the authors concluded that the effect of H^+ ions on the ventral medullary structures is related to the release of acetylcholine in the synaptic regions there. These assumptions are supported by the presence of a nonspecific cholinesterase on glial membranes (*Brightman* and *Albers* 1959; *Koelle* 1954).

3.3 Central Chemosensitivity: Unspecific–Specific

Dev and *Loeschcke* (1979a) found a very remarkable effect on resting ventilation when they applied either physostigmine or atropine locally to the chemosensitive areas. This led them to the assumption that the respiratory drive under resting conditions is continuously under the tonic influence of a cholinergic input. The authors, on the base of their data, those of *Fukuda* and *Loeschcke* (1979) and *Fukuda* et al. (1978), as well as in the light of what is known about the action of H^+ ions on the cholinergic mechanism (*Augustinsson* 1948; *MacIntosh* and *Perry* 1950; *Lüllmann* and *Peters* 1967; *Cohen* and *Osterbaan* 1963; *Hubbard* et al. 1968; *Eyzaguirre* and *Zapata* 1968), concluded that an increase in the H^+ ion concentration in the extracellular fluid of the brain might contribute to an increase in sensitivity of cholinoceptive neurons to acetylcholine, and/or to an augmented release of acetylcholine from presynaptic terminals due to a greater ionization of calcium, and/or to an inhibition of acetylcholine esterase activity. The possibility of the latter effect being present is strongly supported by a significant increase in the content of acetylcholine, and a decrease in acetylcholine esterase activity in the medulla oblongata of dogs during hypercapnia or acidosis (*Metz* 1962, 1966).

An involvement of cholinergic substances in the respiratory system has been discussed for a long time (*Comroe* and *Schmidt* 1938; *Dautrebande* and *Marechal* 1933; *Dikshit* 1934; *Eyzaguirre* and *Zapata* 1968; *Gesell* 1940; *Gesell* and *Frey* 1950; *Gesell* and *Hansen* 1945; *Gesell* et al. 1942; *Heymans* et al. 1936; *Landgren* et al. 1952; *Liljestrand* and *Zotterman* 1954; *Mitchell* et al. 1963a, b; *Philipot* 1937; *Schweitzer* and *Wright* 1938; *Trzebski* et al. 1980; *Winder* 1937; *Winterstein* 1955) and also in connection with the peripheral chemoreceptor mechanism. *Salmoiraghi* and *Steiner* (1963) stated that most of the respiratory neurons in the medulla oblongata could not be excited by acetylcholine; however, the cholinergic transmitter system is widely engaged in the brain and spinal cord (*Hoover* et al. 1978; *Kirsten* et al. 1978; *McLennan* and *Hicks* 1978) and even in the medulla oblongata as has been described recently by *Jordan* and *Spyer* (1979) for an expiratory population within the nucleus retroambigualis. This again raises the question why the specialization of central chemo-

sensitivity as a cholinergic apparatus is so much restricted to structures within the ventral medullary surface layer. This question is credited by further observations, e.g. that various unspecific stimuli with their effect on respiration seem to be dependent upon ventral structures (*Schlaefke* et al. 1969; *Schlaefke* 1973; *Spode* and *Schlaefke* 1975; *Spode* 1980). *Gesell* (1940), in suggesting the involvement of a cholinergic system in the neuronal mechanism of respiration, advanced the opinion that CO_2 would merely act on the interneurons of the respiratory reflex and coordinating centers, at the same time allowing for an activation by afferent impulses. In addition, *Gesell* believed that CO_2 would act by impulse discharge modulation which is evidently the hypothesis of *Loeschcke* and co-workers (*Dev* and *Loeschcke* 1979a, b; *Fukuda* and *Loeschcke* 1979). Some recent data (*Schlaefke* and *See* 1978; *Schlaefke* et al. 1979c; *See* and *Schlaefke* 1980) may be interpreted partly in the sense of *Gesell*'s hypothesis. The authors identified superficially located chemosensitive neurons within the ventral part of the NPG by local changes in pH on the ventral medullary surface and electrically stimulated the ipsilateral tibial nerve. The same H^+ ion sensitive neurons (Figs. 8, 9) changed their discharge frequency in response to stimulation in the same course as the phrenic nerve discharge.

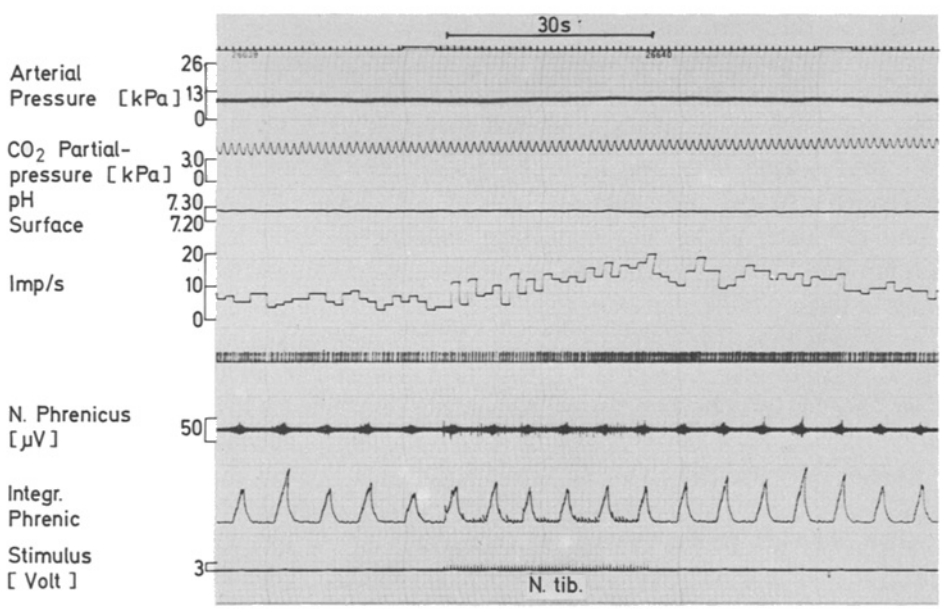


Fig. 10. Same experiment and neuron as Fig. 8. The cut central end of the ipsilateral tibial nerve was electrically stimulated. Stimulation caused increase of neuronal discharge and phrenic nerve activity. From top to bottom: Arterial pressure, CO_2 partial pressure (endtidal), pH measured on the surface, medullary neuron impulses per sec, phrenic nerve discharge, integrated phrenic nerve activity, electrical stimulus

Latencies from the onset of a single electrical stimulus were 22 ms for the ventral medullary neurons, the response of the phrenic nerve activity following after another 22 ms (Fig. 10, same neuron as Fig. 8). From these experiments *Schlaefke* et al. (1979) and *See* and *Schlaefke* (1980) assumed a polysynaptic connection between the chemosensitive neurons within the ventral surface layer and the phrenic motoneurons. In addition the authors interpreted their data in connection with previous findings obtained by bilateral cold block of the intermediate area in peripherally chemodenervated cats. When this is performed, any increase in ventilation induced by mechanical stimulation of the cornea, or by electrical stimulation of the femoral nerve, or by electrical stimulation of cut ventral roots (the latter producing hindlimb movements), is abolished, and apnea is the consequence of cooling even during continuous stimulation (*Schlaefke* 1973; *Schlaefke* et al. 1969; *Spode* 1980; *Spode* and *Schlaefke* 1975). If however, instead of the "nonspecific" afferents, the central cut end of the sinus nerve was electrically stimulated, respiration continued during cold block (*Loeschcke* et al. 1979; *Schlaefke* et al. 1969). The intactness of the function of peripheral chemoreceptors during bilateral cold block or coagulation of the intermediate area has been shown already by *Schlaefke* and *Loeschcke* (1967), *Schlaefke* (1972, 1973, 1976), *Schlaefke* et al. (1970, 1974, 1979a, b) and was recently confirmed by *Cherniak* et al. (1978, 1979a, b). From all these studies it seems very likely that the respiratory centers are principally intact during cold block of the intermediate area. However, the influence on ventilation of nonchemosensitive afferents is dependent upon the respiratory centers being sufficiently activated by the chemosensitive afferent system. This may include synaptic input from various "unspecific" afferent systems, and can be abolished with local cold block of the intermediate area, under the condition that peripheral chemoreceptors are eliminated. These "unspecific" systems may include afferent signals originating during muscular exercise.

In case that this concept will hold true, previous arguments of *Bisgard* et al. (1978) and *Kao* et al. (1965), that due to CSF alkalosis during exercise the central chemosensitive apparatus may not be involved in exercise hyperpnoea, have to be reexamined. The afferent activation by muscular exercise may use the chemosensitive structures, e.g. by changing its threshold, a concept which was supported by *Guenard* et al. (1976). In a recent paper, *Kao* et al. (1979) support the idea of an involvement of C-fibre afferents in the hyperpnoea of muscular exercise. The authors suggest the tractus spinothalamicus as a possible pathway. This is in accordance with the concept of the spinothalamic tract being a likely candidate for the formation of synaptic connections within the ventral medullary surface layer (Fig. 11).

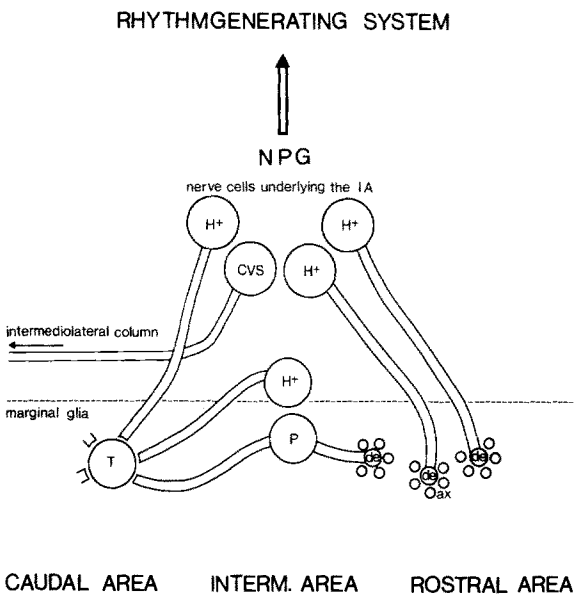


Fig. 11. H^+ ion sensitive cholinergic synapses formed by dendrites of nerve cells underlying the intermediate area (including the NPG) with superficially located neurons within the caudal and rostral area, and with afferent and efferent pathways. The NPG also contains nerve cells (*CVS*, cardiovascular system) which project to the intermediolateral column and are supposed to inhibit sympathetic activity (*Amendt* 1978; *Schlaefke* and *See* 1980). *T*, nerve cells described by *Trouth* et al. (1973b). *P*, nerve cells described by *Petrovický* (1968). Dendrites *(de)* are surrounded by axons *(ax)*, a typical formation within the rostral area (*Dermietzel* 1976)

The neurogenic component in the respiratory drive during muscular exercise is a generally accepted concept (*Asmussen* and *Nielsen* 1948; *Comroe* and *Schmidt* 1943; *Comroe* 1944, 1974; *Cunningham* and *Lloyd* 1963; *Cunningham* 1963; *Dejours* 1959, 1963, 1964; *Dejours* et al. 1955; *Flandrois* et al. 1974a; *Kao* 1965; *Krogh* and *Lindhard* 1913; *McCloskey* and *Mitchell* 1972; *Rodgers* 1968; *Whipp* 1978). In the light of the present view on central chemosensitivity and its mechanism, this neurogenic component may at least partly arise from possible synaptic connections within the superficial structure, in which acetylcholine and H^+ ions may act synergistically.

These observations on the behaviour of ventral medullary structures in connection with "nonspecific" or nonfeedback afferents lead to an understanding of the central chemosensitive apparatus in the meaning portrayed by *Gesell* (1940), namely that afferent impulse transmission is modulated by H^+ ions. However, the recent observations differ from the opinion of *Gesell* in that this chemosensitive system is not within the relais chain of the respiratory neural system. This argument has already been expressed

by *v. Euler* and *Söderberg* (1952a, b) and was substantiated by the verification of the intactness of various other respiratory drives in the case of cold block or coagulation of the superficial structure of the intermediate area, e.g. as the peripheral chemoreceptors (*Cherniak* et al. 1979; *Schlaefke* et al. 1969, 1979b; *See* 1973), the vagal reflexes (*Schlaefke* and *Loeschcke* 1967; *Cherniak* et al. 1979a; *Purves* and *Schlaefke,* unpublished; *Schlaefke* et al. 1979b), and hypothalamic drive, as produced during hyperthermia or by local hypothalamic warming (*See* 1976a, b).

The specificity of central respiratory response to CO_2 was also questioned on the grounds of previous experiments of *Dell* and *Bonvallet* (1954) who stated that CO_2 would act directly on the ascending reticular activating system in contrast to hypoxia, the latter causing arousal mediated by the peripheral chemoreceptors. In the event of their elimination only the central depressant hypoxic effect would remain. The observations of *Dell* and *Bonvallet* have been supported by *Hugelin* and *Cohen* (1963) who considered the respiratory activation to be a component of the generalized activating response. *Cozine* and *Ngai* (1967) believed the location of the central respiratory sensor of CO_2 on the ventral medullary surface area to be an artefact of anesthesia inhibiting structures sensitive to CO_2 which should be elsewhere located. In the light of these arguments, *Schlaefke* et al. (1969) performed various studies on decerebrate unanesthetized or awake cats in which the respiratory response to inhalation of CO_2 was tested when the intermediate area was inhibited by cold block or when it was coagulated bilaterally. With such manipulations the respiratory response to CO_2 would disappear completely, while in the case of additional peripheral chemodenervation, apnea ensued. In contrast to the loss of a stimulating action on respiration, CO_2 inhalation during cold block was accompanied by an arousal in the cortical EEG measured on the anterior sigmoid gyrus and the medial ectosylvian gyrus although the apnea persisted (*Hukuhara* et al. 1976). Electrical stimulation of the femoral nerve, producing an increase in ventilation together with an arousal reaction, lost its augmenting effect on respiration, apnea occurring during cold block, the cortical arousal however following stimulation (*Hukuhara* et al. 1976). These experiments demonstrated that the ability of the respiratory system to respond to CO_2 or unspecific stimuli after peripheral chemodenervation, must be dependent on the intactness of the ventral medullary surface structure. Any other CO_2 sensitive structures, or the ascending activating system, or structures responsible for the EEG arousal, exhibit no effect on respiration in the absence of peripheral chemoreceptors and the structure within the ventral medullary surface layer.

The search for actions of CO_2 on the central nervous system in a variety of studies revealed that its effect is not a generally activating one. Low concentrations of CO_2 caused desynchronisation of EEG patterns in the

cortex and thalamic areas and slow wave activity in the medulla (*Ivanov* 1963). Inhalation of 10% CO_2 decreased the responsiveness of the specific sensory projection areas to auditory and visual stimuli but increased the reactivity of the hypothalamic cortical projections (*Gellhorn* 1953). Inhalation of 6–20% CO_2 was followed by shifts in the DC potential of the brain. With high CO_2 concentrations the electrocorticographic activity was found reduced (*Woody* et al. 1970). The excitability of some cortical cells to L-glutamate was increased by CO_2, whereas in other cases it was reduced. Increases in membrane potential were found to be due to an increase in CO_2 by *Krnjević* et al. (1965). In general the excitability of neurons in the intact mammalian CNS is decreased by CO_2. Even at rather low concentrations (e.g. 5%), it may act as an anesthetic for various reflexes (*Poulsen* 1952; *Jurna* and *Söderberg* 1963) and 5%–10% CO_2 may act as an analgesic in man (*Dundee* et al. 1962). A variety of investigators could demonstrate the predominantly depressive action of CO_2 on spinal cord neurons (*Brooks* and *Eccles* 1947; *Kirsten* 1951), while in other experiments no effects at all were seen (*Krnjević* et al. 1965). *Gill* and *Kuno* (1963) found that the responsiveness of phrenic motor neurons was dramatically decreased by 6% CO_2. *Washizu* (1960) considered a hyperpolarizing effect of 15% CO_2 on the isolated toad spinal cord to be primarily a pH effect. *Speckmann* and *Caspers* (1969a, b) and *Speckmann* et al. (1970) saw 90% of spinal neurons hyperpolarized and 10% depolarized by an increase of P_ACO_2. Cortical DC reactions were comparable with the membrane potential shifts in those spinal neurons which were inhibited by CO_2.

The mechanisms of CO_2 effects on the nervous function have been comparatively studied in the giant cells of Aplysia which has been reviewed by *Carpenter* et al. (1974), *Chalazonitis* (1974), and *Brown* (1974). *Brown* and *Berman* (1970) found that H^+ ions could mediate the effect of CO_2 by increasing the Cl^- ion conductivity of the membrane. According to differences in the internal Cl^- ion activity of the neurons, both could occur, excitation by CO_2 in cases of a Cl^- ion potential less than the resting potential, and inhibition when the Cl^- ion potential was more negative than the membrane potential. *Chalazonitis* (1974) and *Carpenter* et al. (1974) hesitated to ascribe all CO_2 effects merely to the action of H^+ ions. The proof for this hypothesis is, however, still missing.

Regardless of whether central chemosensitivity is constituted by a specific or an unspecific action of H^+ ions or whether a cholinergic mechanism or direct action on the membrane or both may be involved, the described and discussed possibilities of an excitatory action of H^+ ions on the respiratory system, remain ineffective as long as the structure of the intermediate area is eliminated, in the absence of peripheral chemoreceptors.

3.4 The "Sense-Less" Centers

Our knowledge of the respiratory rhythm generator changed from the beginning of respiratory physiology along with the methodological improvements by which respiratory neurons became more easily detectable (*Cohen* 1979; *Mitchell* 1977; *Mitchell* and *Berger* 1975; *Wyss* 1964). Still, we seem to be far from an unanimous opinion (*v. Euler* und *Trippenbach* 1976; *Hugelin* 1977; *Koepchen* 1973, 1976; *Koepchen* et al. 1977). Regarding experimental conditions, two extremes of a distribution of active respiratory neuron populations may be drawn, by comparing the results of *Salmoiraghi* and *Burns* (1960) from the study of the completely isolated brainstem of the cat with results from the study of the intact brainstem of the cat when unanesthetized and paralyzed, as obtained by *Hukuhara* (1973) and *Hukuhara* et al. (1969, 1979). The first set of workers found a fairly reduced activity in their preparation, while the latter found a wide representation of respiratory related units. *Hukuhara* and his associates considered themselves able to recognize primary respiratory neurons or candidates for the rhythm generating population by injection of pentobarbital. The primary type shows stability in phasic discharge and is rather unaffected by anesthesia in contrast to the other type.

The classic fight of physiologists believing in the reflexogenic driven respiratory centers (*Hall* 1837; *Volkmann* 1841; *Vierordt* 1944; *Schiff* 1858/59; *Rach* 1963, *Wittich* 1866) with those believing in a primary central cause of respiration (*Müller* 1837) was neither decided beyond doubt by *Salmoiraghi* and *Burns* (1960) nor by further studies of *Hukuhara* (1974, 1976) using a section technique in combination with single unit recording. As long as ventral medullary surface areas had been left intact, afferent drive was produced by acidity.

Recently, *Cohen* (1979), presented in a review a picture of the distribution of respiratory neurons and their position in the central organization of respiratory neuron populations. Areas of high density in respiratory related neurons form a ventral respiratory group, located in the ventrolateral region of the brainstem, and extending from the level of the first cervical roots to the level of the middle pons. This group includes the nucleus ambiguus and the nucleus retroambigualis. Inspiratory and expiratory neurons are intermingled in this group, but the I-type predominates. A dorsal respiratory group, mostly consisting of inspiratory neurons, is situated 1.5–2.5 mm ventrally from the dorsal medullary surface, and extends from the obex to 2 mm rostrally and 2.0–2.8 mm lateral to the midline. It includes the ventrolateral nucleus of the solitary tract. Further areas of high density in respiratory activity are located within the pons, and include the nucleus parabrachialis, the Kölliker Fuse nucleus, and areas near several cranial motor nuclei such as the nucleus ambiguus and the retrofascial

nucleus, the hypoglossal, facial, and trigeminal nuclei. According to their change in discharge pattern following a change in CO_2 from normocapnia to hypocapnia, *Cohen* (1968) classified three types of responses: Type I, comprising almost all inspiratory and inspiratory/expiratory neurons, and two thirds of the expiratory neurons, reduced its discharge frequency in all portions of the respiratory cycle or ceased firing entirely. Type II, comprising about half of the medullary expiratory neurons, most expiratory/inspiratory neurons and most expiratory motoneurons, increased its frequency in the low-frequency portion of the respiratory cycle and could become completely tonic. Type III, comprising neurons with tonic respiratory modulated activity answered with a reduction of discharge in the high-frequency portion of the respiratory cycle, with relatively little change in the low-frequency portion. The neurons could lose their respiratory modulation but continued to fire tonically. *Cohen* concluded for type III, which was observed in 70% of tonically firing inspiratory- and expiratory modulated neurons, typical for the pons, that the respiratory modulated portion may arise from other CO_2 sensitive respiratory neurons, and that the tonic portion may stem from sources outside the primary respiratory rhythm generating system. Nothing, however, could be said about the origin of the CO_2 induced changes in discharge.

A more recent major contribution to the problem of a self-exciting rhythm generator (*Salmoiraghi* and *v. Baumgarten* 1961) and to the question of whether CO_2 would act on its neurons directly or through some synaptic input (*Pappenheimer* et al. 1965; *Walker* and *Brown* 1970) has been made by *Mitchell* and *Herbert* (1974a, b) using intracellular recording techniques. The authors recorded from the ventral respiratory group, including the nucleus retroambigualis and confined their findings to the inspiratory and expiratory populations. CO_2 induced two kinds of reactions: 1. it hyperpolarized inspiratory neurons during expiration and 2. it caused an increase in the rhythmic slow depolarization potentials proportional to the level of CO_2. The authors suggested that the hyperpolarization was due to the direct effect of CO_2 on the cell, and that the increase in the slow depolarizing potential resulted from increased rhythmic excitatory synaptic input. Peripheral chemoreceptor stimulation acted on the slow depolarization potential similarly to CO_2 but did not cause hyperpolarization. No large differences in the inspiratory discharge pattern were recorded from various parts, however, differences were seen in the expiratory populations. In the nucleus ambiguus CO_2 hyperpolarized expiratory cells and low CO_2 caused continuous firing with respiratory modulation. With an increase in CO_2 the spike activity decreased and became rhythmic. High CO_2 could lead to such a degree of hyperpolarization that the neuron stopped firing at any point of the respiratory cycle. Expiratory cells recorded from the nucleus retroambigualis reacted similarly. The authors

concluded from their results that the respiratory neurons behave similarly to the cortical and spinal neurons as observed by *Speckmann* and *Caspers* (1969a, b), and *Krnjević* et al. (1965). *Mitchell* and *Herbert* (1974b) concluded from their observations on inspiratory neurons, recorded in the nucleus ambiguus, the nucleus retroambigualis, and from the ventrolateral portion of the nucleus tractus solitarii, that these neurons receive a highly synchronized excitatory input, and that synchronized potentials in the expiratory cells arise from the medullary inspiratory cells via inhibtory interneurons. The magnitude of the synchronized synaptic input was decreased by hypocapnia and anesthesia.

The studies of *Mitchell* and *Herbert* were followed by observations by *Marino* and *Lamb* (1975) made on the effect of iontophoretically applied H^+ ions on respiratory neurons of peripherally chemodenervated cats. The authors used extracellular techniques. All respiratory neurons increased their firing during CO_2 inhalation but not one responded to the extracellularly increased H^+ ion concentration. The authors also recorded from 74 neurons with nonphasic discharge patterns, 71 of these being insensitive to CO_2 inhalation as well as to locally applied H^+ ions. Only three neurons of this type were found in the depth of the medulla oblongata which responded to CO_2 inhalation and to an increase in H^+ ions. The data strongly indicate that respiratory populations being engaged in rhythmogenesis or in the coordinating function of the respiratory system are not specifically sensitive to their chemical surrounding, but are dependent on the input from some specialized mechanism mediating chemical drive. *Kim* and *Carpenter* (1961) observed respiratory neuron responses to direct chemical stimuli, and interpreted the reactions, as being not a consequence of pH changes but an unspecific response following changes of the dissociation of calcium due to higher or lower acidity.

The aforementioned studies were performed with the ventral surface structure intact. Up to the present, only two research teams cooled or blocked the intermediate area during simultaneous recording from central respiratory neurons. Such studies have been presented by *Schwanghart* et al. (1974), *Koepchen* (1976), *Koepchen* et al. (1976a,b, 1977, 1979), and by *Peskow* and *Piatin* (1976), and *Peskow* et al. (1980). *Koepchen* and co-workers showed that 1%–5% procaine applied to the ventral medullary surface caused a decrease in the neuronal activity of inspiratory, expiratory and reticular neurons. With sinus nerves intact *Schwanghart* et al. (1974) found activation of nonrespiratory neurons in some cases. *Peskow* and *Piatin* (1976) found a complete cessation of inspiratory activity, brought about by cold block of the intermediate area. They observed the strongest inhibition in the discharge of early and late inspiratory neurons. In both studies it was found that expiratory units could become silent or contin-

ued to exhibit tonic firing and reduced impulse frequency during cessation of phrenic activity.

Loeschcke et al. (1979) performed an indirect test to determine whether or not the respiratory rhythm generator is chemosensitive. The authors blocked or coagulated both intermediate areas with cut sinus and aortic nerves which produced apnea. Intermittant stimulation of the afferent sinus nerves provoked respiration. This however, was practically insensitive to an elevation of PCO_2, on the contrary, CO_2 rather led to a reduction in ventilation. In general it can be said that circumstantial evidence from experiments in which complete chemodenervation in spontaneously breathing cats was performed, corroborate these findings. In these cats P_aCO_2 rose to values of about 9.31 kPa and pH_a was 7.08 (*See* 1976a, b) which, if the "centers" were chemosensitive, whould have constituted a major drive for producing ventilation.

The data provide substantial evidence for the fact that the CO_2 induced discharge or changes in discharge of respiratory neurons is dependent upon influences mediated by the ventral medullary surface structures. However, there are only very preliminary data at hand giving evidence of neuronal connections. *Davies* and *Loeschcke* (1977a) compared latencies of the phrenic nerve response to electrical stimulation of either the sinus nerve or the caudal or rostral medullary surface areas. The response to sinus nerve stimulation consisted of an initial excitation followed by a period of inhibition and then a delayed excitation. Similar responses were observed when the caudal area was stimulated, although the latencies differed. The initial excitation appeared with a latency of 5 ms after sinus nerve stimulation and with only 2–5 ms after stimulation of the caudal area, the delayed excitation following with a latency of 15–30 ms. Rostral area stimulation induced an initial inhibition and delayed excitation only. The authors considered the initial fast response of the phrenic discharge to stimulation of the caudal area, to be a consequence of stimulating efferent fibers directly, whereas the later response might indicate a polysynaptic pathway from the ventral surface structures to the phrenic motoneurons. *Cohen* (1973) measured latencies between peak to peak activities from respiratory units and the phrenic nerve and found them to be 3–5 ms. Interpretation of short latencies when stimulating the chemosensitive areas should therefore be considered with caution.

Davies and *Loeschcke* (1977b) first reported results from recordings within the nucleus of the solitary tract, where they found responses when electrically stimulating the caudal area with latencies of 5–15 ms. When recording from a ventrolateral region, lateral to the hypoglossal root, probably the lateral reticular nucleus, latencies to stimulation of the caudal or rostral areas were found between 2–4 ms. Since the evoked potentials

could not be seen when using frequencies higher than $100 \cdot s^{-1}$ the authors took this as circumstantial evidence for the involvement of polysynaptic pathways.

At present no definitive neurophysiological data are available on connections between the ventral medullary surface (the tonic afferent system located here), to the various parts of the medulla involved in respiratory reflexes, coordination and rhythm generation (*Cohen* 1979; *v. Euler* 1977). The most likely candidates for such connections might be the ventrolateral portion of the nucleus tractus solitarii (*Davies* and *Loeschcke* 1977a, b), the infrasolitary nucleus (*Koepchen* et al. 1974), the nuclei ambiguus and retroambigualis (*Mitchell* and *Herbert* 1974a, b) and pontine nuclei (*Cohen* 1971; *Cohen* and *Hugelin* 1965; *Cohen* et al. 1976; *v. Euler* 1979; *v. Euler* et al. 1976; *Hugelin* 1977a, b).

3.5 Some Remarks on the Role of the Ventral Medullary Surface for the Cardiovascular and Other Efferent Systems

Contrary to *Feldberg* and his group who have rediscovered the ventral medullary surface areas for the purpose of cardiovascular responses (*Bousquet* 1974; *Bousquet* et al. 1975; *Edery* and *Guertzenstein* 1974; *Feldberg* 1976, 1980; *Feldberg* and *Guertzenstein* 1972, 1976; *Feldberg* and *Wei* 1977; *Feldberg* et al. 1978; *Guertzenstein* 1973; *Guertzenstein* and *Silver* 1974), *Trzebski* and his associates investigated correlations of the H^+ ion sensitive apparatus of the respiratory system with the sympathetic system as well as the ascending reticular system (*Trzebski* et al. 1971, 1972, 1980). Furthermore *Trzebski* and his co-workers and also *Willshaw* described an excitatory influence on efferent connections to the carotid body from the ventral medullary surface, induced by an increase in the local pH by means of superfusion or artificial hyperventilation (*Majcherczyk* and *Willshaw* 1973, 1977, 1980; *Trzebski* et al. 1974, 1976; *Willshaw* 1975, 1977). *Schlaefke* et al. (1980), using bilateral cold block of the intermediate area, confirmed the activating effect on efferent pathways within the sinus nerve and the cervical sympathetic nerve, whereas an inverse reaction in the splanchnic nerve activity was observed at the same time. A stimulating effect of hypercapnia on efferent pathways to the carotid body had already been established by *Biscoe* and *Sampson* (1968). These findings indicate the involvement of different systems in the efferent neuronal supply to the carotid body. Nothing can be said so far about the role of such efferent pathways in the likely interaction between peripheral and central chemoreceptive systems. In this connection some preliminary data may be mentioned, indicating that ventral medullary neurons within the intermediate area are influenced by pH changes and also by electrical

stimulation of the afferent sinus nerve (*See* and *Schlaefke* 1978). Such findings are contradicted by the data of *Biscoe* and *Sampson* (1970) and by *Lipski* et al. (1975). There is however, support from some recent data on the degeneration of a few fibers within the ventral medullary area after intracranial section of the ipsilateral glossopharyngeal nerve (*Kille* and *Schlaefke* 1978). It should be worthwhile testing whether both the chemosensitive neuron responses to electrical stimulation of the sinus nerve as well as the fiber degeneration may be due to the involvement of efferent pathways.

During investigations of the central chemosensitive mechanism of respiration, many simultaneous observations on the circulatory system led to the assumption that there also exists an influence from the ventral medullary surface structure on the cardiovascular system. There is considerable evidence, however, that the morphological correlates are not identical (*Dev* and *Loeschcke* 1979a, b; *Loeschcke* et al. 1958; *Schlaefke* and *Loeschcke* 1967; *Schlaefke* et al. 1979b; *Trouth* et al. 1973c). Some more support for this view comes from recent recordings from ventral medullary neurons underlying the intermediate area, located within the ventral region of the NPG (*Schlaefke* and *See* 1978, 1980; *Schlaefke* et al. 1977, 1980b, d). The neurons increased their firing frequency when an antihypertensive drug (imidazolidine derivative) or acetylcholine was applied locally to the intermediate area, but did not respond to either CO_2 inhalation or to local ventral medullary superfusion with solutions of varied pH. Since the simultaneously recorded splanchnic nerve and cervical sympathetic activity were depressed at the same time, the authors suggested that the statement of *Feldberg* (1976) and *Shahar* and *Edery* (1976), namely that the superficial nerve cells within the intermediate area would be responsible for both the chemosensitive respiratory as well as the "drug-sensitive" cardiovascular reactions, should be considered with caution. One has to be aware that besides the deeper lying drug-sensitive neurons in the NPG, the ventral part of the lateral reticular nucleus is also superficially located, a nucleus which has been shown by *Coote* and *McLeod* (1974) to be related to the cardiovascular system.

Recent tracer studies with horseradish peroxidase (HRP) revealed multiple connections from the ventral medullary surface travelling in different directions. Convincing evidence for a direct connection between the ventral surface and the intermediolateral column of the thoracic spinal cord was provided by the labelling of neurons within the NPG and very close to the ventral medullary surface of the intermediate and rostral area, when HRP was injected at the level of T 3 (*Amendt* et al. 1978). This pathway may be a potential candidate for a tonic inhibitory effect on the sympathetic system (*Dembowski* et al. 1980). Tracer studies of *Loewy* and *McKellar* (1980) show that central autonomic pathways with excitatory

effects on the cardiovascular system, pass through the ventral region of the medulla oblongata. They mediate interconnections from the catecholamine cell group of the A 5 region lateral to the superior olivary nucleus to the intermediolateral column, and from the raphe nuclei, the Kölliker Fuse nucleus, and the hypothalamic paraventricular nucleus to the intermediolateral column. In addition the authors found multiple interconnections between the nucleus of the solitary tract, the parabrachial nucleus, the paraventricular nucleus of the hypothalamus, the central nucleus of the amygdala, and the bed nucleus of the stria terminalis. Their course runs very close to the ventral medullary surface. Ventral to the lateral reticular nucleus a pathway was traced by ^{3}H labelled proteins, when injected into the hypothalamus (*Saper* et al. 1976). *Errington* and *Dashwood* (1979) found HRP labelled axons within the ventral medullary surface with somata in the nucelus of the solitary tract and in the dorsal motor nucleus. The latter was shown to be involved in heart contractility (*Geis* and *Wurster* 1980).

An example of the complexity of the neuronal network within the ventral medullary surface layer is given by the following: Cold block or procaine on the intermediate area causes a fall in blood pressure (*Berndt* et al. 1970; *Dev* and *Loeschcke* 1979b; *Peskow* and *Piatin* 1976; *Koepchen* et al. 1977; *Schlaefke* and *Loeschcke* 1967; *Schlaefke* et al. 1969). Recent histochemical and neurophysiological experiments (*Amendt* et al. 1978; *Schlaefke* and *See* 1980; *Seller* et al. 1980) as have been discussed above, suggest the existence of neurons in the ventral part of the NPG with a tonic inhibitory function on the sympathetic system. Electrical microstimulation within the same area causes a decrease in splanchnic nerve activity and a fall in arterial blood pressure (*Schlaefke* et al. 1978). Thus one would expect cold block to provoke a rise in blood pressure. Simultaneous recordings from the splanchnic and the cervical sympathetic nerves during cold block show however, that excitatory as well as inhibitory pathways acting on the sympathetic system must be influenced through structures underlying the intermediate area. This leaves us with the proposal that cold block, reaching a larger substrate than microstimulation, and besides blocking central chemosensitivity of respiration, may affect neuron populations with inhibitory and excitatory characteristics for the cardiovascular system. These may include descending pathways as described by *Loewy* and *McKellar* (1980).

Another open question is the cause for the opposite effect on respiration of H^{+} ions when locally applied to a small spot within the intermediate area (*Schlaefke* et al. 1970). This little spot projects to the area of highest density of small vessels (*Cragg* et al. 1977; *Schlaefke* 1972; Figs. 1, 3) which, with their accompanying sheaths could prevent the bicarbonate buffer solutions from reaching the underlying sensor elements. Instead, alkaline solutions, when only applied locally to this little spot may cause

vasoconstriction, which would mean that the sensor elements are exposed to a reduced tissue perfusion. Consequently they might increase their firing frequency. The opposite reaction would follow local superfusion with acid buffer (*Betz* 1972, 1976a, b; *Betz* and *Csornai* 1978). This interpretation of an opposite response of respiration is supported by the fact that no paradoxical response to H^+ ions recorded from neurons below the intermediate area have been found so far. On the contrary the highest density of neurons excited by pH changes, projects to this area. The neurons which responded to drugs affecting the cardiovascular system were found underneath the rostral part of the intermediate area (Fig. 1). These neurons did not show any response to either inhalation of CO_2 nor to local changes in pH by superfusion. So there is no high probability that these same neurons, which are supposed to project to the intermediolateral column, are also the substrate responsible for the inhibitory effect of H^+ ions on the sympathetic system, as far as can be seen from the few data available at present.

The findings demonstrate that the question of an interconnection between H^+ ion sensitive neurons presumed to belong to the respiratory system and neurons presumed to influence the cardiovascular system, both populations within the ventral medullary surface layer, is completely open.

3.6 A Wanted Model: Or a Model of How I Want it to be

Summarizing the symposium on "acid base homeostasis in the brain extracellular fluid" held in Bochum (1976) Dr. *Cunningham* unsuccessfully challenged some speakers to give a model of the pathways responsible for the various effects producible from the three areas on the ventral medullary surface by different experimental approaches. Considering the recent findings on the network mentioned above, one may feel even more discouraged than four years ago to continue building a model. Focusing on the neurophysiological data of *Davies* and *Loeschcke* (1977a, b), *Davies* (1980), *Pokorski* (1976), *Prill* (1977), *Schlaefke* (1976a, b), *Schlaefke* et al. (1975, 1979c), *Schlaefke* and *See* (1980), and *Shimada* et al. (1969), the morphological data of *Dermietzel* (1976), *Dermietzel* et al. (1977, 1978), *Leibstein* (1979), *Luber* (1976) the data on bicarbonate exchange mechanisms by *Ahmad* et al. (1976, 1978), *Loeschcke* and *Ahmad* (1980), and *Loeschcke* and *Sugioka* (1969), and finally the studies obtained by stimulation and elimination (*Berndt* et al. 1972a–d; *Loeschcke* et al. 1970, 1979; *Schlaefke* and *Loeschcke* 1967; *Schlaefke* et al. 1969, 1970, 1979a, b; *See* 1973, 1976a, b), one may venture to portray the following hypothetical picture:

There is the marginal glia, which contains a high internal concentration of chloride (*Reed* et al. 1967), as well as carbonic anhydrase (*Maren* and *Broder* 1970; *Wichser* and *Kazemi* 1975). The glia is involved in acid base balance in the following way, as described by *Ahmad* et al. (1978): Respiratory acidosis is reflected by an increase in the PCO_2 in the brain extracellular fluid, a decrease in pH, and an increase in bicarbonate, all changing within about a minute. The increase in bicarbonate in the extracellular fluid reflects the increase in local PCO_2 according to *Ahmad* (1976), *Ahmad* et al. (1976), *Granholm* and *Ponten* (1969), *Loeschcke* and *Ahmad* (1980), *Loeschcke* and *Sugioka* (1969), and is the consequence of a quick exchange of bicarbonate between the extracellular fluid and the glia cells in analogy to the Hamburger shift in the blood. This means that there is a rapid exchange of bicarbonate and chloride between the extracellular fluid and the glia cells. In metabolic disturbances the following happens: When simulating a metabolic alkalosis by intravenous injection of $NaHCO_3$ at constant PCO_2 a rapid migration of bicarbonate ions occurs from the plasma to the brain extracellular fluid which was measured on the ventral medullary surface by *Ahmad* et al. (1978). A rapid decrease in extracellular chloride follows the injection within 30–40 sec. An intravenous injection of NaCl results in a rapid increase in extracellular chloride concentration, leading the authors to conclude that the blood/extracellular fluid barrier may be permeable to HCO_3^- and Cl^- ions. In their three compartment model, *Ahmad* et al. (1978) make evident the free permeability of CO_2 through all three compartments: 1. blood, 2. extracellular fluid, and 3. the brain cell compartment and also the exchange of bicarbonate against blood chloride as well as against the glia cell chloride.

Coming back to our draft, we find within the superficial layer a large number of synapses, central dendrites surrounded by axons like a rosette, or somata surrounded by dendrites (according to the various synaptic formations described by *Dermietzel* 1976; *Shahar* and *Edery* 1976; *Luber* 1976). This neuronal formation is embedded in a large number of capillaries. The subarachnoid space, the extracellular spaces (which are characteristically wide within the chemosensitive areas) and the capillaries, constitute almost no diffusion barrier for CO_2 and bicarbonate. There is close contact between the cholinergic synapses in question and the glia formation. Whether or not this may be of importance for the synaptic transmission within the chemosensitive mechanism is not yet clear. Nothing can be said beyond the fact that in the brain slice preparation (which may be more an artifical than a physiological preparation), the glia in this particular area responds with a reduction of its membrane potential to acid shifts of pH (*Fukuda* et al. 1978). The somata of the dendrites forming synapses within the rostral, the caudal, and the intermediate area we tentatively allocate to the NPG, superficially underlying the intermediate area

(Fig. 11). The synaptic connections within the three areas may be of afferent and efferent nature. One potential candidate forming synaptic input might be the spinothalamic afferent system, and could account for part of an "unspecific" synaptic activity, being modified by the actual acid base conditions. According to *Saper* et al. (1976), descending hypothalamic pathways reach the ventral medullary surface layer. Their possible contribution to an inhibitory modulation of central chemosensitivity in the respiratory defense against high body temperature, as studied by *See* (1976a, b) may at least provide us with an attractive working hypothesis. This is supported by the fact that the drive mediated by the intermediate area produces mainly an increase in tidal volume but not in frequency (*Cherniak* et al. 1979a; *Loeschcke* et al. 1958; *Schlaefke* and *Loeschcke* 1967; *Schlaefke* et al. 1979a, b). There may exist ascending as well as descending pathways to and from pontine structures (*v. Euler* et al. 1970; *St. John* and *Wang* 1976, 1977), and these may be involved in the same mechanism of frequency modulation. If now the various synaptic inputs are controlled by the H^+ ion concentration, the signals will be integrated within the NPG and be relayed to the various respiratory neuron complexes, i.e. the CIA (centrally generated inspiratory activity) of *v. Euler* (1976) and *v. Euler* et al. (1976), and the inspiratory and expiratory populations, as well as other types of respiratory neurons showing a characteristic response to CO_2 (*Cohen* 1979; *Koepchen* 1976) mediated by synaptic input (*Mitchell* and *Herbert* 1974a, b). If this convergence of H^+ ion controlled input within the NPG, or whatever is cooled or blocked with the structure in the intermediate area, is facilitatorily linked to the process of synchronization of inspiratory neuron excitation (*Mitchell* and *Herbert* 1974b), this may be considered as one possible explanation for the poorly understood phenomenon that respiratory sensitivity to acids or CO_2 seems to be concentrated on "some kind of a vital point". Since little or nothing is known about central connections of ventral medullary H^+ ion sensitive structures, the draft of a model of central chemosensitivity ends with the NPG. From here the big arrow to the rhythm generator may be taken as a symbol pointing to future research. The superficial cells within the three areas may or may not be regarded as reticular neurons, mediators in the connection with various systems which constitute a disturbance for the homeostatic apparatus. Central chemosensitivity of respiration acts as a stabilizer within the respiratory control system and through it for the brain extracellular fluid pH in the sense of *Winterstein* (1911).

4 Loss of Function – Loss of Acid Base Homeostasis

4.1 Respiratory Response to CO_2 After Elimination of Central Chemosensitivity

The superficial position of the central chemosensitive structure and the observation that cold block or the relatively small intermediate area abolishes its function, may be considered as a chance to add some more respiratory response curves to the numerous others, which were measured under various conditions with intact and cut sinus and aortic nerves. They could display the ability of peripheral chemosensors to signalize hypercapnia and hypoxia without interaction with the central chemosensitive input to the respiratory rhythm generator. This has been tried by *Cherniak* et al. (1978a, 1979a, b) using bilateral cold block of the intermediate area in the anesthetized cat and by *Schlaefke* (1972, 1976a, b) and *Schlaefke* et al. (1974, 1979a, b) in the awake cat, using coagulation techniques. The latter technique does not produce a fall in blood pressure which usually occurs during cold block, or application of procaine (*Berndt* et al. 1970; *Koepchen* et al. 1979; *Peskow* and *Piatin* 1976; *Schlaefke* and *Loeschcke* 1967; *Schlaefke* et al. 1969). Such respiratory responses to CO_2 may deviate from controls dependent upon the completeness of coagulation or the degree of cold block. In the first case the focus can be evaluated afterwards by histological techniques. For comparison of such curves, obtained from either peripherally or centrally chemodenervated preparations, one has to be aware that in the intact animal reticular neurons respond to an increase in peripheral chemoreceptor activity, as well as to central chemoreceptor stimulation (*Hukuhara* 1973, 1976; *Hukuhara* et al. 1969; *Koepchen* et al. 1979; *Schwanghart* et al. 1974). Peripheral and central chemosensitive input increases the magnitude of rhythmic depolarization and spike frequency of inspiratory neurons (*Mitchell* and *Herbert* 1974a, b). The interruption from either the peripheral or the central chemosensor changes the level of activity of the whole respiratory system (*Wiemer* et al. 1965). Furthermore, the type and stage of anesthesia produces inhibition especially of the central chemosensitive mechanism (*Mitchell* and *Herbert* 1974b). The conditions become further complicated by the central depressant action of hypoxia, or the stimulating action of hyperoxia (*Cherniak* et al. 1970/71; *Miller* and *Tenney* 1975) and the central depressant action of CO_2, namely the unspecific component, which in respiratory neurons causes hyperpolarization (*Mitchell* and *Herbert* 1974a). Data from both peripherally or centrally chemodenervated groups, under "comparable" conditons, are available from studies of respiratory response to CO_2 in hyperoxia in awake cats by *Miller* and *Tenney* (1975) and *Schlaefke* et al. (1979a, b) and are shown in Table 2. Under the condition

Table 2. Endtital PCO_2 (Torr), tidal volume (ml), ventilation (ml) and respiratory frequency ($f \cdot min^{-1}$) from six awake cats before and after coagulation of areas S in hyperoxia (*Schlaefke* et al. 1979b). The corresponding data from six awake cats before and after peripheral chemodenervation in hyperoxia (*Miller* and *Tenney* 1975)

Chemodenerv.	PCO_2 (Torr)		V_T (ml)		V (ml)		$f \cdot min^{-1}$	
	before	after	before	after	before	after	before	after
Central	27.0	43.0	26.0	18.0	847	428	36.4	24.6
Peripheral	31.4	31.1	24.0	25.1	698	547	29.3	23.1

of hyperoxia the two series seem to demonstrate a remarkably stronger loss of respiratory drive after central than after peripheral chemodenveration. However, the peripherally chemodenervated cats hyperventilate in hyperoxia. The data previously presented by *Bouverot* et al. (1965), *Gautier* (1976a,b), *Miller* and *Tenney* (1975), *Mitchell* (1965) and *Mitchell* et al. (1964) make evident that ventilation after peripheral chemodenervation decreases during air breathing. Thus cutting sinus and aortic nerves causes a loss of a drive component which is strengthened during air breathing and reduced in hyperoxia. The respiratory depression elicited during

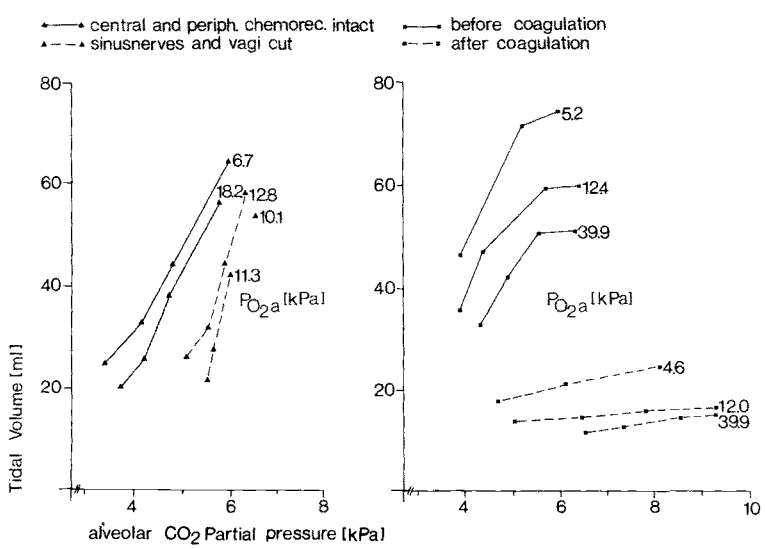


Fig. 12. Tidal volume response to hypercapnia and hypoxia before and after cutting both sinus nerves and vagi *(left)*, and before and after bilateral coagulation of the intermediate area. Mean values of steady states. Both cats anesthetized with ketamine hydrochloride and chloralose urethane

air breathing in peripherally chemodenervated cats, in principle has an analogy in the central respiratory depression by CO_2 after central chemodenervation (*Loeschcke* et al. 1979; *Schlaefke* et al. 1979a), although the mechanisms may be quite different. The main features resulting from peripheral and central chemodenervation may be demonstrated with two examples (Fig. 12), comparing respiratory responses from peripherally and centrally chemodenervated and anesthetized cats, with controls. Both cats were anesthetized with chloralose urethane and were under similar surgical and experimental conditons. In accordance with the results from anesthetized cats or rabbits obtained by *Katsaros* (1965), *Kiwull* et al. (1972) and *Borison* and *McCarthy* (1973), respiratory response curves after peripheral chemodenervation (in the example of Fig. 12 sinus nerves and vagi had been cut) are shifted to higher CO_2 values but no change of slope could be observed. Hypoxia caused a further shift to the right, indicating depression of respiratory activity. Central chemodenervation goes along with a severe respiratory acidosis and strongly reduced or abolished response to inhalation of CO_2 (*Cherniak* et al. 1979a; *Schlaefke* 1972; *Schlaefke* et al. 1979a). Air breathing or hypoxia indicates an intact peripheral chemoreceptor response (Fig. 12), activation of peripheral chemoreceptor drive diminishing the respiratory acidosis. After central chemodenervation the maintained ventilation in hyperoxia is completely dependent upon the intact sinus nerves, which may include a hypothetical unspecific drive component (*Katsaros* 1965) as well as a CO_2 dependent part (*Fitzgerald* and *Parks* 1971). Cutting of sinus and aortic nerves causes respiratory arrest even when trying to produce respiratory drive by unspecific stimulation. However, by stimulation of both sinus nerves spontaneous breathing is induced. The respiratory response curve to CO_2 of such an evoked respiration is completely flat (*Loeschcke* et al. 1979).

Cherniak et al. (1979a) applied local temperature changes to the intermediate area in the artificially or spontaneously breathing cat. Focal cooling of the intermediate area to 303–305 K caused a parallel shift in the CO_2 response curves to the right, while cooling below 303 K caused a decrease in slope and further shifts to the right. Additional hypoxia increased respiration in an additive manner, irrespective of whether CO_2 was changed or the intermediate area was cooled. *Loeschcke* (1979), by local application of atropine found resting ventilation and the slope of the CO_2 response curve diminished.

4.2 Breathing After Loss of Central Chemosensitivity

Awake cats, surviving for days or weeks following bilateral coagulation of the intermediate area, showed the following respiratory characteristics,

which were observed during oxygen breathing: A great loss of resting respiratory drive and an ensuing severe respiratory acidosis were predominantly due to a reduction in tidal volume (Fig. 13). The reduced ability to respond to a further increase in PCO_2 has been demonstrated in Fig. 12 already.

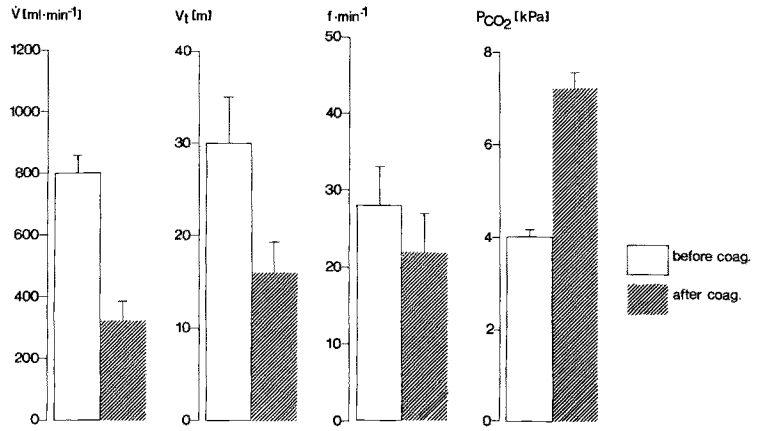


Fig. 13. Means of respiratory values from three awake cats before and after central chemodenervation by bilateral coagulation of the intermediate area

Periodic breathing occurred under the condition of hyperoxia. *Cherniak* et al. (1979b, c) found periodic breathing in anesthetized cats when graded cooling was applied to the intermediate area and when the "controller gain" was increased, e.g. by hypoxia or lung deflation. The effect of wakefulness of the animal may be interpreted in the same sense (*Schlaefke* 1972, 1976a; *Schlaefke* et al. 1979a). The awake cats breathing without central chemosensitivity generally reacted with a surprisingly strong ventilatory response to unspecific stimuli. This phenomenon may or may not reflect an increase in reticular neuronal activity similar to that seen in cats with procaine block of the ventral medullary surface as long as the peripheral chemoreceptors were intact (*Schwanghart* et al. 1974). Apneic phases could be interrupted by waking the cats. In cases of artificial ventilation, stimulation by pinching the paw produced an EEG arousal and onset of spontaneous breathing (*Schlaefke* et al. 1980a, b). *Sullivan* et al. (1978) give support to the idea that chemosensitive afferents are necessary for sufficient activation of the respiratory centers, and that in the event of them being insufficient, wakefulness is necessary for compensation. Peripheral chemoreceptors however, seem to be dependent on the cooperation with central chemosensitivity for the continuation of ventilation during sleep. The observations indicate that the stabilizing function of CO_2,

as considered by *Loeschcke* (1960), is closely associated with the function which can be eliminated by interference with the superficial layer of the intermediate area.

After central chemodenervation the respiratory system of neither awake nor anesthetized cats was unable to respond to an additional charge with fixed acids induced by orally administered acetazolamide or by intravenous injections of HCl. The cats stopped breathing due to a further fall in arterial pH to values of about 6.96 (*Schlaefke* 1972; *Schlaefke* et al. 1974) which had not yet affected the arterial blood pressure. The strong reduction in the respiratory response to inhaled CO_2 and the inability to compensate metabolic disturbances by respiration, may be regarded as a confirmation of former observations by *Katsaros* (1965). He described peripheral chemoreceptors as playing a minor role in the respiratory compensation of metabolic acidosis. In centrally chemodenervated cats one has to be aware however, that H^+ ions may directly inhibit respiratory neurons (*Marino* and *Lamb* 1975; *Mitchell* and *Herbert* 1974a), so that a strong acidosis may cause respiratory arrest after the loss of the central excitatory component of H^+ ions.

Therefore any quantitative comparison with peripheral chemoreceptor sensitivity from these studies is questionable.

In this situation the mathematical studies of *Middendorf* and *Loeschcke* (1976a, b; 1978) are valuable. Using the data of *Loeschcke* and *Sugioka* (1969), *Kronenberg* and *Cain* (1968), *Fencl* (1971) and *Mitchell* (1965), the authors calculated the quantitative contribution of the peripheral and the central chemosensitivity in nonrespiratory acidosis. They found an optimal agreement between calculated and experimental data when the central system was 25 times more sensitive than the peripheral system under steady state conditions and in normoxia. The authors attributed the reason for small deviations of ECF pH in non respiratory disturbances (*Fencl* et al. 1966; *Fencl* 1971; *Pappenheimer* 1967; *Robin* et al. 1958) to the cooperation of the two sensors in different locations, where the more sensitive one measures the controlled value (ECF pH) and the less sensitive one measures the disturbance before it reaches the sensor of the controlled value. The precise control seems to be necessary for an equally precise coordination and function of the respiratory "centers". The studies in centrally chemodenervated cats show that the intact control function of peripheral chemoreceptors can not guarantee acid base homeostasis.

4.3 Loss of Central Chemosensitivity: A Basic Model for Ondine's Curse and Sudden Infant Death Syndrome

The centrally chemodenervated cats in the chronic experiment showed symptoms which are observed in patients whose disease is called Ondine's curse syndrome. Its manifestations are periodic breathing, alveolar hypoventilation, respiratory acidosis, and sleep apnea (*Chiesa* et al. 1970; *Comroe* 1975; *Deonna* et al. 1974; *Fishman* et al. 1965, 1966; *Flandrois* et al. 1974b; *Fruhmann* et al. 1961; *Gerardy* et al. 1960; *Giroud* et al. 1974; *Granholm* 1973; *Grant* 1968; *Guilleminault* et al. 1974; *Kafer* and *Leigh* 1972; *Richter* et al. 1957; *Severinghaus* and *Mitchell* 1962). In combination with obesity the same symptoms are described as Pickwick syndrome (*Addington* et al. 1969; *Burwell* et al. 1956; *Doll* 1968; *Drachman* and *Gumnit,* 1962; *Geisler* 1971; *Geisler* et al. 1967; *Kaemmerer* and *Dolce* 1967; *Kuhlo* 1968; *Jung* and *Kuhlo* 1965; *Lugaresi* et al. 1968, 1972; *Terzian* 1966; *Unterberg* 1971; *Ward* and *Kelsey* 1962). Since there is a relationship between sleep phases and a higher or lower respiratory sensitivity to inhalation of CO_2 (*Bertrand* et al. 1967; *Birchfield* et al. 1958; *Bülow* 1963; *Duron* 1972; *Guazzi* 1969; *Guazzi* and *Freis* 1969; *Ingvar* and *Bülow* 1963; *Jung* 1965; *Netick* et al. 1977; *Orem* 1978; *Orem* et al. 1977; *Phillipson* 1977, 1978a, b; *Phillipson* et al. 1977), which in addition is different in the various stages of maturation (*Bolton* and *Herman* 1974; *Bryan* and *Bryan* 1979; *Bryan* et al. 1976; *Carrol* 1974; *Daily* et al. 1969; *Fagenholtz* et al. 1976; *Fenner* et al. 1973; *Finer* et al. 1976; *Frantz* et al. 1976; *Gabriel* et al. 1976; *Guthrie* et al. 1980; *Haddad* et al. 1979; *Hathorn* 1974; *Krauss* et al. 1975; *Meier* and *Berger* 1965; *Reite* et al. 1974; *Rigatto* 1979; *Rigatto* et al. 1975a–c; *Thach* et al. 1978; *Wyszogrodski* et al. 1978), the chemosensitive mechanism was considered to be an important factor involved in the sudden infant death syndrome (SIDS) (sudden death of apparently healthy babies during sleep) by *Gabriel* and *Albani* (1976), *Guilleminault* et al. (1974, 1975), *Hasselmeyer* and *Hunter* (1975), *Hunt* et al. (1978), *Krauss* et al. (1977), *Naeye* (1978, 1980), *Rigatto* and *Brady* (1972), *Shannon* and *Kelly* (1977), *Shannon* et al. (1977), *Steinschneider* (1972), and *Wennergren* and *Wennergren* (1980).

The causes for the pathogenesis of syndromes within the two groups (Ondine's curse and SIDS) may be apparently different for the individual, but under precisely determined conditions one should be able to discriminate patients of both groups in which a malfunction of central chemosensitivity may be causally involved.

The fault in the central chemosensitive mechanism may develop through various causes. In the adult and adolescent group, genetic factors, neurological diseases, cerebrovascular diseases, infections, neoplasms, and trauma are discussed.

Krieger (1973) described patients who suffer from respiratory failure during sleep, after high anterior spinal surgery. The respiratory response to inhaled CO_2 in these patients is flattened. From the clinical impressions an interruption of ascending pathways was suggested. *Schlaefke* et al. (1980a) studying a 12 year old girl found peripheral chemoreceptor reflexes intact as well as voluntary breathing, but a complete loss of respiratory response to CO_2 after the patient had undergone surgergy for an anomaly of the first and second vertebra (os odontoideum). The girl was breathing periodically and had long apneic phases. The values of arterial PCO_2 varied between 58 to 77 Torr (7.7 to 10.2 kPa). When the girl was pushed to wake up for breathing, the EEG showed arousal before the onset of breathing. An unexpected an inexplicable death of a 37 year old man was described by *Zink* et al. (1977). The authors verified an anomaly of the first and second vertebra as the only possible cause of death. The case reports indicate a ventral position at the lower part of the medulla oblongata for the defect, the involvement of an afferent pathway, and show analogies to the symptoms observed in centrally chemodenervated cats.

Recent studies of *Folgering* et al. (1979) and *Schlaefke* et al. (1980a–c) indicate that in cases of SIDS the ventral medullary surface layer shows the absence of neurons medial to the rostral part of the hypoglossal root and rostromedial to it, which equals the topography of the intermediate area of the cat. Sleep apnea, no respiratory response to inhaled CO_2 and intact peripheral chemoreflexes could be measured in a child before the absence of ventral medullary neurons was established (the child died within 9 months) (*Folgering* et al. 1979). *Wells* et al. (1980) reported the case of a 19 months old child which showed no response to inhaled CO_2, whose ventilation increased however, with a reduction of PO_2. The ventilatory response curves of this child are strikingly similar to those obtained from centrally chemodenervated cats.

In some of the cases of SIDS in which the absence of ventral medullary neurons was established, macrophages were found in peripheral lung alveoli as well as an increased amount of muscles in the pulmonary arteries, both indicating chronic hypoventilation (*Naeye* et al. 1976).

These first comparative studies indicate that a relevant experimental model for Ondine's curse syndrome and SIDS can be developed by the elimination of central chemosensitivity in the animal. Further basic research depends upon a close cooperation between clinicians, pathologists, and physiologists. It could lead a step forward in the understanding of central chemosensitivity and its interconnection with various other homeostatic mechanisms such as sleep and the cardiovascular system; but it would also help in the clarification of a poorly understood and often fatal disease.

5 Conclusion

The H^+ ion concentration of the brain extracellular fluid is required to be precisely controlled in order to guarantee the basic functional conditions of the central nervous system. This control is provided by a contribution of the brain buffer system and by the cooperation between peripheral and central chemosensitive mechanisms influencing the respiratory system by more or less afferent input. There is an almost free exchange of Cl^- and HCO_3^- between the three compartments, blood/brain extracellular fluid/brain (glia) cells. CO_2 diffuses extremely rapidly through all compartments. Any change in pH, induced by metabolic or respiratory disturbances is mirrored in the brain extracellular fluid pH. In contrast, however, to respiratory acidosis, in which the release of CO_2 is limited, in metabolic acidosis the extracellular pH of the brain is maintained close to normal. This is due to the rapid response of the peripheral chemoreceptors, controlling the pH value before the disturbance reaches the central sensor. The latter measures the controlled value and is 25 times more sensitive to pH deviations than the peripheral one. It is responsible for the precision of the H^+ control mechanism. The morphological correlate is a structure, possibly involving cholinergic mechanisms, located within the ventral medullary surface layer in close contact with the marginal glia cells and a dense capillary network. The responsible structure is underlying three areas, which differ in nerve cell and synaptic formations as well as in their responses to drugs and H^+ ions. A simplified model has been proposed in order to summarize the as yet incomplete experimental results.

The neuronal elements of central chemosensitivity discharge tonically. They display a characteristic response to small changes of pH measured on the ventral medullary surface, either produced via the blood, or by superfusing the medullary surface with mock CSF of varied pH. There are only a few preliminary data indicating that the surface neurons may project to the nucleus of the solitary tract via a polysynaptic pathway.

Since the phasic respiratory neurons in deeper parts of the medulla are inhibited by acids or CO_2, the elimination of the central chemosensitive apparatus on the ventral medullary surface is accompanied by a strong depression of respiratory drive; a loss of brain acid base homeostasis, and a loss of the normal breathing pattern.

In SIDS, ventral medullary neurons were absent in an area corresponding to the intermediate area medial to the rostral part of the hypoglossal root and rostromedial to it. Since the symptoms in awake cats after destruction of cells in the intermediate area equal those of SIDS and Ondine's curse syndrome, one feels tempted to ascribe the human central chemosensitive system of respiration to a morphological and functional correlate comparable to that described in the cat.

There is much evidence that another tonically firing system is located in the ventral medullary surface layer with an inhibitory action on the sympathetic system. However, its sensitivity to H^+ ions has not yet been convincingly demonstrated. Nevertheless the ventral medullary surface also seems to be important for the control of sympathetic outflow.

Studies on the structure and function of central chemosensitivity have shown that it is not only a morphological structure different from the respiratory neuron populations and contributes to the respiratory drive to a large extent (in the sense of *Wyss* 1964, as a neural input into the respiratory centers), but is also a decisive component for the precise regulation of the environment of the brain cells in the sense of *Winterstein* (1955).

This review does not give a complete account of all data obtained on central chemosensitivity up until now. In particular the interaction of central chemosensitivity with other respiratory drives received relatively little attention. The intention of this review was to demonstrate the problems and limitations of interpretation, using more recent results. It should serve the purpose of stimulating further research in the field of central chemosensitivity.

Acknowledgement. I wish to express my gratitude to my co-worker Dr. W.R. See, for reading the manuscript, for language corrections, and discussions, as well as for his help in compiling the literature. I have also to thank Mr. F. Burghardt and Mr. B. Reitemeyer for their most helpful engagement during the preparation of the manuscript, and Mr. J.F. Kille for his expert histological work. I would further like to thank Professor Dr. Hukuhara Jr. for reading the manuscript and his helpful suggestions.

References

Addington WW, Pfeffer SH, Gaensler EA (1969) Obesity and alveolar hypoventilation. Respiration 26:214–225

Adolph EF, Naberschnig A, Orchard DP (1961) Ventilation of lung in deeply hypothermic rats. J Appl Physiol 16:819–826

Ahmad HR (1976) Nicht-respiratorische Mechanismen der Säure-Basen-Homoiostase der extracellulären Flüssigkeit des Gehirns. Ph D Dissertation (Biology). Ruhr-Universität Bochum

Ahmad HR, Berndt J, Loeschcke HH (1976) Bicarbonate exchange between blood, brain extracellular fluid and brain cells at maintained pCO_2. In: Loeschcke HH (ed) Acid base homeostasis of the brain extracellular fluid and the respiratory control system. Thieme, Stuttgart, pp 19–27

Ahmad HR, Loeschcke HH, Woidtke HH (1978) Three compartments model for the bicarbonate exchange of the brain extracellular fluid with blood and cells. In: Fitzgerald RS, Gautier H, Lahiri S (eds) Regulation of respiration during sleep and anaesthesia. Plenum Press, New York, London, pp 195–209

Alexander JK, West JR, Wood JA, Richards DW (1955) Analysis of respiratory response to carbon dioxide inhalation in varying clinical states of hypercapnia, anoxia, and acid-base derangement. J Clin Invest 34:511–532

Amendt K, Czachurski J, Dembowski K, Seller H (1978) Neurones within the 'chemosensitive area' on the ventral surface of the brainstem which project to the intermediolateral column. Pfluegers Arch 375:289–292
Anitschkow SV (1936) Atemreflexe bei intravenöser Einführung von Ammoniumchlorid. Arch Int Pharmacodyn Ther 54:193–200
Asmussen E, Nielsen M (1948) Studies on the initial changes in respiration at the transition from rest to work and from work to rest. Acta Physiol Scand 16:270–285
Åström A (1952) On the action of combined carbon dioxide excess and oxygen deficiency in the regulation of breathing. Acta Physiol Scand (Suppl) 27:98
Augustinsson KB (1948) Cholinesterase. A study in comparative enzymology. Acta Physiol Scand (Suppl) 17:52
Banus MG, Cormann HH, Perto VP, Popkin GL (1944) The sensitivity of the respiration center to hydrogen ion concentration. Am J Physiol 142:121
Bellville JW, Howland WS, Seed JC, Houde RW (1959) Effect of sleep on the respiratory response to CO_2. Anesthesiology 20:628–634
Benzinger Th, Opitz E, Schoedel W (1938) Atmungserregung durch Sauerstoffmangel. Pfluegers Arch 241:71–77
Berger AJ, Krasney JA, Dutton RE (1973) Respiratory recovery from CO_2 breathing in intact and chemodenervated awake dogs. J Appl Physiol 35:35–41
Berger AJ, Mitchell RA, Severinghaus JW (1977a) Regulation of respiration (First of three parts). N Engl J Med 297:92–97
Berger AJ, Mitchell RA, Severinghaus JW (1977b) Regulation of respiration (Second of three parts). N Engl J Med 297:138–143
Berger AJ, Mitchell RA, Severinghaus JW (1977c) Regulation of respiration (Third of three parts). N Engl J Med 297:194–201
Berger W, Berndt J, Berger K (1971) Die Antwort der Atmung auf isolierte Änderung des Liquor-pH: Untersuchungen an anaesthesierten und decerebrierten Katzen vor und während Vagusblockade. Pfluegers Arch 325:287–304
Berman AL (1968) The brain stem of the cat. A cytoarchitectonic atlas with coordinates. University of Wisconsin Press, Madison
Berndt J, Fenner A, Berger K (1969) Influence of calcium and magnesium on the respiratory response to changes in CSF pH. Respir Physiol 7:216–229
Berndt J, Berger W, Trouth CO (1970) Respiratory and circulatory effects of 100 meq/l potassium or 2% procaine in the cerebrospinal fluid of cats. Pfluegers Arch 321: 346:363
Berndt J, Berger W, Mückenhoff K (1972a) Untersuchungen zum zentralen chemosensiblen Mechanismus der Atmung. I. Die Bestimmung des extracellulären pH im Gehirngewebe. Pfluegers Arch 332:127–145
Berndt J, Berger W, Berger K, Schmidt M (1972b) Untersuchungen zum zentralen chemosensiblen Mechanismus der Atmung. II. Die Steuerung der Atmung durch das extracelluläre pH im Gewebe der Medulla oblongata. Pfluegers Arch 332:146–170
Berndt J, Berger W, Berger K, Schmidt M (1972c) Untersuchungen zum zentralen chemosensiblen Mechanismus der Atmung. III. Die Wirkung starker Änderungen des Liquor-pH (pH 5.4–7.7) bei Katzen vor und nach Vagotomie. Pfluegers Arch 332:171–183
Berndt J, Berger W, Berger K, Schmidt M (1972d) Untersuchungen zum zentralen chemosensiblen Mechanismus der Atmung. IV. Beobachtungen an anaesthesierten und dezerebrierten Katzen. Pfluegers Arch 332:184–197
Bertrand F, Hugelin A, Jutier P (1967) Analyse quantitative des effects du réveil et de P_{ACO_2} sur les ondes électrocortigraphiques dans la bande de 20 á 70 Hz. J Physiol (Paris) (Suppl 19) 59:215
Betz E (1972) Cerebral blood flow: Its measurement and regulation. Physiol Rev 52: 595–630
Betz E (1976a) Cerebral blood flow and acid-base homeostasis of the cerebral tissue. In: Loeschcke HH (ed) Acid-base homeostasis of the brain-extracellular fluid and the respiratory control system. Thieme, Stuttgart, pp 44–49

Betz E (1976b) Ionic interaction in pial vascular smooth muscles. In: Betz E (ed) Ionic action on vascular smooth muscle. Springer, Berlin Heidelberg New York, pp 75–77
Betz E, Csornai M (1978) Action and interaction of perivascular H^+, K^+ and Ca^{++} on pial arteries. Pfluegers Arch 374:67–72
Birchfield R, Sieker H, Heyman A (1958) Alterations in blood gases during natural sleep and narcolepsy. Neurology (Minneap) 8:107
Biscoe TJ, Sampson SR (1968) Rhythmical and non-rhythmical spontaneous activity recorded from the central cut end of the sinus nerve. J Physiol (Lond) 196:327–338
Biscoe TJ, Sampson SR (1970) Responses of cells in the brain stem of the cat to stimulation of the sinus, glossopharyngeal, aortic and superior laryngeal nerves. J Physiol (Lond) 209:359–373
Biscoe TJ, Taylor A (1963) The discharge pattern recorded in chemoreceptor afferent fibres from the cat carotid body with normal circulation and during perfusion. J Physiol (Lond) 168:332–334
Bisgard GE, Vogel JH (1971) Hyperventilation and pulmonary hypertension in calves after carotid body excision. J Appl Physiol 31:431–437
Bisgard GE, Forster HV, Byrnes B, Stanek K, Klein J, Manohar M (1978) Cerebrospinal fluid acid-base balance during muscular exercise. J Appl Physiol 45/1:94–101
Bishop PO (1964) Properties of afferent synapses and sensory neurones in the lateral geniculate nucleus. Int Rev Neurobiol 6:191–255
Bolton DPB, Herman S (1974) Ventilation and sleep state in the newborn. J Physiol (Lond) 240:67–77
Borison HL, McCarthy LE (1973) CO_2 ventilatory response time obtained by inhalation step forcing in cerebrate cats. J Appl Physiol 34/1:1–7
Bousquet P (1974) Etude des mécanismes de l'action hypotensive de la Clonidine: approche pharmacologique des centres vasomoteurs bulbaires. MD dissertation, University Louis Pasteur, Strasbourg
Bousquet P, Feldman J, Velly J, Block R (1975) Role of the ventral surface of the brain stem in the hypotensive action of clonidine. Eur J Pharmacol 34:151–156
Bouverot P (1976) Arterial chemosensitivity and CSF acid-base status of awake dogs at low and high altitude. In: Loeschcke HH (ed) Acid-base homeostasis of the brain extracellular fluid and the respiratory control system. Thieme, Stuttgart, pp 81–87
Bouverot P, Bureau M (1975) Ventilatory acclimatization and csf acid-base balance in carotid chemodenervated dogs at 3550 m. Pfluegers Arch 361:17–23
Bouverot P, Flandrois R, Grandpierre R (1961) A propos des méchanism d'action chemoreflex ou reflex central du "stimulus CO_2" de la ventilation. CR Acad Sci (D) (Paris) 252:790
Bouverot P, Puccinelli R, Flandrois R, Vaurelle A (1963) Effects respiratoires de la section chronique des nerfs de Hering cher le chien éveillé. CR Soc Biol (Paris) 157:2045–2047
Bouverot P, Flandrois R, Puccinelli R, Dejours P (1965) Etude du role des chémorecepteurs artériels dans la régulation de la respiration pulmonaire chez le chien éveillé. Arch Int Pharmacodyn Ther 157:253–271
Brettschneider H, Ullah Z, Dermietzel R (1974) Die Feinstruktur eines zentralen Chemo-Rezeptors der Atmung. Verh Anat Ges 68:409–417
Brightman MW, Albers RW (1959) Species differences in the distribution of extraneuronal cholinesterase within the vertebrate central nervous system. J Neurochem 4:244–250
Brooks CMcC, Eccles JC (1947) A study of the effects of anaesthesia and asphyxia on the monosynaptic pathway through the spinal cord. J Neurophysiol 10:349–360
Brown AM (1974) Carbon dioxide action on neuronal membranes. In: Nahas G, Schaefer KE (eds) Carbon dioxide and metabolic regulations. Springer, Berlin Heidelberg New York, pp 81–86
Brown AM, Berman PR (1970) Mechanism of excitation of aplasia neurons by carbon dioxide. J Gen Physiol 56:543–558

Bryan HM, Hagan R, Gulston G, Bryan AC (1976) CO_2 response and sleep state in infants. Clin Res 24:689

Bryan MW, Bryan AC (1979) Respiration during sleep in infants. Wenner-Gren Cent Int Symp Ser 32:457–465

Buelow K (1963) Respiration and wakefulness in man. Acta Physiol Scand 59:1–74

Burwell CS, Robin ED, Whaley RD, Bickelmann AG (1956) Extreme obesity associated with alveolar hypoventilation – pickwickian syndrome. Am J Med 811–818

Cakar L, Terzioglu M (1976) The response of the chemosensitive areas of the cat to the breathing of hypercapnic gas mixtures. Bull Physiol Pathol Resp 12(6):224

Carpenter DO, Hubbard JH, Humphrey DR, Thompson H, Marshall WH (1974) Carbon dioxide effects on nerve cell function. In: Nahas G, Schaefer KE (eds) Carbon dioxide and metabolic regulations. Springer, Berlin Heidelberg New York, pp 63–80

Carroll D (1974) Sleep periodic breathing and snoring in the aged: Control of ventilation in the aging and diseases respiratory system. J Am Geriatr Soc 22/7:307–315

Chalazonitis N (1974) Simultaneous recordings of pH, PCO_2, an neuronal activity during hypercapnic transients (identifiable neurons of aplasia). In: Nahas G, Schaefer KE (eds) Carbon dioxide and metabolic regulations. Springer, Berlin Heidelberg New York, pp 63–80

Cherniak NS, Edelman NH, Lahiri S (1970/71) Hypoxia and hypercapnia as respiratory stimulants and depressants. Respir Physiol 11:113–126

Cherniak NS, Euler C v, Homma I, Kao FF (1978a) Some effects of graded changes in central chemoreceptor input by local temperature changes on the ventral surface of medulla. In: Fitzgerald RS, Gautier H, Lahiri S (eds) The regulation of respiration during sleep and anesthesia. Adv Exp Med Biol 99:397–402

Cherniak NS, Euler C v, Homma I, Kao FF (1978b) Effects of increased respiratory controller gain during hypoxia and hypercapnia on periodic breathing in cats. In: Fitzgerald RS, Gautier H, Lahiri S (eds) The regulation of respiration during sleep and anesthsia. Adv Exp Med Biol 99:423–431

Cherniak NS, Euler C v, Homma I, Kao FF (1979a) Graded changes in central chemoreceptor input by local temperature changes on the ventral surface of medulla. J Phyisol (Lond) 287:191

Cherniak NS, Euler C v, Homma I, Kao FF (1979b) Animal models of Cheyne-Stokes breathing. Wenner-Gren Cent Int Symp Ser 32:417–425

Cherniak NS, Euler C v, Homma I, Kao FF (1979c) Experimentally induced Cheyne-stokes breathing. Respir Physiol 37:185–200

Chiesa A, Balbi L, Dolcetti A, Motta PE (1970) Un caso di ipoventilazione alveolare primitiva in sogetto non obeso. Minerva Med 61 (33):1755–1761

Clark SL (1934) Innervation of the choroid plexuses and to blood vessels within the central nervous system. J Comp Neurol 60:21–35

Cohen JA, Osterbaan RA (1963) The interaction of acetylcholinesterase with substrate. In: Koelle GB (ed) Cholinesterase and Anticholinesterase agents. Chapter 7, The active side of acetylcholinesterase and related esterases and its reactivity towards substrates and inhibitors. Springer, Berlin Göttingen Heidelberg (Handbuch der experimentellen Pharmakologie XV, S 300–316)

Cohen MI (1968) Discharge patterns of brain-stem respiratory neurons in relation to carbon dioxide tension. J Neurophysiol 31:142–165

Cohen MI (1971) Switching of the respiratory phases and evoked phrenic responses produced by rostral pontine electrical stimulation. J Physiol (Lond) 217:133–158

Cohen MI (1973) Synchronization of discharge, spontaneous and evoked, between inspiratory neurons. Acta Neurobiol Exp (Warsz) 33:189–218

Cohen MI (1979) Neurogenesis of respiratory rhythm in the mammal. Physiol Rev 59: 1105–1173

Cohen MI, Hugelin A (1965) Suprapontine reticular control of intrinsic respiratory mechanisms. Arch Ital Biol 103:317–334

Cohen MI, Piercey MF, Gootman PM, Wolotsky P (1976) Respiratory rhythmicity in the cat. Fed Proc 35:1967–1974
Comroe JH (1943) The effects of direct chemical and electrical stimulation of the respiratory center in the cat. Am J Physiol 139:490–498
Comroe JH (1944) The hyperpnoea of muscular exercise. Physiol Rev 24:319
Comroe JH (1975) Frankenstein, Pickwick, and Ondine. Am Rev Respir Dis 111:689–692
Comroe JH, Schmidt CF (1938) The part played by reflexes from the carotid body in the chemical regulation of respiration in the dog. Am J Physiol 121:75
Comroe JH, Schmidt CF (1943) Reflexes from the limbs as a factor in the hyperpnea of muscular exercise. Am J Physiol 138:536–547
Comroe JH, Bahnson ER, Coates EO (1950) Mental changes occurring in chronically anoxemic patients during oxygen therapy. JAMA 143:1044–1048
Coote JH, McLeod VH (1974) The influence of bulbospinal monoaminergic pathways on sympathetic nerve activity. J Physiol (Lond) 241:453–475
Cozine RA, Ngai SH (1967) Medullary surface chemoreceptors and regulation of respiration in the cat. J Appl Physiol 22:117–121
Cragg P, Patterson L, Purves MJ (1977) The pH of brain extracellular fluid in the cat. J Physiol (Lond) 272:137–166
Cunningham DJC (1963) Regulation of breathing in exercise. Circ Res (Suppl) 1:20–21
Cunningham DJC (1973) The control system regulating breathing in man. Q Rev Biophys 6(4):433–483
Cunningham DJC, Lloyd BB (eds) (1963) The regulation of human respiration. Blackwell, Oxford
Daily WIR, Klaus M, Meyer HBP (1969) Apnea in premature infants: monitoring, incidence, heart rate changes, and an effect of environmental temperature. Pediatrics 43:510–518
Dautrebande L, Maréchal R (1933) L'action d'un nouvel éther de al choline chez le chien. CR Soc Biol (Paris) 113:76
Davies RO (1980) Evidence that neural paths from the caudal and cranial chemoreceptor zones of the ventral medulla converge in the intermediate zone. Proc Int Un Physiol Sci 14:371
Davies RO, Loeschcke HH (1977a) Response of phrenic nerve to electrical stimulation of medullary chemosensitive areas and carotid sinus nerve. Pfluegers Arch 368:R39
Davies RO, Loeschcke HH (1977b) Neural activity evoked by electrical stimulation on the chemosensitive areas on the ventral medullary surface. Proc Int Un Physiol Sci 13:164
Dejours P (1959) La régulation de la ventilation au cours de l'exercise musculaire chez l'homme. J Physiol (Paris) 51:163–261
Dejours P (1962) Chemoreflexes in breathing. Physiol Rev 42:335–358
Dejours P (1963) The regulation of breathing during muscular exercise in man. A neurohumoral theory. In: Cunningham DJC, Lloyd BB (eds) The regulation of human respiration. Blackwell, Oxford, pp 535–547
Dejours P (1964) Control of respiration in muscular exercise. Handb Physiol 1/3: 631–648
Dejours P, Raynaud J, Guenod CL, Labrousse Y (1955) Modifications instantanées de la ventilation au début et à l'arrêt de l'exercise musculaire. Interprétation. J Physiol (Paris) 47:155–159
Del Castillo J, Engbaek L (1954) The nature of neuromuscular block produced by magnesium. J Physiol (Lond) 124:370–384
Dell P, Bonvallet M (1954) Contrôle direct et réflexe de l'activité du système réticulé activateur ascendant du tronc cérébral par l'oxygène et le gaz carbonique du sang. CR Soc Biol 148:855–858
Dembowski K, Lackner K, Czachurski Z, Seller H (in press) Tonic catecholaminergic inhibition of the spinal somato-sympathetic reflex with origin in the ventro-lateral medulla oblongata. J Auto Nerv Syst

Deonna T, Arczynska W, Torrado A (1974) Congenital failure of automatic ventilation (Ondine's curse). J Pediatr 84(5):710–714
Dermietzel R (1976) Central chemosensitivity, morphological studies. In: Loeschcke HH (ed) Acid-base homeostasis of the brain extracellular fluid and the respiratory control system. Thieme, Stuttgart, pp 52–66
Dermietzel R, Luber J, Leibstein A, Schlaefke ME, Kille JF, See WR (1974) Morphology of central chemosensitivity. Proc Int Un Physiol Sci 11:88
Dermietzel R, Leibstein A, Willenberg I, See WR, Schlaefke ME (1977) In vivo labelling of neurons in the chemosensitive areas of the ventral surface of the medulla oblongata with horse-radish peroxidase. Proc Int Un Physiol Sci 13:180
Dermietzel R, Willenberg IM, Leibstein AG (1978) In vivo labelling of neurons in the chemosensitive fields of the ventral surface of the medulla oblongata. Drug Res 28/II, 121:2366
Dev NB, Loeschcke HH (1979a) Topography of the respiratory and circulatory responses to Acetylcholine and Nicotine on the ventral surface of the medulla oblongata. Pfluegers Arch 379:19–27
Dev NB, Loeschcke HH (1979b) A cholinergic mechanism involved in the respiratory chemosensitivity. Pfluegers Arch 379:29–36
Dikshit BB (1934) Action of acetylcholine on the brain and its occurrence therein. J Physiol (Lond) 80:409–421
Doll E, Kuhlo W, Steiner H, Keul J (1968) Zur Genese des Cor pulmonale beim Pickwick-Syndrom. Dtsch Med Wochenschr 49:2361–2364
Drachman DB, Gumnit RJ (1962) Periodic alteration of consciousness in the "pickwickian" syndrome. Arch Neurol 6:63–69
Dripps RD, Dumke PR (1943) The effect of narcotics on the balance between central and chemoceptor control of respiration. J Pharmacol 77:290–299
Dripps RD, Severinghaus JW (1955) General anaesthesia and respiration. Physiol Rev 35:741–777
Duffin J (1971) The chemical regulation of ventilation. Anaesthesia 26:142–154
Dundee JW, Black GW, Nicholl RM (1962) Alteration in response to somatic pain associated with anaesthesia. Br J Anaesth 34:24–30
Duron B (1972) La fonction respiratoire pendant le sommeil physiologique. Bull Eur Physiopathol Respir 8:1031–1057
Duron B (ed) (1976) Respiratory centers and afferent systems. INSERM, Paris
Edery H, Guertzenstein PG (1974) A central vasodepressor effect of dyflos. Br J Pharmacol 50:481–487
Elmqvist D, Feldmann DS (1965) Mammalian neuromuscular junction in tetrodotoxin. Acta Physiol Scand 64:475–476
Errington ML, Dashwood MR (1979) Projections to the ventral surface of the cat brainstem demonstrated by horseradish peroxidase. Neurosci Lett 12:153–158
Euler C v (1976) Origin of the respiratory rhythm. Verh Ges Lungen- und Atmungsforschg 6:21–32
Euler C v (1977) The functional organization of the respiratory phase-switching mechanisms. Fed Proc 36:2375–2380
Euler C v (1979) On the neural organization of the motor control of the diaphragma. Amer Rev Respir Dis 119:45–50
Euler C v, Soederberg U (1952a) Medullary chemosensitive receptors. J Physiol (Lond) 118:545–559
Euler C v, Soederberg U (1952b) Slow potentials in the respiratory centers. J Physiol (Lond) 118(4):555–564
Euler C v, Trippenbach T (1976) On the respiratory phase-switching mechanisms. In: Duvon B (ed) Respiratory centers and afferent systems. INSERM, Paris, pp 1–18
Euler C v, Lagercrantz H (1979) Central nervous control mechanisms in breathing. Wenner-Gren Cent Int Symp Ser 32:1–508

Euler C v, Herrero F, Wexler I (1970) Control mechanisms determining rate and depth of respiratory movements. Respir Physiol 10:93–108
Euler C v, Marttila I, Remmers JE, Trippenbach T (1976) Effects of lesions in the parabrachial nucleus on the mechanisms for central and reflex termination of inspiration in the cat. Acta Physiol Scand 96:324–337
Euler US v, Liljestrand G (1940) The effect of carotid sinus denervation on respiration during rest. Acta Physiol Scand 1:93
Eyzaguirre C, Koyano H (1965) Effect of some pharmacological agents on chemoreceptor discharges. J Physiol (Lond) 178:410–437
Eyzaguirre C, Zapata P (1968) A discussion of possible transmitter or generator substances in carotid body chemoreceptors. In: Torrance RW (ed) Arterial chemoreceptors. Blackwell, Oxford, pp 213–251
Fagenholtz SA, O'Connell K, Shannon DC (1976) Chemoreceptor function and sleep state in apnea. Pediatrics 58:31–36
Feldberg W (1976) The ventral surface of the brain stem: a scarcely explored region of pharmacological sensitivity. Neuroscience 1:427–441
Feldberg W (1980) Cardiovascular effects of drugs acting on the ventral surface of the brain stem. In: Koepchen HP, Hilton SM, Trzebski A (eds) Central interaction between respiratory and cardiovascular control systems. Springer, Berlin Heidelberg New York, pp 45–55
Feldberg W, Guertzenstein PG (1972) A vasodepressor effect of pentobarbitone sodium. J Physiol (Lond) 224:83–103
Feldberg W, Guertzenstein PG (1976) Vasodepressor effects obtained by drugs acting on the ventral surface of the brain stem. J Physiol (Lond) 258:337–355
Feldberg W, Rocha de Silva M Jr (1978) Vasopressin release produced in anaesthetized cats by antagonists of GABA and glycine. Br J Pharmacol 62:99–106
Feldberg W, Wei W (1977) The central origin and mechanism of cardiovascular effect of morphine as revealed by naloxone in cats. J Physiol (Lond) 272:99–100
Fencl V (1971) Distribution of H^+ and HCO_3^- in cerebral fluids. In: Siesjoe OK, Sørensen SC (eds) Ion homeostasis of the brain. Munksgaard, Kopenhagen, pp 175–195
Fencl V, Miller TB, Pappenheimer JR (1966) Studies on the respiratory response to disturbances of acid-base balance, with deductions concerning the ionic composition of cerebral interstitial fluid. Am J Physiol 210:459–472
Fenner A, Schalk U, Hoenicke H, Wendenburg A, Roehling T (1973) Periodic breathing in premature and neonatal babies: incidence, breathing pattern, respiratory gas tensions, response to changes in the composition of ambient air. Pediatr Res 7:174–183
Finer NN, Abroms IF, Taeusch MW Jr (1976) Ventilation and sleep states in newborn infants. J Pediatr 89:100–108
Fishman AP, Goldring RM, Turino GM (1966) General alveolar hypoventilation: a syndrome of respiratory and cardiac failure in patients with normal lungs. Q J Med 35(138):261–275
Fishman LS, Samson JH, Sperling DR (1965) Primary alveolar hypoventilation syndrome (Ondine's curse). Am J Dis Child 110:155–161
Fitzgerald RS, Parks DC (1971) Effect of hypoxia on carotid chemoreceptor response to carbon dioxide in cats. Respirat Physiol 12:218–229
Fitzgerald RS, Gautier H, Lahiri S (eds) (1978) The respiratory regulation during sleep and anaesthesia. Adv Exp Med Biol 99:1–448
Flandrois R, Lacour JR, Eclache JP (1974a) Control of respiration in exercising dog: Interaction of chemical and physical humoral stimuli. Respir Physiol 21:169–181
Flandrois R, Guerin JC, Kalb JC (1974b) Le controle chémoréflexe de la ventilation chez le malade hypoxique chronique. Poumon coeur 30(4):301–311
Fleischhauer K, Petrovický R (1968) Über den Bau der Wandungen des Aquaeductus cerebri und des IV. Ventrikels der Katze. Z Zellforsch 88:113–125
Florez J, Borison HL (1969) Effect of central depressant drugs on respiratory regulation in the decerebrate cat. Respir Physiol 6:318–329

Florez J, Borison HL (1971) Tidal volume in CO_2 regulation: peripheral denervations and ablation of area postrema. Am J Physiol 212:985–991
Folgering H, Kuyper F, Kille JF (1979) Primary alveolar hypoventilation (Ondine's curse syndrome) in an infant without external arcuate nucleus. Case report. Bull Physico-Pathol Resp 15/4:659–666
Frantz ID III, Adler SM, Thach BT, Taeusch HW Jr (1976) Maturational effects on respiratory responses to carbon dioxide in premature infants. J Appl Physiol 41: 41–45
Frédéric QL (1901) Sur la cause de l'apnée. Arch Biol (Paris) 17:561–576
Fruhmann G, Bloemer H, Kolb P (1961) Primäre alveolare Hyperventilation infolge einer Funktionsstörung des Atemzentrums unbekannter Genese. Klin Wochenschr 39/12:618–625
Fukuda Y, Honda Y (1975) pH-sensitive cells at ventrolateral surface of the rat medulla oblongata. Nature New Biol 256:317–318
Fukuda Y, Loeschcke HH (1977) Effect of H^+ on spontaneous neuronal activity in the surface layer of the rat medulla oblongata in vitro. Pfluegers Arch 371:125–134
Fukuda Y, Loeschcke HH (1979) A cholinergic mechanism involved in the neuronal exciation by H^+ in the respiratory chemosensitive structures of the ventral medulla oblongata of rats in vitro. Pfluegers Arch 379:125–135
Fukuda Y, Honda Y, Schlaefke ME, Loeschcke HH (1978) Effect of H^+ on the membrane potential of silent cells in the ventral and dorsal surface layers of the rat medulla in vitro. Pfluegers Arch 376:229–235
Fukuda Y, See WR, Schlaefke ME, Loeschcke HH (1979) Chemosensitivity and rhythmic activity of neurons in the ventral surface layer of the rat medulla oblongata in vitro and in vivo. Pfluegers Arch 379:50
Gabriel M, Albani M (1976) Cardiac slowing and respiratory arrest in preterm infants. Pediatrics 57:257–261
Gabriel M, Albani M, Schulte FJ (1976) Apnoeic spells and sleep states in preterm infants. Pediatrics 57:142–147
Gautier H (1976a) Pattern of breathing during hypoxia or hypercapnia of the awake or anaesthetized cat. Respir Physiol 27:193–206
Gautier H (1976b) Respiratory activity and peripheral chemosensitivity in the cat. In: Duron B (ed) Respiratory centers and afferent systems. INSERM, Paris, pp 221–229
Geis GS, Wurster RD (1980) Cardiac responses during stimulation of the dorsal motor nucleus and nucleus ambiguus in the cat. Circ Res 46:606–611
Geisler LS (1971) Das Pickwick-Syndrom. Dtsch Med Wochenschr 5:212–216
Geisler LS , Herberg D, Schönthal H (1967) CO_2-Antwortkurven bei Patienten mit Pickwick-Syndrom. Verh Dtsch Ges Inn Med 73:864–868
Gellhorn E (1953) On the physiological action of carbon dioxide on cortex and hypothalamus. Electroencephalogr Clin Neurophysiol 5:401–413
Gerardy W, Herberg D, Kuhn HM (1960) Chronische primäre alveoläre Hypoventilation. Klin Wochenschr 38/12:583–589
Gesell R (1923) On the chemical regulation of respiration. I. The regulation of respiration with special reference to the metabolism of the respiratory center etc. Am J Physiol 66:5
Gesell R (1925) The chemical regulation of respiration. Physiol Rev 5:551
Gesell R (1939) Respiration and its adjustments. Am Rev Physiol 1:185–216
Gesell R (1940) A neurophysiological interpretation of the respiratory act. Erg Physiol 43:477–639
Gesell R, Frey JS (1950) Temporal summation of stimuli studied with the aid of anticholinesterases. Am J Physiol 160:375–384
Gesell R, Hansen ET, Worzniak JJ (1942/43) Humoral intermediation of nerve cell activation in the central nervous system. Am J Physiol 138:776–791
Gesell R, Hansen ET (1945) cH and nervous integration. Am J Physiol 144:126–163

Gill PK, Kuno M (1963) Properties of phrenic motoneurones. J Physiol (Lond) 168: 258–273
Giroud M, Flandrois R, Calamai M, Buffat JJ, Quiriger P, Mouret JR, Fisher C (1974) L'hypoventilation alveolaire primaire "Malediction d'Ondine". Poumon Coeur 30/2:101–108
Granholm L (1973) Pulmonary ventilation in neurological disorders. Bull Physio-Pathol Resp 9/3:593–602
Granholm L, Pontén U (1969) The in vivo CO_2 buffer curve of the intracellular space of cat cerebral cortex. Acta Neurol Scand 45:493–501
Grant JL, Arnold W (1968) Idiopathic hypoventilation. JAMA 194:119–122
Gray EG (1959) Axosomatic and axodendritic synapses of the cerebral cortex: an electron microscope study. J Anat 94:345–356
Gray JS (1950) Pulmonary ventilation and its physiological regulation. Thomas, Springfield
Grodinski M. Beber M, Backer CP (1933) A test for the response of novocaine in nervous tissue. Science 77:450
Grossmann RG, Hampton T (1968) Depolarization of cortical glial cells during electrocortical activity. Brain Res 11:316–324
Guazzi M (1969) Importanza di reflezzi zeno-aortici nel controllo di pH, pO_2 e pCO_2 arteriozi durante la veglia e il zonno. Bol Soc Ital Biol Sper 45/2:108–111
Guazzi M, Freis E (1969) Sino-aortic reflexes and arterial pH, and P_{CO_2} in wakefulness and sleep. Am J Physiol 217/6:1623–1627
Guertzenstein PG (1973) Blood pressure effects obtained by drugs applied to the ventral surface of the brain stem. J Physiol (Lond) 229:395–408
Guertzenstein PG, Silver A (1974) Fall in blood pressure produced from discrete regions of the ventral surface of the medulla by glycine and lesions. J Physiol (Lond) 242:489–503
Guenard H, Chambille B, Bargeton D (1976) Characteristics of the regulation of respiration during moderate muscular exercise. In: Loeschcke HH (ed) Acid base homeostasis of the brain extracellular fluid and the respiratory control system. Thieme, Stuttgart, pp 203–209
Guilleminault CR, Eldridge FL, Dement WC (1974) Human pathology – sleep – induced apneas and cardiovascular changes. Bull Physio-Pathol Resp 10/2:244–247
Guilleminault CR, Peraita R, Souquet M, Dement WC (1975) Apneas during sleep in infants: possible relationship with sudden infants death syndrome. Science 190: 677–679
Guthrie RD, Standaert TA, Hodson WA, Woodrum DE (1980) Sleep and maturation of encapnic ventilation and CO_2 sensitivity in the premature primate. J Appl Physiol 48/2:347–354
Guz A (1975) Regulation of respiration in man. Am Rev Physiol 37:303–323
Guz A, Noble MJM, Widdicombe JG, Trenchard DW, Mushin WW (1966) The effect of bilateral block of vagus and glossopharyngeal nerves on the ventilatory response to CO_2 of conscions man. Respir Physiol 1:206–210
Haddad G, Epstein R, Epstein M, Leistner H, Mariono P, Mellins R (1979) Maturation of ventilatory pattern in normal sleeping infants. J Appl Physiol 46:998–1002
Haldane JS, Priestley JG (1905) The regulation of the lung ventilation. J Physiol (Lond) 32:225–266
Hall M (1837) Memoirs on the nervous system. Sherwood, Gilbert & Piper, London
Hasselmeyer EG, Hunter JC (1975) The sudden infant death syndrome. Obstet Gynecol Annu 4:213–236
Hathorn MDS (1974) The rate and depth of breathing in newborn infants in different sleep states. J Physiol (Lond) 243:101–113
Heeringa J (1979) Peripheral chemoreceptors and the ventilatory response to CO_2 during hyperoxia. Dissertation, University Leiden

Heinemann H, Goldring R (1974) Bicarbonate and the regulation of ventilation. Am J Med 57:361–370

Hesser CM (1949) Central and chemoreflex components in the respiratory activity during acid-base displacements in the blood. Acta Physiol Scand (Suppl) 18:64

Heymans C (1951) Chemoreceptors and regulation of respiration. Acta Physiol Scand 22:4–13

Heymans C, Bouckaert JJ (1930) Sinus caroticus and respiratory reflexes. I. Cerebral blood flow and respiration adrenaline apnoea. J Physiol 69:254

Heymans C, Bouckaert JJ (1939) Les chémo-récepteurs du sinus carotidien. Erg Physiol 41:28–55

Heymans C, Bouckaert JJ, Farber S, Hsu FJ (1936) Influence réflexogène de l'acéthylcholine sur les terminaisons nerveuses chimiosensitives du sinus caroticien. Arch Int Pharmacodyn Ther 54:129

Heymans JF, Heymans C (1927) Sur les modifications directes et sur la régulation réflexe de l'activité du centre respiratoire de la lête isolée du chien. Arch Int Pharmacodyn Ther 33:273

Heymans JF, Bouckaert JJ, Dautrebande L (1931) Sinus carotidien et réflexes respiratoires. II. Sensibilité de sinus carotidien au substances chimiques etc. Arch Int Pharmacodyn Ther 40:54

Hoover DB, Muth EA, Jacobowitz DM (1978) A mapping of the distribution of acetylcholine, choline acetyltransferase and acetylcholinesterase in discrete areas of rat brain. Brain Res 153:295–306

Hori T, Roth GI, Yamamoto WS (1970) Respiratory sensitivity of rat brain-stem surface to chemical stimuli. J Appl Physiol 6:721–724

Hubbard JI, Jones SF, Landau EM (1968) On the mechanism by which calcium and magnesium affect the spontaneous release of transmitter from mammalian motor nerve terminals. J Physiol (Lond) 194:355–380

Hugelin A (1977a) Anatomical organization of bulbopontine respiratory oscillators. Fed. Proc 36:2390–2394

Hugelin A (1977b) L'organisation des oscillateurs respiratoires. J Physiol (Paris) 73: 511–521

Hugelin A, Cohen MI (1963) The reticular activating system and respiratory regulation in the cat. Ann NY Acad Sci 109:568–603

Hukuhara T Jr (1973) Neuronal organization of the central respiratory mechanism in the brain stem of the cat. Acta Neurobiol Exp (Warsz) 33:219–244

Hukuhara T Jr (1974) Functional organization of brain stem respiratory neurons and rhythmogenesis. In: Umbach W, Koepchen HP (eds) Central rhythmic and regulation, circulation, respiration, extrapyramidal motor system. Hippokrates, Stuttgart, pp 35–52

Hukuhara T Jr (1976) Functional organization of brain stem respiratory neruones and its modulation induced by afferences. In: Duron B (ed) Respiratory centres and afferent systems. INSERM, Paris, pp 41–53

Hukuhara T Jr, Saji Y, Kumadaki N, Kojima H, Tamaki H, Takeda R, Sakai F (1969) Die Lokalisation von atemsynchron entladenden Neuronen in der retikulären Formation des Hirnstammes der Katze unter verschiedenen experimentellen Bedingungen. Naunyn Schmiedebergs Arch Pharmakol 263:462–484

Hukuhara T Jr, See WR, Fritsche P, Schlaefke ME (1976) Respiratory drives in the ascending reticular activating system. Pfluegers Arch 365:39

Hukuhara T Jr, Goto K, Kiguchi Y, Kazuo T (1979) Unterschiedliche Stabilität respiratorischer Einzelneuronenaktivität im Hirnstamm der Katze. Jikeikai Med J 26: 245–261

Hunt CE, Matalon SV, Thompson TR, Demuth S, Loew JM, Liu HH, Mastri A, Burke B (1978) Central hypoventilation syndrome. Experience with bilateral phrenic nerve pacing in 3 neonates. Am Rev Respir Dis 118:23–28

Hutter OF, Kostial K (1954) Effect of magnesium and calcium ions on the release of acetylcholine. J Physiol (Lond) 124:234–241
Ingvar DH, Buelow KB (1963) Respiratory regulation in sleep. Ann NY Acad Sci 109/2: 870–881
Ivanov YN (1963) Changes in electrical activity of different brain regions in cats and dogs exposed to carbon dioxide. Fed Proc 22:13–18
Jacobs MH (1920a) To what extent are the physiological effects of carbon dioxide due to the hydrogen ions? Am J Physiol 51:321–331
Jacobs MH (1920b) Production of intracellular acidity by neutral and alkaline solutions containing carbon dioxide. Am J Physiol 53:457
Jones DR, Purves MJ (1970) The effect of carotid body denervation upon the respiratory response to hypoxia and hypercapnia in the duck. J Physiol (Lond) 211:295–310
Jordan D, Spyer KM (1979) The effects of acetylcholine on respiratory neurones of the nucleus retroambigualis. J Physiol (Lond) 300:37–38P
Jung R (1965) Physiologie und Pathophysiologie des Schlafes. Verh Dtsch Ges Inn Med 7:787–797
Jung R, Kuhlo W (1965) Neurophysiological studies of abnormal night sleep and the Pickwickian syndrome. Progr Brain Res 18:141–159
Jurna I, Soederberg U (1963) The effect of carbon dioxide, anesthetics and strychnine on jaw reflexes. Arch Int Pharmacodyn Ther 142:323–338
Kaemmerer E, Dolce G (1967) Pickwickian-Syndrom. Münch Med Wochenschr 109: 220–223
Kafer ER, Leigh J (1972) Recurrent respiratory failure associated with the absence of ventilatory response to hypercapnia and hypoxemia. Am Rev Resp Dis 106:100–103
Kao FF, Wang CW, Mei SF, Michel CC (1965) The relationship of exercise hyperpnea to csf-pH. In: Brooks C McC, Kao FF, Lloyd BB (eds) Cerebrospinal fluid and the regulation of respiration. Blackwell, Oxford, pp 269–275
Kao FF, Mei SS, Kalia M (1979) Interaction between neurogenic exercise drive and chemical drive. Wenner-Gren Cent Int Symp Ser 32:75–90
Karahashi Y, Goldring S (1966) Intracellular potentials from 'Idle' cells in cerebral cortex of cat. Electroencephalogr Clin Neurophysiol 20:600–607
Karczewski WA (1974) Organization of the brain stem respiratory complex. In: Guyton AC, Widdicombe JG (eds) MTP international review of science, physiology series. I. Respiratory physiology I. University Park Press, Baltimore, pp 197–219
Karczewski WA, Widdicombe JG (1973) Neural control of breathing. Acta Neurobiol Exp (Warsz) 33/1:1–432
Katsaros B (1965) Die Rolle der Chemorezeptoren des Carotisgebietes der narkotisierten Katze für die Antwort der Atmung auf isolierte Änderung der Wasserstoffionenkonzentration und des CO_2-Druckes des Blutes. Pfluegers Arch 282:157–178
Katz B, Miledi R (1963) A study of spontaneous miniature potentials in spinal motoneurones. J Physiol (Lond) 168:389–422
Katzman R, Graziani L, Ginsburg S (1968) Cation exchange in blood, brain and csf. In: Lajtha A, Ford DH (eds) Brain barrier systems, vol 29, Progress in brain research. Elsevier, Amsterdam London New York, pp 283–294
Kelly JP, van Essen DC (1974) Cellstructure and function in the visual cortex of the cat. J Physiol (Lond) 238:515–547
Kille JF (1969) Ergebnisse von Gefäßfüllungen mit Plastogen G an Nieren verschiedener Tierarten. Praeparator 15(3/4):129–136
Kille JF, Schlaefke ME (1974) Histological investigations in the chemosensitive areas on the ventral surface of the medulla oblongata. Pfluegers Arch 347:38
Kille JF, Schlaefke ME (1978) Histological studies on the medullary chemosensitive areas after chronic denervation of a carotid body. Neurosci Lett (Suppl) 1:16

Kim JK, Carpenter FG (1961) Excitation of medullary neurons by chemical agents. Am J Physiol 201/6:1187–1190

Kirsten EB, Satayavivad J, John WMSt, Wang SC (1978) Alteration of medullary respiratory unit discharge by iontophoretic application of putative neurotransmitters. Br J Pharmacol 63:275–283

Kirstein L (1951) Early effects of oxygen lack and carbon dioxide excess on spinal reflexes. Acta Physiol Scand (Suppl) 23:80

Kiwull-Schoene H, Kiwull P (1979) The role of the vagus nerves in the ventilatory response to lowered P_aO_2 with intact and eliminated carotid chemoreflexes. Pfluegers Arch 381:1–9

Kiwull-Schoene H, Kiwull P, Mueckenhoff K, Both W (1976) The role of carotid chemoreceptors in the regulation of arterial oxygen transport under hypoxia with and without hypercapnia. In: Grote J, Reneau D, Thews G (eds) Oxygen transport to tissue – II. Plenum Press, New York, pp 469–476

Kiwull P, Wiemer W, Schoene H (1972) The role of the carotid chemoreceptors in the CO_2-hyperpnea under hypoxia. Pfluegers Arch 336:171–186

Kiwull P, Kiwull-Schoene H, Klatt H (1976) Interaction of central and peripheral respiratory drives: differentiation between the role of stimuli and afferents. In: Loeschcke HH (ed) Acid-base homeostasis of the brain-extracellular fluid. Thieme, Stuttgart, pp 146–154

Koelle GB (1954) The histochemical localization of cholinesterase in the central nervous system of the cat. J Comp Neurol 100:211–235

Koepchen HP (1976) Quantitative approach to neural control of ventilation. In: Loeschcke HH (ed) Acid-base homeostasis of the brain extracellular fluid and the respiratory control system. Thieme, Stuttgart, pp 164–186

Koepchen HP, Klüssendorf D, Philipp U (1973) Mechanisms of central transmission of respiratory reflex. Acta Neurobiol Exp (Warsz) 33/1:287–300

Koepchen HP, Lazar H, Borchert J (1974) On the role of nucleus infrasolitarius in the determination of respiratory periodicity. In: Proc Int Un physiol sci, vol XI (243):81

Koepchen HP, Borchert J, Frank Ch, Klüssendorf D, Kolbe K, Sommer D (1976a) Respiratory centre input-output relations in response to different afferences. In: Duron B (ed) Respiratory centres and afferent systems. INSERM, Paris, pp 73–84

Koepchen HP, Kolbe K, Klüssendorf D, Schroeter R, Schwanghart F (1976b) CO_2 and the brain stem respiratory complex. Bull Physio-Pathol Respir 12/6:216–217

Koepchen HP, Klüssendorf D, Borchert J, Lessmann DW, Dinter A, Frank Ch, Sommer D (1977) Type of respiratory neuronal activity pattern in reflex control of ventilation. In: Paintal AS, Gill Kumar P (eds) Respiratory adaptation, capillary exchanges and reflex mechanisms. Navchetan Press, New Dehli, pp 291–311

Koepchen HP, Sommer D, Frank Ch, Klüssendorf D, Krämer A, Rosin P, Forstreuter K (1979) Characteristics and functional significance of the expiratory bulbar neuron pools. Wenner-Gren Cent Int Symp Ser 32:217–232

Koepchen HP, Hilton SM, Trzebski A (eds) (1980) Central interactions between respiratory and cardiovascular control systems. Springer, Berlin Heidelberg New York

Krauss AN, Klain DB, Waldman S, Auld PAM (1975) Ventilatory response to carbon dioxide in newborn infants. Pediatr Res 9:46–50

Krauss A, Solomon GE, Auld PAM (1977) Sleep state, apnea and bradycardia in preterm infants. Dev Med Child Neurol 19:160–168

Krieger AJ (1973) Respiratory failure after ventral spinal surgery: A clinical and experimental study. J Surg Res 14:512–517

Krnjević K (1974) Chemical nature of synaptic transmission in vertebrates. Physiol Rev 54:418–540

Krnjević K, Schwartz S (1967) Some properties of unresponsive cells in the cerebral cortex. Exp Brain Res 3:306–319

Krnjević K, Randić M, Siesjoe B (1965) Cortical CO_2 tension and neuronal excitability. J Physiol (Lond) 176:105–122

Krogh A, Lindhard J (1913) The regulation of respiration and circulation during the initial stages of muscular work. J Physiol 47:112–136
Kronenberg RS, Cain SM (1968) Effects of acetazolamide and hypoxia on cerebrospinal fluid bicarbonate. J Appl Physiol 24:17–20
Kuffler SW, Nicholls JG (1966) The physiology of neuroglial cells. Erg Physiol Biol Chem Exp Pharmacol 57:1–90
Kuffler SW, Nicholls JG (1976) From neuron to brain: A cellular approach to the function of the nervous system. Sinauer Assoc, Sunderland, MA, pp 255–288
Kuhlo W (1968) Neurophysiologische und klinische Untersuchungen beim Pickwick-Syndrom. Arch Psychiatr Nervenkr 211:170–192
Lambertsen CJ, Semple SJG, Smyth MG, Gelfand R (1961) H^+ and pCO_2 as chemical factors in respiratory and cerebral circulatory control. J Appl Physiol 16:473–484
Landgren S, Liljestrand G, Zotterman Y (1952) The effect of certain autonomic drugs on the action potentials of the sinus nerve. Acta Physiol Scand 26:264
Leibstein AG (1979) Vergleichende elektronenmikroskopische Untersuchungen der chemosensiblen Areale an der ventralen Oberfläche der Medulla oblongata. Dissertation, Gesamthochschule Essen
Leibstein A, Luber J, Dermietzel R, Brettschneider H, Schlaefke ME, See WR (1975) Elektronenoptische Untersuchungen eines für die Regulation der Atmung bedeutsamen Gebietes der Medulla oblongata. Verh Anat Ges 69:601–606
Leonhardt H (1967) Über die Blutkapillaren und perivaskulären Strukturen der area postrema des Kaninchens und über ihr Verhalten im Pentamethylentetrazol-(Cardiazol)-Krampf. Z Zellforsch Mikroskop Anat 76:511–524
Leonhardt H (1970) Subependymale Basalmembranlabyrinthe im Hinterhorn des Seitenventrikels des Kaninchengehirns. Z Zellforsch Mikroskop Anat 105:595–604
Leusen IR (1954a) Chemosensitivity of the respiratory center. Influence of CO_2 in the cerebral ventricles on respiration. Am J Physiol 176:39–44
Leusen IR (1954b) Chemosensitvity of the respiratory center. II. Influences of changes in the H^+- and total buffer concentrations in the cerebral ventricles on respiration. Am J Physiol 176:45–51
Leusen IR (1972) Regulation of CSF composition with reference to breathing. Physiol Rev 52:1–56
Liljestrand Å (1953) Respiratory reactions elicited from medulla oblongata of the cat. Acta Physiol Scand (Suppl 106) 29:321–393
Liljestrand Å (1958) Neural control of breathing. Physiol Rev 38:691–708
Liljestrand G, Zotterman Y (1954) The effect of certain choline esters on the chemoreceptor activity in the carotid body. Acta Physiol Scand 31:203
Lipscomb WT, Boyarski LL (1972) Neurophysiological investigations of medullary chemosensitive areas of respiration. Respir Physiol 16:362–376
Lipski J, McAllen RM, Spyer KM (1975) The sinus nerve and baroreceptor input to the medulla of the cat. J Physiol (Lond) 251:61–78
Loeschcke HH (1957) Intracraniale Chemoreceptoren mit Wirkung auf die Atmung? Helv Physiol Pharmacol Acta 15:C25–C26
Loeschcke HH (1960) Beziehung zwischen CO_2 und Atmung. Anaesthesist 9:38–46
Loeschcke HH (1965) A concept of the role of intracranial chemosensitivity in respiratory control In: Brooks C McC, Kao FF, Lloyd BB (eds) Cerebrospinal fluid and the regulation of ventilation. Blackwell, Oxford, pp 183–210
Loeschcke HH (1969) On specificity of CO_2 as a respiratory stimulus. Bull Physio-Pathol Respir 5:13–25
Loeschcke HH (1971) DC potentials between CSF and blood. In: Siersjö BK, Sørensen SC (eds) Ion homeostasis of the brain. Alfred Benzon Symposium III. Munksgaard, Copenhagen, pp 77–96
Loeschcke HH (1972) Der Säure-Basenstatus des Liquor cerebrospinalis und seine Regulation durch die Lungenventilation. Klin Wochenschr 50:581–593

Loeschcke HH (1973a) Control of the acid base status of brain extracellular fluid by the ventilation. Clin Physiol (Jpn) 3:156–169
Loeschcke HH (1973b) Respiratory adaptations to changes in the acid base status of CSF and brain. Bull Physio-Pathol Respir 9:647–656
Loeschcke HH (1973c) Respiratory chemosensitivity in the medulla oblongata. Acta Neurobiol Exp (Warsz) 33:97–112
Loeschcke HH (1973d) The respiratory control system: analysis of steady state solutions for metabolic and respiratory acidosis-alkalosis and increased metabolism. Pfluegers Arch 341:23–42
Loeschcke HH (1974a) Central nervous chemoreceptors. In: Widdicombe JG (ed) Physiology, Series One, vol 2, Respiration physiology. Butterworths, London, pp 167–196
Loeschcke HH (1974b) Regulation of the extracellular pH of the brain by the lung ventilation. In: Umbach W, Koepchen HP (eds) Central rhythmic and regulation. Hippokrates, Stuttgart, pp 85–92
Loeschcke HH (ed) (1976) Acid base homeostasis of the brain extracellular fluid and the respiratory control system. Thieme, Stuttgart
Loeschcke HH (1977) Neuere Aspekte der Atmungsregulation. Wiener Klin Wochenschr 89/13:429–435
Loeschcke HH (1979) Functional aspects of central chemosensitivity. Wenner-Gren Cent Int Symp Ser 32:13–24
Loeschcke HH, Ahmad HR (1980) Transients and steady state of chloride-bicarbonate relationship of brain extracellular fluid. In: Bauer C, Gros G, Bartels H (eds) Biophysics and physiology of carbon dioxide. Springer, Berlin Heidelberg New York, pp 439–448
Loeschcke HH, Koepchen HP (1958a) Beeinflussung von Atmung und Vasomotorik durch Einbringen von Novocain in die Liquorräume. Pfluegers Arch 266:611–627
Loeschcke HH, Koepchen HP (1958b) Über das Verhalten der Atmung und des arteriellen Druckes bei Einbringen von Veratridin, Lobelin und Cyanid in den Liquor cerebrospinalis. Pfluegers Arch 266:586–610
Loeschcke HH, Koepchen HP (1958c) Versuch zur Lokalisation des Angriffsortes der Atmungs- und Kreislaufwirkung von Novocain im Liquor cerebrospinalis. Pfluegers Arch 266:628–641
Loeschcke HH, Schlaefke ME (1976) Central chemosensitivity. In: Paintal AS (ed) Morphology and mechanisms of chemoreceptors. Vallabhbahai Patel Chest Institute, New Delhi, pp 282–298
Loeschcke HH, Sugioka K (1969) pH of cerebrospinal fluid in the cisterna magna and on the surface of the choroid plexus of the 4th ventricle and its effect on ventilation in experimental disturbances of acid-base balance. Pfluegers Arch 312:161–188
Loeschcke HH, Koepchen HP, Gertz KH (1958) Über den Einfluß der Wasserstoffionenkonzentration und CO_2-Druck im Liquor cerebrospinalis auf die Atmung. Pfluegers Arch 266:569–585
Loeschcke HH, De Lattre J, Schlaefke ME, Trouth CO (1970) Effects on respiration and circulation of electrically stimulating the ventral surface of the medulla oblongata. Respir Physiol 10:184–197
Loeschcke HH, Schlaefke ME, See WR, Herker-See A (1979) Does CO_2 act on the respiratory centers? Pfluegers Arch 381:249–254
Loewy AD, McKellar S (1980) The neuroanatomical basis of central cardiovascular control. Fed Proc 39:2495–2503
Luber J (1976) Elektronenmikroskopische Untersuchungen eines für die Regulation der Atmung bedeutsamen Gebietes der Medulla oblongata der Katze, rostromedial des Austritts des Nervus hypoglossus. Dissertation, Gesamthochschule Essen
Lüllmann H, Peters T (1967) Influence of pH on the action of parasympathomimetic drugs. Eur J Pharmacol 2:106–111

Lugaresi E, Coecagna G, Ceroni B (1968) Syndrome de Pickwick et syndrome d'hypoventilation alvéolaire. Act Neurol Belg 68:15–29
Lugaresi E, Coecagna C, Mantovani M (1972) Hypersomnia with periodic breathing: Periodic apneas and alveolar hypoventilation during sleep. Bull Physio-Pathol Respir 8:1103–1113
MacIntosh FC, Perry WLM (1950) Biological estimation of acetylcholine. Methods Med Res 3:76–92
Majcherczyk S, Willshaw P (1973) Inhibition of peripheral chemoreceptor activity during superfusion with an alkaline c.s.f. of the ventral brainstem surface of the cat. J Physiol (Lond) 231:26–27P
Majcherczyk S, Willshaw P (1977) The influence of hyperventilation on efferent control of peripheral chemoreceptors. Brain Res 124:561–564
Majcherczyk S, Willshaw P (1980) Central chemosensitive mechanisms involved in a feedback control of peripheral chemoreceptors. In: Koepchen HP, Hilton SM, Trzebski A (eds) Central interaction between respiratory and cardiovascular control systems. Springer, Berlin Heidelberg New York, pp 76–81
Maren TH, Broder LE (1970) The role of carbonic anhydrase in anion secretion into cerebrospinal fluid. J Pharmacol Exp Ther 172:197–202
Marino PL, Lamb TW (1975) Effects of CO_2 and extracellular H^+ iontophoresis on single cell activity in the cat brainstem. J Appl Physiol 38/4:688–695
Marshall EK Jr, Rosenfeld M (1936) Depression of respiration by oxygen. J Pharmacol Exp Ther 57:437–457
McCloskey PI, Mitchell JH (1972) Reflex cardiovascular and respiratory responses originating in exercising muscle. J Physiol (Lond) 224:173–186
McLennan H, Hicks TP (1978) Pharmacological characterization of the excitatory cholinergic receptors of rat central neurones. Neuropharmacology 17:329–334
Meier GW, Berger RJ (1965) Development of sleep and wakefulness patterns in the infant rhesus monkey. Exp Neurol 12:257–277
Metz B (1962) Correlation between respiratory reflex and acetylcholine content of pons and medulla. Am J Physiol 202:80–82
Metz B (1966) Hypercapnia and acetylcholine release from the cerebral cortex and medulla. J Physiol (Lond) 186:321–332
Middendorf T, Loeschcke HH (1976a) Analysis of the respiratory control system. In: Loeschcke HH (ed) Acid-base homeostasis of the brain extracellular fluid and the respiratory control system. Thieme, Stuttgart, pp 190–202
Middendorf T, Loeschcke HH (1976b) Mathematische Simulation des Respirationssystems. J Math Biol 3:149–177
Middendorf T, Loeschcke HH (1978) Cooperation of the peripheral and central chemosensitive mechanisms in the control of the extracellular pH in brain in non-respiratory acidosis. Pfluegers Arch 375:257–260
Miledi R, Slater SR (1966) The action of calcium on neuronal synapses in the squid. J Physiol (Lond) 184:473–498
Miller MJ, Tenney SM (1975) Hyperoxic hyperventilation in carotid-deafferentiated cats. Respir Physiol 23:23–30
Mitchell RA (1965) The regulation of respiration in metabolic acidosis and alkalosis. In: Brooks C McC, Kao FF, Lloyd BB (eds) Cerebrospinal fluid and the regulation of respiration. Blackwell, Oxford, pp 109–132
Mitchell RA (1977) Location and function of medullary respiratory neurons. Am Rev Respir Dis (Suppl) 115:209–216
Mitchell RA, Berger AJ (1975) Neural regulation of respiration. Am Rev Respir Dis 111:206–224
Mitchell RA, Herbert DA (1974a) The effect of carbon dioxide on the membrane potential of medullary respiratory neurons. Brain Res 75:345–349
Mitchell RA, Herbert DA (1974b) Synchronized high frequency synaptic potentials in medullary respiratory neurons. Brain Res 75:350–355

Mitchell RA, Massion W, Carman CT, Severinghaus JW (1960) 4th Ventricle respiratory chemosensitivity and the area postrema. Fed Proc 19:374
Mitchell RA, Loeschcke HH, Massion WH, Severinghaus JW (1963a) Respiratory responses mediated through superficial chemosensitive areas on the medulla. J Appl Physiol 18:523–533
Mitchell RA, Loeschcke HH, Severinghaus JW, Richardson BW, Massion WH (1963b) Region of respiratory chemosensitivity on the surface of the medulla. Ann NY Acad Sci 109:661–681
Mitchell RA, Bainton CR, Edelist G (1964) Respiratory response and CSF pH during disturbances in blood acid-base balance in awake dogs with denervated aortic and carotid bodies. Physiologist 7:208
Mitchell RA, Carman CJ, Severinghaus JW, Richardson BW, Singer MM, Schnider S (1965) Stability of cerebrospinal fluid pH in chronic acid-base disturbances in blood. J Appl Physiol 20:443–452
Mueller J (1837) Handbuch der Physiologie des Menschen für Vorlesungen. Zweiten Bandes erste Abtheilung. J Hölscher, Coblenz
Naeye RL (1978) The sudden infant death syndrome. In: Thurlbeck WM (ed) The lung. Structure, function and disease. Williams and Wilkins, Baltimore, pp 262–270
Naeye RL (1980) Sudden infant death. Sci Am 242/4:52–59
Naeye RL, Whalen P, Ryser M, Fisher R (1976) Cardiac and other abnormalities in the sudden infant death syndrome. Am J Pathol 82:1–8
Nakayama S, Baumgarten R v (1964) Lokalisierung absteigender Atmungsbahnen im Rückenmark der Katze mittels antidromer Reizung. Pfluegers Arch 281:231–244
Neil E (1973) Chemical regulation of ventilation. Bull Physio-Pathol Respir 9:587–591
Neil E, O'Regan RG (1969) Effects of sinus and aortic efferents and arterial chemoreflex function. J Physiol (Lond) 200:69
Netick A, Orem J, Dement W (1977) Neuronal activity specific to REM sleep and its relationship to breathing. Brain Res 120:197–207
Ngai SH, Wang SC (1957) Organization of central respiratory mechanisms in the brain stem of the cat: localization by stimulation and destruction. Am J Physiol 190/2: 343–349
Nicholson HC (1936) Localization of the central respiratory mechanisms as studied by local cooling of the surface of the brain stem. Am J Physiol 115:402–409
Nicholson HC, Sobin S (1938) Respiratory effects from the application of cocaine, nicotine, and lobeline of the floor of the fourth ventricle. Am J Physiol 123:766–774
Orem J, Netick A, Dement W (1977) Breathing during sleep and wakefulness in the cat. Respir Physiol 30:265–289
Orem J (1978) Some observations on breathing during sleep in the cat. In: Guilleminault C, Dement WC (eds) Sleep apnea syndromes. Troc Foundat Series vol 11. Liss, New York, pp 65–91
Paintal AS (ed) (1976) Morphology and mechanisms of chemoreceptors. Vallabhbhai Patel Chest Institute, New Delhi
Paintal AS, Gill Kumar P (eds) (1977) Respiratory adaptation, capillary exchange and reflex mechanisms. Vallabhbhai Patel Chest Institute, New Delhi
Pappenheimer JR (1967) The ionic composition of cerebral extracellular fluid and its relation to control of breathing. Harvey Lect 61:71–94
Pappenheimer JR, Fencl V, Hasey SR, Held D (1965) Role of cerebral fluids in the control of respiration as studied in unanaesthetized goats. Am J Phyisol 208:436–450
Perkins JF Jr (1968) The contribution of the peripheral respiratory chemoreceptors to pulmonary ventilation – a historical and experimental approach. In: Torrance RW (ed) Symposium on arterial chemoreceptors. Blackwell, Oxford, pp 335–352
Peskow BJ, Piatin WF (1976) Reactions of neurons of the respiratory center to local cooling of the ventral surface of the medulla oblongata. Sechenov Physiol J UDSSR 62/7

Peskow BJ, Piatin BF, Kulchitsky NA, Pekarskaya TK, Ovchinnikova EV (1980) Functional significance of the intermedial chemosensitive significance of the intermedial chemosensitive area of the medulla oblongata ventrale surface. Proc Int Un Physiol Sci 14:636

Petrovický R (1968) Über die Glia marginalis und oberflächliche Nervenzellen im Hirnstamm der Katze. Z Anat Entw 127:221–231

Pflüger E (1868) Über die Ursache der Atembewegungen sowie der Dyspnoe und Apnoe. Pfluegers Arch 1:61–106

Philipot E (1937) Action réspiratoire et circulatoire sino-carotidienne réflexe de quelques dérivés de la choline. Arch Int Pharmacodyn 57:357

Phillipson EA (1977) Regulation of breathing during sleep. Am Rev Respir Dis (Suppl) 115:217–224

Phillipson EA (1978a) Control of breathing during sleep. Am Rev Respir Dis 118:909–939

Phillipson EA (1978b) Respiratory adaptations in sleep. Annu Rev Physiol 40:133–156

Phillipson EA, Kozar LF, Rebuck AS, Murphy E (1977) Ventilatory and waking responses to CO_2 in sleeping dogs. Am Rev Respir Dis 115:251–259

Phillipson EA, Sullivan CE, Read JC, Murphy E, Kozar LF (1978) Ventilatory and waking responses to hypoxia in sleeping dogs. J Appl Physiol 44/4:512–520

Pitts RF (1940) The respiratory center and its descending pathways. J Comp Neurol 72:605–625

Pitts RF (1946) Organization of the respiratory center. Physiol Rev 26:609–630

Pitts RF, Magoun HW, Ranson SW (1939a) Localization of the medullary respiratory centers in the cat. Am J Physiol 126:673–688

Pitts RF, Magoun HW, Ranson SW (1939b) Interrelations of the respiratory centers in the cat. Am J Physiol 126/3:689–707

Pitts RF, Magoun HW, Ranson SW (1939c) The origin of respiratory rhythmicity. Am J Physiol 127:654–670

Pokorski M (1976) Neurophysiological studies on central chemosensor in medullary ventrolateral areas. Am J Physiol 230:1288–1295

Pokorski M, Schlaefke ME, See WR (1975) Neurophysiological studies on the central chemosensitive mechanism (rostral area). Pfluegers Arch 355:R33

Porter R (1963) Unit responses evoked in the medulla oblongata by vagus nerve stimulation. J Physiol (Lond) 168:717–735

Poulsen T (1952) Investigation into the anaesthetic properties of carbon dioxide. Acta Pharmacol Toxicol (Copenh) 8:30–46

Prill RK (1977) Das Verhalten von Neuronen des caudalen chemosensiblen Feldes in der Medulla oblongata der Katze gegenüber intravenösen Injektionen von $NaHCO_3$ und HCl. Dissertation, Ruhr-Universität, Bochum

Prill RK, Pokorski M, See WR, Schlaefke ME (1975) Neurophysiological studies on the central chemosensitive mechanism (caudal area). Pfluegers Arch 355:33

Prime FJ, Westlake EK (1954) The respiratory response to CO_2 in emphysema. Clin Sci 13:321–332

Rach E (1863) Quo modo medulla oblongata, ut respirandi motus efficiat, incitetur. Inauguraldissertation, Regiomonti

Ranson BR, Goldring S (1973a) Ionic determinants of membrane potential of cells presumed to be glia in cerebral cortex of cat. J Neurophysiol 36:855–868

Ranson BR, Goldring S (1973b) Slow depolarization in cells presumed to be glia in cerebral cortex of cat. J Neurophysiol 36:869–878

Reed DJ, Kellog RH (1958) Changes in respiratory response to CO_2 during natural sleep at sea level and at altitude. J Appl Physiol 13:325

Reed DJ, Kellog RH (1960) Effect of sleep on hypoxic stimulation of breathing at sea level and altitude. J Appl Physiol 15:1130–1134

Reed DJ, Withrow CD, Woodbury DM (1967) Electrolyte and acid-base parameters of rat cerebrospinal fluid. Exp Brain Res 3:212–219

Reite M, Pauley JD, Kaufman IC, Stynes AJ, Marker V (1974) Normal physiological patterns and physiological behavioral correlations in unrestrained monkey infants. Physiol Behav 12:1021–1033

Richards CD, Sercombe R (1970) Calcium, magnesium and the electrical activity of guinea-pig olfactory cortex in vitro. J Physiol (Lond) 211:571–584

Richter T, West JR, Fishman AP (1957) The syndrome of alveolar hypoventilation and diminished sensitivity of the respiratory center. The New England J Med 256:1165–1170

Rigatto H (1979) A critical analysis of the development of peripheral and central respiratory chemosensitivity during the neonatal period. Wenner-Gren Cent Int Symp Ser 32:137–148

Rigatto H, Brady JP (1972) Periodic breathing and apnea in preterm infants. I Evidence for hypoventilation possibly due to central respiratory depression. Pediatrics 50:202–218

Rigatto H, Brady J, de la Torre Verduzco R (1975a) Chemoreceptor reflexes in preterm infants: I. The effect of gestational and postnatal age on the ventilatory response to inhalation of 100% and 15% oxygen. Pediatrics 55:604–614

Rigatto H, Brady JP, de la Torre Verduzco R (1975b) Chemoreceptor reflexes in preterm infants. II. The effect of gestational and postnatal age on the ventilatory response to inhaled carbon dioxide. Pediatrics 55:614–620

Rigatto H, de la Torre Verduzco R, Cates D (1975c) Effects of O_2 on the ventilatory response to CO_2 in preterm infants. J Appl Physiol 39:896–899

Robin RD, Whaley RD, Crump CH, Bickelman AG, Travis DM (1958) Acid base relations between spinal fluid and arterial blood with special references to control of ventilation. J Appl Physiol 13:385–392

Rodgers SH (1968) Ventilatory response to ventral root stimulation in the vertebrate cat. Respir Physiol 5:165–174

Rosenstein R, McCarthy LE, Borison HC (1974) Influence of hypoxia on tidal volume response to CO_2 in decerebrate cats. Respir Physiol 20:239–250

Rutherford SG, Cuthbertson EM, Hansen JT, Pace N (1967) Surgical excision of the canine carotied bodies and denervation of the aortic bodies. J Surg Res 7/10:457–463

Salmoiraghi GC, Baumgarten Rv (1961) Intracellular potentials from respiratory neurons in brain stem of cat and mechanism of rhythmic respiration. J Neurophysiol 24:203

Salmoiraghi GC, Burns BD (1960) Notes on mechanism of rhythmic respiration. J Neurophysiol 23:14–26

Salmoiraghi GC, Steiner FA (1963) Acetylcholine sensitivitiy of cat's medullary neurones. J Neurophysiol 26:581–597

Saper CB, Swanson LW, Cowan WM (1976) The efferent connections of the ventromedial nucleus of the hypothalamus of the rat. J Comp Neurol 169:409–442

Schiff JM (1858/9) Lehrbuch der Muskel- und Nervenphysiologie. Schauenburg, Lahr

Schlaefke ME (1972) Untersuchungen zur Funktion und Struktur der zentralen Chemosensibilität, ihre Bedeutung für die Lungenventilation und die Regelung des extracellulären pH im Zentralnervensystem. Habilitationsschrift Medizin, Ruhr-Universität Bochum

Schlaefke ME (1973) 'Specific' and 'non-specific' stimuli in the drive of respiration. Acta Neurobiol Exp (Warsz) 33:149–154

Schlaefke ME (1974) Role of central chemosensitivity for the respiratory system during respiratory and metabolic acidosis. In: Viswanathan R, Jaggi OP (eds) Advances in chronic obstructive lung disease. Atma Ram, New Delhi, pp 223–227

Schlaefke ME (1976a) Central chemosensitivity: Neurophysiology and contribution to regulation. In: Loeschcke HH (ed) Acid-base homeostasis of the brain extracellular fluid on the respiratory control system. Thieme, Stuttgart, pp 66–80

Schlaefke ME (1976b) Central chemosensitivity. In: Ulmer WT (ed) Verh Ges Lungen- und Atmungsforschung 6:14–20

Schlaefke ME, Loeschcke HH (1967) Lokalisation eines an der Regulation von Atmung und Kreislauf beteiligten Gebietes an der ventralen Oberfläche der Medulla oblongata durch Kälteblockade. Pfluegers Arch 297:201–220
Schlaefke ME, See WR (1978) Origin and modification of central chemosensitive drive. Drug Res 28/II 12:2365–2367
Schlaefke ME, See WR (1979) Ventral medullary neurons modified by H^+-ions and peripheral nerve stimulation. Pfluegers Arch 379:R50
Schlaefke ME, See WR (1980a) Ventral surface stimulus response in relation to ventilatory and cardiovascular effects. In: Koepchen HP, Hilton SM, Trzebski A (eds) Central interaction between respiratory and cardiovascular control systems. Springer, Berlin Heidelberg New York, pp 56–64
Schlaefke ME, See WR (1980b) Loss of central chemosensitivity in the respiratory control system – a model for studying Ondine's curse and sudden infant death syndrome. Bull Europ Physio-Pathol Resp 16/5:220P
Schlaefke ME, See WR, Massion WH, Loeschcke HH (1969) Die Rolle ‚spezifischer' und ‚unspezifischer' Afferenzen für den Antrieb der Atmung, untersucht durch Reizung und Blockade von Afferenzen an der decerebrierten Katze. Pfluegers Arch 312:189–205
Schlaefke ME, See WR, Loeschcke HH (1970) Ventilatory response to alterations of H^+-ion concentration in small areas of the ventral medullary surface. Respir Physiol 10:198–212
Schlaefke ME, Kille JF, Folgering H, Herker A, See WR (1974) Breathing without central chemosensitivity. In: Umbach W, Koepchen HP (eds) Central rhythmic and regulation. Hippokrates. Stuttgart, pp 97–104
Schlaefke ME, Pokorski M, See WR, Prill RK Kille JF, Loeschcke HH (1975a) Chemosensitive neurons on the ventral medullary surface. Bull Physio-Pathol Respir 11: 277–284
Schlaefke ME, See WR, Herker A (1975b) Response of neurons in the ventral medullary surface to alterations of H^+ ion concentration in the cerebrospinal fluid. Pfluegers Arch (Suppl) 359:49
Schlaefke ME, See WR, Kille JF (1977) Untersuchungen zum Angriffsort antihypertensiv wirkender Imidazolinderivate. Therapiewoche 27:44
Schlaefke ME, See WR, Fukuda Y (1978) An attempt to differentiate chemosensitive neurones in the ventral medulla oblongata. Neurosci Lett (Suppl) 1:18
Schlaefke ME, Kille JF, Loeschcke HH (1979a) Elimination of central chemosensitivity by coagulation of a bilateral area on the ventral medullary surface in awake cats. Pfluegers Arch 379:231–241
Schlaefke ME, See WR, Herker-See A, Loeschcke HH (1979b) Respiratory response to hypoxia and hypercapnia after elimination of central chemosensitivity. Pfluegers Arch 381:241–248
Schlaefke ME, See WR, Kille JF (1979c) Origin and afferent modification of respiratory drive from ventral medullary areas. Wenner-Gren Cent Int Symp Ser 32:25–34
Schlaefke ME, Luttmann A, Dermietzel R, Kille JF, Hukuhara T Jr, Tinti M, Wierich W, See WR (1980a) Zur Pathophysiologie der zentralen Chemosensibilität der Atmung. Atemwegs- und Lungenkrankheiten 6/3:165–169
Schlaefke ME, Hukuhara T Jr, See WR (1980b) Loss of central chemosensitivity, experimental studies on a clinical problem. Proc Int Un Physiol Sci 14:686
Schlaefke ME, Kille JF, Wierich W (1980c) Comparative studies on central chemosensitivity in cat and in man. Pfluegers Arch 384:R30
Schlaefke ME, See WR, Burghardt F (1980d) Influence of central chemosensitivity upon respiratory and sympathetic efferent pathways. Neurosci Lett (Suppl) 18:S140
Schmidt CF, Comroe JH Jr (1940) Functions of the carotid and aortic bodies. Physiol Rev 20:115–157
Schwanghart F, Schroeter R, Klüssendorf D, Koepchen HP (1974) The influence of novocaine block of superficial brain stem structures on discharge pattern of specific

respiratory and unspecific reticular neurons. In: Umbach W, Koepchen HP (eds) Central rhythmic and regulation. Hippokrates, Stuttgart, pp 104–110
Schweitzer A, Wright S (1938) Action of prostigmine and acetylcholine on respiration. Quart J Exp Physiol 28:33–47
Scott RW (1920) Observations on pathologic physiology of chronic pulmonary emphysema. Arch Intern Med 26:544
See WR (1973) Die Rolle chemischer und nicht chemischer Afferenzen des Nervus vagus als Atmungsantriebe. Biology thesis, Ruhr Universität Bochum
See WR (1976a) Über den Beitrag des Hypothalamus zur Atmung nach Ausschaltung der chemischen Atemantriebe. Ph D Dissertation, Ruhr Universität Bochum
See WR (1976b) Respiratory drive in hyperthermia, interaction with central chemosensitivity. In: Loeschcke HH (ed) Acid-base homeostasis of the brain extracellular fluid and the respiratory control system. Thieme, Stuttgart, pp 122–129
See WR, Schlaefke ME (1978) The influence of sinus-nerve stimulation on neuronal activity of ventral medullary neurons. Neurosci Lett (Suppl) 1:519
See WR, Schlaefke ME (1980) Convergence of afferents on H^+-modulated neurones in central chemosensitive structures. Proc Int Un Physiol Sci 14:692
See WR, Schlaefke ME, Herker-See A (1977) The effect of local hypothalamic heating on respiration after elimination of chemical afferences to the respiratory system. Proc IV Iranian Congr Physiol Pharmacol pp 117–118
See WR, Folgering H, Schlaefke ME (1978) Central respiratory and cardiovascular effects of the ACh-releaser 4-Aminopyridine. Pfluegers Arch 377:R20
See WR, Hukuhara T Jr, Schlaefke ME (1980) Efferent sinus nerve activity before and during elimination of central chemosensitivity. Pfluegers Arch 384:R30
Segundo JP, Perkel DH (1969) The nerve cell as an analyzer of spike trains. Med Sci 11:349–390
Segundo JP, Takenaka T, Eucabo H (1967a) Electrophysiology of bulbar reticular neurons. J Neurophysiol 30:1194–1220
Segundo JP, Takenaka T, Eucabo H (1967b) Somatic sensory properties of bulbar reticular neurons. J Neurophysiol 30:1221–1238
Severinghaus JW, Mitchell RA (1962) Ondine's curse – failure of respiratory center automaticity while awake. Clin Res 20:122
Shahar A, Edery H (1976) Large multipolar nerve cells in the ventral medulla oblongata of the cat. Proc VI Europ Congr on Electron Microscopy, Jerusalem, pp 614–616
Shannon DC, Kelly D (1977) Impaired regulation of alveolar ventilation and the sudden infant death syndrome. Science 197:367–368
Shannon DC, Kelly DH, O'Connell K (1977) Abnormal regulation of ventilation in infants of risk for sudden infant death syndrome. N Engl J Med 297:747
Shimada K, Trouth CO, Loeschcke HH (1969) Von der H^+-Ionenkonzentration des Liquors abhängige Aktivität von Neuronen im Gebiet der chemosensiblen Zonen der Medulla oblongata. Pfluegers Arch 312:R55
Siesjö BK, Sørensen SC (eds) (1971) Ion homeostasis of the brain. The regulation of hydrogene and potassium ion concentrations in cerebral intra- and extracellular fluids. Alfred Benzon Symposium III. Munksgaard, Copenhagen
Soerensen SC, Mines AH (1970) Ventilatory response to acute and chronic hypoxia in goats after sinus nerve section. J Appl Physiol 28:832–835
Somjen GG (1975) Electrophysiology of neuroglia. Am Rev Physiol 37:163–190
Speckman EJ, Caspers H (1969a) Verschiebungen des corticalen Bestandspotentials bei Veränderungen der Ventilationsgröße. Pfluegers Arch 310:235–250
Speckman EJ, Caspers H (1969b) Inhibitorische CO_2-Wirkungen auf spinale Zwischen- und Motoneurone bei der Ratte. Pfluegers Arch 307:R119–120
Speckmann EJ, Caspers H, Sokolov W (1970) Aktivitätsänderungen spinaler Neurone während und nach einer Asphyxie. Pfluegers Arch 319:122–138
Spode R (1980) Ausschaltung der zentralen und peripheren chemosensiblem Atemantriebe bei der anaesthesierten Katze während Muskelarbeit. Medical Dissertation, Ruhr-Universiät Bochum

Spode R, Schlaefke ME (1975) Influence of muscular exercise on respiration after central and peripheral chemodenervation. Pfluegers Arch 359:R49
Steinschneider A (1972) Prolonged apnea and the sudden infant death syndrome. Clinical and laboratory observations. Pediatrics 50/4:646–654
Stella G (1938a) On the mechanism of production and the physiological significance of 'apneusis'. J Physiol (Lond) 93:10–23
Stella G (1938b) The dependence of the activity of the 'apneustic centre' on the carbon dioxide of the arterial blood. J Physiol 93:263–275
St. John WM, Wang SC (1976) Integration of chemoreceptor stimuli by caudal pontile and rostral medullary sites. J Appl Physiol 41/5:612–622
St. John WM, Wang SC (1977a) Response of medullary respiratory neurons to hypercapnia and isocapnic hypoxia. J Appl Physiol 43/5:812–821
St. John W, Wang SC (1977b) Alterations from apneusis to more regular rhythmic respiration in decerebrate cats. Respir Physiol 31:91–106
Sullivan CE, Kozar LF, Murphy E, Phillipson EA (1978) Primary role of respiratory afferents in sustaining breathing rhythm. J Appl Physiol 45/1:11–17
Taber E (1961) The cytoarchitecture of the brain stem of the cat. I. Brain stem nuclei of cat. J Comp Neurol 116:27–70
Terzian H (1966) Syndrome de Pickwick el. nercolepsie. Rev Neurol (Paris) 115:184–188
Thach B, Frantz I, Adler S, Taeusch HW Jr (1978) Maturation of reflexes influencing inspiratory duration in human infants. J Appl Physiol 45:203–211
Trouth CO, Loeschcke HH, Berndt J (1973a) A superficial substrate on the ventral surface of the medulla oblongata influencing respiration. Pfluegers Arch 339:135–152
Trouth CO, Loeschcke HH, Berndt J (1973b) Histological structures in the chemosensitive regions on the ventral surface of the cat's medulla oblongata. Pfluegers Arch 339:171–183
Trouth CO, Loeschcke HH, Berndt J (1973c) Topography of the circulatory responses to electrical stimulation in the medulla oblongata. Pfluegers Arch 339:185–201
Trzebski A, Zielinski A, Lipski J, Majcherczyk S (1971) Increase of sympathetic preganglionic discharges and of the peripheral resistance following stimulation by H^+ ions of the superficial chemosensitive areas in the medulla oblongata in cats. Proc Int Un Physiol Sci 9:571
Trzebski A, Zielinski A, Majcherczyk S, Lipski J, Szulczyk P (1974) Effect of chemical stimulation and depression of the medullary superficial areas on the respiratory motoneurones discharges, sympathetic activity and efferent control of carotid area receptors. In: Umbach W, Koepchen HP (eds) Central rhythmic and regulation. Hippokrates, Stuttgart, pp 170–177
Trzebski A, Majcherczyk S, Szulczyk P, Chruscielewski L (1976) Direct nervous mechanisms as the possible pathways of interaction of the central and peripheral chemosensitive areas. In: Loeschcke HH (ed) Acid-base homeostasis of the brain extracellular fluid and the respiratory control system. Thieme, Stuttgart, pp 130–146
Trzebski A, Mikulski A, Przybyszewski A (1977) Influence of the chemosensitive areas on the ventral surface of the medulla oblongata on the EEG activity in encéphale isolé preparations in cats. Proc Int Un Physiol Sci 13:764
Trzebski A, Mikulski A, Przybyszewski A (1980) Effects of stimulation of chemosensitive areas by superfusion on ventral medulla and by infusion into vertebral artery of chemical stimuli in non-anaesthetized "encéphale isolé" preparations in cats. In: Koepchen HP, Hilton S, Trzebski A (eds) Central interaction between respiratory and cardiovascular control systems. Springer Berlin Heidelberg New York, pp 65–75
Ullah Z (1973) Elektronenmikroskopische Untersuchungen über ein chemosensibles Areal für die Atmungsregulation im Bereiche der Medulla oblongata der Hauskatze. Medical Disseration, Gesamthochschule Essen
Umbach W, Koepchen HP (eds) (1974) Central rhythmic and regulation. Hippokrates, Stuttgart

Unterberg W (1971) Das Pickwick-Syndrom. Dtsches Ärtzeblatt 14:1009–1012
Vierordt K (1844) Respiration. In: Wagner (ed) Handwörterbuch Physiologie II, pp 828–919
Volkmann AW (1841) Über die Bewegungen des Athmens und Schluckens, mit besonderer Berücksichtigung neurologischer Streitfragen. Arch Anat Physiol 332–360
Walker JL, Brown AM (1970) Unified account of variable effects of carbon dioxide on nerve cells. Science 167:1502–1504
Ward RA, Kelsey WM (1962) The pickwickian syndrome. J Pediatr 61:745–749
Warsaw Symposium (1976) Carbon dioxide and breathing. Bull Physio-Pathol Respir 12:213–255
Washizu Y (1960) Effect of CO_2 and pH on the responses of spinal motoneurons. Brain Nerve 12:757–766
Wells HH, Kattwinkel J, Morrow JD (1980) Control of ventilation in Ondine's curse. J Pediatr 96/5:865–867
Wennergren G, Wennergren M (1980) Respiratory effects elicited in newborn animals via the central chemoreceptors. Acta Physiol Scand (Copenh) 108:309–311
Whipp BJ (1978) Trends of the exercise hyperpnea and their degree of corroboration. Chest73:274–283
Wichser J, Kazemi H (1975) CSF bicarbonate regulation in respiratory acidosis and alkalosis. J Appl Physiol 38/3:504–511
Wiemer W, Ott N, Winterstein H (1964) Reflektorische und zentrale Anteile der Hyperpneo bei HCl-Acidose. Z Biol (Munich) 114:299–308
Wiemer W, Winterstein H, Kiwull P, Ott N (1965) Interaction of intracranial and extracranial respiratory mechanisms. In: Brooks C McC, Kao FF, Lloyd BB (eds) Cerebrospinal fluid and the regulation of ventilation. Blackwell, Oxford, pp 303–321
Willshaw P (1975) Sinus nerve efferents as a link between central and peripheral chemoreceptors. In: Purves MT (ed) The peripheral arterial chemoreceptors. Cambridge University Press 253–268
Willshaw P (1977) Mechanism of inhibition of chemoreceptor activity by sinus nerve efferents. In: Acker H, Fidone S, Pallot D, Eyzaguirre C, Lübbers DW, Torrance RW (eds) Chemoreception in the carotid body. Springer, Berlin Heidelberg New York, pp 168–174
Willshaw P, Majcherczyk S (1979) The peripheral arterial chemoreceptors as detectors of oxygen flow to the brain. Wenner-Gren Cent Int Symp Ser 32:25–34
Winder CV (1937a) Pressoreceptor reflexes from the carotid sinus. Am J Physiol 118: 379–388
Winder CV (1937b) On the mechanism of stimulation of carotid gland chemoreceptor. Am J Physiol 118:389–398
Winterstein H (1910) Die Regulierung der Atmung durch das Blut. Centralbl Physiol 24:811
Winterstein H (1911) Die Regulierung der Atmung durch das Blut. Pfluegers Arch 138: 167
Winterstein H (1923) Atmungsregulation und Reaktionsregulation. Naturwissenschaften 625:644
Winterstein H (1950) Die Atmung ohne Chemorezeptoren. Arch Intern Pharmacodyn Ther 83:80–90
Winterstein H (1955) Die chemische Steuerung der Atmung. Erg Physiol Biol Chem Exp Pharmakol 48:328–528
Witnerstein H (1960) Die Entdeckung neuer Sinnesflächen für die chemische Steuerung der Atmung. Naturwissenschaften 47:99–103
Winterstein H, Goekhan N (1953) Chemoreceptoren-Reizstoff und Blut/Hirn-Schranke. Arch Exp Pathol Pharmakol 219:192–196
Wittich CW von (1866) Über die Beziehung der Medulla oblongata zu den Atembewegungen bei Fröschen. Virchows Arch Pathol Anat 37:322–345

Woody CD, Marshall WH, Besson JM, Brain HK (1970) Brain potential shift with respiratory acidosis in the cat and monkey. Am J Physiol 218:275–283
Wyss OAM (1964) Die nervöse Steuerung der Atmung. Erg Physiol Biol Chem Exp Pharmakol 54:1–413
Wyszogrodski I, Thach B, Milic-Emili J (1978) Maturation of respiratory control in unanesthetized newborn rabbits. J Appl Physiol 44:304–310
Zink P, Hach B, Hillemacher A (1977) Plötzlicher unerwarteter Tod bei seltener Halswirbelsäulenmißbildung. Z Rechtsmed 79:63–67

Author Index

Page numbers in *italics* refer to the bibliography.

Subject Index