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Catecholamine Receptors*

ALEXANDER LEVITZKI **

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I. Introduction

Catecholamines elicit a multitude of biochemical, physiologic, and pharmacologic effects. The large variety of activities induced at the different target cells is brought about by the interaction of these compounds with specific catecholamine receptors. The active catecholamine can either function as a hormone at different organs or as a neurotransmitter at a

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* *Dedication.* This article is dedicated to my noble teacher *Arieh Berger* wo died prematurely six years ago at the age of 51.

Arieh Berger established the molecular basis for the understanding of ligand-macromolecule recognition through his brilliant studies on the mapping of the active sites of proteolytic enzymes. His work should be a source of inspiration to all molecular pharmacologists as it is for us.

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postsynaptic membrane. Catecholamine receptors appear in the periphery as well as in the CNS and belong to a number of classes. The different classes of catecholamine receptors are distinguished from each other in ligand specifity, as well as in the nature of the biochemical response elicited subsequent to catecholamine binding to its specific receptor.

II. Classification of Catecholamine Receptors

Catecholamine receptors are classified according to their ligand specificity and not according to the physiologic or pharmacologic activity resulting from receptor occupancy. A brief summary of catecholamine receptors is given in Table 1. The most studied catecholamine receptors are the β-adrenergic receptors, whereas the α-adrenergic receptors and dopamine receptors are less well characterized. Pharmacologically and physiologically, α-adrenergic receptors and β-adrenergic receptors are the most important catecholamine receptors and are responsible for a large spectrum of physiologic effects (Table 2). The distinction between α-adrenergic and β-adrenergic receptors is based on a number of criteria which were defined in the work of *Ahlquist* (1948, 1967). It should, however, be noted that *Dale* (1906) already noted the existence of two types of adrenergic receptors and formulated the experimental basis for the distinction between the two types of receptors.

Table 1. The main classes of catecholamine receptors

Type of receptor	Ligand specifity	Specific blockers	Second messenger
α (Alpha)	Norepinephrine > epinephrine > > phenylephrine ≫ isoproterenol	Phentolamine, erogotamine, phenoxybenzamine, dibenamine	Ca^{2+} cAMP [a] Phospholipid effect
β (Beta)	Isoproterenol > epinephrine > > norepinephrine ≫ phenylephrine	Dichloroisoproterenol Propranolol Alprenolol Pindolol	cAMP
Dopamine	Dopamine > norepinephrine	Haloperidol Chloropromazine Some α-blocking agents	cAMP [b]

[a] In the central nervous system.
[b] A secondary biochemical response other than cAMP formation has also been postulated recently (*H. Shepard,* personal communication). Thus, there may be more than one class of dopamine receptors.
Comment: α-adrenergic receptors and β-adrenergic receptors are specific for the R stereoisomers (or the l-stereoisomer if the optical rotatory power is considered).

Table 2. Typical physiologic actions of adrenergic receptors

System or tissue	Action	Receptor
Cardiovascular system:	Increased force of contraction	β
Heart	Increased rate	β
Blood vessels	Constriction	α
	Dilation	β
Respiratory system, tracheal and bronchial muscle	Relaxation	β
Iris (radial muscle)	Pupil dilated	α
Smooth muscle, uterus	Contraction	α
Spleen	Relaxation	β
	Contraction	α
Bladder	Contraction	α
	Relaxation	β
Skeletal muscle	Changes in twitch tension	β
	Increased release of acetylcholine	α
	Increased glycogenolysis	β
Adipose tissue	Increased lipolysis	β

The present characterization of adrenoreceptors (*Furchgott,* 1972; *Jenkinson,* 1973) is based on a twofold procedure: (1) the relative potency (potency ratio) of a series of adrenergic agonists for eliciting the specific response, and (2) the potency of an antagonist for blocking the response to a given agonist.

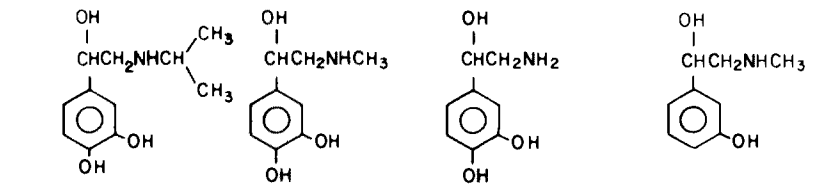
Based on these two principles *Furchgott* (1972) gave a more general definition of α- and β-receptors:

β-receptor: a β-receptor is one which mediates a response pharmacologically characterized by: (1) a relative potency series: isoprenaline > adrenaline (epinephrine) ⩾ noradrenaline (norepinephrine) ⩾ phenylephrine, and (2) a susceptibility to specific blockade by pindolol, propranolol, or alprenolol at relatively low concentrations;

α-receptors: an α-receptor is one which mediates a response pharmacologically characterized by: (1) a relative potency series in which noradrenaline > adrenaline > phenylephrine ⩾ isoprenaline, and (2) a susceptibility to specific blockade by phentolamine, dibenamine or phenoxybenzamine at low concentrations. A summary of agonist specificity of α- and β-receptors is given in Table 3 and a summary of the chemical formulas of α-antagonists and β-antagonists is given in Table 4.

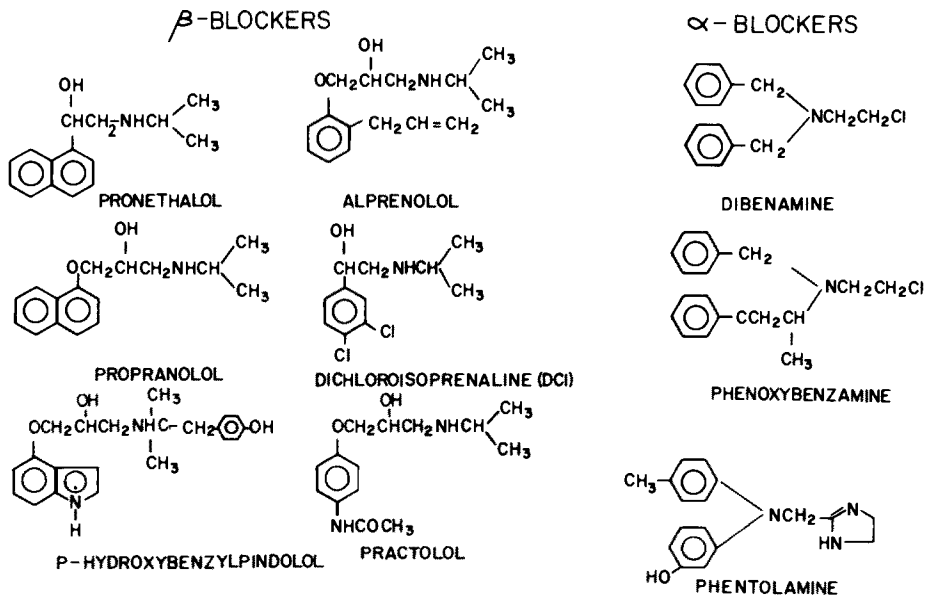
Both types of receptors are stereospecific for the R-stereoisomer of either the agonist or the antagonist. As is seen from Table 1 dopamine receptors are blocked by still another class of blockers, namely, by com-

Table 3. Agonist specificity for α-adrenergic and β-adrenergic receptors



α	1	<	2	<	3	<	4
β	1	>	2	≫	3	≫	4

Table 4. α-adrenergic blockers and β-adrenergic blockers



pounds of the phenothiazine family and haloperidol. It should, however, be emphasized that lysergic acid derivatives interact with dopamine receptors as well as with α-receptors. Thus, ergocryptine was found to be an α-blocker (*Williams* and *Lefkowitz*, 1976) and d-LSD was found to bind to dopamine receptors (*Burt* et al., 1976).

III. Biochemical Signals Coupled to Catecholamine Receptors

A. β-Adrenergic Receptors

Of all catecholamine receptors the β-adrenergic receptors have been given the most attention. This may be due to the fact that the primary biochemical signal elicited upon agonist binding to the surface β-receptors has been identified and found to be the activation of adenylate cyclase producing the "second messenger" cAMP from ATP within the target cell (*Sutherland* et al., 1965; *Robison* et al., 1968):

$$\text{ATP} \xrightarrow{\text{l-catecholamine}} \text{cAMP} + \text{PPi} \qquad (1)$$

In this respect, the coupling between the β-adrenergic receptor and the enzyme adenylate cyclase is similar to the coupling between adenylate cyclase and hormone receptors to certain polypeptide hormones such as glucagon, ACTH, and secretin (*Perkins,* 1973; *Cuatrecasas,* 1974). In certain cells, such as the liver cell and the fat cell, β-adrenergic receptors as well as receptors of polypeptide hormones are coupled to the enzyme adenylate cyclase. The second messenger cAMP, produced intracellularly by the enzyme adenylate cyclase, triggers a large variety of biochemical events typical to the cell, usually through the activation of protein kinase (*Walsh* et al., 1968), as a first step. The activation of adenylate cyclase by β-adrenergic agonists is mediated by the nucleotide GTP which acts in a synergistic fashion with the catecholamines (*Levitzki* et al., 1976; *Sevilla* et al., 1976; *Bilezikian* and *Aurbach*, 1974; *Schramm* and *Rodbell,* 1975; *Pfeuffer* and *Helmreich*, 1975). This type of synergistic action of hormones with GTP was first observed in adenylate cyclase activated by glucagon (*Salomon* et al., 1975; *Lin* et al., 1975; *Rendell* et al., 1975). Thus, both the occupancy of the β-adrenergic receptor with agonists and the level of intracellular GTP determine the final output of cAMP by the enzyme adenylate cyclase. The role of GTP in the activation of adenylate cyclase has been extensively studied using the nonhydrolyzable analogs Gpp(NH)p and GTPγS. It is not as yet clear whether the activity of the enzyme is absolutely dependent on GTP and exhibits no activity in its absence or possesses basal activity in the presence of a β-adrenergic agonist and in the absence of GTP. Recently it was found that turkey erythrocytes possess a specific β-adrenergic receptor dependent GTPase (*Cassel* and *Selinger,* 1976):

$$\text{GTP} \xrightarrow{\text{l-catecholamine}} \text{GDP} + \text{Pi} \qquad (2)$$

It was suggested that the activity of this enzyme is closely associated with adenylate cyclase and determines the steady state level of GTP and thus,

the level of adenylate cyclase activity. A general model for the interrelationship between GTP and β-agonists was recently proposed (*Levitzki*, 1976; *Sevilla* and *Levitzki*, 1977). This model can be summarized by the following scheme:

$$R \cdot E \overset{GTP}{\rightleftharpoons} R \cdot E \cdot GTP \overset{H}{\rightleftharpoons} H \cdot R \cdot E \cdot GTP \overset{k_3}{\rightarrow} H \cdot R' \cdot E' \cdot GTP \overset{k_4}{\rightarrow} HR \cdot E + GDP + Pi \quad (3)$$

Where R is the receptor and E is the enzyme adenylate cyclase coupled to it. When both GTP and the agonist H are bound to their respective regulatory sites the enzyme is converted to its active form E′. The E′ state exhibits GTPase activity and is responsible for the termination of the hormonal signal once the bound GTP is hydrolyzed and the enzyme reverts to its inactive state E. The adenylate cyclase catalytic moiety and the GTPase catalytic moiety probably reside on two separate subunits both of which are coupled to the β-receptor R. Once the enzyme E is converted to its active state E′ the receptor is also modified to a state R′. The state R′ exhibits lower affinity towards the β-agonists (*Maguire* et al., 1976; *Lefkowitz* et al., 1976) but possesses an unaltered affinity towards β-antagonists (*Levitzki* et al., 1975; *Tolkovsky* and *Levitzki*, 1977) The lower affinity of R′ towards β-agonists insures that the dose response for the GTPase step is displaced to higher agonist concentrations as compared to the dose response curve for adenylate cyclase activation. This state of affairs insures both that a finite level of E′ will be achieved and that the hormone induced signal will eventually be terminated. The mechanism of conversion of E to E′, characterized by the rate constant k_3 in equation (3), was studied in detail using the nonhydrolyzable GTP analog Gpp(NH)p for which $k_4 = 0$ (*Levitzki* et al., 1976; *Sevilla* et al., 1976). An alternative explanation is that the receptor and the enzyme are permanently uncoupled to each other and the enzyme becomes activated during the formation of the transient complex H · R · E:

$$H \cdot R + E \cdot GTP \rightarrow H \cdot R \cdot E \cdot GTP \rightarrow HR + E' \cdot GTP \rightarrow E + GDP + Pi \quad (4)$$

The activated enzyme E′ is converted back to E concomitantly with the hydrolysis of GTP at the regularotory site. Evidence favoring the latter mechanism has recently been obtained (*Tolkovsky* and *Levitzki*, 1978). GTP and Gpp(NH)p function as activators in other hormone-sensitive adenylate cyclase from different tissues (*Londos* et al., 1974). It may be, therefore, that the interrelations between hormones and GTP are a general phenomenon. Hormone-dependent GTPase in these systems has not yet been demonstrated. This is probably due to the fact that neither was this enzyme searched for in other systems nor was the methodology

(*Cassel* and *Selinger*, 1976) available. Therefore, it is not clear at this point whether the scheme formulated in equation (3) or (4) represents a general regulatory mechanism for other hormone dependent adenylate cyclases. These studies are in line with the fact that the β-receptor and the enzyme reside on separate subunits both of which can diffuse slowly in the membrane matrix (*Orly* and *Schramm*, 1976). Elegant experiments performed recently by *Orly* and *Schramm* (1976) have demonstrated this fact. In these experiments turkey erythrocytes, in which the catalytic activity of adenylate cyclase had been inactivated by N-ethylmaleimide or by heat, served to contribute the β-adrenergic receptor. Friend erythroleukemic cells (F cells) which possess no β-adrenergic receptors served to contribute the enzyme adenylate cyclase. The erythrocytes in which the enzyme had been inactivated were fused with the F cells by Sendai virus. The cell ghosts of the fused preparation demonstrated isoproterenol-dependent adenylate cyclase. These experiments therefore reveal that the β-adrenergic receptor of the turkey erythrocytes must have become functionally coupled to the adenylate cyclase of the mouse F cells. Activation by isoproterenol was demonstrable within a few minutes after fusion and inhibitors of protein synthesis had no effect. Thus coupling must have occurred between the preexisting components. These hybridization experiments demonstrate that the receptor has become permanently attached to the active adenylate cyclase, thus conferring to it hormone sensitivity. The rate of formation of the newly formed enzyme-receptor complex is slow, but once formed it is quite stable. A recent report (*Insel* et al., 1976) demonstrates that the β-adrenergic receptor and the adenylate cyclase are products of separate genes. The data show that a clone of S49 lymphoma cells devoid of adenylate cyclase, but possesssing β-adrenergic receptors, can be isolated (*Insel* et al., 1976). More recently it was shown that β-receptors can also be coupled to biochemical signals other than adenylate cyclase. We have already mentioned β-receptor-dependent GTPase which is probably part of the β-receptor adenylate cyclase complex. Another biochemical signal coupled to β-receptors found recently is the catecholamine dependent Ca^{2+} efflux (*Steer* and *Levitzki*, 1975; *Steer* et al., 1975; *Rassmussen* et al., 1975). It was shown in both turkey erythrocytes (*Steer* and *Levitzki*, 1975; *Steer* et al., 1975) and in human erythrocytes (*Rasmussen*, 1975) that $^{45}Ca^{2+}$ efflux is enhanced by β-agonists and is blocked by β-antagonists. This effect is not mimicked by cAMP or dibutyryl cAMP and therefore is not mediated by a denylate cyclase (*Steer* and *Levitzki*, 1975). It is interesting that β-receptor-dependent adenylate cyclase from turkey erythrocytes is inhibited by Ca^{2+} by virtue of its interaction with a specific allosteric site (*Steer* and *Levitzki*, 1975; *Hanski* et al., 1977). These findings may suggest (*Steer* and *Levitzki*, 1975; *Steer* et al., 1975) that the first effect of a β-agonist is the deinhibition of the enzyme which

is in the resting state in the inhibited Ca^{2+} bound form. These findings, however, are so far restricted to the turkey erythrocyte system and it is not clear at this point whether the interactions between Ca^{2+} and adenylate cyclase is a general principle in the action of β-adrenergic receptors.

B. α-Adrenergic Receptors

It has been demonstrated that the primary event occurring upon occupation of the α-adrenergic receptor by an α-agonist in the parotid gland is the influx of Ca^{2+} which functions as the second messenger (*Schramm* and *Selinger,* 1975). Furthermore, the specific Ca^{2+} ionophore A-23187, when incorporated into the cell membrane, can substitute for the α-adrenergic ligand and bypass the receptor dependent mechanism (*Selinger* et al., 1974). The influx of Ca^{2+} as the primary event in the salivary gland (rat parotid) causes the efflux of K^+ ions with water (*Schramm* and *Selinger,* 1975; *Batzri* et al., 1975). The efflux of potassium has also been recognized as an α-adrenergic effect in guinea pig liver (*Haylett* and *Jenkinson,* 1972a; *Haylett* and *Jenkinson,* 1972b), and in adipose tissue (*Girardier* et al., 1968).

The stimulation of the pineal gland with l-epinephrine via the α-adrenergic receptor was found to be dependent on the presence of Ca^{2+} in the incubation medium and results in the sevenfold increase of the cGMP level (*O'Dea* and *Zatz,* 1976). It seems from these studies that the influx of Ca^{2+} is the first event induced by the α-agonist. The formation of cGMP seems to be the result of Ca^{2+} influx. This is not surprising since guanylate cyclase is a Ca^{2+} dependent enzyme. As is indicated in Section II α-adrenergic receptors are involved in a variety of physiologic activities and it remains to be seen whether in each case Ca^{2+} functions as the scond messenger. It has been claimed that in the CNS, α-receptors as well as β-receptors are coupled to the enzyme adenylate cyclase (*Perkins* and *Moore,* 1973; *Huang* et al., 1973; *Skolnick* and *Daly,* 1975; *Skolnick* and *Daly,* 1976; *Sattin* et al., 1975). These observations are based on the experimental finding that α-adrenergic blockers inhibit the formation of cAMP, as was found in rat cerebral cortical tissue.

Another biochemical response elicited by the activation of α-adrenergic receptors is the incorporation of inorganic ^{32}Pi into phosphatidylinositol in slices of the parotid gland (*Oron* et al., 1975). This biochemical event is shown to be unrelated to the K^+ efflux and water secretion which is also induced by α-receptor activation. Interestingly enough, the divalent cation ionophore A-23187 which introduces Ca^{2+} into the cell, thus causing K^+ release (*Selinger* et al., 1974), has no significant effect on the incorporation of ^{32}Pi into phosphatidylinositol. Conversely, the α-receptor induced

phospholipid effect (*Oron* et al., 1975) is maximal in the absence of Ca^{2+} in the medium, when there is no K^+ release from the cell. In summary, it can be concluded that α-receptor activation leads to two parallel and independent biochemical events in the rat parotid gland: (1) increase in membrane permeability towards extracellular Ca^{2+} which enters the cell and causes K^+ release; (2) the same interaction with the α-receptor results in the increased incorporation of ^{32}Pi into acidic phospholipids. This latter response is Ca^{2+} independent and, in fact, maximally stimulated in its absence.

It should be noted that the phospholipid effect was shown also to be induced by the activation of the muscarinic receptor in the same preparation of the parotid gland (*Oron* et al., 1975). Phospholipid effects were shown for acetylcholine receptors as well as in response to other stimuli in other tissues (*Oron* et al., 1975, and references therein). The physiologic response, however, in all of these cases, was not dependent on the presence of extracellular Ca^{2+} as in the case of the α-receptor induced response described by *Schramm* and *Selinger* (1975); *Selinger* et al. (1974) and *Batzri* et al. (1975). These observations tend to strengthen *Selinger*'s assertion that the pospholipid effect and the Ca^{2+} dependent K^+ release are two independent biochemical responses to α-receptor stimulation as in the case of some other hormone or neurotransmitter stimuli.

1. The Relationship between α-Receptors and β-Receptors

Almost every organ, tissue or cell which possesses an α-adrenergic receptor possesses also a β-adrenergic receptor. These two receptors elicit opposite physiologic effects in the target organ (Table 2). Thus, it is possible that the final response of the organ in question depends on the relative activity of the two receptors. Since Ca^{2+} functions as the second messenger of α-receptor action and inhibits the β-receptor dependent adenylate cyclase, it may provide the link between α- and β-receptors in systems which possess both types of adrenergic receptors. It is interesting that *Batzri* et al. (1975), find that in the rat parotid gland, the α-blocker phentolamine slows down the fall in the level of cAMP subsequent to epinephrine stimulation, as compared to a system in the absence of the α-blocker. This effect, however, may be due to secondary biochemical events other than the direct effect of Ca^{2+} on the level of adenylate cyclase activity. For example, Ca^{2+} is known to activate cAMP phosphodiesterase (*Appleman* et al., 1972, and references therein) and thus an increase in intracellular Ca^{2+} may result not only in the inhibition of adenylate cyclase, but also in the depletion of the cAMP pool. At this point, it can only be stated that the interaction of α-receptors with β-receptors is still not understood in biochemical terms and requires further investigation.

In the CNS and peripheral tissues it was found that the uptake of norepinephrine in adrenergic synapses is controlled by the interaction of norepinephrine with presynaptic α-receptors (*Kirkekar* and *Puig*, 1971; *Enero* et al., 1972; *Starke*, 1972; *De Potter* et al., 1971). Thus, if the postsynaptic receptor is of the β-type, the level of its activity is determined by the concentration of norepinephrine in the synapse which is controlled by α-receptors. A similar mechanism seems to operate in the rat heart where it was shown (*Eisenfeld* et al., 1967) that occupancy of α-receptors by norepinephrine enhances norepinephrine uptake into the sympathetic neurons. In both systems studied, namely, in the CNS and in the rat heart (*Eisenfeld*, 1967) α-blockers were effective in preventing norepinephrine uptake. The current views on the role of presynaptic α-receptors are summarized in a recent article by *Langer* (1977).

2. Interconversion of α- and β-Receptors?

Some reports in the literature have suggested that α-receptors and β-receptors are two allosteric configurations of the same macromolecule. The experiments upon which this hypothesis is based were performed on the frog heart, where it has been claimed that α-receptors prevail at low temperatures and transform into β-receptors at higher temperatures. It was claimed (*Kunos* et al., 1973; *Kunos* and *Szentivanyi*, 1968; *Buckley* and *Jordan*, 1970) that stimulation of cardiac rate and contractibility by catecholamines has the properties of a classic β-adrenergic response when experiments are performed at warm temperatures (25–37° C), and of an α-adrenergic response when experiments are performed at low temperatures (5–15° C). *Caron* and *Lefkowitz* (1974) examined this hypothesis by looking at the adenylate cyclase activity at a wide range of temperatures. These investigators examined dog heart, rat heart, frog heart, and frog erythrocytes. In all of these cases, it was found that the adenylate cyclase is stimulated by adrenergic ligands typical of β-receptors at a wide range of temperatures. Furthermore, the adrenergic inhibitors affecting cyclase at a wide range of temperatures were always of the β-type. α-Blockers had no effect on adenylate cyclase over a wide range of temperatures. As *Caron* and *Lefkowitz* (1974) pointed out the studies claiming the α to β interconversion were performed on an intact tissue (*Kunos* et al., 1973; *Kunos* and *Szentivanyi*, 1968; *Buckley* and *Jordan*, 1970) whereas the adenylate cyclase measurements were performed on membrane fragments (*Caron* and *Lefkowitz*, 1974). Thus it still remains possible that the interconversion of α and β-receptors requires the intact cellular structure. The integrity of the cellular structure may preserve the biochemical mechanism which may be responsible for α-receptor to β-receptor interconversion. In conclusion, α-receptor to β-receptor intercon-

version still remains possible in view of the pharmacologic experiments (*Kunos* et al., 1973; *Kunos* and *Szentivanyi,* 1968; *Buckley* and *Jordan,* 1970), although it does not, at present, find any support from direct biochemical experiments.

C. Dopamine Receptors

Dopamine, like epinephrine and norepinephrine, is a neurotransmitter in the CNS. In some cases it was demonstrated that the dopamine receptor is coupled to adenylate cyclase (*Kebabian* et al., 1972; *Bockaert* et al., 1976; *Phillipson* and *Horn*, 1976; *Daly* et al., 1972). Dopamine-sensitive adenylate cyclase was also demonstrated in neuroblastoma (*Prasad* and *Gilmer,* 1974). Dopamine receptors are distinct from the β-adrenergic receptor in their ligand specifity and in their response to specific blockers (Table 1, *Kelly* and *Miller,* 1975; *Miller* et al., 1974). Thus, dopamine receptors coupled to adenylate cyclase respond to dopamine better than to norepinephrine (Table 1), whereas in β-receptors the situation is reversed. β-Adrenergic blockers such as propranolol do not affect dopamine-dependent adenylate cyclase whereas phenothiazine-type compounds such as chlorpromazine or butyrophenones such as haloperidol act as specific blockers of dopamine-dependent adenylate cyclase (Table 1) and have no effect on β-adrenergic-receptor-dependent adenylate cyclase. Whether dopamine receptors are coupled to other biochemical signals, such as ion fluxes, is not yet clear. In neuroblastoma, α-adrenergic blockers were found to affect dopamine-dependent adenylate cyclase whereas β-adrenergic blockers were found to have an effect only at very high concentrations (*Prasad* and *Gilmer,* 1974). Dopamine-sensitive adenylate cyclase from the mesolimbic system was found to be inhibited by α-blockers but not at all by β-blockers (*Horn* et al., 1974). Thus, there is some overlap in ligand specifity of the dopamine receptor with the α-adrenergic receptor. Furthermore, binding studies using ^{3}H-dLSD have shown (*Burt* et al., 1976) that the latter cmpound monitors dopamine receptors in the brain. One should, however, remember that other lysergic acid derivatives such as ergocryptine were claimed to be specific α-adrenergic blockers (*Williams* and *Lefkowitz,* 1976). The situation is further complicated by the fact that dLSD is a well-known serotonin antagonist in several smooth muscles (*Gaddum*, 1953; *Gaddum* et al., 1955; *Wooley* and *Shaw,* 1954) and weak mixed agonist at postsynaptic serotonin receptors in the brain (*Haigler* and *Aghajanian,* 1974; *Anden* et al., 1968; *Boakes* et al., 1970). Data is also available to show that dLSD interacts with presynaptic receptors on serotonin neurons which are distinct from the postsynaptic serotonin receptor sites (*Haigler* and *Aghajanian,* 1974; *Aghajanian*, 1972); *Aghajanian* et al., 1973). The

partial overlap between dopamine receptors, α-adrenergic receptors, and serotonin receptors makes the biochemical characterization of dopamine receptors more difficult.

IV. Radioassay of Catecholamine Receptors

Until recently a reliable assay for catecholamine receptors was not available and the use of ^{3}H-catecholamines to monitor β-adrenergic receptors, as well as other catecholamine receptors, was proved to be unreliable (*Levitzki* et al., 1975; *Levitzki* et al., 1974; *Cuatrecasas* et al., 1974). The failure to detect catecholamine receptors using radioactive labeled catecholamines stems from the fact that the receptor concentration accessible experimentally is far below the catecholamine-receptor dissociation constant (*Levitzki* et al., 1974). Furthermore, catecholamines bind to many nonreceptor components in the membrane preparations studied, and thus the signal-to-noise ratio is lowered further (*Levitzki* et al., 1974; *Cuatrecasas* et al., 1974). In 1974 radioactivity labeled β-adrenergic blockers were found to monitor reliably β-adrenergic receptors. The first ligand used was ^{3}H-propanol (*Levitzki* et al., 1974; *Atlas* et al., 1974; *Levitzki* et al., 1975; *Nahorski,* 1976) and shortly thereafter and idependently ^{125}I-hydroxybenzylpindolol (*Aurbach* et al., 1974; *Brown* et al., 1976a; *Brown* et al., 1976b; *Maguire* et al., 1976) and ^{3}H-alprenolol (*Lefkowitz* et al., 1974) were introduced as specific ligands for the radioassay of β-adrenergic receptors. The use of these radioactively labeled ligands has since become a routine procedure to monitor β-adrenergic receptors in nucleated erythrocytes (*Levitzki* et al., 1975; *Levitzki* et al., 1974; *Atlas* et al., 1974; *Aurbach* et al., 1974; *Brown* et al., 1976), lymphocytes (*Williams* et al., 1976), the pineal organ (*Kebabian* et al., 1975; *Romero* et al., 1975; *Zatz* et al., 1976), adipocytes (*Williams* et al., 1976), and brain (*Sporn* and *Molinoff*, 1976; *Alexander* et al., 1975; *Bylund* and *Snyder,* 1976). The affinity of these β-blockers to the β-receptors is very high and is 3–6 orders of magnitude higher than the affinity of β-agonists towards the β-receptors (Table 5). Therefore, one can monitor low concentrations of these receptors using these compounds. The binding of these compounds is stereospecific for the R stereoisomer (*l*) and all of them are displaced from the β-receptor by (−) catecholamines and not by (+) catecholamines. The dissociation constants found for these β-blockers, using binding experiments, match closely with the inhibition constants found from their competition with catecholamines in the adenylate cyclase reaction. So far the use of specific radioactively labeled α-adrenergic blockers is rather limited. *Williams* and *Lefkowitz* (1976) reported on the use of ^{3}H-dihydroergocryptine to monitor α-adrenergic receptors and *Greenberg* et al. (1976) on the use of ^{3}H-clonidine and ^{3}H-WB-4101.

Table 5. Affinity of different β-blockers towards the β-receptors

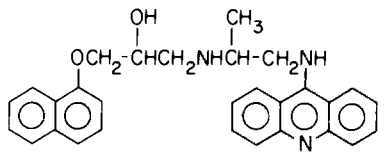
Ligand	$K_{Diss.}$ nM	
	From kinetics [a]	From binding experiments
l-Propranolol	1.3 ± 0.1	1.2 ± 0.1
l-Alprenolol	10 ± 1	10 ± 1
l-^{125}I-hydroxybenzylpindolol	–	0.02 ± 0.002 – 0.5 ± 0.2

Data taken from references cited in the text.
[a] Competetitive inhibition of (−) catecholamines in the adenylate cyclase reaction.

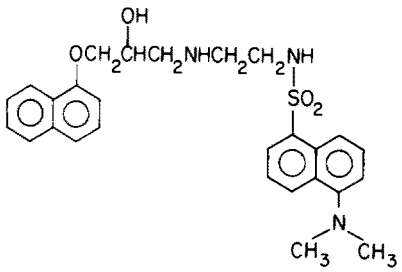
Dopamine receptors can be monitored using radioactively labeled blockers such as ^{3}H-haloperidol (*Creese* et al., 1976) and ^{3}H-dLSD (*Burt* et al., 1976). Radioactively labeled blockers can also, in principle, be used to monitor detergent solubilized receptors, provided the receptor does not denature in the process of solubilization. Such an attempt was recently reported where deoxycholate solubilized β-adrenergic receptors from frog erythrocyte membranes were monitored using ^{3}H-alprenolol (*Caron* and *Lefkowitz*, 1976). Similar attempts using ^{3}H-propranolol on lubrol PX solubilized turkey erythrocyte membranes were unsuccessful (*Steer* and *Levitzki*, unpublished). Attempts are now being made to monitor β-adrenergic receptors in the solubilized state using ^{125}I-hydroxybenzylpindolol. Since ^{125}I-hydroxybenzylpindolol exhibits extremely high affinity towards β-adrenergic receptors it can be used to monitor these receptors in whole cells (*Brown* et al., 1976; *Atlas* et al., 1977). Furthermore, this compound can be used by applying autoradiographic techniques, to localize the β-receptors on the surface of the cell. Such techniques may reveal whether the receptors are clustered or scattered on the cell surface. The availability of specific ligands to monitor β-adrenergic receptors has also made it possible to monitor receptor cryptization due to desensitization. Using radiolabeled β-blockers it was demonstrated that β-receptor desensitization induced by catecholamine is due to the decline in the total number of receptors. This decline is responsible for the decline in the activity of β-receptor-dependent adenylate cyclase (see detailed discussion Section VII A).

V. Fluorescent Antagonists for Mapping Adrenergic Receptors in vivo

Recently two fluorescent β-blockers were synthesized in our laboratory (*Atlas* and *Levitzki*, 1977): 9-AAP and DAPN (Fig. 1). These compounds were shown to bind in a stereospecific manner to β-adrenergic receptors



9-AAP



DAPN

Fig. 1. Fluorescent β-blockers

in vivo upon their injection to rats and mice. Both peripheral β-receptors as well as β-receptors in the CNS bind these fluorescent antagonists and become visible in the fluorescent microscope (*Atlas* and *Levitzki*, 1977; *Melamed* et al., 1976a; *Melamed* et al., 1976b; *Melamed* et al., 1976c; *Atlas* et al., 1977). Prior injection of l-propranolol into the animal prevents the binding of the blocker whereas the injection of d-propranolol is without effect (*Atlas* and *Levitzki*, 1977). Furthermore, treatment of the rat or mice with 6-hydroxydopamine, which causes the disappearance of the catecholamine storage vesicle in the presynaptic region, does not prevent the appearance of the fluorescence pattern in the Purkinje cell layer upon injection of 9-AAP (*Atlas* et al., 1977) or DAPN (*Atlas*, unpublished experiments). These latter results demonstrate that the localization of the fluorescent compound is indeed in the postsynaptic β-receptors. Thus it is now clear that these compounds can be used for the mapping of β-adrenergic receptors in vivo. Experiments with turkey erythrocyte membranes in vitro also reveal that the binding of 9-AAP can be monitored directly in the fluorimeter (*Atlas* and *Levitzki*, 1977). Currently, efforts are being made to synthesize specific fluorescent α-antagonists in order to map α-adrenergic receptors. A similar approach is planned for the mapping of dopamine receptors. The use of specific ligands to probe catecholamine receptors is complementary to the formaldehyde method of *Falck* et al. (1962) and of the glyoxylic acid method (*Lindvall* and *Björklund*, 1974) for the mapping of *l*-catecholaminergic pathways. These methods (*Falck* et al., 1962; *Lindvall* and *Björklund*, 1974) do not discriminate between the

different types of catecholaminergic neurons since all catecholamines condense with formaldehyde and glyoxylic acid to yield a fluorescent derivative. Furthermore, serotonin also yields a fluorescent derivative upon condensation with formaldehyde or glyoxylic acid and thus serotonergic pathways also become visible in the fluorescence microscope. The development of specific fluorescent ligands for each type of receptor may become a powerful tool for mapping the different types of catecholaminergic receptors. Obviously, this approach can be extended for the study of other receptors for neurotransmitters and hormones.

The availability of fluorescent agonists or antagonists makes it also possible to study directly the receptor in vitro if high enough concentration can be generated in the test tube. Preliminary experiments (*Atlas* and *Levitzki*, 1977) using 9-AAP, do indeed demonstrate that its binding to β-adrenergic receptor in turkey erythrocyte membranes can be monitored in vitro. It is hoped that such compounds will become a powerful tool for monitoring the state of the receptor under different conditions.

VI. Affinity Labeling of the β-Adrenergic Receptor

Affinity labeling of the β-adrenergic receptor has recently been achieved by using a reversible β-blocker to which the reactive group bromoacetyl was attached (*Atlas* and *Levitzki*, 1976; *Atlas* et al., 1976). The compound N-(2-hydroxy-3-naphthyloxypropyl)-N′-bromoacetylethylenediamine (Figure 2) has been shown to inhibit irreversibly the epinephrine-dependent

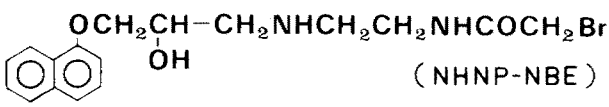


Fig. 2. An affinity label for the β-adrenergic receptor

adenylate cyclase activity without damaging the F^--dependent activity in turkey erythrocyte membranes (*Atlas* and *Levitzki*, 1976; *Atlas* et al., 1976). Furthermore, propranolol and l-epinephrine offer protection against the affinity-labeling reaction. Similarly, the compound was shown to inhibit irreversibly the hormone-stimulated activity in a whole turkey red cell (*Atlas* et al., 1976). The loss of epinephrine-dependent activity is accompanied by the loss of ^{3}H-propranolol binding (*Atlas* and *Levitzki*, 1976; *Atlas* et al., 1976), thus demonstrating directly the loss of the β-receptor subsequent to treatment with the affinity label. More recently, the ^{3}H-affinity label has been synthesized (*Atlas* and *Levitzki*, 1978) and

used to identify the subunits of the β-adrenergic receptor (*Atlas* and *Levitzki*, 1978). The availability of a β-receptor affinity label will also help to establish whether the hormone receptor and the enzyme activity reside on separate polypeptide chains. Since the β-receptor-activated enzymes from pigeon erythrocytes (*Pfeuffer* and *Helmreich*, 1975) and turkey erythrocytes (*Hanski* et al., 1977) have already been solubilized by Lubrol PX and partially purified, an attempt is being made to use the ^{3}H-affinity label and identify the β-receptor in the solubilized state. This procedure can be of use since in the solubilized form, the adenylate cyclase from pigeon erythrocytes or turkey erythrocytes, is hormone insensitive. The loss of hormone sensitivity of adenylate cyclase upon solubilization is well known, not only for catecholamine stimulated adenylate cyclase, but also for adenylate cyclase activated by polypeptide hormones. This uncoupling event leaves the investigator with no direct means to monitor the hormone receptor unless an affinity label or a reversible ligand possessing high affinity are available.

In a recent report (*Takayanagi* et al., 1976) it was claimed that β-adrenergic receptors mediating the relaxation of the guinea pig taenia coli were photoaffinity labeled with *l*-isoproterenol or 2-(2-hydroxy-3-isopropylaminopropoxy-iodobenzene). The irradiation of the guinea pig taenia coli in the presence of either compound resulted in the irreversible loss of response to β-adrenergic agonists such as *l*-isoproterenol. Chloropractolol was also claimed to be an irreversible β-agonist (*Erez* et al., 1975). However, the blocking action of chloropractolol was slowly reversible; therefore, it may very well be that this compound dissociates slowly from the receptor rather than attach to it covalently. *Wrenn* and *Haber* (1976), reported recently on the preparation of an antibody against the β-receptor. Rabbits were immunized with a deoxycholate solubilized fraction from dog heart and the serum isolated from these rabbits was found to inhibit specific ^{3}H-propranolol binding (*Wrenn* and *Haber*, 1976), as well as l-isoproterenol-dependent adenylate cyclase activity in dog heart. So far affinity labels for the α-adrenergic receptor or the dopamine receptor have not been prepared.

VII. Self-Regulation of β-Adrenergic Receptors

A. Desensitization

A decrease in the responsiveness to catecholamines as a result of repeated exposure to catecholamines was found in cultured pineal organs (*Deguchi* and *Axelrod*, 1972). In that system the ability of isoproterenol to induce N-acetylserotonin-transferase activity was observed to decrease upon

repeated isoproterenol stimulation. Similar observations were made with slices of the cerebral cortex. In this case cAMP synthesis as a response to norepinephrine is also decreased upon repeated stimulations (*Kakiachi* and *Rall,* 1968; *Schultz* and *Daly,* 1973a,b,c). It should be emphasized that in the cerebral cortex both α- and β-receptors are claimed to be coupled to adenylate cyclase (see Section III and ref. *Daly*, 1975). Catecholamine-induced refractoriness was observed also in frog erythrocytes (*Mukherjee* et al., 1975; *Mickey* et al., 1975; *Mickey* et al., 1976; *Mukherjee* and *Lefkowitz,* 1976) and with lymphoid cells (*Makman,* 1971), human leukocytes (*Morris* et al., 1975), and macrophages (*Remold-O'Donnel,* 1974). Similar effects were demonstrated in cultured fibroblasts (*Franklin* and *Foster,* 1973; *Franklin* et al., 1975), and glioma cells (*Franklin* and *Foster,* 1973).

The availability of direct means for probing the β-adrenergic receptors led to the discovery that the concentration of a ligand can regulate the concentration or the binding properties of the receptors on the target cell. In the case of β-receptors it has been known for some time that β-adrenergic agonists can induce functional desensitization (tachyphylaxis, tolerance) of target tissue in vivo and in vitro. Using ^{3}H-alprenolol, *Mukherjee* et al. (1975) have shown that prolonged exposure of frog erythrocytes to β-adrenergic catecholamines in vivo or in vitro (*Mickey* et al., 1975) lead to a decrease of 50–70% in the number of alprenolol binding sites without a change in affinity towards the β-antagonists. The order of potency of the catecholamines is isoproterenol > epinephrine > norepinephrine. These investigators also find that the β-blocker propranolol inhibits this action of agonists but does not by itself cause any decrease in the number of receptors.

The down regulation of β-receptors in frog erythrocytes differs from that of insulin or growth hormone in that the treatment in vivo with the protein biosynthesis inhibitor cycloheximide, does not prevent recovery after down regulation (*Lefkowitz,* 1976). These observations suggest that the β-receptors are reversibly inactivated or masked and not lost as in the case of insulin receptors and growth hormone receptors. Similar down regulation of β-receptors was recently reported by *Kebabian* et al. The system studied by *Kebabian* and his colleagues is the β-receptors on the rat pineal gland (*Kebabian* et al., 1975). When the receptors were stimulated physiologically in vivo by keeping the animals in the dark or pharmacologically by injecting *l*-isoproterenol, a rapid fall in the number of ^{3}H-alprenolol binding sites resulted. The fall amounts to 70% reduction in response within 2 h subsequent to stimulation. Within 4 h a recovery in the number of ^{3}H-alprenolol binding sites was found. Exposing the rats to light, thus decreasing the sympathetic activity, results in the increase of the number of β-receptors as measured by ^{3}H-alprenolol binding. These

investigators also reported (*Kebanian* et al., 1975) that the number of β-receptors on the pineal gland normally varies with a circadian periodicity which is inversely related to the cycle of neurotransmitter release (*Romero* et al., 1975).

Down regulation is an efficient mechanism to regulate receptor response especially when the number of receptors is such that only fractional occupancy of these receptors results in maximal response. Under these circumstances, namely in the presence of "spare receptors", a decrease in the number of receptors does not cause a decrease in the potential *maximal* response but will shift the dose response curve to higher agonist concentrations. The reduction in the number of β-receptors can, in principle, account for the functional desensitization of target tissues to repeated agonist stimulation. However, it should be stressed that other mechanisms such as the reduction in receptor affinity may also be responsible for desensitization. Recently it was reported that desensitized catecholamine receptors can be resensitized by purine nucleotide triphosphates, especially GTP and Gpp(NH)p (*Mukherjee* and *Lefkowitz*, 1976). The molecular events which are responsible for the desensitization phenomenon are not at all clear. It is interesting that β-receptor desensitization occurs in frog erythrocytes but not in turkey erythrocytes (*Hanski* and *Levitzki*, 1978).

Whether membrane-bound cytoskeletal elements are involved in receptor-to-enzyme coupling and in the events leading to desensitization is not yet clear. What is clear, however, from the study of β-receptor desensitization in S-49 lymphoma cells, is that cAMP-dependent phosphorylation is not involved (*Shear* et al., 1977). This assertion is made on the basis of the finding that mutant S-49 cells lacking protein kinase but which contain β-receptor dependent adenylate cyclase undergo desensitization. On the other hand, S-49 cells possessing the β-adrenergic receptor but which are devoid of adenylate cyclase do not undergo desensitization (*Shear* et al., 1977). Thus, it is clear that the process of desensitization involves processes which depend on an actively functioning enzyme.

Postreceptor biochemical mechanisms may also operate in the phenomenon of desensitization. Thus, for example, the number of opiate receptors in the brain is not decreased by chronic exposure to high opiate levels (*Pert* et al., 1973; *Klee* and *Streaty*, 1974; *Sharma* et al., 1975). The effect of the opiate ligand is the fast and reversible decrease in cAMP levels; both basal acitivity and PGE-stimulated activity are inhibited. The cells compensate by a subsequent increase of the cAMP levels, back to the level found in the absence of the opiate ligand. In this case, either the adenylate cyclase activity or its amount is increased (*Sharma* et al., 1975). The removal of the opiate ligand results in an immediate further increase of the cAMP levels, *above* the level found in the absence of the drug.

De Vellis and *Brooker* (1974) reported that the generation of cAMP in response to catecholamines in the 2B subclone of RC6G rat glioma cells, previously exposed to norepinephrine and refractory to further norepinephrine addition, is substantially increased by the addition of inhibitors of RNA and protein synthesis. They conclude that formation of a protein (or proteins), important for catecholamine refractoriness, affects synthesis rather than degradation of cAMP.

B. Supersensitivity

Supersensitization to β-adrenergic agonists is observed in different systems and is manifested in both increased cAMP synthesis and in increased level of β-receptors. It was found that after adrenalectomy of the rat, the responsiveness of rat liver adenylate cyclase to catecholamines was enhanced 3–5-fold (*Wolfe* et al., 1976). This increase in adenylate cyclase activity is accompanied by a 3–5-fold increase in the number of β-adrenergic receptors as revealed by direct binding studies using ^{125}I-hydroxybenzylpindolol (*Wolfe* et al.,1976). These changes were reversed by the administration of cortisone. It was suggested (*Wolfe* et al., 1976) that this increase in β-receptors and adenylate cyclase activity may be a compensatory response to the impairment in gluconeogenesis and glycogenolysis which occur after adrenalectomy.

Changes in catecholamine responsiveness in the mammalian brain occur as a result of intraventricular injection of 6-hydroxydopamine (*Sporn* et al., 1976; *Palmer,* 1972; *Kalisker* et al., 1973). Using ^{125}I-iodohydroxybenzylpindolol, an increase in the number of β-receptors in the cerebral cortex could be measured (*Sporn* et al., 1976). Parallel to the increase in the number of β-receptors an increase in the *l*-isoproterenol-dependent adenylate cyclase was detected.

VIII. Catecholamines as Neurotransmitters

β-Adrenergic receptors, α-adrenergic receptors, and dopamine receptors are involved in catecholaminergic innervation. Some of these activities are due to adrenergic stimulation, where the catecholamine functions as a neurotransmitter released from presynaptic vesicles within the adrenergic synapse, acting at a postsynaptic adrenoreceptor. While there is much biochemical data concerning the action of adrenergic agonists as hormones at α- and β-receptors, little is known about their mechanism of action as neurotransmitters. It seems, however, that β-receptor activity at β-adrenergic synapses involves the activation of adenylate cyclase, and that

α-receptor activity involves changes in ion permeability where the first event is the influx of Ca^{2+}. For example, certain cells in the CNS such as the Purkinje cells of the cerebellum are innervated by noradrenergic neurons. The neurotransmitter acting at the Purkinje cells is noradrenaline. It was found that the noradrenaline transmitter is responsible for the sustained depression of spontaneous firing of the rat cerebellar Purkinje cells (*Siggins* et al., 1969; *Hoffer* et al., 1971). It was suggested that this noradrenaline action is mediated by cAMP (*Siggins* et al., 1969; *Siggins* et al., 1971; *Gähwiler,* 1976). Indeed phosphodiesterase inhibitors such as papaverine were found also to depress the firing of Purkinje cells (*Siggins* et al., 1971). In conclusion, it seems that intracellular cAMP levels influence noradrenergic neurotransmission. This conclusion actually classifies these noradrenergic receptors to belong to the β-type. It is therefore possible that other adrenergic synapses, not yet characterized may involve receptors of the β-type which function via the formation of cAMP. The presence of β-adrenoreceptors in cerebral tissue has been confirmed by electrophysiologic techniques (*Siggins* et al., 1969) and by direct measurements of ^{3}H-propranolol binding (*Nahorski,* 1976), ^{3}H-alprenolol binding (*Alexander* et al., 1975; *Bylund* and *Snyder,* 1976), and ^{125}I-hydroxybenzylpindolol binding (*Sporn* and *Molinoff,* 1976). Another example where noradrenaline acts as a neurotransmitter is the rat pineal gland. In this case the noradrenaline neurotransmitter is released from the sympathetic nerves and stimulates adenylate cyclase within the pineal gland (*Brownstein* and *Axelrod,* 1974; *Deguchi* and *Axelrod,* 1973). Studies on this system were reported by *Kebabian* et al. (1975), *Romero* et al. (1975) and *Zatz* et al. (1976). This latter case is another example where the noradrenaline neurotransmitter acts on a postsynaptic membrane receptor of the β-type.

As discussed in Section III it was found that the activation of α-receptors causes changes in the ion permeability of cell membranes of several tissues including intestinal smooth muscle, guinea pig liver and adipose tissue. It is likely that the ion selectivity of the permeability increase varies between tissues. Thus, for example, in the inhibition of longitudinal muscle of the intestine by α-agonists, mainly K^{+} ions are involved. It is possible that the K^{+} ion effects are secondary to Ca^{2+} influx as found in the parotid gland by *Selinger* et al. (1974. Section III b). Indeed, it was suggested by *Triggle* (1972) that α-adrenergic receptors may initiate contraction of certain smooth muscle, mostly arteries, by release of Ca^{2+} from an intracellular store such as the sarcoplasmic reticulum. This mechanistic aspect was proposed mainly on the basis of the finding that the contractile response of such muscles falls only slowly when external Ca^{2+} is withdrawn.

Claims have been made that α-adrenergic receptors in the CNS are also linked to the activation of adenylate cyclase (*Perkins* and *Moore,* 1973;

Huang et al., 1973; *Skolnick* and *Daly*, 1975; *Skolnick* and *Daly*, 1976; *Daly*, 1975). Pharmacologic evidence seems to suggest that α-adrenergic receptors reside in the presynaptic region of a catecholaminergic synapse where the β-receptor is the primary postsynaptic receptor. It was suggested that the extent of norepinephrine release from the presynaptic vesicle is controlled by the presynaptic α-receptor (*Kirkekar* and *Puig*, 1971; *Enero* et al., 1972; *Starke*, 1972; *De Potter* et al., 1971; *Langer*, 1977), thus controlling the accessibility of norepinephrine to the postsynaptic β-receptor. This interplay between α-adrenergic receptors and β-adrenergic receptors may be the basis for the control mechanism of catecholamine action at nerve terminals.

IX. Spare Receptor Control of Catecholamine Action

It was pointed out that the level of the circulating catecholamine is far below its dissociation constant to the receptor (*Levitzki*, 1976). Therefore, only a small fraction of the receptors become occupied at physiologic levels of the hormone. A direct comparison can be made between the dependence of the adenylate cyclase reaction on hormone concentration and the dependence of the hormone-mediated effect on hormone concentration (Table 6). This can be done only in cases where the two sets of data are available. It can be seen from Table 6 that 50% of the biochemical response is achieved at hormone concentrations two orders of magnitude below the hormone concentrations required for 50% saturation

Table 6. The $[H]_{0.5}$ values for hormone in cAMP-mediated processes

The cAMP-mediated biochemical process measured	$[H]_{0.5}$ for the hormone mediated effect	$[H]_{0.5}$ for the hormone in the adenylate cyclase reaction
	(M)	(M)
Epinephrine-stimulated Na^+ outflux in turkey erythrocytes	1×10^{-8} [a]	6×10^{-6} [b]
Epinephrine-stimulated α-amylase secretion by the rat parotid gland	2×10^{-7} [c]	1×10^{-5} [c]

[a] *Gardner* et al. (1974); *Gardner* et al. (1973). $[H]_{0.5}$ refers to the hormone concentration yielding 50% of the effect.
[b] *Levitzki* et al. (1975); *Levitzki* et al. (1974); *Atlas* et al. (1974); *Gardner* et al. (1974); *Gardner* et al. (1973).
[c] *Batzri* et al. (1975); *Schramm* and *Naim* (1970).

of the adenylate cyclase reaction. It appears therefore that only a small fraction of the β-adrenergic receptors must be occupied by the hormone in order to saturate the subsequent biochemical response. Since both biochemical signals quoted in Table 6 are cAMP dependent one can calculate the level of cAMP attained in these systems at different receptor occupancies (*Levitzki,* 1976). It was shown that occupation of 0.016% of the β-adrenergic receptors in the systems described in Table 6 is sufficient to produce 2×10^{-8} *M* to 2×10^{-7} *M* cAMP within one minute (*Levitzki,* 1976). This concentration of cAMP is sufficient to saturate protein kinase and protein kinase-dependent processes such as the processes depicted in Table 6. From this example it is quite clear that only a small fraction of the receptors becomes occupied upon hormone stimulation, and therefore most of the receptors are spare receptors. These receptors are actually *not "spare"* since the necessary small number of receptors which must become occupied in order to produce the necessary signal depends on the *total* number of receptors. The fraction of receptors saturated will provide the level of second messenger necessary to saturate the cAMP dependent biochemical processes. It seems, therefore, that the terminology of spare receptors is inadequate since all the receptors are an integral part in generating the response.

It seems that some hormone-mediated processes and neurotransmitter-mediated responses operate by a mechanism involving the interaction of a ligand possessing relatively low affinity to the receptor, with a large excess of the receptor. The end result of such a situation is similar to that obtained when a high affinity ligand interacts with a smaller number of receptors, since the signal always depends on the product of the two. However, there is a possible advantage to a mechanism in which the agonist has low affinity to the receptor and, therefore, the extent of the signal elicited becomes dependent on the total number of receptors per cell. Such a mechanism allows for a discriminatory action of the same hormone on different tissues possessing the same receptor. Namely, whether or not different tissues will respond to the same hormone concentration depends on the total number of receptors per cell in each of the target tissues. Catecholamines, glucagon, and histamine are responsible for a multitude of biochemical processes in different tissues, and in all three cases there is evidence for the existence of spare receptors. We would therefore like to suggest that for ligands acting on a variety of tissues, the spare receptor mechanism is likely to operate.

Two approaches can be used to analyze the existence of spare receptors. One is a quantitative comparison between receptor occupancy and the dose-response curve. This approach can be used when quantitative measurements of receptor occupancy can be performed. Another approach, which was originally used by *Nickersen* (1956), is to block irreversibly

a fraction of the receptors and determine whether full response can still be obtained. Using this technique, *Nickersen* was able to show that the blocking of 99% of the histamine receptors by an irreversible blocker shifts the dose-response curve with respect to the histamine agonist to higher concentrations but does not reduce the maximal response.

X. Conclusion

In the last few years the characterization of catecholamine receptors has developed considerably. The primary biochemical signals occurring subsequent to catecholamine binding are better understood today than a few years ago. Specific ligands can now be used to monitor catecholamine receptors both in vitro and in vivo. This applies especially to the field of β-adrenergic receptors. Even attempts to solubilize and affinity label the β-adrenergic receptor have met with initial success. It is to be expected that within a few years the mechanism of β-receptor activity will be understood more fully and that further progress will be made in the field of α-adrenergic and dopamine receptors.

References

Aghajanian, G.K.: Fed. Proc. **31**, 91–96 (1974)
Aghajanian, G.K., Foote, W.E., Sheard, M.H.: J. Pharmacol. Exp. Ther. **171**, 171, 178–187 (1970)
Aghajanian, G.K., Foote, W.E., Sheard, M.H.: Science **161**, 706–708 (1973)
Aghajanian, G.K., Haighler, H.J., Bloom, F.E.: Life Sci. **17**, 615–622 (1972)
Ahlquist, R.P.: Am. J. Physiol. **153**, 586–598 (1948)
Ahlquist, R.P.: Ann. N. Y. Acad. Sci. **139**, 549–552 (1967)
Alexander, R.W., Davis, J.N., Lefkowitz, R.J.: Nature **258**, 437–440 (1975)
Anden, N.E., Corrodi, H., Fuxe, K., Hökfelt, T.: Br. J. Pharmacol. **34**, 1–7 (1968)
Appleman, A.M., Thompson, W.J., Russel, T.R.: Adv. Cycl. Nucleotide Res. **3**. Greengard, P., Robison, G.A. (eds). New York: Raven Press, pp. 65–98, 1972
Atlas, D., Hanski, E., Levitzki, A.: Nature **268**, 144–146 (1977)
Atlas, D., Levitzki, A.: Biochem. Biophys. Res. Commun. **69**, 397–403 (1976)
Atlas, D., Levitzki, A.: Proc. Natl. Acad. Sci. USA **74**, 5290–5294 (1977)
Atlas, D., Levitzky, A.: Nature, in press (1978)
Atlas, D., Steer, M.L., Levitzki, A.: Proc. Natl. Acad. Sci. USA **71**, 4246–4248 (1974)
Atlas, D., Steer, M.L., Levitzki, A.: Proc. Natl. Acad. Sci. USA **73**, 1921–1925 (1976)
Atlas, D., Teichberg, V.I., Changeux, J.P.: Brain Res. **28**, 532–536 (1977)
Aurbach, G.D., Fedak, S.A., Woodward, C.J., Palmer, J.S., Hauser, D., Troxler, F.: Science **186**, 1223–1225 (1974)
Batzri, S., Selinger, Z., Schramm, M., Robinovitch, M.R.: J. Biol. Chem. **248**, 361–368 (1975)
Bilezikian, J.P., Aurbach, G.D.: J. Biol. Chem. **249**, 157–161 (1974)
Boakes, R.J., Bradley, P.B., Briggs, I., Dray, A.: Br. J. Pharmacol. **40**, 202–218 (1970)

Bockaert, J., Premont, J., Glowinski, J., Thierry, A.M., Tassin, J.P.: Brain Res. **107**, 303–315 (1976)
Brown, E.M., Gardner, J.D., Aurbach, G.D.: Endocrinology **99**, 1370–1376 (1976)
Brown, E.M., Hauser, D., Toxler, F., Aurbach, G.D.: J. Biol. Chem. **251**, 1232–1238 (1976a)
Brown, E.M., Rodbard, D., Fedak, S.A., Woodward, C.J., Aurbach, G.D.: J. Biol. Chem. **251**, 1239–1246 (1976b)
Brownstein, M.J., Axelrod, J.: Science **184**, 163–165 (1974)
Buckley, G.A., Jordan, C.C.: Br. J. Pharmacol. **38**, 394–398 (1970)
Burt, D.R., Creese, I., Snyder, S.H.: Mol. Pharmacol. **12**, 631–638 (1976)
Bylund, D.B., Snyder, S.H.: Mol. Pharmacol. **12**, 568–580 (1976)
Caron, M.G., Lefkowitz, R.J.: Nature **249**, 258–260 (1974)
Caron, M.G., Lefkowitz, R.J.: J. Biol. Chem. **251**, 2374–2384 (1976)
Cassel, D., Selinger, Z.: Biochim. Biophys. Acta **452**, 538–551 (1976)
Creese, I., Burt, D.R., Snyder, S.H.: Science **192**, 481–483 (1976)
Cuatrecasas, P.: Ann. Rev. Biochem. **43**, 169–214 (1974) and references therein
Cuatrecasas, P., Tell, G.P.E., Sica, V., Parikh, I., Change, K.J.: Nature **247**, 92–97 (1974)
Dale, H.H.: J. Physiol. (Lond.) **34**, 163–206 (1906)
Daly, J.: Role of Cyclic Nucleotides in the Nervous System. In: Handbook of Psychopharmacology. Iversen, L.L., Iversen, S.D., Snyder, S.H. (eds.), Vol. 5, pp. 47–130, New York: Plenum Press 1975
Daly, J.W., Huang, H., Shimuzu, H.: Nature **255**, 163–166 (1976)
Deguchi, T., Axelrod, J.: Proc. Natl. Acad. Sci. USA **69**, 2208–2211 (1972)
Deguchi, T., Axelrod, J.: Mol. Pharmacol. **9**, 612–618 (1973)
De Potter, W.P., Chubb, I.W., Put, A., De Schaepdryver, A.F.: Arch. Int. Pharmacodyn. Ther. **193**, 191–197 (1971)
De Vellis, J., Brooker, G.: Science **186**, 1221–1223 (1974)
Eisenfeld, A.J., Landsberg, L., Axelrod, J.: J. Pharmacol. Exp. Ther. **158**, 378–385 (1967)
Enero, M.A., Langer, S.Z., Rothlin, R.P., Stefano, E.J.E.: Br. J. Pharmacol. **44**, 672–688 (1972)
Erez, M., Weinstock, M., Cohen, S., Stacher, G.: Nature **255**, 635-636 (1975)
Falck, B., Hillarp, N.A., Thieme, G., Torp, A.: J. Histochem. Cytochem. **10**, 348–354 (1962)
Franklin, T.J., Foster, S.J.: Nature [New Biol.] **246**, 119–120 (1973)
Franklin, T.J., Morris, W.P., Twose, P.A.: Mol. Pharmacol. **11**, 485–491 (1975)
Furchgott, R.F.: In: Handbook of Experimental Pharmacology XXXIII. Blaschko, H., Muscholl, E. (eds). Berlin–Heidelberg–New York: Springer 1972, pp. 283–335
Gaddum, J.H.: J. Physiol. (Lond.) **121**, 15 p. (1953)
Gaddum, J.H., Hameed, K.A., Hathaway, D.E., Stephens, F.F.: Q. J. Exp. Physiol. **40**, 49–74 (1955)
Gähwiler, B.H.: Nature **259**, 483–484 (1976)
Gardner, J.D., Klaeveman, H.L., Bilezikian, J.P., Aurbach, G.D.: J. Biol. Chem. **248**, 5590–5597 (1973)
Gardner, J.D., Klaeveman, H.L., Bilezikian, J.P., Aurbach, G.D.: J. Biol. Chem. **249**, 516–520 (1974)
Girardier, L., Seydoux, G., Clausen, T.: J. Gen. Physiol. (Lond.) **52**, 925–940 (1968)
Greenberg, D.A., O'Prichard, D.C., Snyder, S.H.: Life Sci. **19**, 69–76 (1976)
Haigler, H.J., Aghajanian, G.K.: J. Pharmacol. Exp. Ther. **188**, 688–699 (1974)
Hanski, E., Sevilla, N., Levitzki, A.: Eur. J. Biochem. (1977) in press
Hanski, E., Levitzki, A.: Life Sci. **22**, 53–60 (1978)
Haylett, D.G., Jenkinson, D.H.: J. Physiol. (Lond.) **225**, 721–750 (1972a)
Haylett, D.G., Jenkinson, D.H.: J. Physiol. (Lond.) **225**, 752–772 (1972b)
Hoffer, B.J., Siggins, G.R., Bloom, F.E.: Brain Res. **25**, 523–534 (1971)

Horn, A.S., Cuello, A.C., Miller, R.J.: J. Neurochem. **22**, 265–270 (1974)
Huang, M., Mo, A.K.S., Daly, J.W.: Mol. Pharmacol. **3**, 711–717 (1973)
Insel, P.A., Maguire, M.E., Gilman, A.G., Bourne, H.R., Coffino, P., Melbron, K.L.: Mol. Pharmacol. **12**, 1062–1069 (1976)
Jenkinson, D.H.: Brit. Med. Bull. **29**, 142–147 (1973)
Kakiachi, S., Rall, T.W.: Mol. Pharmacol. **4**, 367–378 (1968)
Kalisker, A., Rutledge, C.O., Perkins, J.P.: Mol. Pharmacol. **9**, 619–629 (1973)
Kebabian, J.W., Petzold, G.L., Greengard, P.: Proc. Natl. Acad. Sci. USA **63**, 2145–2149 (1972)
Kebabian, J.W., Zatz, M., Romero, J.A., Axelrod, J.: Proc. Natl. Acad. Sci. USA **72**, 3735–3739 (1975)
Kelly, P.H., Miller, R.J.: Br. J. Pharmacol. **54**, 115–121 (1975)
Kirkekar, S.M., Puig, M.: Br. J. Pharmacol. **43**, 359–369 (1971)
Klee, W.A., Streaty, R.A.: Nature **248**, 61–63 (1974)
Kunos, G., Young, M.S., Nickersen, M.: Nature **241**, 119–120 (1973)
Kunos, G., Szentivanyi, M.: Nature **217**, 1077–1078 (1968)
Langer, S.Z.: Br. J. Pharmacol. **60**, 481–497 (1977)
Lefkowitz, J.F., Mukherjee, C., Coverston, M., Caron, M.G.: Biochem. Biophys. Res. Commun. **60**, 703–709 (1974)
Lefkowitz, R.J., Mukherjee, C., Limbrid, L.E., Caron, M.G., Williams, L.T., Alexander, R.W., Mickey, J.V., Tate, R.: Recent Progress in Hormone Research **32**, 597–632 (1977)
Lefkowitz, R.J., Mulliken, D., Caron, M.G.: J. Biol. Chem. **251**, 4686–4692 (1976)
Levitzki, A.: In: Receptors and Recognition. Cuatrecasas, P., Greaves, M.F. (eds.), Vol. 2, Ser. A., pp. 199–229. London: Chapman and Hall 1976
Levitzki, A.: Biochem. Biophys. Res. Commun. **74**, 1154–1159 (1977)
Levitzki, A., Atlas, D., Steer, M.L.: Proc. Natl. Acad. Sci. USA **71**, 2773–2776 (1975)
Levitzki, A., Sevilla, N., Atlas, D., Steer, M.L.: J. Mol. Biol. **97**, 35–53 (1975)
Levitzki, A., Sevilla, N., Steer, M.L.: J. Supramol. Struct. **4**, 405–418 (1976)
Lin, M.C., Salomon, Y., Rendell, M., Rodbell, M.: J. Biol. Chem. **250**, 4246–4252 (1975)
Lindvall, O., Björklund, A.: Acta Physiol. Scand. (Suppl.) **92**, 1–48 (1974)
Londos, C., Salomon, Y., Lin, M.C., Harwood, J.P., Schramm, M., Wolff, Y., Rodbell, M.: Proc. Natl. Acad. Sci. USA **71**, 3087–3090 (1974)
Maguire, M.E., Van Arsdale, P.M., Gilman, A.G.: Mol. Pharmacol. **12**, 335–339 (1976)
Maguire, M.E., Wiklund, R.A., Anderson, H.J., Gilman, A.G.: J. Biol. Chem. **251**, 1221–1231 (1976)
Makman, M.H.: Proc. Natl. Acad. Sci. USA **68**, 885–889 (1971)
Melamed, E., Lahav, M., Atlas, D.: Nature **261**, 420–422 (1976a)
Melamed, E., Lahav, M., Atlas, D.: Brain Res. **116**, 511–515 (1976b)
Melamed, E., Lahav, M., Atlas, D.: Experientia **32**, 1387–1389 (1976c)
Mickey, J., Tate, R., Lefkowitz, R.J.: J. Biol. Chem. **250**, 5727–5729 (1975)
Mickey, J.W., Tate, R., Mulliken, D., Lefkowitz, R.J.: Mol. Pharmacol. **12**, 409–419 (1976)
Miller, R.J., Kelly, P.H.: Nature 163–166 (1975)
Morris, H.G., De Roche, G.B., Caro, C.M.: Adv. Cyclic Nucleotide Res. **5**, Ab 812 (1975)
Mukherjee, C., Lefkowitz, R.J.: Proc. Natl. Acad. Sci. USA **73**, 1494–1498 (1976)
Nahorski, S.R.: Nature **259**, 488–489 (1976)
Nickersen, M.: Nature **178**, 697–698 (1956)
O'Dea, R.F., Zatz, M.: Proc. Natl. Acad. Sci. USA **73**, 3398–3402 (1976)
Orly, J., Schramm, M.: Proc. Natl. Acad. Sci. USA **73**, 4410–4414 (1976)
Oron, Y., Löwe, M., Selinger, Z.: Mol. Pharmacol. **11**, 79–86 (1975)
Palmer, G.C.: Neurpharmacology **11**, 145–149 (1972)
Perkins, J.P.: Adv. Cycl. Nucleotide Res. **3**, 1–64 (1973) and references therein

Perkins, J.P., Moore, M.M.: J. Pharmacol. Exp. Ther. **185**, 371–378 (1973)
Pert, C.B., Pasternak, G., Snyder, S.H.: Science **182**, 1359–1361 (1973)
Pfeuffer, T., Helmreich, E.J.M.: J. Biol. Chem. **250**, 867–876 (1975)
Phillipson, O.T., Horn, A.S.: Nature **261**, 418–420 (1976)
Prasad, K.N., Gilmer, K.N.: Proc. Natl. Acad. Sci. USA **71**, 2525–2529 (1974)
Rassmussen, M., Lake, W., Allen, J.E.: Biochim. Biophys. Acta **411**, 63–73 (1975)
Remold-O'Donnel, E.: J. Biol. Chem. **249**, 3615–3621 (1974)
Rendell, M., Salomon, Y., Lin, M.C., Rodbell, M., Berman, M.: J. Biol. Chem. **250**, 4253–4260 (1975)
Robison, G.A., Butcher, R.W., Sutherland, E.W.: Ann. Rev. Biochem. **37**, 149–174 (1968)
Romero, J.A., Zatz, M., Kebabian, J.W., Axelrod, J.: Nature **258**, 435–436 (1975)
Salomon, Y., Lin, M.C., Londos, C., Rendell, M., Rodbell, M.: J. Biol. Chem. **250**, 4239–4245 (1975)
Sattin, A., Rall, T.W., Zanella, J.: J. Pharmacol. Exp. Ther. **135**, 371–378 (1975)
Schramm, M., Naim, E.: J. Biol. Chem. **245**, 3225–3231 (1970)
Schramm, M., Rodbell, M.: J. Biol. Chem. **250**, 2232–2237 (1975)
Schramm, M., Selinger, Z.: J. Cycl. Nucl. Res. **1**, 181–192 (1975)
Schultz, J., Daly, J.W.: Mol. Pharmacol. **4**, 367–378 (1973a)
Schultz, J., Daly, J.W.: J. Neurochem. **21**, 537–579 (1973b)
Schultz, J., Daly, J.W.: J. Neurochem. **21**, 1319–1326 (1973c)
Selinger, Z., Eimerl, S., Schramm, M.: Proc. Natl. Acad. Sci. USA **71**, 128–131 (1974)
Sevilla, N., Levitzki, A.: FEBS Lett. **76**, 129–134 (1977)
Sevilla, N., Steer, M.L., Levitzki, A.: Biochemistry **15**, 3493–3499 (1976)
Sharma, S.K., Nirenberg, M., Klee, W.A.: Proc. Natl. Acad. Sci. USA **72**, 590–594 (1975)
Shear, M., Insel, P.A., Melmon, K.L., Coffino, P.: J. Biol. Chem. **251**, 7572–7576 (1976)
Siggins, G.R., Hoffer, B.J., Bloom, F.E.: Mol. Pharmacol. **9**, 619–629 (1969)
Siggins, G.R., Hoffer, B.J., Bloom, F.E.: Brain Res. **25**, 535–553 (1971)
Skolnick, P., Daly, J.W.: Mol. Pharmacol. **11**, 535–551 (1975)
Skolnick, P., Daly, J.W.: Life Sci. **19**, 497–504 (1976)
Sporn, J.R., Harden, T.K., Wolfe, B.B., Molinoff, P.B.: Science **194**, 624–626 (1976)
Sporn, J.R., Molinoff, P.B.: J. Cyclic Nucl. Res. **2**, 149–161 (1976)
Starke, K.: Naunyn-Schmiedebergs Arch. Pharmac. **274**, 18–45 (1972)
Steer, M.L., Atlas, D., Levitzki, A.: New Engl. J. Med. **292**, 409–414 (1975)
Steer, M.L., Levitzki, A.: Arch. Biochem. Biophys. **167**, 371–375 (1975a)
Steer, M.L., Levitzki, A.: J. Biol. Chem. **250**, 2080–2084 (1975b)
Sutherland, E.W., Øye, I., Butcher, R.W.: Recent Progr. Horm. Res. **21**, 623–646 (1965)
Takayanagi, I., Yoshioka, M., Takagi, K., Tamura, Z.: Eur. J. Pharmacol. **35**, 121–125 (1976)
Tolkovsky, A.M., Levitzki, A.: Biochemistry (1977). In press
Triggle, D.J.: Ann. Rev. Pharmacol. **12**, 185–196 (1972)
Walsh, D.A., Perkins, J.D., Krebs, E.G.: J. Biol. Chem. **243**, 3763–3765 (1968)
Williams, L.T., Jarett, L., Lefkowitz, R.J.: J. Biol. Chem. **251**, 3096–3104 (1976)
Williams, L.T., Lefkowitz, R.J.: Science **192**, 791–793 (1976)
Williams, L.T., Snyderman, R., Lefkowitz, R.J.: J. Clin. Invest. **57**, 149–155 (1976)
Wolfe, B.B., Harden, T.K., Molinoff, P.B.: Proc. Natl. Acad. Sci. USA **73**, 1343–1347 (1976)
Wooley, D.W., Shaw, E.: Proc. Natl. Acad. Sci. USA **40**, 228–231 (1954)
Wrenn, S.M., Haber, E.: Fed. Proc. **35** Ab 1410 (1976)
Zatz, M., Kebabian, J.W., Romero, J.A., Lefkowitz, R.J., Axelrod, J.: J. Pharmacol. Exp. Ther. **196**, 714–722 (1976)

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The Synthesis and Secretion of Albumin

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Abbreviations

ATP	adenosine triphosphate
DNP	dinitrophenol
dpm	disintegrations per minute
GTP	guanosine triphosphate
mRNA	messenger ribonucleic acid
PEP	phosphoenol pyruvate
RNA	ribonucleic acid
Tris	Tris(hydroxymethyl)aminomethane
tRNA	transfer ribonucleic acid

I. Introduction

Knowledge presently available on albumin and its metabolism has been summarized in various reviews (*Foster*, 1960; *Ogata*, 1966, 1967, 1973; *Schultze* and *Heremans*, 1966; *Freeman*, 1967; *Peters*, 1970, 1975, 1977). Some reviews have concentrated on special points such as biosynthesis of albumin (*Campbell*, 1961b, 1967, 1970, 1975; *Papaconstantinou* and *Ledford*, 1973; *Weigand*, 1977b), binding of fatty acids (*Spector*, 1975), pinocytotic uptake (*Holtzer* and *Holtzer*, 1960), catabolism (*Bocci*, 1970), and clinical aspects (*Sandor*, 1966; *Rothschild* et al., 1970, 1972a,b, 1973, 1975; *Hitzig*, 1977; *Weigand*, 1977a). Two recent developments require a critical reevaluation of hitherto reported work on the synthesis and secretion of albumin:

1. The isolation of radiochemically pure albumin was found to be necessary in studies involving incorporation of radioactive amino acids (*Schreiber* et al., 1969; *Rotermund* et al., 1970; *Urban* et al., 1974b). Radioactively labeled albumin must be purified to constant specific radioactivity to distinguish between unspecific binding and biosynthetic incorporation of amino acids.

2. Antiserum against albumin does not precipitate only albumin but also at least two other proteins, namely pre-proalbumin (*Strauss* et al., 1977a,b; *Yu* and *Redman*, 1977) and proalbumin (*Urban* et al., 1974a;

Russell and *Geller,* 1975; *Edwards* et al., 1976c; *Patterson* and *Geller,* 1977; *Millership,* 1977). This is important when immunoprecipitation is used for determination or isolation of albumin or related proteins.

Particulars of the isolation of albumin from various sources will be discussed in greater detail in Section II. In subsequent chapters it will be shown how our current ideas on the site, mechanism, and regulation of albumin synthesis and its secretion need to be changed or supplemented.

II. Isolation and Determination of Albumin: Implications on Studies of Synthesis and Secretion

The study of protein synthesis and secretion is often based on two different experimental techniques used either independently or in conjunction. Incubation in the presence of radioactively labeled amino acids and/or precipitation with an antiserum are used to monitor the rate of synthesis of specific proteins.

The following will describe the major pitfalls of these techniques and stress the precautions necessary in interpreting the obtained results.

A. Binding of Amino Acids to Albumin in the Absence of Ribosomes and mRNA

1. Unspecific Binding of Amino Acids

To test the binding of amino acids, *Rotermund* et al. (1970) incubated purified rat serum albumin with radioactive lysine or with various radioactive leucine preparations. The radioactivity bound to albumin was determined according to *Mans* and *Novelli* (1960, 1961). The method involves application of the sample to filter paper disks which are subsequently immersed in ice-cold 10% trichloroacetic acid. The disks are then extensively washed with cold and hot 5% trichloroacetic acid, warm and cold ethanol/ether (1:1, vol/vol) and ether. Albumin in the incubation medium became radioactive in spite of the complete absence of a protein synthesizing system. The radioactivity bound by albumin was proportional to the amount of free radioactive amino acid in the medium (Fig. 1). Furthermore, the uptake of radioactive amino acid by rat serum albumin increased with time and appeared to be more than doubled if the incubation was carried out in the presence of an ATP-generating system (Fig. 2). The radioactivity was bound tightly to albumin since neither dialysis, addition of a large excess of the respective nonradioactive amino acid, nor precipitation of albumin with trichloroacetic acid followed by the washing

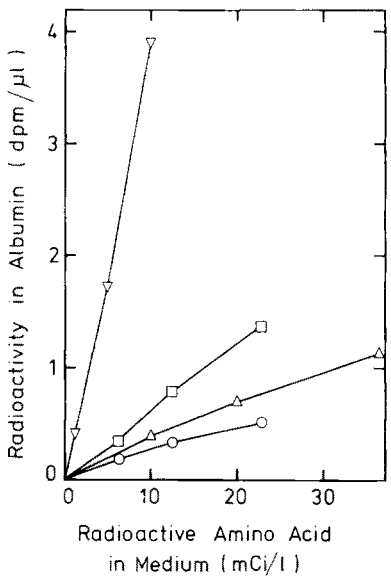

Fig. 1. Binding of radioactivity to albumin upon incubation with radioactive amino acid preparations in the absence of a protein synthesizing system. Albumin-bound radioactivity is plotted against the amount of free radioactivity in the incubation medium. Purified rat serum albumin (340 µg) was incubated with the labeled amino acid for 30 min at 37°C in 1 ml of 50 m*M* Tris-HCl buffer, pH 7.4, containing 75 m*M* sucrose, 45 m*M* KCl, 7.5 m*M* $MgCl_2$, 1 m*M* ATP, 0.5 m*M* GTP, 10 m*M* phosphoenolpyruvate, and 50 µg of pyruvate kinase. The radioactive amino acid preparations were: (▽) *L*-[4,5-$^3H_2$]leucine (40,000 Ci · mol^{-1}); (□) *L*-[U-^{14}C]lysine (310 Ci · mol^{-1}); (△) *L*-[l-^{14}C] leucine (51 Ci · mol^{-1}; (○) *L*-[U-^{14}C] leucine (311 Ci · mol^{-1}). Modified from *Rotermund* et al. (1970)

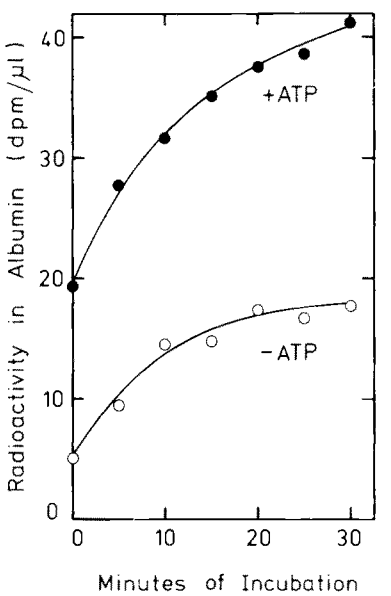

Fig. 2. Binding of radioactivity to albumin during incubation with *L*-[4,5-$^3H_2$] leucine in the absence of a protein-synthesizing system. Albumin-bound radioactivity is plotted against incubation time. Incubation was carried out at 37°C in the medium described in the legend of Fig. 1 either in the presence of (●) or in the absence of (○) ATP, GTP, phosphoenolpyruvate. Modified from *Rotermund* et al. (1970)

procedure described above removed the bound radioactivity. However, about 90% of the bound radioactivity could be removed by extensive purification (Table 1). This involved treatment of the radioactive albumin with 0.7% deoxycholate, precipitation with 10% trichoroacetic acid, solubilization of the precipitated albumin in absolute ethanol containing 1% trichloroacetic acid, dialysis against Tris-HCl buffer, precipitation with ammonium sulfate and resolubilization in Tris-HCl buffer, ion-exchange

Table 1. Removal of radioactivity nonenzymatically bound to rat serum albumin

Purification step	Specific radioactivity of albumin (dpm · μg^{-1})
Incubation medium + 0.7% deoxycholate	170
Trichloroacetic acid-ethanol	200
Ammonium sulfate	70
DEAE-Sephadex A-50	39
Polyacrylamide gel electrophoresis	
at pH 9.3	21
at pH 2.7	20

Purified albumin (18.2 mg) from rat serum was incubated with 2.75 mCi of *L*- [4,5-$^3H_2$] leucine (40 Ci · mol^{-1}) for 30 min at 37°C in 55 ml of 50 m*M* Tris-HCl buffer of pH 7.5 in the presence of GTP, ATP, and an ATP-generating system (see Fig. 1). At the end of the incubation period, the radioactive leucine in the incubation mixture was diluted 12,200-fold with nonradioactive *L*-leucine. Deoxycholate was added to a final concentration of 0.7% before albumin was purified from the mixture.
Modified from *Rotermund* et al. (1970).

chromatography on DEAE-Sephadex A-50, and finally preparative electrophoresis in polyacrylamide gel at both an alkaline and an acidic pH. Binding of radioactivity to protein during incubation with radioactive amino acid preparations in the absence of protein biosynthesis has also been observed by other workers (*Brunish* and *Luck*, 1952; *Cornwell* and *Luck*, 1958; *Samarina* et al., 1960; *Kritzman* et al., 1962; *Hochberg* et al., 1972).

Two methods are commonly used to determine the sites of incorporation of the radioactive amino acid into the polypeptide chain. The first is proteolytic digestion of the radioactive protein followed by chromatographic separation of the peptides. Association of the radioactivity with several peptides together with the identification of the radioactive amino acid has been interpreted as indicating incorporation of the radioactivity into several interior positions of the polypeptide chain, suggesting de novo biosynthesis through peptide bond formation. The second method is to react the protein with ninhydrin. Provided the amino acid used was labeled in the α-carboxyl group, this treatment would effect the release of radioactivity from a free carboxyl group but not from an amino acid incorporated through peptide bonds.

Using both methods, *Kritzman* et al. (1962) showed that the incubation of zinc-free insuline with ^{14}C-phenylalaline resulted in an incorporation of the amino acid into internal positions of the polypeptide chain, possibly through peptide bonds. Other authors found only an insignificant release by ninhydrin of radioactivity from proteins spontaneously labeled with various ^{14}C-amino acids (*Brunish* and *Luck*, 1952; *Samarina* et al.,

1960). *Cornwell* and *Luck* (1958) incubated purified insulin as well as histones from calf thymus nuclei with either *DL*- [3-^{14}C] phenylalanine or *DL*- [2-^{14}C] lysine. The acid-insoluble material was then treated with 1-fluoro-2,4-dinitro-benzene and hydrolyzed with acid. Amino acids and DNP amino acids were separated and counted. About equal amounts of the bound radioactivity were recovered as amino acids and as their DNP derivatives. The total recovery of bound ^{14}C-amino acid was about 83%. The results demonstrate that about half of the bound ^{14}C-amino acid was linked to the N-terminus of the tested proteins.

The observations described above demonstrate that in vitro, in spite of the absence of a biologically active protein synthesizing system, radioactively labeled amino acids may be bound very tightly to various sites of a polypeptide chain, thus simulating de novo biosynthesis of a protein. A common feature of the above binding experiments is the very small proportion of radioactivity bound, compared to the amount of free radioactive amino acid in the incubation medium. Depending on the proteins and amino acids studied, the proportion of radioactivity found in acid-insoluble material ranged from about 0.7% to as little as 0.002% of the applied dose for incubation periods of 30 min to 120 min, usually at 37°C. On the other hand, such low incorporation rates were also obtained in supposedly biologically active in vitro systems. A recent example is a report by *Goussault* et al. (1976) claiming the synthesis and secretion of serum albumin by human peripheral lymphocytes in culture. After an incubation period of 72 h only about 0.2% of the radioactive dose was found associated with the albumin fraction. No information was given as to the nature of the bound radioactivity.

With such low "incorporation" rates, the possible presence of radiochemical impurities in the tracer amino acids used must be born in mind. ^{14}C- and especially ^{3}H-labeled compounds are vulnerable to decomposition by self-radiolysis (see review by *Bayly* and *Evans,* 1966). Most methods of analysis of the radioactive amino acid are not sensitive enough to detect impurities of less than 1%.

2. Specific Binding of Amino Acids

One of the physiologic functions ascribed to albumin is the transport of a variety of ions (for review see *Peters,* 1970, 1975). The specific binding to albumin in vivo of certain amino acids or amino acid derivatives appears to have physiologic implications. The free thiol group of serum albumin can form mixed disulfides with other free SH-group-containing substances such as cysteine (*Eagle* et al., 1960; *King,* 1961; *Szentivanyi* et al., 1961; *Isles* and *Jocelyn,* 1963; *Harrap* et al., 1973) and γ-glutamylcysteinylglycine (*King,* 1961; *Harrap* et al., 1973). The mixed disulfide bond could

not be cleaved by precipitating albumin with 8% trichloroacetic acid; however, heating to 100°C or treatment with dithionite liberated protein-bound cysteine (*Eagle* et al., 1960). Other binding sites of the albumin molecule have a specific affinity for tryptophan (*McMenamy* and *Oncley*, 1958; *McMenamy*, 1963, 1965; *Fairclough* and *Fruton*, 1966; *King* and *Spencer*, 1970; *Stewart* and *Doherty*, 1973) and for the tyrosine derivative thyroxine (*Sterling* and *Tabachnik*, 1961; *Tritsch* et al., 1961; *Sterling* et al., 1962; *Sterling*, 1964; *Tabachnik*, 1964; *Steiner* et al., 1966). At low pH, binding of tryptophan or thyroxine to albumin is negligible. Thus, the specific binding to albumin of these amino acids or amino acid derivatives would hardly interfere with the study of amino acid incorporation into albumin since treatment of albumin with trichloroacetic acid would remove these compounds from the protein, for example, as used in the procedure of *Mans* and *Novelli* (1960, 1961).

3. Enzyme-Catalyzed Amino Acid Transfer

Another complication in interpreting incorporation data is the transfer of amino acids to proteins by certain cytoplasmic enzymes. Such enzymes have been found in bacteria and in mammals (*Soffer* et al., 1969). The catalyzed reaction does not require the presence of ribosomes, magnesium ions, or GTP. An aminoacyl-tRNA protein transferase from *Escherichia coli* (*Kaji* et al., 1965; *Soffer* et al., 1969) has been shown in vitro to specifically transfer leucine or phenylalanine from tRNA to NH_2-terminal arginine, lysine, or histidine of peptides or proteins (*Leibowitz* and *Soffer*, 1971; *Soffer*, 1973). The reaction was inhibited by puromycin but not by chloramphenicol (*Leibowitz* and *Soffer*, 1970). An analogous mammalian enzyme catalyzes the transfer of arginine from arginyl-tRNA into peptide linkage with an NH_2-terminal dicarboxylic acid of an acceptor peptide or protein, for example, serum albumin (*Kaji*, 1968, 1976; *Soffer* and *Horinishi*, 1969; *Soffer*, 1970, 1971, 1973). This enzyme has been found in various mammalian tissues including liver (*Kaji* et al., 1963; *Soffer* and *Mendelsohn*, 1966; *Soffer*, 1968; *Tanaka* and *Kaji*, 1974). In vitro, as much as 90% of the added [^{14}C] arginine could be incorporated into serum albumin, bovine thyroglobulin, human Bence-Jones proteins, or soybean trypsin inhibitor (*Soffer* et al., 1969; *Soffer*, 1970). *Kaji* (1968) reported that ribosome-free rat liver extracts could also incorporate ^{14}C-labeled glutamic acid, glycine, methionine, and tryptophan into as yet unidentified acceptor protein in the liver extracts. The enzymic reactions described above may be involved in posttranslational modification of proteins.

Another class of enzymes found in a variety of mammalian tissues catalyzes the exchange of amide groups of glutamine in a polypeptide

chain with a variety of compounds containing a primary amino group (*Sarkar* et al., 1957; *Neidle* et al., 1958; *Clarke* et al., 1959; *Mycek* et al., 1959). It was shown that this reaction could simulate efficient incorporation of [^{14}C] lysine into serum albumin as well as into various other proteins (*Sarkar* et al., 1957; *Clarke* et al., 1959).

B. Immunoprecipitation is Inappropriate for the Isolation of Radiochemically Pure Albumin

1. Preclearing of Samples with Non-Cross-Reacting Albumin from a Different Species

Pure albumin can be obtained easily from serum for the preparation of monospecific antiserum. Nevertheless, immunochemically isolated radioactive albumin, whether labeled in vivo or in vitro, from sources other than blood, contained radioactive contaminants (*Campbell* and *Stone,* 1957; *Peters,* 1957, 1962a; *Campbell* et al., 1960; *Gordon* and *Humphrey,* 1961; *Decken* and *Campbell,* 1962). Similar observations had been made with other proteins. *Askonas* et al. (1954) and *Keston* and *Katchen* (1956) introduced an additional immunoprecipitation to remove coprecipitable contaminants. The procedure has been called "preclearing". An immunochemically unrelated protein is precipitated in the sample by adding the appropriate antiserum. Then, the protein to be isolated is immunoprecipitated. The method has since been widely used to study the incorporation of radioactive amino acids into protein.

More recent investigations have shown that this procedure is inappropriate for the isolation of radiochemically pure albumin from various rat tissues (*Urban* et al., 1974b). In these experiments rats were injected intravenously with *L*- [1-^{14}C] leucine and the tissues removed 12 min later, that is, before secretion of radioactive albumin into the bloodstream (see Sect. IV, A). Albumin was immunoprecipitated from the tissue extracts. Incorporated radioactivity was measured in the thoroughly washed antigen-antibody precipitate as well as in albumin further purified from the antigen-antibody complex (Table 2). The radioactivity found in the immunoprecipitate after preclearing seemed to indicate that not only the liver and an experimental hepatoma but also testis and kidney apparently had incorporated substantial amounts of radioactivity into albumin (Table 2, Column A). However, the specific radioactivity of protein precipitable with antialbumin decreased considerably during further purification: virtually no radioactivity at all was associated with the albumin from testis, the value for albumin from kidney was reduced sevenfold (Table 2, Column B). The results demonstrated that preclearing is not sufficient to eliminate radiochemical contaminants. Further purification of the antigen-antibody precipitate is required to remove these radioactive contaminants.

Table 2. Treatment of rat tissue extracts with bovine serum albumin and its antiserum prior to immunoprecipitation of the rat serum albumin is inappropriate to remove radioactive contaminants

Deoxycholate extract of	Specific radioactivity of protein isolated with method A or method B (dpm · mg^{-1})	
	A	B
Testis	120	6
Kidney	2,800	410
Morris hepatoma 5123TC	8,800	3,500
Liver	61,000	46,000

Tissues were removed 12 min after intracaval injection of 0.1 mCi *L*- [1-^{14}C] leucine per rat. Rat serum albumin was isolated by two different methods: (A) The tissue extract was "precleared" by precipitating added bovine serum albumin with corresponding anti-serum. Then, rat serum albumin was immunoprecipitated. (B) Rat serum albumin was immunoprecipitated directly and then further purified by acid dissociation of the antigen-antibody complex, extraction of the rat serum albumin in acidic absolute ethanol, precipitation with ether and resolubilization in buffer.
Data from *Urban* et al. (1974b).

2. *Existence of Precursor Albumins*

Recently it has been demonstrated that albumin is synthesized via slightly larger precursor proteins. The details will be discussed in Section IV, B. Antiserum against serum albumin also precipitates the precursor albumins. Therefore, the sum of albumin and its precursor proteins is obtained when antiserum against serum albumin is used for determination and isolation. The term antialbumin-precipitable protein has been introduced to refer to the sum of albumin and its precursor proteins (*Edwards* et al., 1976a,b,c; *Urban* et al., 1976). Albumin can be separated from albumin-like proteins (precursors) by ion-exchange chromatography on DEAE-cellulose or on CM-cellulose (*Schreiber* et al., 1969; *Rotermund* et al., 1970; *Judah* and *Nicholls*, 1971a; *Geller* et al., 1972; *Judah* et al., 1973; *Urban* et al., 1974a,b), electrophoresis in polyacrylamide gel (*Schreiber* et al., 1969; *Rotermund* et al., 1970; *Urban* et al., 1974b), or cellulose acetate (*Peters*, 1977) and isoelectric focusing (*Geller* et al., 1972; *Russell* and *Geller*, 1975). An example for the separation of albumin and precursor albumin by chromatography on DEAE-cellulose is shown in Figures 3 and 4. Prior to the ion-exchange chromatography depicted in Figure 3, the albumin plus precursor albumin had been isolated from liver microsomes by precipitation with antialbumin. Identification of the first peak as precursor albumin is described in detail in Section IV, B.

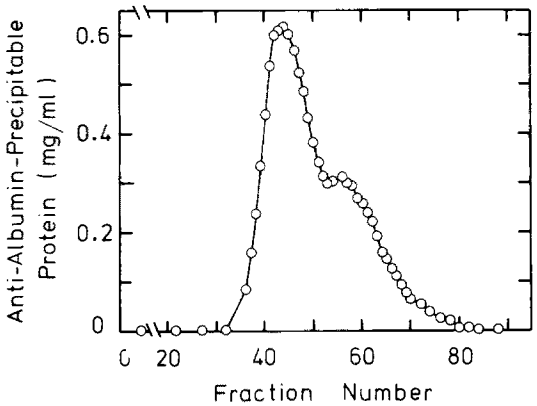

Fig. 3. Separation of microsomal albumin and precursor albumin by chromatography on DEAE-cellulose. Albumin plus precursor albumin had been isolated from the microsomes of 268 g perfused rat liver by homogenization in 0.7% deoxycholate, removal of insoluble material by centrifugation, precipitation in the supernatant with anti-albumin, and subsequent separation from the antibody with trichloroacetic acid, acid ethanol, and ether. The buffer extract of the ether precipitate contained 26 mg of anti-albumin-precipitable protein. The sample was chromatographed on a 1 x 31 cm DEAE-cellulose column with Tris-HCl, pH 7.6, at a flow rate of 19 ml/h. Buffer volumes and concentrations were (1) 60 ml of 10 m*M*, (2) 35 ml of 50 m*M*, and (3) a linear gradient of 200 ml 50–300 m*M*. The gradient was started upon collection of the first fraction. The concentration in each fraction of antialbumin-precipitable protein was determined according to *Mancini* et al. (1965). Modified from *Urban* et al. (1974a)

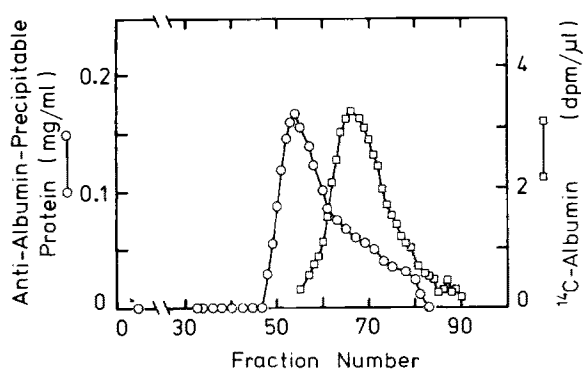

Fig. 4. Cochromatography of precursor albumin obtained from liver microsomes and [^{14}C] albumin obtained from serum on DEAE-cellulose. A 3.6 mg sample of the combined fractions 33–47 of the chromatography depicted in Fig. 3 was mixed with 1.25 mg biosynthetically labeled [^{14}C] albumin which had been purified from rat serum. The mixture was chromatographed at conditions similar to those described in Fig. 3. The peak of radioactivity represents the albumin obtained from rat serum. The peak of antialbumin-precipitable protein represents predominantly precursor albumin obtained from liver microsomes. Positions of peaks in Figs. 3 and 4 are slightly different due to different fraction sizes in the two chromatographies. Modified from *Urban* et al. (1974a)

C. Solubility of Albumin in Organic Solvents

A method long used to measure the concentration of total protein in blood plasma involves precipitation of the protein with trichloroacetic acid. *Race* (1932) noticed substantial losses of protein from the precipitate upon washing with ethanol or acetone. Further investigation revealed that this was due to the complete solubilization of albumin in the organic solvents. Utilizing this unusual solubility, *Race* separated albumin and globulin in order to measure their concentrations in blood plasma. The presence of at least 0.5% trichloroacetic acid in the organic solvent was found to be essential for solubility of serum albumin. The water content of the solvents must not exceed 20%–25% (vol/vol). In the absence of acid, albumin is insoluble in acetone or in ethanol at solvent concentrations exceeding 75% (*Race,* 1932). *Race*'s findings have been confirmed by other workers (*Levine,* 1954; *Delaville* et al., 1954a,b; *Korner* and *Debro,* 1956). In the presence of acid, albumin could also be dissolved in methanol (*Levine,* 1954; *Michael,* 1962), but the separation of albumin and globulin appeared to be less efficient than in ethanol or acetone (*Fernandez* et al., 1966). Albumin which had been precipitated with trichloroacetic acid did not dissolve in benzene, chloroform, 1,4-dioxane, ethyl acetate, nitrobenzene, carbon tetrachloride, or in n-propyl, isopropyl, or n-butyl alcohol regardless of the presence or absence of acid (*Levine,* 1954). The nature of the acid in any of the above systems is important. Precipitation with perchloric acid or with benzene sulfonic acid rendered serum albumin insoluble in each of the solvents mentioned above (*Levine,* 1954). However, albumin remained soluble if serum was mixed directly with ethanolic solutions of 1% perchloric acid (*Gandolfi* and *Fabrini,* 1966) or 0.05% hydrochloric acid (*Race,* 1932; *Fernandez* et al., 1966). *Michael* (1962) tested the effect of various acids added to methanol and found that 0.2 *N* formic acid, for example, was a good solubilizer whereas 2 *N* acetic acid was poor.

The albumin recovered after precipitation with trichloroacetic acid and solubilization in organic solvents appeared to have the same physicochemical characteristics as native albumin (*Schwert,* 1957; *Kallee* et al., 1957). The above principle has been used to separate serum albumin from immunoglobulin in specific immunoprecipitates, by utilizing their different solubilities in acidic ethanol (*Kallee* et al., 1957; *Peters,* 1958; *Gordon* and *Humphrey,* 1960, 1961). The method was recently applied in the purification of precursor albumin from liver extracts (*Judah* and *Nicholls,* 1971a; *Urban* et al., 1974a). Precursor albumin seems to have a similar solubility in acidic ethanol as albumin.

III. Albumin Synthesis

A. Site of Synthesis

1. Liver as the Main Site of Albumin Synthesis

Madden and *Whipple* (1940) summarized early observations which were best explained by assuming that the liver is the main site of albumin synthesis. In 1947, *Tarver* and *Reinhardt* reported that removal of the liver from dogs reduced the incorporation of radioactive methionine into albumin 20-fold or more. This was confirmed by *Kukral* et al. in 1961. *Peters* and *Anfinsen* (1950a,b) incubated tissue slices from chicken liver in a $^{14}CO_2$-bicarbonate medium and were able to isolate a protein with a specific radioactivity which was 8–16 times higher than the average for the slice proteins. The isolated protein was indistinguishable from serum albumin by immunologic, ultracentrifugal, and electrophoretic methods. *Miller* et al. (1951) demonstrated the dominant role of the liver in albumin synthesis by studying albumin synthesis in perfused rat liver, and *Goldsworthy* et al. (1970) obtained similar results with isolated perfused bovine liver. The values measured in these and other studies (e.g., *Gordon* and *Humphrey*, 1960; *Katz* et al., 1967) for the amount of albumin synthesized per h per g of isolated liver were in the same range as in vivo turnover rates found with biosynthetically labeled [^{14}C] albumin injected into living rats (*Schreiber* et al., 1971).

Schreiber et al. (1966) compared the incorporation of a mixture of radioactive *L*-leucine, *L*-histidine, and *L*-lysine into serum protein in normal and in hepatectomized rats. The hepatectomized rats were kept alive by repeated injections of glucose. After intraperitoneal injection of the amino acid mixture, the concentration of the label in the serum from the hepatectomized rats was more than ten times higher than in normal rats (Fig. 5, upper half). However, only minimal incorporation into serum protein was observed (Fig. 5, lower half), suggesting that extrahepatic tissues contributed only very little or not at all to serum protein synthesis.

The synthesis of serum albumin was also demonstrated in liver slices from fetal rats (*Wise* and *Oliver*, 1966), in suspensions of hepatocytes from adult rats (*Weigand* et al., 1971; *Edwards* et al., 1976c), and in cell-free systems from wheat germ, programmed with albumin-mRNA from rat liver (for references see Sect. III, B). Using fluorescence microscopy, albumin was detected over hepatocytes in histologic slices, but not over Kupffer cells or the cells of the bile duct epithelium (*Feldmann* et al., 1972). In a study by other authors (*Hamashima* et al., 1964), albumin was observed only over a few hepatocytes and also over Kupffer cells and cell nuclei. These workers used 95% ethanol plus 1% glacial acetic acid for

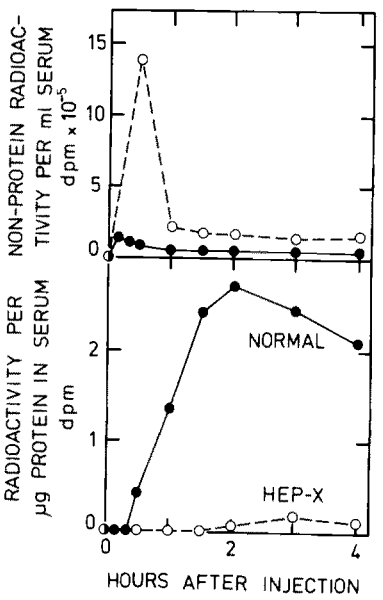

Fig. 5. *Upper half:* shape of labeling pulse after intraperitoneal injection of a mixture of *L*- [U-^{14}C] leucine, *L*- [U-^{14}C] histidine, and *L*- [U-^{14}C]lysine into normal (●—●) and hepatectomized rats bearing hepatomas Morris 9121 implanted into the hind legs (○—○). *Lower half:* incorporation of the radioactive amino acids into serum proteins. Total radioactivity injected per 100 g body weight was 7.89 µCi, dissolved in 0.9% NaCl. Modified from *Schreiber* et al. (1966)

fixation, which may have caused the albumin to dissolve and be redistributed due to its solubility in acid ethanol. Albumin synthesis seems to be distributed uniformly throughout the liver lobule (*Schreiber* et al., 1970; *Wachsmuth* and *Jost*, 1976).

2. *Albumin Synthesis in Hepatomas*

It is not possible to study nonhepatic albumin synthesis in living animals without special precautions. Labeled amino acids injected into animals will be incorporated into albumin in the liver and the newly synthesized radioactive albumin will be secreted into the bloodstream. Its subsequent distribution throughout the body will lead to the appearance of radioactive albumin in organs which do not synthesize serum proteins. Therefore, most of the work on albumin synthesis in hepatomas has been carried out with in vitro systems.

Campbell and *Stone* (1957) described the synthesis of albumin in slices from a primary hepatoma induced by feeding 4-dimethylaminoazobenzene. Tissue fragments obtained from primary hepatocellular carcinomas produced with N-2-fluorenylacetamide seemed to synthesize albumin when incubated in an appropriate medium (*Becker* et al., 1972).

Cell cultures from various mouse hepatomas were found to manufacture albumin (*Breslow* et al., 1973; *Papaconstantinou* and *Ledford*, 1973), and albumin synthesis was also observed in cell cultures or suspensions derived from the transplantable rat hepatomas Reuber H35TC (*Ohanian* et al., 1969; *Deschatrette* and *Weiss*, 1974), Morris 7795 (*Bancroft* et al.,

1969; *Richardson* et al., 1969; *Tashjian* et al., 1970; *Gaudernack* et al., 1973), and Morris 5123TC (*Edwards* et al., 1976b). The structural genes for rat and mouse albumin were both expressed in hybrids of rat hepatoma cells with mouse fibroblasts (*Peterson* and *Weiss*, 1972) or lymphoblasts (*Malawista* and *Weiss*, 1974).

Interference by albumin synthesized in the liver can be avoided in studies on albumin synthesis in transplanted tumors in vivo by removing the liver within the secretion time or minimal transit time for albumin in liver. The secretion or minimal transit time is the time required for uptake of injected amino acids by the liver, their binding to tRNA and incorporation into albumin, transport of the newly synthesized albumin from the rough endoplasmic reticulum to the cell membrane, and its release into the bloodstream. In the rat it is about 14–15 min (see Sect. IV). After application of labeled amino acids, no radioactive albumin, labeled in the liver, will appear in the plasma within this time period. Any labeled albumin found in the tumor within 14 min after i.v. injection of radioactive amino acids must have been synthesized by the tumor.

After carefully measuring the secretion time for albumin in tumor-bearing rats, albumin synthesis was studied in vivo in liver and hepatomas Morris 5123TC and 9121. Liver and tumors were excised within 10–14 min after injection of *L*- [1-^{14}C] leucine. Albumin was found to be formed in vivo in hepatomas Morris 5123TC (*Schreiber* et al., 1969) and 9121 (*Rotermund* et al., 1970). As an example, the purification of albumin to constant specific radioactivity from hepatoma 5123TC is summarized in Table 3. The rate of albumin synthesis was lower in the hepatomas than in liver.

Morris hepatomas 7800 and 7777 were reported to synthesize albumin at a rate of about 10% of that found in liver (*Ove* et al., 1972). Antirat serum albumin Fab fragment, which was labeled with ^{125}I, associated preferentially with membrane-bound polyribosomes isolated from liver and with free, nonmembrane-bound polyribosomes isolated from hepatomas Morris 5123TC, 7800, and 7777 (*McLaughlin* and *Pitot*, 1976).

3. Extrahepatic Albumin Synthesis (Other than in Hepatoma)

Very little radioactivity was incorporated into serum protein in hepatectomized rats (*Schreiber* et al., 1966) or into albumin in hepatectomized rhesus monkeys (*Mullins* et al., 1966). No radioactivity was found in albumin when the lower half of the rat carcass was perfused for 5–6 h with [^{14}C] lysine (*Miller* et al., 1954). The overwhelming majority of relevant experiments suggests that the liver is the main and, with the exception of hepatomas, the only site of synthesis of serum albumin. "Thyroid albumin", a protein synthesized by slices from human thyroid tissue

Table 3. Purification of albumin from hepatomas 5123TC from 20 rats after injection of *L*-[1-^{14}C] leucine, 50 Ci · mol^{-1}, 16 μCi · 100 g body wt^{-1}

Purification step	Total protein (mg)	Albumin in total protein (%)	Specific radioactivity in protein (dpm · mg^{-1})
Homogenate	52,200	4.3	2,500
Partial heat denaturation (10 min at + 69°C)	3,520	41	2,140
Precipitation with 10% trichloroacetic acid, extraction with acid ethanol, fractionation with ammonium sulfate	476	74	2,550
Molecular sieve chromatography on Sephadex G-100	297	115	2,220
First ion-exchange chromatography on DEAE-cellulose	117	96	687
Second ion-exchange chromatography on DEAE-cellulose	65.7	101	541
Preparative electrophoresis on polyacrylamide gel at pH 10.3	33.8	99	485
Preparative electrophoresis on polyacrylamide gel at pH 2.7	10.0	89	404
Charcoal treatment	3.3	108	515

Modified from *Schreiber* et al. (1969).

(*Otten* et al., 1971), differs from serum albumin in its amino acid composition, containing more glycine and less methionine than serum albumin (*Jonckheer* and *Karcher,* 1971). Thyroid albumin and serum albumin are probably two different proteins, and the synthesis of thyroid "albumin" can, therefore, not be regarded as an example of extrahepatic albumin synthesis.

A small amount of radioactivity was found in albumin from cultures of chick embryo and HeLa cells incubated for 48 h with [^{14}C] tyrosine and [^{14}C] leucine (*Abdel-Samie* et al., 1959, 1960). However, the measurement of an increase of incorporation with time of incubation was not attempted. Serum albumin with low radioactivity could also be isolated from resting and lectin-stimulated human lymphocytes incubated for 72 h with 2 μCi of [^{3}H] leucine (30 Ci/mmol) (*Goussault* et al., 1976). Precipitation of radioactivity with trichloroacetic acid was assumed to prove biosynthetic incorporation of the labeled leucine into protein. As outlined in Section II, this is not acceptable as proof for biosynthesis of radioactive albumin.

The conclusion of other authors (*Sandor* et al., 1967) that albumin was probably not synthesized in hepatocytes since its concentration in the serum from patients with viral hepatitis did not decrease, did not take into account that albumin has a longer half-life than most other plasma proteins. An overall change in protein synthesis rates in the liver would influence plasma albumin concentration later than that of other plasma proteins. It is not indicated by *Sandor* et al. when plasma albumin levels were measured during the disease or whether sequential measurements were made.

B. Mechanism

The general principles of protein synthesis in eukaryotes (for recent review see, e.g., *Lucas-Lenard* and *Lipmann*, 1971; *Schreiber*, 1971; *Haselkorn* and *Rothman-Denes*, 1973; *Lodish*, 1976) appear to apply also to the synthesis of albumin. Knowledge about the mechanism of albumin synthesis relies largely on observations made with in vitro systems, particularly cell-free systems. Early work has been hampered by a low capacity of the cell-free systems to incorporate radioactively labeled amino acids into specific proteins. Therefore, early data concerning albumin synthesis in cell-free systems have to be interpreted with caution. The pitfalls in the explanation of data on the incorporation of radioactive amino acids into albumin have been discussed in detail in Section II.

Albumin synthesis requires phosphate bond energy. Synthesis in cell-free systems proceeds only in the presence of GTP, ATP, and an ATP-generating system. Similarly, incubation in vitro of hepatocytes in suspension under nitrogen (*Schreiber* and *Schreiber*, 1973) or addition of 2,4-dinitrophenol or rotenone (*Schreiber* et al., 1977a; *Edwards*, 1978) inhibited the incorporation of labeled amino acid into protein.

The serum albumin molecule is assembled by polyribosomes attached to membranes of the endoplasmic reticulum. This was first indicated in results obtained by *Peters* (1957) who studied the incorporation of [^{14}C] leucine or [^{14}C] glycine into subcellular fractions of chick liver in vivo and of liver slices in vitro. Albumin in the microsomal fraction became labeled first and showed the greatest amount of radioactivity of all subcellular fractions during the first 20 min after addition of the radioactive amino acid. These results were confirmed by similar studies with rat liver in vivo [1], with isolated perfused rat liver [2], with liver microsomes from rat or chick in vitro [3], and with liver ribosomes in vitro [4].

[1] *Takanami*, 1960; *Campbell*, 1961b; *Peters*, 1962b; *Hirokawa* and *Ogata*, 1962; *Takagi* et al., 1969; *Glaumann*, 1970; *Glaumann* and *Ericsson*, 1970; *Peters* et al., 1971; *Redman* and *Cherian*, 1972; *Redman* et al., 1972; *Ikehara* and *Pitot*, 1973; *Jamieson* and *Ashton*, 1973.

Conflicting views are held as to whether the membrane attached ribosomes are the only intracellular site of albumin synthesis. It has been suggested (*Zähringer* et al., 1977) that those preparations of free ribosomes which apparently could synthesize albumin were contaminated by membrane-bound ribosomes released from the membrane during cell fractionation.

The synthesis of albumin proceeds from the NH_2-terminus toward the COOH-terminus of the molecule (*Sargent* and *Campbell,* 1965). *Jungblut* (1963b) demonstrated that the C-terminus of the albumin molecule was synthesized last. He used the isolated perfused rat liver and compared the specific radioactivity of the carboxyl terminal leucine after a single intraportal injection of [^{14}C] leucine with the average specific radioactivity of all 55 leucine residues in the albumin molecule. At 30 s after injection, the carboxyl terminal leucine had a specific radioactivity ten times higher than the average. At about 2 min it had approached the average specific radioactivity. Therefore, *Jungblut* concluded that the synthesis of albumin is completed within 2–2.5 min. Using a different approach, *Peters* and *Morgan* (1971b) estimated about 1 min for the synthesis time of albumin in vivo.

Attempts to demonstrate the existence of a messenger RNA for albumin have been made repeatedly. RNA of high molecular weight was isolated from rat liver and shown to stimulate albumin synthesis in vitro (*Hirokawa* et al., 1961; *Decken* and *Campbell,* 1961a; *Ogata* et al., 1963; *Marsh* and *Drabkin,* 1965). Convincing evidence for an albumin messenger RNA was obtained only recently, when it became possible to translate rat liver mRNA in heterologous protein-synthesizing systems. Such systems used in albumin synthesis were prepared from rabbit reticulocyte lysates (*Taylor* and *Schimke,* 1973; *Shafritz,* 1974; *Shore* and *Tata,* 1977; *Strauss* et al., 1977a,b) and wheat germ lysates (*Nardacci* et al., 1975; *Peterson,* 1976; *Zähringer* et al., 1976, 1977; *Tse* and *Taylor,* 1977; *Strauss* et al., 1977a,b; *Yu* and *Redman,* 1977; *Sonenshein* and *Brawerman,* 1977). Translated rat serum albumin was identified by immunoprecipitation and electrophoresis in polyacrylamide containing sodium dodecylsulfate.

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[2] *Jungblut,* 1963a.

[3] *Campbell* et al., 1960; *Ogata* et al., 1960, 1961; *Campbell,* 1961a,b,c; *Lingrel* and *Webster,* 1961; *Hirokawa* et al., 1961; *Braun* et al., 1962a; *Decken* and *Campbell,* 1961a, 1962; *Campbell* and *Kernot,* 1962; *Sargent* and *Campbell,* 1965; *Ganoza* et al., 1965; *Marsh* and *Drabkin,* 1965; *Williams* et al., 1965; *Rothschild* et al., 1968; *Ove* et al., 1972.

[4] *Korner,* 1960; *Decken* and *Campbell,* 1961b, 1962; *Lingrel* and *Webster,* 1961; *Decken,* 1963a,b; *Ogata* et al., 1961, 1963; *Marsh* et al., 1966; *Takagi* and *Ogata,* 1968, 1971; *Ganoza* and *Williams,* 1969; *Hicks* et al., 1969; *Redman,* 1969; *Rao* and *Tarver,* 1970; *Takagi* et al., 1970; *Ikehara* and *Pitot,* 1973; *Koga* and *Tamaoki,* 1974; *Faber* et al., 1974.

Taylor and *Tse* (1976) succeeded in isolating albumin messenger RNA from liver in apparently homogeneous form. Translation of the purified messenger RNA in wheat germ and in reticulocyte lysates yielded "albumin" as the only translation product. The results obtained with the heterologous systems demonstrated a remarkable uniformity in the ability of ribosomes from animals and even plants to translate the messenger RNA from a different animal species.

From the work of *Strauss* et al. (1977a,b) and *Yu* and *Redman* (1977), it appears that methionine is the initiating amino acid in the translation of the albumin messenger RNA. These authors found that translation of albumin messenger RNA in a heterologous cell-free system yielded albumin molecules with 24 additional amino acids at the amino terminus. This protein is thought to represent the initial translation product of the messenger RNA for albumin. It has been termed pre-proalbumin, relating it to other precursor albumins found earlier in experiments in vivo or in cell suspensions. Precursor albumins will be discussed in detail in the following Sections.

C. Quantitative Aspects

1. Absolute Rate of Albumin Synthesis

The rate of albumin synthesis depends on several factors. For healthy individuals, the age of the animals and the protein content of their diet are the most important. The largest amount of data is available for young adult rats (140–360 g) which were fed a diet containing 17%–30% protein (see Table 4). Different methods have been used to determine rates of albumin synthesis; however, some of the underlying assumptions made may not be valid. In growing animals, for example, the rates of synthesis and catabolism are not in a steady state and this may affect the accuracy of the values shown in Part A of Table 4. Furthermore, recent work (e.g., *Airhart* et al., 1974; *Tavill* et al., 1975) has cast some doubt on the assumption of homogeneous amino acid pools in the liver. This assumption has been made in the direct determination of the rate of synthesis of albumin (Part B of Table 4). In spite of this, the rates of synthesis obtained with different methods for young adult rats from various sources are in remarkably good agreement. In adult rats of about 500 g body weight and 40 weeks of age (last line in Table 4), the production of albumin is reduced to about one-third of that in the young adult rats which were from 6–15 weeks old.

Similar tables for the rates of albumin synthesis in man have been published (*Takeda* and *Reeve*, 1963; *Schultze* and *Heremans*, 1966;

Rossing, 1967; *Rothschild* et al., 1972a). However, only two investigations involved a controlled diet for the subjects studied: in adults with a daily intake of 70 g protein, *Hoffenberg* et al. (1966) found a rate of synthesis of 151 mg (day · kg body wt)$^{-1}$, and *Kelman* et al. (1972a) one of 245 ± 98 mg (day · kg body wt)$^{-1}$ which was averaged from 14 and 8 subjects, respectively. This corresponded to 7.1 mg (day · g liver wt)$^{-1}$ and 11 mg (day · g liver wt)$^{-1}$. Albumin is produced at a similar rate in the liver of the adult rat, for which *Peters* and *Peters* (1972) reported 8.4 mg (day · g liver wt)$^{-1}$. Values for normal rats are listed in Table 4. Fasting for 18 h reduced the rate of albumin synthesis by 40% (*Peters* and *Peters,* 1972). In another study, protein depletion for 3 days had no effect, but depletion for 10 days caused a reduction by 40% (*Kirsch* et al., 1968) or by 66% (*Morgan* and *Peters,* 1971a). *Haider* and *Tarver* (1969) found half the normal rate of synthesis in rats fed no protein for 35–49 days. After protein depletion for 10–12 days, the rate of synthesis returned to normal within 4 h after feeding a complete mixture of amino acids (*Morgan* and *Peters,* 1971a) or within less than a day on normal diet (*Kirsch* et al., 1968). Albumin production was reduced by 72% if rats were fed a 5% protein diet for 60 days. Similar results were obtained for man on a reduced protein diet (*Hoffenberg* et al., 1966; *Kelman* et al., 1972a). Conflicting results were reported for experiments with diets of high protein content. *Peters* and *Peters* (1972) found a reduction of the rate of synthesis by 23% in rats which had received a 64% protein diet for 14–21 days, whereas *Haider* and *Tarver* (1969) found an increase to 127% of the normal rate in rats fed a diet with the same content of protein for 35–49 days. It is desirable that food intake be recorded in addition to the composition of the diet. Unfortunately, actual food intake was not reported in any of the papers discussed. The sensitive response of albumin synthesis to changes in amino acid supply has been confirmed in studies with isolated perfused liver (*Gordon,* 1966; *Rothschild* et al., 1968, 1969; *Kirsch* et al., 1969; *John* and *Miller,* 1969; *Hoffenberg* et al., 1971; *Kelman* et al., 1972b). Besides amino acid supply, the concentration of albumin and of hormones in the blood also appear to influence the rate of albumin synthesis. The effect of the latter two factors is less well understood and will not be discussed here (for a more detailed discussion of the conditions affecting the rate of albumin synthesis, see other reviews by *Waterlow,* 1969; *Peters,* 1970; *Rothschild* et al., 1972a,b, 1973, 1975).

Precise details for the control mechanism of the rate of albumin synthesis are not known. Alteration of rates of synthesis could be caused, e.g., by changes in the concentration of amino-acyl-tRNA, the availability of ribosomes, and the amount of messenger RNA. Changes in amino acid supply were found to be correlated with changes in polyribosome aggregation and RNA turnover (*Fishmàn* et al., 1969; *Jefferson* and *Korner,* 1969; *Henshaw*

Table 4. Absolute rates of albumin synthesis in normal rats with free access to food

Strain [a]	Body weight	Number of rats	Protein content of diet	Method and isotope	Albumin in serum	Pool of albumin in		Half-life in plasma	Synthesis rate	Ref.
						serum	whole body			
	g		%		mg · ml^{-1}	mg (100 g body wt)$^{-1}$		days	mg · day^{-1} (100 g body wt)$^{-1}$	
A. Determined from the turnover and pool size of albumin or from excretion of iodine originating from iodine-labeled albumin										
Hooded	240–260	4	17	^{131}I [f]	38	154	309	2.5	108	1
Holtzman	175	1	18	*L*-[^{35}S] methionine [g]			386	2.3	118	2
Holtzman	159–191	3	30	*L*-[^{35}S] methionine [g]			399	2.3	118	2
Slonakar-Addis	137–258	6	[d]	^{131}I [g]	29	104	228–260	2.2	82	3
Slonakar-Addis	137–258	7	[d]	^{131}I [f]					94	3
Hooded	296	4	17	^{131}I or ^{125}I [f]				2.7	84	4
Sprague-Dawley	359	4	[e]	^{125}I [f]	34	102	276		70	5
Wistar	[b]	20	20	^{131}I [f]	33				89	6
Buffalo	220–300	70	18–20	*L*-[1-^{14}C] leucine [g]	29	100	350	2.7	91	7
Wistar	230–260	16	20	^{131}I and ^{125}I [f]	26	100			82	8
Wistar	250–300	6	[e]	^{131}I and ^{125}I [f]	30				76	9
B. Determined from the incorporation of radioactive amino acid										
Wistar	[b]	20	20	[^{14}C] carbonate [h]	33				94	6
Sprague-Dawley	[c]	10	27	*DL*-[1-^{14}C] lysine [i]	31	100			90	10
Wistar	230–260	36	20	[^{14}C] carbonate [h]	26				81	8
Wistar	278	10	24	*L*-[U-^{14}C] leucine [i]	32				74	11
Wistar	212	15	24	*L*-[U-^{14}C] leucine [i]	33				75	12
Wistar	497	7	24	*L*-[U-^{14}C] leucine [i]	30				26	12

References and footnotes on opposite page.

1. *Cohen* (1957); 2. *Jeffay* and *Winzler* (1958); 3. *Katz* et al. (1963); 4. *Freeman* and *Gordon* (1964); 5. *Morgan* (1966); 6. *Kirsch* et al. (1968); 7. *Schreiber* et al. (1971); 8. *Kernoff* et al. (1971); 9. *Lloyd* et al. (1975); 10. *Haider* and *Tarver* (1969); 11. *Morgan* and *Peters* (1971a); 12. *Peters* and *Peters* (1972).

[a] With the exception of References 3 and 10, only male rats were used in the experiments.
[b] The weight was presumably about 300 g since the authors presented the synthesis rate as mg · h^{-1} (300 g of rat)$^{-1}$.
[c] The weight was presumably about 200 g since the authors presented the synthesis rate as mg · h^{-1} (200 g of rat)$^{-1}$.
[d] Protein content not specified. In other experiments the authors used a diet containing 23% (*Katz* et al., 1967) or 20% protein (*Katz* et al., 1968).
[e] "Standard diet" or "balanced diet", protein content not specified.
[f] Albumin labeled with radioactive iodine was injected intravenously and the specific radioactivity in the plasma and the urinary loss of iodine were monitored for a period of several days. Calculations were based on the ratio of free iodine radioactivity in the urine to specific radioactivity of albumin in plasma. Steady state of catabolism and synthesis was assumed.
[g] Albumin, either labeled with radioactive iodine or labeled biosynthetically with radioactive amino acids, was injected intravenously. Calculations were based on the kinetics of the decay of radioactive albumin in plasma and the size of the exchangeable albumin pool. Catabolism and synthesis were assumed to be in a steady state.
[h] The method was based on the relative rates of incorporation of ^{14}C into urea and into the arginine in albumin, assuming a common precursor pool of arginine for both urea and albumin synthesis.
[i] Calculations were based on the ratio of the radioactivity incorporated into albumin to free amino acid radioactivity, integrated over the experimental period. The pool of free amino acid was assumed to be homogeneous.

et al., 1971; *McGown* et al., 1973; for review of other work see *Munro,* 1970; *Rothschild* et al., 1972a, 1973, 1975).

Using biochemical methods, it is not possible to decide whether alterations of the rate of synthesis are due to changes in the number of cells engaged in synthesis or due to changes in the amount produced by each cell. The few histochemical studies have produced conflicting results. *Chandrasekharan* et al. (1967) and *Peters* et al. (1968) observed that the number of liver cells staining for albumin varies with the rate of albumin synthesis, whereas *Feldmann* et al. (1972) found no change in the number of cells. The histochemical findings allow several explanations as discussed by *Chandrasekharan* et al. (1967) and by *Feldmann* et al. (1972) and are, therefore, not conclusive.

2. Ratio of Albumin Synthesis to Total Protein Synthesis in the Liver

Age of the animal and availability of amino acids affect the rates of synthesis of albumin and total protein to a different extent. The data reviewed below were obtained for young adult rats of 150–350 g body weight which had been starved or fed a diet containing 20%–24% protein.

Urban et al. (1976) measured the kinetics in vivo of the incorporation of *L*- [1-^{14}C] leucine into total liver protein and into antialbumin-precitable protein. During the first 15 min, the proportion of the total protein radioactivity which was found in antialbumin-precipitable protein increased with time after intraportal injection of the label. The proportion was 3.8%, 8.2%, 11%, and 13% at 2.5, 5, 10, and 15 min after injection, respectively. Based on our present knowledge, it is not possible to decide to what extent the increase in the ratio of albumin to total protein labeling was caused by turnover of proteins (or segments thereof) with very short half-lives, such as ornithine decarboxylase, or presegments of secretory proteins. A further contribution to the change in the proportion of albumin to total protein labeling is that incomplete polypeptide chains contribute to measured radioactivity in total protein but not in albumin. At times later than 15 min after injection, the proportion of the total protein radioactivity found in antialbumin-precipitable protein decreased. The proportion was 9.6%, 5.3%, 4.4%, and 2.9% at 30, 50, 80, and 120 min after injection, respectively. The decrease is caused by the release of radioactive albumin into the bloodstream. The albumin released will be replaced in the liver cell by antialbumin-precipitable protein of lower radioactivity due to the decrease of radioactivity in the amino acid pool of the liver cell. Radioactive proteins secreted from the liver into the bloodstream must be included in the calculation of albumin to total liver protein synthesis ratios for time points after the secretion of radioactive protein has begun. A constant value of 13% is then obtained for the ratios at

15–120 min after injection of *L*- [1-^{14}C] leucine. The rats used had been on a 20% protein diet and were starved overnight before the experiment. For rats with free access to food, a ratio of 23% can be calculated from the data of *Urban* et al. (1974b) for a labeling period of 12 min. *Peters* and *Peters* (1972) reported a ratio of 11% in normal rats and 6.6% in rats starved for 18 h, labeling for 14 min. The rate of total protein synthesis in the liver was not affected by the 18 h fast. A value of 11% for fed rats can also be calculated from the data of *Morgan* and *Peters* (1971a), who used a labeling period of 16 min. *Shapiro* et al. (1974) and *Keller* and *Taylor* (1976) arrived at a similar value using a labeling period of 10 min. Furthermore, using indirect immunoprecipitation, they were able to isolate those rat liver polysomes which contained nascent albumin chains. The activity of the polysomes in protein synthesis was assayed in a cell-free system from rabbit reticulocytes. The purified polysomes seemed to produce no other proteins but albumin in appreciable amounts. They synthesized albumin with a ninefold greater specific activity than did total liver polysomes. Thus, it appears that 11% of the total protein synthesizing machinery in the liver was engaged in albumin production.

In suspensions of rat liver cells, radioactivity in albumin represented 7% of the total protein radioactivity after a labeling period of 55 min (*Edwards* et al., 1976c). For a homologous cell-free system from rat liver and a heterologous system from wheat germ lysate with rat liver mRNA added, values of 2.3%–5.8%, and 0.56%–7% have been reported, respectively (*Zähringer* et al., 1976, 1977; *Strauss* et al., 1977b; *Sonenshein* and *Brawerman*, 1977). The rats used for the preparation of polyribosomes had been starved overnight. The values for the ratio of albumin to total protein synthesis in Morris hepatoma 5123TC in vivo and in cell suspensions was 1.8% and 1.2%, respectively (*Edwards* et al., 1976b).

IV. Albumin Secretion

A. Kinetics

In the studies of *Peters* and *Anfinsen* (1950b) with incubated rat liver slices, albumin seemed to increase in the medium without any lag, whereas in perfused liver systems albumin was found to be secreted into the medium with a delay. Values have been reported for this delay ranging from 8 min (*Jungblut*, 1963a) to about 1 h (*Miller* et al., 1951). In the living rat, radioactivity appeared in albumin in the bloodstream about 15 min after intravenous injection of [^{14}C] leucine (*Peters*, 1962b). This lag time could not be shortened by increasing the rate of labeling of plasma proteins by either augmenting the dose of injected radioactive amino

acid or the rate of albumin synthesis (*Schreiber* et al., 1971; *Morgan* and *Peters,* 1971a; *Peters* and *Peters,* 1972). The lag period observed in vivo is the sum of the times required for the injected amino acid to travel to the liver, its uptake into the cell and its binding to tRNA, synthesis and posttranslational modification of the protein to be exported, transport of the protein from the rough endoplasmic reticulum via the Golgi apparatus to the cell membrane, and its release into the bloodstream. It has been called "secretion time" (*Schreiber* et al., 1971) or "minimum transit time" (*Peters* and *Peters,* 1972). An increase and a decrease of the "secretion time" have been reported. It is shorter after partial hepatectomy, when the endoplasmic reticulum is rearranged in the liver cell (*Schreiber* et al., 1971). On the other hand, intracellular albumin transport was found to be delayed in choline-deficient rats (*Oler* and *Lombardi,* 1970).

There seems to be little or no delay in the passage of secreted albumin through the DISSE space before entering the general circulation. Sinusoids in the liver and DISSE space have been suggested as forming a single mixing pool for newly secreted albumin and for albumin from plasma (*Smallwood* et al., 1968).

In patients with liver cirrhosis, newly synthesized albumin was found to enter ascites fluid and the lymph in the thoracic duct faster than albumin from the bloodstream. This led to the suggestion of a direct pathway from the liver to the peritoneal cavity which bypasses the systemic circulation (*Zimmon* et al., 1969).

Albumin secretion can be affected by various agents. Colchicine has been reported to inhibit the secretion of total serum protein (*Le Marchand* et al., 1973) and of albumin (*Dorling* et al., 1975; *Redman* et al., 1975). Long-term feeding of ethanol to rats, producing an alcoholic hepatomegaly, led to intrahepatic accumulation of protein, in particular of albumin and transferrin (*Baraona* et al., 1975). Stimulation of albumin secretion in incubated rat liver slices was reported for potassium ions (*Judah* and *Nicholls,* 1970).

Protein secretion can be studied conveniently in liver cell suspensions (for review, see *Schreiber* and *Schreiber,* 1973, 1975, and *Schreiber* et al., 1976b). Using this system, a secretion time of about 10 min was obtained for total protein (*Schreiber* and *Schreiber,* 1973) and albumin (*Weigand* and *Otto,* 1974). *Weigand* and *Otto* (1974) described a linear increase in albumin concentration in the medium of hepatocyte suspensions 30–180 min after the start of incubation, with constant intracellular levels of albumin. *Crane* and *Miller* (1974) found an almost constant concentration of albumin 5–24 h in long-time incubation experiments with isolated liver cells. They detected secretion only by an increase of the specific radioactivity of albumin in the medium.

B. Mechanism

An increase in the rate of labeling did not shorten the secretion time for serum protein (*Schreiber* et al., 1971). One may conclude that serum proteins cannot simply enter a free intracellular pool and, after mixing in this pool, be secreted. If a newly synthesized serum protein was to mix with a free intracellular pool, it would be expected that the observed secretion time would be lowered for increased labeling. The existence of a definite minimum of the secretion time for albumin suggests an ordered mechanism of secretion. This may involve a series of chemical modifications, albumin transport via various intracellular compartments, or a combination of both.

Peters suggested in 1957 that albumin bound to cytoplasmic particles is the precursor of soluble albumin. This albumin could be released from microsomes with sodium deoxycholate and was precipitable with an antiserum against albumin. It was indistinguishable from native albumin from serum by ultracentrifugation, electrophoresis, absorption spectrum, analysis of N-terminal and C-terminal residues, and precipitation with a specific antiserum (*Peters,* 1959).

Microsomal albumin was not bound to RNA. The RNA content of microsomal albumin, based on the determination of ribose by the orcinol procedure (*Volkin* and *Cohn,* 1954), was less than 0.2% (*Peters,* 1959).

As albumin isolated from cellular organelles and serum was found to have the same chemical composition and structure, the basis for the sequential organization of albumin secretion ("pipe line-like" mechanism of *Gordon* and *Humphrey,* 1961) seemed to be structural compartmentation. After intravenous injection of [^{3}H] leucine (*LeBouton,* 1968), the kinetics of labeling of intrahepatic albumin and plasma albumin showed the relationship postulated for a precursor and its product by *Zilversmit* et al. in 1943. The kinetics of labeling of albumin in various subcellular compartments presented a more complicated pattern (*Peters,* 1962b; *Peters* et al., 1971; *Glaumann,* 1970; *Glaumann* and *Ericsson,* 1970; *Redman* and *Cherian,* 1972; *Redman* et al., 1972; *Jamieson* and *Ashton,* 1973). Maximal labeling of albumin occurred first in the smooth, then in the rough endoplasmic membranes, and finally in the Golgi apparatus. The relative specific radioactivities of albumin from the various subcellular fractions did not satisfy the criteria of *Zilversmit* et al. (1943) for precursor-product relationships. *Peters* et al. (1971) therefore suggested that the isolated albumin fractions may contain some albumin not normally involved in the process of active synthesis and secretion. This could represent albumin in temporarily quiescent membrane channels or in pinocytotic vesicles.

As discussed in Section II, the most relevant purification criterion for the isolation of radioactive proteins in studies on incorporation of labeled amino acids is radiochemical purity. Albumin is usually believed to be a protein which can be easily purified; however, in studies on albumin synthesis in liver, hepatomas, and cell-free systems, radiochemically pure albumin was very difficult to obtain (*Schreiber* et al., 1969; *Rotermund* et al., 1970; *Maeno* et al., 1970; *Judah* and *Nicholls*, 1971a; *Schreiber*, 1972). Highly radioactive protein very similar to albumin seemed to contaminate the isolated albumin fractions. The contaminating protein was not present in the plasma or tissues such as testis and kidney, which do not synthesize albumin (*Urban* et al., 1974b). Furthermore, immunochemical precipitation with antiserum against albumin gave albumin preparations of much higher specific radioactivity than those obtained by extensive nonimmunochemical purification (*Rotermund* et al., 1970; *Judah* and *Nicholls*, 1971a; *Schreiber*, 1972; *Urban* et al., 1974b). An example is illustrated in Table 5.

Table 5. Comparison of albumin radioactivity measured by immunoprecipitation with that calculated from the specific radioactivity of purified albumin

Purification step	A. Radioactivity found in albumin by immunoprecipitation (dpm · ml^{-1})	B. Radioactivity in albumin calculated from the specific radioactivity of purified albumin (dpm · ml^{-1})	A/B
Homogenate (liver)	43,400	8,620	5.0
Postmitochondrial supernatant treated with deoxycholate	20,100	7,320	2.7
Trichloroacetic acid-ethanol	9,640	3,490	2.8
Ammonium sulfate fractionation	142,000	49,300	2.9
Sephadex G-100	11,900	4,790	2.5
DEAE-cellulose	40,800	20,200	2.0
Preparative electrophoresis on polyacrylamide gel at pH 10.3	3,550	2,360	1.5

From *Rotermund* et al. (1970).

After interruption of radioactive protein synthesis with an excess of unlabeled amino acid or with 100 μM cycloheximide, radioactivity continued to rise in albumin in rat liver slices (*Judah* and *Nicholls*, 1971b). The protein contaminating the immunochemically isolated albumin preparations could be separated from albumin by isoelectric focusing (*Geller* et al., 1972). It was converted into albumin in vitro by trypsin treatment, with

the concomitant release of a small arginine-containing peptide (*Judah* et al., 1973). Thus, whereas the early studies seemed to suggest that serum albumin and its intracellular precursor(s) were chemically identical (*Peters,* 1959), the separation of albumin-like protein(s) and albumin by electrophoresis, ion-exchange chromatography (*Decken,* 1963b; *Schreiber* et al., 1969; *Rotermund* et al., 1970; *Maeno* et al., 1970; *Judah* and *Nicholls,* 1971a; *Urban* et al., 1974b), or isoelectric focusing (*Geller* et al., 1972; *Quinn* et al., 1975), as well as the tryptic conversion of albumin-like protein into albumin (*Judah* et al., 1973), were better explained by assuming a difference in the chemical structure of albumin and its precursor(s). This difference did not seem to be in the N-terminal amino acid of the molecule as *Russell* and *Geller* (1973) reported the N-terminal amino acid of both the albumin-like protein and albumin to be glutamate (or glutamine).

Possibly albumin and albumin-like protein differed in a region other than the N-terminus. A sequential degradation from the N-terminus of a large amount of albumin-like protein from rat liver, using the method described by *Edman* and *Begg* (1967), revealed that the albumin-like protein differed from albumin by an N-terminal oligopeptide extension (*Urban* et al., 1974a). The data of *Urban* et al. (1974a) are summarized in Figure 6. After five degradation cycles of the albumin-like protein, the same amino acid sequence was obtained for albumin and albumin-like protein. The removed amino acids were glycine, valine, phenylalanine, serine (identified only by thin layer chromatography of the phenylthiohydantoin amino acid derivatives of cycle 4), and arginine. Thus, in contrast to the results of *Russell* and *Geller* (1973), albumin and albumin-like proteins were found to have different N-terminal amino acids.

In 1975, *Quinn* et al. and *Russell* and *Geller* also reported an oligopeptide extension at the N-terminus of the albumin-like protein. Two different albumin-like proteins were found, one (about 60% of total) with a hexapeptide, the other with a pentapeptide extension at the N-terminus. The amino acids released by sequential degradation were arginine, glycine, valine, phenylalanine, arginine, and arginine (*Russell* and *Geller,* 1975). A similar sequence was obtained for bovine precursor albumin (*Patterson* and *Geller,* 1977). *Peters* (1977) found that the hexapeptide precursor albumin was obtained preferentially if protease inhibitors were added at an early step of purification. Separation of the hexapeptide and the pentapeptide precursors by electrophoresis on cellulose acetate at pH 8.6 has been reported (*Peters,* 1977). The erroneous finding of identical N-termini for albumin and albumin-like protein by *Russell* and *Geller* in 1973 may probably be explained by insufficient separation of the two proteins during the isolation. Any albumin still present in the albumin-like protein preparation would have given glutamate at the N-terminus. It has not yet been clarified whether the amino acid in position -2 of the oligopeptide

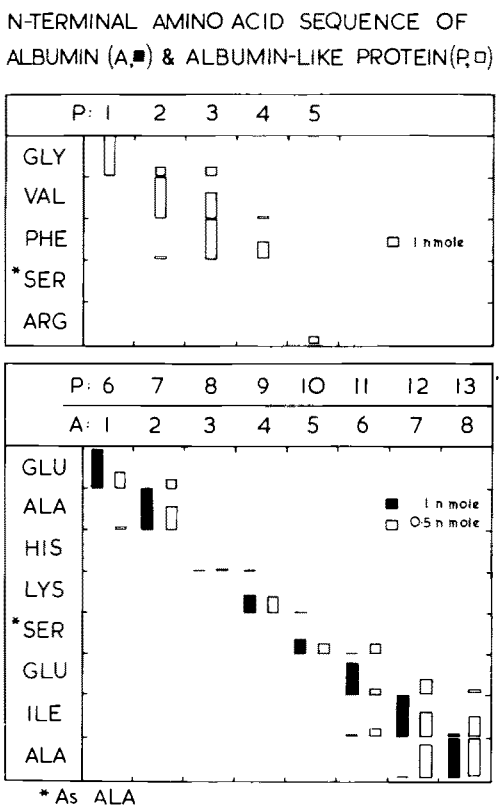

Fig. 6. Amount of amino acids obtained by hydriodic acid hydrolysis (*Inglis* et al., 1971) of the phenylthiohydantoin derivatives produced in sequence analysis of 7.5 mg of albumin or 3.7 mg of albumin-like protein (proalbumin) from rat liver according to *Edman* and *Begg* (1967). One initial cycle was performed without adding phenylisothiocyanate. The phenylthiohydantoin derivatives were also identified by thin layer chromatography with the method described by *Inglis* and *Nicholls* (1973). Thirteen degradation steps were performed for albumin-like protein and eight for albumin. Step numbers are given on top of the two Figure halves. Serine at step 4 in the albumin-like protein was identified by the latter method only. From *Urban* et al. (1974a)

extension is serine or arginine or whether both amino acids can occur in this position.

The conversion of precursor albumin into albumin was demonstrated convincingly both in hepatocyte suspensions (*Edwards* et al., 1976c) and in hepatoma cell suspensions (*Edwards* et al., 1976b). The data for hepatocytes are illustrated in Figure 7. The kinetics of the conversion in the living animal was investigated by various authors (*Urban* and *Schreiber*, 1975; *Dorling* et al., 1975; *Urban* et al., 1976). A summarizing illustration is presented in Figure 8.

The question arises in which intracellular compartment precursor albumin is converted into albumin. *Edwards* et al. (1976a) isolated various subcellular fractions from liver and determined their content of precursor albumin and albumin by adding radioactive precursor albumin or albumin and measuring the dilution of the specific radioactivity. Rough endoplasmic reticulum contained only precursor albumin. About equal proportions of precursor albumin and albumin were found in smooth endoplasmic reticulum. The Golgi apparatus contained two times more albumin than precursor, and albumin only was detected in the cytoplasmic fraction.

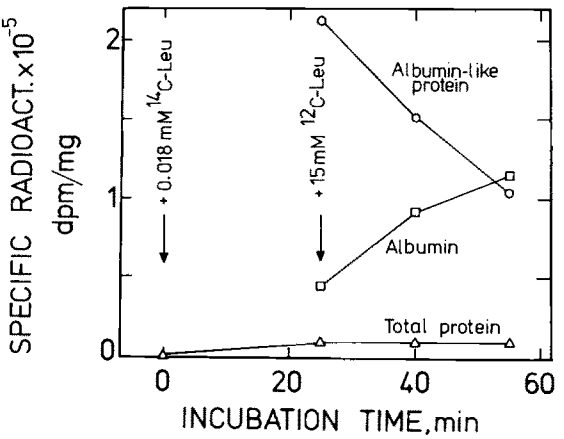

Fig. 7. Conversion of albumin-like protein into albumin in rat hepatocyte suspensions. The specific radioactivities are given for total protein, albumin, and albumin-like protein (proalbumin) isolated from liver cells incubated for 25 min with *L*-[1-^{14}C] leucine followed by a chase with excess nonlabeled leucine. Modified from *Edwards* et al. (1976c)

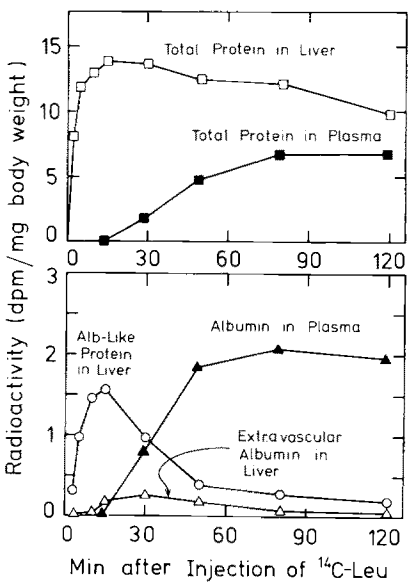

Fig. 8. Conversion of albumin-like protein into albumin in living rats. Radioactivity in total protein of liver and plasma (upper half) and in albumin-like protein in liver, extravascular albumin in liver, and in albumin in plasma is shown at various times after injection of *L*-[1-^{14}C] leucine into the portal vein of male Buffalo rats. Radioactivity values are given per unit body weight. Modified from *Urban* et al. (1976)

Thus, the site of the conversion of precursor into albumin seems to be the region of the smooth endoplasmic reticulum-Golgi apparatus. *Ikehara* et al. (1976) also found predominantly precursor albumin in microsomes. According to their study the conversion occurs in the so-called "secretory vesicles".

The transport of precursor albumin plus its conversion into albumin is an energy-dependent process (*Schreiber* et al., 1977a; *Edwards*, 1978). Both protein synthesis and secretion in hepatocyte suspensions decreased upon inhibiting ATP synthesis with 2,4-dinitrophenol and rotenone, or upon lowering intracellular ATP levels with fructose and glycerol. Higher doses were required for inhibition of secretion than those which inhibited protein synthesis (Table 6, Fig. 9).

It is not known yet whether the amino acids of the oligopeptide extension are removed sequentially during conversion or whether one or several oligopeptides are released en bloc.

The analysis of the amino acid sequence of the N-terminus of precursor albumin gave arginine or glycine as first amino acid. However, the initiating amino acid in the assembly of the polypeptide chain of eucaryotic proteins is methionine (for review, see *Schreiber*, 1971). It follows that the precursor albumin discussed above is probably not the first product in the translation of albumin mRNA. It might have arisen from partial specific hydrolysis of an earlier precursor protein with methionine as N-terminus. Recently, such "pre-precursor" albumin has indeed been found

Table 6. Effect of inhibitors of oxidative phosphorylation, of sugars, and of other compounds on protein synthesis and secretion in rat liver cell suspensions

Compound added	Concentration range tested (μM)	Dose (μM) producing an inhibition of		
		25%	50%	75%
A. Protein Synthesis				
2,4-Dinitrophenol	2 – 500	14	26	35
Rotenone	0.0025 – 25	0.85	2.0	4.6
D-Fructose	10 – 500,000	2,600	4,700	6,500
Glycerol	10 – 500,000	1,500	2,400	4,300
D-Glucose	1,000 – 320,000	no decrease of protein synthesis observed		
D-Galactose	1 – 100,000			
B. Protein Secretion				
2,4-Dinitrophenol	1 – 500	40	65	101
Rotenone	0.00075 – 75	3.0	6.6	14
D-Fructose	1,000 – 180,000	2,500	10,000	50,000
Glycerol	1,000 – 58,000	2,000	40,000	not measured
D-Glucose	1,000 – 320,000	no inhibition of protein secretion observed		

Modified from *Edwards* (1978), with permission.

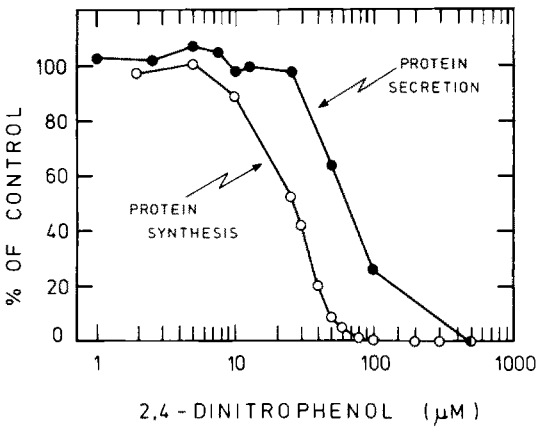

Fig. 9. Synthesis and secretion of protein by suspensions of liver cells in the presence of 2,4-dinitrophenol. For studies on protein synthesis, cell suspensions (1.2 x 10^6 cells/ml) were incubated at + 37°C for 50 min with 1 μCi *L*- [1-^{14}C] leucine (62 Ci/mol) per ml of incubation medium in the presence of varying concentrations of 2,4-dinitrophenol. Incubation was terminated by cooling to + 2°C. Radioactivity in protein was determined in each sample and expressed as a percentage of the value measured in the control sample, which did not contain 2,4-dinitrophenol.

For studies on protein secretion, cell suspensions were incubated at + 37°C for 25 min with 1 μCi *L*- [1-^{14}C] leucine (62 Ci/mol) per ml of incubation medium. Cells were then cooled to + 2°C and 22.9 μ*M* cycloheximide was added. One portion was kept at + 2°C as zero sample and the remainder dispensed into tubes containing varying concentrations of 2,4-dinitrophenol. Incubation of the cells (2.8 x 10^6 cells/ml) was then continued at + 37°C for 30 min and terminated by rapid cooling. Cells were separated from the medium by centrifuging for 15 min at 1300 x *g* and radioactivity in protein in the supernatant was determined. The radioactivity in protein which appeared in the medium during the 30 min incubation is plotted as a percentage of the value measured in the control sample which contained no 2,4-dinitrophenol. From *Edwards* (1978), with permission

when albumin mRNA was translated in a cell-free protein-synthesizing system from wheat germ (*Strauss* et al., 1977a,b; *Yu* and *Redman,* 1977). The wheat germ system has a low proteinase content (*Roberts* et al., 1974), in particular, it lacks the proteinase converting pre-precursor albumin into precursor albumin. The early precursor albumin was termed pre-proalbumin. It contained 24 additional amino acids beyond the sequence of albumin and had methionine as N-terminus (*Strauss* et al., 1977a,b; *Yu* and *Redman,* 1977). Pre-proalbumin has not been found in liver homogenates.

In summary, the experimental evidence presently available suggests the following sequence of events in the synthesis and secretion of albumin:

1. The assembly of the polypeptide chain is initiated with methionine, as in other proteins in eucaryotic cells.

2. Soon after the start of the translation of albumin mRNA, the first 18 amino acids are removed from the N-terminus.
3. The polypeptide chain of precursor albumin is completed within 2 min, the site of synthesis being the rough endoplasmic reticulum.
4. Precursor albumin is transported from the rough to the smooth endoplasmic reticulum and Golgi apparatus, where it is converted into albumin. Conversion begins after about 10 min, and the first albumin is released into the bloodstream after about 14 to 15 min.

C. Regulation

Data on the regulation of secretion are scarce. It is not coupled to continued synthesis. Upon interruption of albumin synthesis in living rats by cycloheximide, albumin was lost from hepatic microsomes at an unchanged rate until the liver was nearly depleted of intracellular albumin (*Peters* and *Peters,* 1972). The addition of cycloheximide (0.1 mg/ml) or puromycin (0.133 mg/ml) to suspensions of hepatocytes (*Schreiber* and *Schreiber,* 1973) or hepatoma cells (*Schreiber* et al., 1974) interrupted protein synthesis, but had no effect on the transfer of newly synthesized protein from the cells to the medium.

D. Secretion in Hepatomas

The Morris hepatomas 5123TC (*Schreiber* et al., 1969; *Edwards* et al., 1976b) and 9121 (*Rotermund* et al., 1970) are able to synthesize albumin, but failed to secrete serum protein into the bloodstream during growth in living rats (*Schreiber* et al., 1966). This seemed to lead to an intracellular accumulation of albumin in the hepatomas with an ensuing enlargement of the cisternae of the endoplasmic reticulum (*Urban* et al., 1972).

In contrast to the lack of secretion observed in living rats, hepatoma cells in vitro have been reported to secrete albumin. Slices from primary hepatomas induced by feeding 4-dimethylaminoazobenzene (*Campbell* and *Stone,* 1957) and cell cultures derived from Morris hepatoma 7795 (*Richardson* et al., 1969; *Gaudernack* et al., 1973), Reuber hepatoma H35 (*Deschatrette* and *Weiss,* 1974), or mouse hepatoma (*Ledford* and *Papaconstantinou,* 1973; *Breslow* et al., 1973; *Ledford* et al., 1977) were found to release albumin into the culture medium. The rate of albumin synthesis and secretion in the hepatoma cells in culture depended on the stage of the cell cycle (*Schreiber* et al., 1977b). The rate of albumin synthesis and secretion was higher in resting cells than in rapidly growing cells.

However, despite continued secretion, an intracellular accumulation of albumin occurred when the rate of albumin synthesis was high, indicating a limited secretory capacity of hepatoma cells in culture (*Schreiber* et al., 1977b).

The experiments with in vitro systems demonstrated that the pathway for the synthesis of albumin, its conversion, and the secretion of albumin can operate in hepatoma cells. Why did intramuscularly implanted hepatomas growing in living rats then fail to secrete serum proteins? An explanation may possibly be found in the difference between the histologic structures of liver and hepatoma tissue (Fig. 10). In the liver a sophisticated vascular system distributes nutrients to and removes compounds from virtually all parenchymal cells. A functional polarity exists in hepatocytes with different uptake and excretory processes occurring at the biliary and sinusoidal poles. As discussed previously, no delay in the mixing of newly secreted albumin with plasma albumin was observed (*Smallwood* et al., 1968), indicating that released protein is efficiently removed from the immediate environment of the hepatocyte. The highly ordered pericellular architecture existing in liver is not present in the tumors. Only a few cells are still arranged in trabeculas. Perhaps the lack of an efficient draining system can contribute to a failure of secretion. The orderless growth of the tumors might also reduce possibilities for communication between cells and pericellular environment by decreasing the accessibility of the tumor cells to regulatory compounds present in tissue and vascular fluids (hormones, chalones, etc.).

V. Possible Function of the Oligopeptide Extensions in Precursor Proteins

A. The "Pre"-Segment

The synthesis of proteins for extracellular use such as serum proteins (*Redman*, 1968, 1969) and in particular albumin (*Takagi* and *Ogata*, 1968; *Takagi* et al., 1969; *Hicks* et al., 1969; *Takagi* et al., 1970) has been reported to occur on polyribosomes bound to membranes of the endoplasmic reticulum (for review, see *Campbell*, 1970). Proteins for intracellular use are believed to be made on free polyribosomes. However, no differences in composition or other properties of ribosomes were observed which would permit the distinction of two types of ribosomes; one being involved in the synthesis of proteins for export from the cells, the other involved in the synthesis of proteins for intracellular use. *Blobel* and *Sabatini* (1971) and *Milstein* et al. (1972) suggested that the message for the ribosomes to bind to membranes is contained in the nascent peptide chain. According to *Blobel* and *Sabatini* translation of the mRNA for

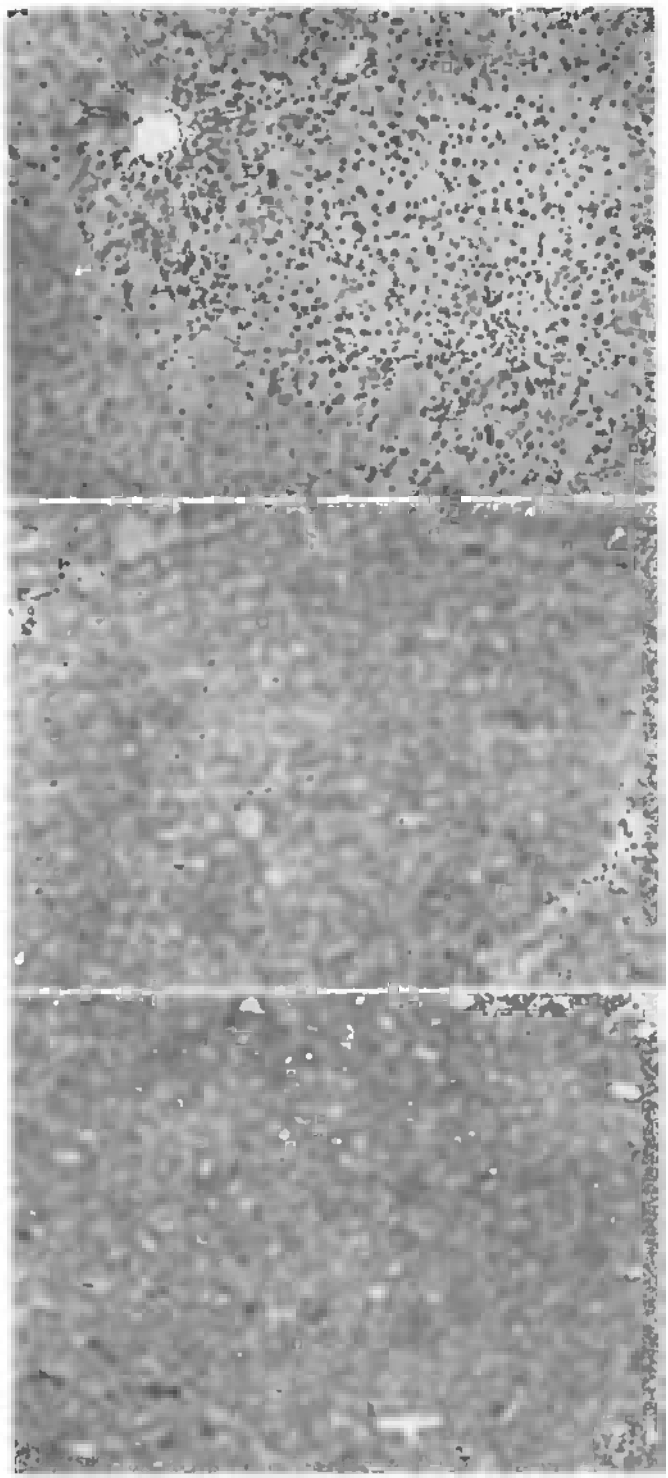

Fig. 10. Histologic structure of normal rat liver (*top*), Morris hepatoma 9121 (*middle*), and Morris hepatoma 5123TC (*bottom*). Staining with hematoxylin-eosine, magnification, x 115. From *Schreiber* (1970)

export proteins begins on free polyribosomes. A nascent polypeptide segment of about 10–40 amino acids emerges from the ribosome which is then recognized by the membranes of the endoplasmic reticulum, leading to the binding of the polyribosomes (*Milstein* et al., 1972; *Blobel* and *Dobberstein,* 1975). Since the original proposal of this "signal theory", a number of mRNAs for export proteins have been isolated and translated in cell-free systems lacking the ability for the proteolytic processing of the newly synthesized peptide chains, such as the system derived from wheat germ. The N-terminal sequences of amino acids found in the products of cell-free translation are summarized in Table 7. A possible mechanism for the interaction of the presegment with the membrane of the endoplasmic reticulum is illustrated schematically in Figure 11.

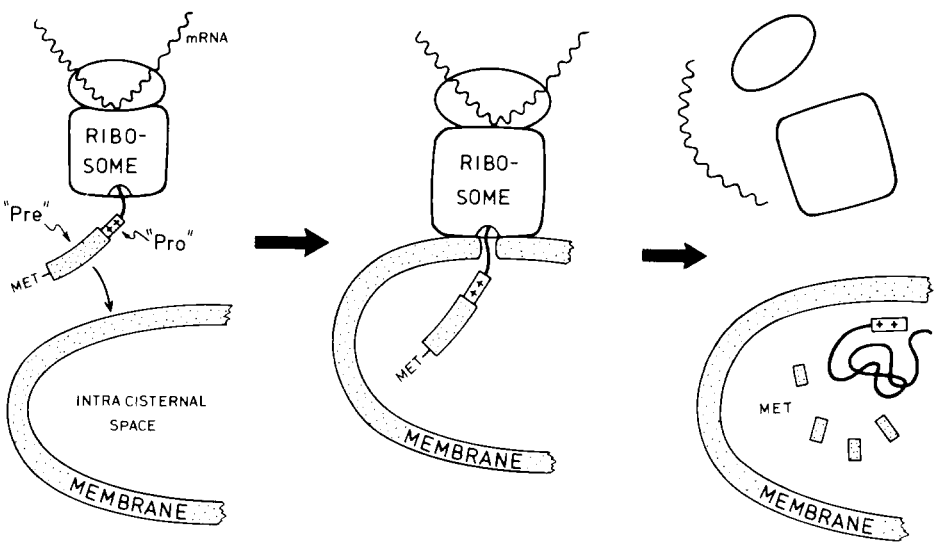

Fig. 11. Function of the presegment in precursor proteins. Hydrophobic segment and membrane of the endoplasmic reticulum are indicated by dotting. The basic prosegment is indicated by ++. The presegment mediates the initial contact of the precursor-protein-synthesizing ribosome with the membrane. It is removed by partial specific hydrolysis after transfer of the synthesized polypeptide chain into the intracisternal space of the rough endoplasmic reticulum

The average length of the oligopeptide extension in the presegments listed in Table 7 is 21.6 amino acid residues. There seems to be no sequence of amino acids which is common to all presegments listed. The proportion of hydrophobic amino acid residues, however, is clearly very high. For example, the average proportion of leucine in the sequences given in Table 7 is 24.4% compared with a leucine content of about 12% in total

Table 7. Amino acid sequence in the presegment of the precursors for various proteins in cell-free systems

Precursor for	Amino acid sequence in the presegment (−25 … −20 … −15 … −10 … −5 … −1)
a) Immunoglobulins (Ig);	
κ *L*-chain of Ig from myeloma MOPC-321 (mouse)	(M) X T X T *L* *L* *L* *W* *V* *L* *L* *L* *W* *V* P X X T X
κ *L*-chain of Ig from myeloma MOPC-41 (a) (mouse)	(M) D M R A P A Q *I* *F* G *F* *L* *L* *L* *L* *F* P G T R C
κ *L*-chain of Ig from myeloma MOPC-41 (b) (mouse)	(M) R A P A Q *I* *F* G *F* *L* *L* *L* *L* *F* P G T R C
κ *L*-chain of Ig from myeloma MOPC-63 (mouse)	(M) X X X X *L* *L* *L* X X *L* *L* *L* X X P X S X X
λ_1 *L*-chain of Ig from myeloma MOPC-104E (mouse)	(M) A *W* *I* S *L* *I* *L* S *L* *L* A *L* S S G A *I* S
b) Other proteins from eucaryotes:	
Serum albumin (rat)	(M) K *W* *V* T *F* *L* *L* *L* *L* *F* *I* S G S A *F* S
Parathyroid hormone (bovine)	(M) M X A K D M X K X M *I* X M *L* A *I* X X *L* A R X D X
Trypsin (dog)	A K *L* *F* *L* *F* *L* A *L* *L* *L* A Y *V* A *F*
Insulin (rat)	X *L* K M X *F* *L* *F* *L* *L* K *L* *L* X *L* X X X X X X X X
Lactogen from placenta (human)	(M) P X X X X X *L* *L* *L* X X X *L* *L* X *L* X P X X X X X X X
Prolactin (rat)	(M) X X X X X X X X X X X *L* *L* *L* *L* M M X X *L* *L* X C X X X X X
Lysozyme (chicken)	(M) R S *L* *L* *I* *L* *V* *L* C *F* *L* P *L* A A *L* G
Ovomucoid (chicken)	A M A G *V* *F* *V* *L* *F* S *F* *V* *L* X G *F* *L* P D A A *F* G
c) Proteins from bacteria:	
Outer membrane lipoprotein in *E. coli*	(M) K A T K *L* *V* *L* G A *V* *I* *L* G S T *L* *L* A G

The amino acid immediately preceding the N-terminus of the final form, or the proform, of the product protein has the number – 1, the amino acid 5 positions apart from the N-terminus of the product protein, or its proform, has the number – 5, and so on. Amino acids with hydrophobic side-chains are underlined. Methionine is encircled if it occurs at the N-terminus of a polypeptide chain.

The code used to indicate the amino acids is A, alanine; V, valine; L, leucine; I, isoleucine; P, proline; F, phenylalanine; W, tryptophan; M, methionine; G, glycine; S, serine; T, threonine; C, cysteine; Y, tyrosine; N, asparagine; Q, glutamine, D, aspartic acid; E, glutamic acid; K, lysine; R, arginine; H, histidine.

The amino acid sequences for the various precursor proteins are taken from: κ *L*-chain of immunoglobulin from myeloma MOPC-321, *Schechter* and *Burstein* (1976b,c); Ig light chain from MOPC-41 (a) and (b), *Schechter* and *Burstein* (1976a,b), *Burstein* and *Schechter* (1977); Ig light chain from MOPC-63, *Burstein* and *Schechter* (1976); Ig light chain from MOPC-104E, *Burstein* et al. (1976), *Burstein* and *Schechter* (1977); serum albumin, *Strauss* et al. (1977a); parathyroid hormone, *Habener* et al. (1975); *Kemper* et al. (1976); trypsin, *Devillers-Thiery* et al. (1975); insulin, *Chan* et al. (1976); lactogen, *Birken* et al. (1977); prolactin, *Maurer* et al. (1977); lysozyme and ovomucoid, *Thibodeau* et al. (1977); outer membrane lipoprotein in *E. coli, Inouye* et al. (1977).

liver protein (*Schreiber* et al., 1971). Amino acids with hydrophobic side chains are underlined in Table 7. A high proportion of hydrophobic amino acids has also been reported for the presegment in pre-promelittin (*Suchanek* et al., 1975).

Another common feature of many of the proteins listed in Table 7 is a methionine residue at the N-terminus. This is the case in 11 of the 13 listed polypeptides. It strongly suggests that the cell-free systems used for translating the various mRNAs initiated translation in the correct place and that the obtained amino acid sequences were those of the N-termini of the first precursors in the formation of export proteins.

A third property common to the proteins listed in Table 7 (except the immunoglobulins) is a high incidence of glycine at the carboxy-terminus of the presegment. Glycine occurs in four of the six cases where the carboxy-terminal amino acid of the presegment was determined. Glycine differs from other amino acids in three aspects:

1. It lacks a bulky side-chain.
2. It is frequently found in turns or reversals of the polypeptide chain backbone (*Crawford* et al., 1973; *Tanaka* and *Scheraga,* 1976) Turns occur predominantly on the surface of globular proteins (*Kuntz,* 1972).
3. It has a strong helix "breaking" effect (*Pain* and *Robson,* 1970; *Lewis* and *Scheraga,* 1971).

The other amino acids at the carboxyl-end of the presegments are also "helix breakers" (serine) or "helix indifferent" (cysteine and phenylalanine). Thus, the amino acid composition of the carboxy-terminus of the presegment seems to indicate a "hinge-like" structure. One may speculate that this hinge-like structure is involved in the intracellular transport of the protein and could be the site which is recognized by the enzyme for proteolytic cleavage of the presegment.

B. The "Pro"-Segment

The function of the "pro"-segment in the albumin precursor is not known. Possible functions could be:

1. Mediating the Binding to Membranes

In pre-proalbumin, a hydrophobic section of the polypeptide chain is followed by a short segment rich in positively charged amino acids. This is also a feature of the parathyroid hormone precursor (*Hamilton* et al., 1974; *Kemper* et al., 1976) and glycophorin (*Bretscher,* 1975). The amino acid

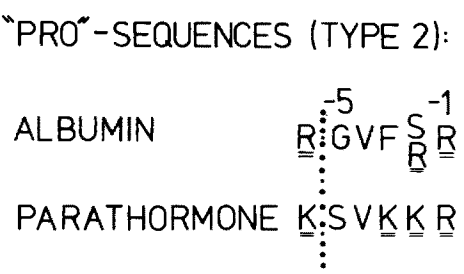

Fig. 12. Amino acid sequence in the prosegment of the precursor proteins for albumin and parathyroid hormone. The code used to indicate the amino acids is explained in the legend to Table 7. The amino acid immediately preceding the N-terminus of the final protein product has the number − 1. A dotted line indicates that the prosegment has been reported to consist of either five or six amino acids. For types of "pro"-sequences see Fig. 16

sequences of the prosegment in the precursors for albumin and parathyroid hormone are compared in Figure 12. Glycophorin is the major sialoglycoprotein of the erythrocyte membrane. In its polypeptide chain, an extracellular hydrophilic section is followed by an intramembranous hydrophobic sequence and finally an intracellular hydrophilic segment. This starts with the sequence Arg-Arg-Leu-Ile-Lys-Lys (*Segrest* et al., 1972, 1973; *Bretscher,* 1975). *Bretscher* suggested that the highly positively charged amino acid segment could form tight salt linkages and thereby anchor the polypeptide chain to negatively charged phosphatidylserine in the membrane. Phosphatidylserine seems to be located exclusively in the inner layer of the erythrocyte membrane (*Bretscher,* 1972; *Verkleij* et al., 1973). The relationship between glycophorin and the cell membrane is illustrated schematically in Figure 13. It may be possible that the positively charged oligopeptide extensions in proalbumin and proparathyroid hormone promote binding of the precursor proteins to the membrane of the endoplasmic reticulum in a similar fashion. Thus, the transport of the newly synthesized protein for export from the cells could be coupled to membrane flow.

2. Masking Binding Sites on Albumin

One of the functions of albumin in the bloodstream is the transport of various substances such as fatty acids, metal ions, bilirubin, and tryptophan (for review, see *Peters,* 1970). Copper and nickel ions, in particular, are tightly bound to the N-terminus of albumin. The masking of binding sites might be necessary to prevent an inappropriate export from the liver cell of substances bound to and secreted with albumin. The N-terminal oligopeptide extension might be involved in such masking.

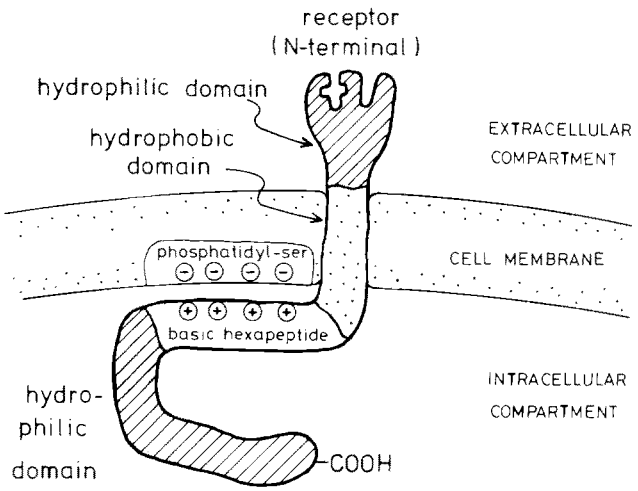

Fig. 13. The structural relationship between glycophorin and the cell membrane of erythrocytes. The receptor is involved in cell recognition in the MN-blood group system and in binding of other specific agents, e.g., viruses

3. Regulation of the Synthesis or of Degradation of Albumin

The albumin level in the bloodstream of healthy individuals varies so little that monitoring albumin concentration in serum is used in clinical diagnosis. The constancy of the albumin level suggests an efficient regulation of the rates of synthesis or degradation of albumin, or both, with the blood albumin level as the regulating parameter. Increased albumin synthesis has been described repeatedly for conditions with decreased blood albumin concentration (*Drabkin* and *Marsh,* 1955; *Wasserman* et al., 1956; *Marsh* and *Drabkin,* 1958, 1960; *Rothschild* et al., 1961; *Braun* et al., 1962a,b; *Marsh* et al., 1966; *Tracht* et al., 1967; *Katz* et al., 1967, 1968; *Rothschild* et al., 1969). Furthermore, intracellular albumin concentration does not seem to be related directly to the regulation of the rate of albumin synthesis. Albumin synthesis was decreased 60%–70% by feeding a protein-free diet and increased again by refeeding a complete mixture of amino acids by gavage. Intracellular albumin reached its normal level before the pool of circulating albumin was restored, indicating that hypoalbuminemia can elicit increased albumin synthesis without maintaining a decrease in the intracellular pool (*Morgan* and *Peters,* 1971a). It follows that an information-transfer mechanism should exist between the concentration in blood and the intracellular sites of synthesis or processing of either albumin or its mRNA, or of both. The albumin molecule itself is too large to freely pass through the cell membrane. Liver cells in suspension take up albumin only to a limited extent and at a low rate (*Schreiber* and *Schreiber*

1973, 1975). A small molecule released during the conversion of precursor albumin into albumin immediately before the release into the bloodstream would be more appropriate for the transfer of information than a macromolecule. In such a scheme, albumin need influence only the converting reaction. The hypothesis was discussed previously in greater detail (*Schreiber* et al., 1967a,b). It can be easily modified to account also for the regulation of albumin degradation.

Apart from the albumin system, the linking of the site of function with the site of synthesis is important in the regulation of the synthesis and/or degradation rates of many other proteins. A similar problem would occur, for example, if sites of function and synthesis for a protein are located in different tissues or compartments or in different subcellular organelles. Synthesis via a protein precursor may be a possible way of regulating the rates of synthesis of enzyme subunits which are coded for by different genomes (for review, see *Bogorad,* 1975). For example, ribulose-1,5-biphosphate carboxylase is composed of small and large subunits (*Rutner* and *Lane,* 1967; *Moon* and *Thompson,* 1969; *Kawashima* and *Wildman,* 1970; *Givan* and *Criddle,* 1972; *Iwanij* et al., 1974). The synthesis of the enzyme is inhibited by both chloramphenicol and cycloheximide (*Margulies,* 1971) since the large subunit is made in the chloroplast (*Blair* and *Ellis,* 1973; *Morgenthaler* and *Mendiola-Morgenthaler,* 1976) and the small subunit is assembled on cytoplasmic ribosomes (*Gray* and *Kekwick,* 1974; *Roy* et al., 1976). Synthesis of the small subunit via a precursor may be necessary for the transfer of the subunit into the chloroplast (*Dobberstein* et al., 1977). Simultaneously, a smaller molecule might be released during conversion of precursor protein into product, thereby adapting the rate of synthesis of the protein on the cytoplasmic ribosomes to the required supply.

4. Stabilizing the Albumin Molecule

The stabilities of albumin and precursor albumin during prolonged storage and upon heating appear to differ considerably (Table 8 and Fig. 14). It may be possible that protection of albumin against degradation during intracellular transport is also a biologically important feature of the N-terminal oligopeptide extension in precursor albumin. Albumin or its precursor(s) could become more susceptible to degradation if they have to partially unfold during secretion. Protection of partially unfolded proteins during excretion has been shown to be important in studies of protein excretion in microorganisms. Polysaccharide (*Braatz* and *Heath,* 1974) or calcium ions (*May* and *Elliott,* 1968) seem to be involved in such protection. In *Sarcina* cultures Ca^{2+} was not required for synthesis or excre-

tion of protein (*Bissell* et al., 1971); however, Ca^{2+} seemed to protect the protein excreted by *Sarcina* after it had been released from the cell (*Sarner* et al., 1971).

Table 8. Stability upon storage of proalbumin (from microsomes), liver albumin (from microsomes), and albumin (from serum)

Sample	Storage time at − 18°C (of purified sample in 10–100 m*M* Tris-HCl, pH 7.7)	Concentration of purified sample (mg · ml^{-1}) measured by:		
		Optical density at 277.5 nm [a]	Biuret reaction	Immunodiffusion in agar plates
Serum albumin (isolated from serum)	14 months	1.8	2.0	2.2
	no storage	ND [b]	ND [b]	2.1
Liver albumin (isolated from microsomes)	3 months	5.2	5.5	3.2
	before storage	ND [b]	5.4	5.3
Proalbumin (isolated from microsomes)	6 months	1.6	ND [b]	1.4
	no storage	1.5	ND [b]	1.5

[a] Molar concentrations of bovine serum albumin were estimated from the optical density at the wavelength of maximum absorption using $E^{1\%}_{1\ cm}$ (277.5 nm) = 6.67 (*Foster* and *Sterman*, 1956).

[b] ND = not determined.

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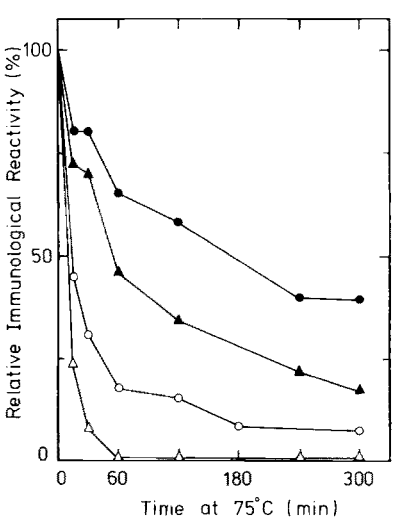

Fig. 14. Heat stability of proalbumin (*circles*) and liver albumin (*triangles*) before (*filled symbols*) and after treatment with activated charcoal (*open symbols*). Proalbumin and liver albumin had been isolated from bovine liver microsomes. Immunologic reactivity was measured according to *Mancini* et al. (1965). Incubation was at a protein concentration of 0.5 mg/ml in 50 m*M* Tris-HCl buffer of pH 7.7. Charcoal treatment was performed as described by *Chen* (1967). From *Millership* (1977), with permission

5. Facilitating the Formation of Tertiary Structure

The main function of the prosegment in proinsulin seems to be facilitating the formation of the correct tertiary structure of insulin (for review, see *Steiner* et al., 1974). The question arises of whether there is a major rearrangement of the protein molecule during conversion of precursor albumin into albumin in which the precursor segment may possibly be involved. No significant difference could be detected in the conformations of albumin isolated from liver microsomes or serum and precursor albumin by circular dichroic spectra (Fig. 15). The α-helix contents calculated from the circular dichroic spectra were 62.8%, 58%, and 58.5% for serum albumin, liver microsomal albumin, and precursor albumin, respectively.

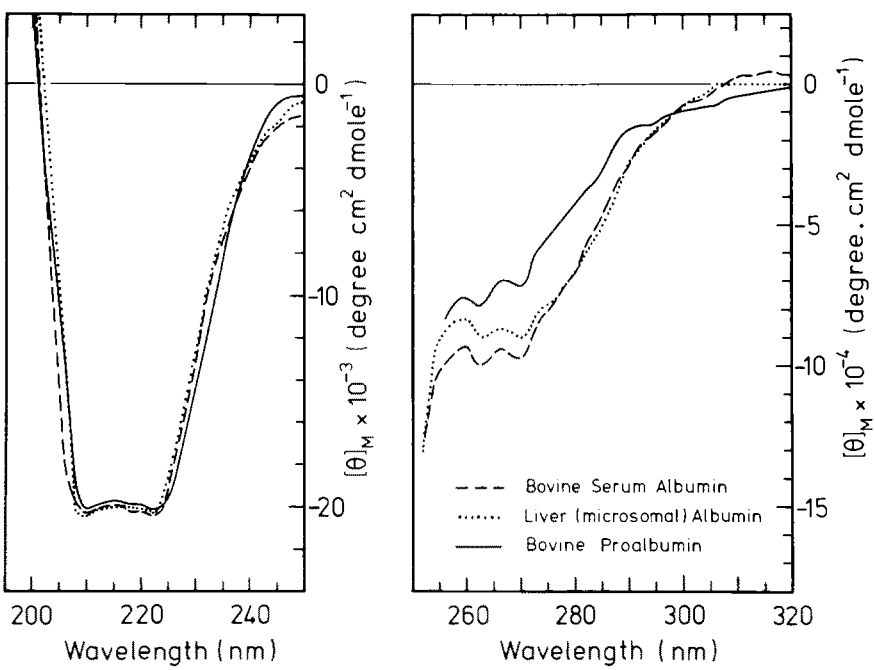

Fig. 15. Near UV and far UV circular dichroic spectra of albumin isolated from bovine serum and from bovine liver microsomes, and of proalbumin from bovine liver microsomes. Tris-HCl buffer, pH 7.7, 10 m*M*. Sample concentration was 0.05%–0.59%. From *Millership* (1977), with permission

VI. Oligopeptide Extensions as a General Principle in the Interaction of Proteins with Supramolecular Structures. Evolution of Precursor Proteins

Synthesis via precursor proteins appears to be a general principle perhaps valid for all secretory proteins. The known precursors for secretory proteins can be arranged into four main groups according to their structure, as illustrated schematically in Figure 16. All possess a hydrophobic presegment at the N-terminus. Its function, as discussed in the previous section, has been suggested to be the binding of polyribosomes to the membrane of the endoplasmic reticulum. Hydrophobic domains of proteins seem to be utilized more generally in linking cell structure and metabolic reactions. *Burstein* and *Schechter* (1977) pointed out that the presegments of immunoglobulin precursors are similar in their hydrophobicity and absence of charged amino acid residues to the hydrophobic domains of proteins in membranes, such as glycophorin (*Segrest* et al., 1972, 1973) and cytochrome b_5 (*Spatz* and *Strittmatter,* 1971). They proposed that not all precursor immunoglobulin molecules are processed proteolytically (*Schechter* and *Burstein,* 1976b). In immature plasma cells and lymphocytes, most precursor molecules could remain uncleaved and be embedded in the cell surface as antigen-recognizing receptors, the hydrophobic presegment anchoring the receptor in the membranes. In mature plasma cells most precursor molecules are cleaved and secreted. Thus, the immunoglobulin precursor protein would be a common intermediate of the secreted antibody molecules and of the antigen-recognizing receptors in the cell membrane (however see addendum for contradictory data).

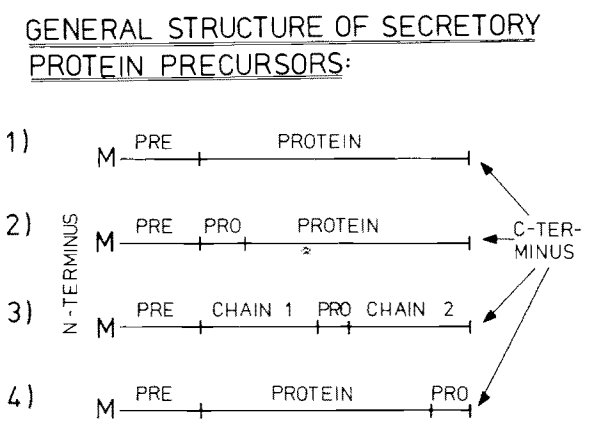

Fig. 16. Arrangement of precursor proteins into four structural classes. Examples are: type 1, precursor for the light chain of various immunoglobulins (see Table 7); type 2, precursors for albumin, parathyroid hormone, and trypsin; type 3, precursor for insulin; type 4, precursor for glucagon (*Tager* and *Steiner,* 1973). A presegment has not yet been identified for the glucagon precursor

Virus procapsids are another example of precursor proteins facilitating the formation of supramolecular structures. The name "procapsid" was introduced for the precursor of the coat protein of poliovirus by *Jacobsen* and *Baltimore* (1968). The poliovirus procapsid is cleaved hydrolytically before assembly with the viral RNA. In the case of coxsackievirus B 1, the entire RNA genome is translated into one single giant polypeptide chain whose molecular weight is greater than 200,000. This giant precursor protein is then split by proteinases which may be coded for by the virus genome or may be host enzymes that are activated upon infection (*Kiehn* and *Holland,* 1970). A total of 15 virus-specific polypeptides was detected in HeLa cells infected with encephalomyocarditis virus. Three primary gene products were cleaved after translation yielding proteins, which included all of the capsid polypeptide chains (*Butterworth* et al., 1971). A mechanism involving precursor proteins was also suggested for the formation of the chloroplast structure (*Dobberstein* et al., 1977). A membrane protein synthesized in a cell-free, membrane-free reticulocyte extract was reported to be modified, probably by the loss of 20–40 amino acids, after its incorporation into the reticulocyte membrane (*Lodish,* 1973).

All examples for precursor proteins discussed fo far have been from eucaryotic systems or viruses in eucaryotes. The question arises of whether evolution of precursor proteins is related to the evolution of a membrane system. It appears that the capsid formation in a bacterial virus also involves precursor proteins. In bacteriophage T 4, the products of genes 22, 23, 24, and one protein of unknown genetic origin were reported to be cleaved during assembly of the phage head (*Laemmli,* 1970; *Hosoda* and *Cone,* 1970; *Kellenberger* and *Kellenberger-van der Kamp,* 1970; *Yanagida,* 1974). Recently, the messenger RNA for the lipoprotein of molecular weight 7200 in the outer cytoplasmic membrane of *E. coli* was isolated and translated in a cell-free protein-synthesizing system. The product of translation was identified as a "prolipoprotein" with the extension of 20 amino acids listed in Table 7 (*Inouye* et al., 1977). One might conclude that formation of proteins via precursor polypeptides seems to be a mechanism which evolved early in the synthesis of proteins related to membranes. The mechanism might then have been adapted to, and modified for, the secretion of proteins.

VII. Nomenclature for Precursor Proteins

No uniform, generally accepted nomenclature exists for precursor proteins. The name "proinsulin" was introduced by *Steiner* et al. in 1967 and the term "proalbumin" was coined accordingly (*Judah* et al., 1973). The expression "proparathyroid hormone" was first used by *Kemper* et al.

(1972) for a precursor of parathyroid hormone which had also been described by *Cohn* et al. (1972). "Procollagen" was the name of a soluble precursor of the collagen fiber which is now called "soluble collagen" or "tropocollagen". *Bellamy* and *Bornstein* (1971) introduced the use of the term "procollagen" for functional precursors of collagen which are converted into collagen by partial proteolysis. The prefix "pro" in prolactin bears no relation to the mode of synthesis of the protein.

Many protein precursors of the "pro" class have been already assigned trivial names. The prefixes "pre" and "post" have also been used to indicate electrophoretic mobility relative to albumin. Prealbumin, for example, is a thyroxin-binding protein of the plasma not related structurally or functionally to albumin (for review, see *Putnam,* 1975). The name "prealbumin" indicates that the protein has a higher electrophoretic mobility than albumin. Postalbumin is a fetal plasma protein migrating electrophoretically between the albumin and the α-globulin fractions (*Wise* et al., 1963, 1966). The fetal postalbumin and adult albumin from rats do not share any antigenic determinants (*Kirsch* et al., 1967), suggesting that postalbumin and albumin are two different, unrelated proteins. Guidelines are needed for the use of the prefixes "pre", "pro", "pre-pro", and "post". Electrophoretic mobility is a rather arbitrary criterion and would change with the conditions of electrophoresis. *Devillers-Thiery* et al. suggested in 1975 that the designation "pre" should be adopted for precursor proteins with a "signal region" cleaved by microsomal proteinases, whereas "pro" should refer to protein segments cleaved by either intracellular (localized in Golgi- and condensing vacuole membranes) or extracellular proteinases. Thus, information about the location and metabolism of protein precursors would have to be available before the final assignment of names.

VIII. Summary

The progress in our understanding of albumin synthesis and secretion made in recent years has been a consequence of work on the purification of albumin from tissue extracts. This purification is complicated by the binding of amino acids to albumin and by the presence of albumin-like proteins. Interference due to the binding of radioactive amino acids is critical in those cases where incorporation into albumin is low. Albumin-like proteins occur in those tissues or systems which are synthesizing albumin. They are precipitated together with albumin by antialbumin and, therefore, protein obtained by immunoprecipitation must be purified further if separation of albumin and albumin-like proteins is required. Investigations on the nature of albumin-like protein led to the discovery of precursor proteins in the synthesis and secretion of albumin.

In the normal animal, albumin is synthesized in the liver. Albumin is synthesized efficiently in isolated perfused liver, but not at all in hepatectomized animals. Cell-free systems derived from liver or programmed with mRNA from liver synthesize antialbumin-precipitable protein in vitro. Various hepatomas form albumin, usually at rates lower than those observed in liver. Observations of extrahepatic albumin synthesis (other than that in hepatomas and in cell-free systems programmed with mRNA from liver) are probably due to binding of amino acids to albumin which is not related to protein biosynthesis. The basic mechanism of the biosynthesis of albumin does not differ from that of other eucaryotic proteins. The polypeptide chain is initiated with methionine and completed within less than 2 min. Albumin synthesis requires energy. Most of the molecule is assembled on polyribosomes bound to the membranes of the endoplasmic reticulum. The mRNA for albumin can be isolated from liver extracts and then translated into protein in cell-free systems. Such systems synthesize a protein larger than albumin, if they lack the proteinases involved in posttranslational modification of the polypeptide chain.

In vivo, rates of albumin synthesis depend on the age of animals and on the protein content of their diet. Rats of 6–15 weeks weighing 140–360 g on a diet containing 17%–30% protein synthesize about 87 mg albumin per 100 g body weight per day. In these rats, albumin synthesis amounts to between 11% and 23% of total liver protein synthesis, compared with 7% in hepatocyte suspensions, and between 0.56% and 7% in cell-free protein synthesizing systems translating liver mRNA. The ratio of albumin synthesis to total protein synthesis in Morris hepatoma 5123TC is 1.8% in vivo, and 1.2% in cell suspensions.

Albumin is secreted via a sequential, "pipe line-like" mechanism, i.e., without random mixing of albumin molecules within the liver cell. The minimum time required for the transport of an albumin molecule from the site of synthesis to the cell membrane is about 14 min. It is slightly shorter in regenerating liver and slightly longer in choline-deficient rats.

It has been suggested that the translation of the mRNA for export proteins begins on free polyribosomes. A polypeptide segment is synthesized which is rich in hydrophobic amino acids; in the case of albumin it is 18 amino acids long. This segment may mediate the binding of the polyribosomes involved in export protein synthesis to the membrane of the endoplasmic reticulum. The nascent polypeptide chain is then transferred through the membrane of the endoplasmic reticulum into the intracisternal space. Here, the hydrophobic segment is probably removed by proteolytic cleavage, while the rest of the polypeptide chain is still being completed. The product is an albumin precursor protein with a short oligopeptide extension at the N-terminus. The name "proalbumin" has been suggested for this precursor. It is transported to the region of the smooth endoplasmic

reticulum and Golgi apparatus where it is converted into albumin by removal of the oligopeptide extension. This occurs about 5–6 min before secretion and is engergy dependent. Albumin secretion is not coupled to continued synthesis of protein.

No serum protein secretion can be detected in hepatoma-bearing and hepatectomized rats; however, hepatoma cells in suspension secrete albumin into the medium. In the incubated cells, albumin accumulates when rates of albumin synthesis are high, indicating a limited secretory capacity also in hepatoma cells in vitro.

Several suggestions are discussed for the function of the prosegment in the albumin precursor. It could mediate the interaction with membranes or mask binding sites for metal ions on the surface of the molecule. Another role of the oligopeptide segment could be in the regulation of the rate of synthesis or breakdown of albumin. Finally, the oligopeptide segment at the N-terminus of albumin might protect albumin against degradation during intracellular transport or facilitate the formation of the tertiary structure.

Oligopeptide extensions seem to be a general principle in the interaction of proteins with supramolecular structures. Synthesis via a precursor protein is important in the assembly of bacteriophages and of many viruses in eucaryotes. The occurrence of a precursor protein for a lipoprotein in the outer cytoplasmic membrane of *E. coli* might indicate that precursor proteins have developed early in evolution, related perhaps to the appearance of membranes and, later, adapted to the secretion of proteins.

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References

Abdel-Samie, Y.M., Broda, E., Kellner, G.: The autonomous production of individual serum proteins by tissue in culture. Biochem. J. **75**, 209–215 (1960)

Abdel-Samie, Y., Broda, E., Kellner, G., Zischka, W.: Production of serum albumin and of globulins by chick mesenchymal tissue and by HeLa tumour tissue in culture. Nature (Lond.) **184**, 361–362 (1959)

Airhart, J., Vidrich, A., Khairallah, E.A.: Compartmentation of free amino acids for protein synthesis in rat liver. Biochem. J. **140**, 539–548 (1974)

Askonas, B.A., Campbell, P.N., Humphrey, J.H., Work, T.S.: The source of antibody globulin in rabbit milk and goat colostrum. Biochem. J. **56**, 597–601 (1954)

Bancroft, F.C., Levine, L., Tashjian, A.H., Jr.: Serum albumin production by hepatoma cells in culture: direct evidence for stimulation by hydrocortisone. Biochem. Biophys. Res. Commun. **37**, 1028–1035 (1969).

Baraona, E., Leo, M.A., Borowsky, S.A., Lieber, C.S.: Alcoholic hepatomegaly: accumulation of protein in the liver, Science **190**, 794–795 (1975)

Bayly, R.J., Evans, E.A.: Stability and storage of compounds labelled with radioisotopes. J. Labelled Comp. **2**, 1–34 (1966)

Becker, F.F., Klein, K.M., Asofsky, R.: Plasma protein synthesis by N-2-Fluorenylacetamide-induced primary hepatocellular carcinomas and hepatic nodules. Cancer Res. **32**, 914–920 (1972)

Bellamy, G., Bornstein, P.: Evidence for procollagen, a biosynthetic precursor of collagen. Proc. Natl. Acad. Sci. USA **68**, 1138–1142 (1971)

Birken, S., Smith, D.L., Canfield, R.E., Boime, I.: Partial amino acid sequence of human placental lactogen precursor and its mature hormone form produced by membrane-associated enzyme activity. Biochem. Biophys. Res. Commun **74**, 106–112 (1977)

Bissell, M.J., Tosi, R., Gorini, L.: Mechanism of excretion of a bacterial proteinase: factors controlling accumulation of the extracellular proteinase of a *Sarcina* strain (Coccus P). J. Bacteriol. **105**, 1099–1109 (1971)

Blair, G.E., Ellis, R.J.: Protein synthesis in chloroplasts I. Light-driven synthesis of the large subunit of fraction I protein by isolated pea chloroplasts. Biochim. Biophys. Acta **319**, 223–234 (1973)

Blobel, G., Dobberstein, B.: Transfer of proteins across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. J. Cell Biol. **67**, 835–851 (1975)

Blobel, G., Sabatini, D.D.: Ribosome – membrane interaction in eukaryotic cells. Biomembranes **2**, 193–195 (1971)

Bocci, V.: Metabolism of plasma proteins (Review). Arch. Fisiol. **67**, Fasc. IV, 315–444 (1970)

Bogorad, L.: Evolution of organelles and eukaryotic genomes. Science **188**, 891–898 (1975)

Braatz, J.A., Heath, E.C.: The role of polysaccharide in the secretion of protein by *Micrococcus sodonensis*. J. Biol. Chem. **249**, 2536–2547 (1974)

Braun, G.A., Marsh, J.B., Drabkin, D.L.: Stimulation of protein and plasma albumin synthesis in a cell-free system from livers of nephrotic rats. Biochem. Biophys. Res. Commun. **8**, 28–32 (1962a)

Braun, G.A., Marsh, J.B., Drabkin, D.L.: Synthesis of plasma albumin and tissue proteins in regenerating liver. Metabolism **11**, 957–966 (1962b)

Breslow, J.L., Sloan, H.R., Ferrans, V.J., Anderson, J.L., Levy, R.I.: Characterization of the mouse liver cell line F L 83 B. Exp. Cell Res. **78**, 441–453 (1973)

Bretscher, M.S.: Phosphatidyl-ethanolamine: differential labelling in intact cells and cell ghosts of human erythrocytes by a membrane-impermeable reagent. J. Mol. Biol. **71**, 523–528 (1972)

Bretscher, M.S.: C-terminal region of the major erythrocyte sialoglycoprotein is on the cytoplasmic side of the membrane. J. Mol. Biol. **98**, 831–833 (1975)

Brunish, R., Luck, J.M.: Amino acid "incorporation" in vitro by desoxypentose nucleoprotein and histone. J. Biol. Chem. **197**, 869–882 (1952)

Burstein, Y., Kantor, F., Schechter, I.: Partial amino-acid sequence of the precursor of an immunoglobulin light chain containing NH_2-terminal pyroglutamic acid. Proc. Natl. Acad. Sci. USA **73**, 2604–2608 (1976)

Burstein, Y., Schechter, I.: Amino acid-sequence variability at the N-terminal extra piece of mouse immunoglobulin light-chain precursors of the same and different subgroups. Biochem. J. **157**, 145–151 (1976)

Burstein, Y., Schechter, I.: Amino acid sequence of the NH_2-terminal extra piece segments of the precursors of mouse immunoglobulin λ_1-type and κ-type light chains. Proc. Natl. Acad. Sci. USA **74**, 716–720 (1977)

Butterworth, B.E., Hall, L., Stoltzfus, C.M., Rueckert, R.R.: Virus-specific proteins synthesized in encephalomyocarditis virus-infected HeLa cells. Proc. Natl. Acad. Sci. USA **68**, 3083–3087 (1971)

Campbell, P.N.: The correlation between morphological structure and the synthesis of serum albumin by the microsome fraction of the rat liver cell. In: Biological Structure and Function. Goodwin, T.W., Lindberg, O. (eds.), Vol. I, pp. 255–259. London–New York: Academic Press 1961a

Campbell, P.N.: The synthesis of serum albumin by the microsome fraction of the liver. In: Protein Biosynthesis. Harris, R.J.C. (ed.), pp. 19–35. New York–London: Academic Press 1961b

Campbell, P.N.: The Biosynthesis of Rat Serum Albumin. Proc. 5th Internatl. Congr. Biochem., Moscow 1961c, Vol. II, pp. 195–203

Campbell, P.N.: The correlation between morphology and protein synthesizing activity in liver. Acta Biol. Med. Germanica **19**, 621–639 (1967)

Campbell, P.N.: Functions of polyribosomes attached to membranes of animal cells. FEBS Lett. **7**, 1–7 (1970)

Campbell, P.N.: The biosynthesis of serum albumin. FEBS Lett. **54**, 119–121 (1975)

Campbell, P.N., Greengard, O., Kernot, B.A.: Studies on the synthesis of serum albumin by the isolated microsome fraction from rat liver. Biochem. J. **74**, 107–117 (1960)

Campbell, P.N., Kernot, B.A.: The incorporation of [^{14}C] leucine into serum albumin by the isolated microsome fraction from rat liver. Biochem. J. **82**, 262–266 (1962)

Campbell, P.N., Stone, N.E.: The synthesis of serum albumin and tissue proteins in slices of rat liver and liver tumour. Biochem. J. **66**, 19–31 (1957)

Chan, S.J., Keim, P., Steiner, D.F.: Cell-free synthesis of rat preproinsulins – characterization and partial amino acid sequence determination. Proc. Natl. Acad. Sci. USA **73**, 1964–1968 (1976)

Chandrasekharan, N., Fleck, A., Munro, H.N.: Albumin content of rat hepatic cells at different levels of protein intake. J. Nutr. **92**, 497–502 (1967)

Chen, R.F.: Removal of fatty acids from serum albumin by charcoal treatment. J. Biol. Chem. **242**, 173–181 (1967)

Clarke, D.D., Mycek, M.J., Neidle, A., Waelsch, H.: The incorporation of amines into protein. Arch. Biochem. Biophys. **79**, 338–354 (1959)

Cohen, S.: Turnover of some chromatographically separated serum protein fractions in the rat. S. Afr. J. Med. Sci. **23**, 245–256 (1957)

Cohn, D.V., Macgregor, R.R., Chu, L.L.H., Kimmel, J.R., Hamilton, J.W.: Calcemic fraction-A: biosynthetic peptide precursor of parathyroid hormone. Proc. Natl. Acad. Sci. USA **69**, 1521–1525 (1972)

Cornwell, D.G., Luck, J.M.: Studies on amino-acid protein interactions. Arch. Biochem. Biophys. **73**, 391–409 (1958)

Crane, L.J., Miller, D.L.: Synthesis and secretion of fibrinogen and albumin by isolated rat hepatocytes. Biochem. Biophys. Res. Commun. **60**, 1269–1277 (1974)

Crawford, J.L., Lipscomb, W.N., Schellman, C.G.: The reverse turn as a polypeptide conformation in globular proteins. Proc. Natl. Acad. Sci. USA **70**, 538–542 (1973)

Decken, A., von der: Labelling of immunologically specific proteins by ribonucleoprotein particles from rat-liver and chick-liver cell sap. Biochem. J. **88**, 385–394 (1963a)

Decken, A., von der: Labelling with ^{14}C amino acids of albumin-like protein by rat liver ribonucleoprotein particles. J. Cell Biol. **16**, 471–481 (1963b)

Decken, A., von der, Campbell, P.N.: The role of soluble ribonucleic acid in the synthesis of serum albumin by the isolated microsome fraction from rat liver. Biochem. J. **80**, 38P–39P (1961a)

Decken, A., von der, Campbell, P.N.: Studies on the synthesis of serum albumin by isolated ribonucleoprotein particles from rat liver. Biochem. J. **80**, 39P (1961b)

Decken, A., von der, Campbell, P.N.: Studies on the synthesis of serum albumin by ribonucleoprotein particles isolated from rat liver. Biochem. J. **84**, 449–455 (1962)

Delaville, M., Delaville, G., Delaville, J.: Caractère de solubilité de la fraction albuminique de sérum sanguin dans les solutions d'éthanol trichloracétique; application au dosage des diverses fractions protéiques du sérum. Ann. Biol. Clin. (Paris) **12**, 320–323 (1954a)

Delaville, M., Delaville, G., Delaville, J.: Caractère de solubilité de la fraction albuminique du sérum sanguin dans l'alcool trichloracétique; son application au dosage des diverses fractions protéiques. Ann. Pharm. Fr. **12**, 109–113 (1954b)

Deschatrette, J., Weiss, M.C.: Characterization of differentiated and dedifferentiated clones from a rat hepatoma. Biochimie **56**, 1603–1611 (1974)

Devillers-Thiery, A., Kindt, T., Scheele, G., Blobel, G.: Homology in amino-terminal sequence of precursors to pancreatic secretory proteins. Proc. Natl. Acad. Sci. USA **72**, 5016–5020 (1975)

Dobberstein, B., Blobel, G., Chua, N.-H.: *In vitro* synthesis and processing of a putative precursor for the small subunit of ribulose-1,5-biphosphate carboxylase of *Chlamydomonas reinhardtii*. Proc. Natl. Acad. Sci. USA **74**, 1082–1085 (1977)

Dorling, P.R., Quinn, P.S., Judah, J.D.: Evidence for the coupling of biosynthesis and secretion of serum albumin in the rat. The effect of colchicine on albumin production. Biochem. J. **152**, 341–348 (1975)

Drabkin, D.L., Marsh, J.B.: Metabolic channeling in experimental nephrosis. I. Protein and carbohydrate metabolism. J. Biol. Chem. **212**, 623–631 (1955)

Eagle, H., Oyama, V.I., Piez, K.A.: The reversible binding of half-cysteine residues to serum protein and its bearing on the cysteine requirement of cultured mammalian cells. J. Biol. Chem. **235**, 1719–1726 (1960)

Edman, P., Begg, G.: A protein sequenator. Eur. J. Biochem. **1**, 80–91 (1967)

Edwards, K.: Biosynthesis of albumin in rat liver. Ph. D. Thesis, University of Melbourne, 1978

Edwards, K., Fleischer, B., Dryburgh, H., Fleischer, S., Schreiber, G.: The distribution of albumin precursor protein and albumin in liver. Biochem. Biophys. Res. Commun. **72**, 310–318 (1976a)

Edwards, K., Schreiber, G., Dryburgh, H., Millership, A., Urban, J.: Biosynthesis of albumin via a precursor protein in Morris hepatoma 5123TC. Cancer Res. **36**, 3113–3118 (1976b)

Edwards, K., Schreiber, G., Dryburgh, H., Urban, J., Inglis, A.S.: Synthesis of albumin via a precursor protein in cell suspensions from rat liver. Eur. J. Biochem. **63**, 303–311 (1976c)

Faber, A.J., Miall, S.H., Tamaoki, T.: Synthesis of albumin with exogenous mouse-liver messenger RNA in a homologous cell-free system. Can. J. Biochem. **52**, 429–432 (1974)

Fairclough, G.F., Fruton, J.S.: Peptide-protein interaction as studied by gel filtration. Biochemistry **5**, 673–683 (1966)

Feldmann, G., Penaud-Laurencin, J., Crassous, J., Benhamou, J.P.: Albumin synthesis by human liver cells: its morphological demonstration. Gastroenterology **63**, 1036–1048 (1972)

Fernandez, A., Sobel, C., Goldenberg, H.: An improved method for determination of serum albumin and globulin. Clin. Chem. **12**, 194–205 (1966)

Fishman, B., Wurtman, R.J., Munro, H.N.: Daily rhythms in hepatic polysome profiles and tyrosine transaminase activity: Role of dietary protein. Proc. Natl. Acad. Sci. USA **64**, 677–682 (1969)

Foster, J.F.: Plasma Albumin. In: The Plasma Proteins. Putman, F.W. (ed.), Vol. I, pp. 179–239. New York–London: Academic Press 1960

Foster, J.F., Sterman, M.D.: Conformation changes in bovine plasma albumin associated with hydrogen ion and urea binding. II. Hydrogen ion titration curves. J. Am. Chem. Soc. **78**, 3656–3660 (1956)

Freeman, T.: The function of plasma proteins. In: Protides of the Biological Fluids. Peeters, H. (ed.), Vol. XV, pp. 1–14. Amsterdam: Elsevier 1967

Freeman, T., Gordon, A.H.: Metabolism of albumin and γ-globulin in protein deficient rats. Clin. Sci. **26**, 17–26 (1964)

Gandolfi, E., Fabrini, G.: A new method for serum albumin determination. Ital. J. Biochem. **15**, 244–249 (1966)

Ganoza, M.C., Williams, C.A.: *In vitro* synthesis of different categories of specific proteins by membrane-bound and free ribosomes. Proc. Natl. Acad. Sci. USA **63**, 1370–1376 (1969)

Ganoza, M.C., Williams, C.A., Lipman, F.: Synthesis of serum proteins by a cell-free system from rat liver. Proc. Natl. Acad. Sci. USA **53**, 619–622 (1965)

Gaudernack, G., Rugstad, H.E., Hegna, I., Prydz, H.: Synthesis of serum proteins by a clonal strain of rat hepatoma cells. Exp. Cell Res. **77**, 25–30 (1973)

Geller, D.M., Judah, J.D., Nicholls, M.R.: Intracellular distribution of serum albumin and its possible precursors in rat liver. Biochem. J. **127**, 865–874 (1972)

Givan, A.L., Criddle, R.S.: Ribulosediphosphate carboxylase from *Chlamydomonas reinhardi*: purification, properties and its mode of synthesis in the cell. Arch. Biochem. Biophys. **149**, 153–163 (1972)

Glaumann, H.: Studies on the synthesis and transport of albumin in microsomal subfractions from rat liver. Biochim. Biophys. Acta **224**, 206–218 (1970)

Glaumann, H., Ericsson, J.L.E.: Evidence for the participation of the Golgi apparatus in the intracellular transport of nascent albumin in the liver cell. J. Cell Biol. **47**, 555–567 (1970)

Goldsworthy, P.D., McCartor, H.R., McGuigan, J.E., Peppers, G.F., Volwiler, W.: Relative albumin, transferrin, and fibrinogen synthesis rates in perfused bovine liver. Am. J. Physiol. **218**, 1428–1433 (1970)

Gordon, A.H.: Synthesis of plasma proteins by the perfused rat liver. Effects of protein free diet, 3′-MDAB and dimethylnitrosamine. Eur. J. Cancer **2**, 19–31 (1966)

Gordon, A.H., Humphrey, J.H.: Methods for measuring rates of synthesis of albumin by the isolated perfused rat liver. Biochem. J. **75**, 240–247 (1960)

Gordon, A.H., Humphrey, J.H.: Measurement of intracellular albumin in rat liver. Biochem. J. **78**, 551–556 (1961)

Goussault, Y., Sharif, A., Bourrilon, R.: Serum albumin biosynthesis and secretion by resting and lectin stimulated human lymphocytes. Biochem. Biophys. Res. Commun. **73**, 1030–1035 (1976)

Gray, J.C., Kekwick, R.G.O.: The synthesis of the small subunit of ribulose 1,5-biophosphate carboxylase in the french bean *Phaseolus vulgaris*. Eur. J. Biochem. **44**, 491–500 (1974)

Habener, J.F., Kemper, B., Potts, J.T., Jr., Rich, A.: Pre-proparathyroid hormone identified by cell-free translation of messenger RNA from hyperplastic human parathyroid tissue. J. clin. Invest. **56**, 1328–1333 (1975)

Haider, M., Tarver, H.: Effect of diet on protein synthesis and nucleic acid levels in rat liver. J Nutr. **99**, 433–445 (1969)

Hamashima, Y., Harter, J.G., Coons, A.H.: The localization of albumin and fibrinogen in human liver cells. J. Cell Biol. **20**, 271–279 (1964)

Hamilton, J.W., Niall, H.D., Jacobs, J.W., Keutmann, H.T., Potts, J.T., Jr., Cohn, D.V.: The N-terminal amino-acid sequence of bovine proparathyroid hormone. Proc. Natl. Acad. Sci. USA **71**, 653–656 (1974)

Harrap, K.R., Jackson, R.C., Riches, P.G., Smith, C.A., Hill, B.T.: The occurrence of protein-bound mixed disulfides in rat tissues. Biochim. Biophys. Acta **310**, 104–110 (1973)

Haselkorn, R., Rothman-Denes, L.B.: Protein synthesis. Annu. Rev. Biochem. **42**, 397–438 (1973)

Henshaw, E.C., Hirsch, C.A., Morton, B.E., Hiatt, H.H.: Control of protein synthesis in mammalian tissues through changes in ribosome activity. J. Biol. Chem. **246**, 436–446 (1971)

Hicks, S.J., Drysdale, J.W., Munro, H.N.: Preferential synthesis of ferritin and albumin by different populations of liver polysomes. Science **164**, 584–585 (1969)

Hirokawa, R., Ogata, K.: *In vivo* evidence for albumin biosynthesis in rat liver ribosomes. J. Biochem.(Tokyo) **52**, 377–378 (1962)

Hirokawa, R., Omori, S., Takahashi, T., Ogata, K.: The transfer of amino acid from

soluble ribonucleic acid to microsomal albumin. Biochim. Biophys. Acta **49**, 612–614 (1961)
Hitzig, W.H.: Plasmaproteine, 2. Aufl. Berlin–Heidelberg–New York: Springer 1977
Hochberg, A.A., Stratman, F.W., Zahlten, R.N., Lardy, H.A.: Artifacts in protein synthesis by mitochondria *in vitro*. FEBS Lett. **25**, 1–7 (1972)
Hoffenberg, R., Black, E., Brock J.F.: Albumin and γ-globulin tracer studies in protein depletion states. J. Clin. Invest. **45**, 143–152 (1966)
Hoffenberg, R., Gordon, A.H., Black, E.G.: Albumin synthesis by the perfused rat liver. A comparison of methods with special reference to the effect of dietary protein deprivation. Biochem. J. **122**, 129–134 (1971)
Holtzer, H., Holtzer, S.: The *in vitro* uptake of fluorescein labelled plasma proteins. I. Mature cells. C.R. Trav. Lab. Carlsberg **31**, 373–408 (1960)
Hosoda, J., Cone, R.: Analysis of T4 phage proteins, I. conversion of precursor proteins into lower molecular weight peptides during normal capsid formation. Proc. Natl. Acad. Sci. USA **66**, 1275–1281 (1970)
Ikehara, Y., Oda, K., Kato, K.: Conversion of proalbumin into serum albumin in the secretory vesicles of rat liver. Biochem. Biophys. Res. Commun. **72**, 319–326 (1976)
Ikehara, Y., Pitot, H.C.: Localization of polysome-bound albumin and serine dehydratase in rat liver cell fractions. J. Cell Biol. **59**, 28–44 (1973)
Inglis, A.S., Nicholls, P.W.: Identification of phenylthiohydantoins of amino acids by thin-layer chromatography. J. Chromatogr. **79**, 344–346 (1973)
Inglis, A.S., Nicholls, P.W., Roxburgh, G.M.: Acid hydrolysis of phenylthiohydantoins of amino acids. Aust. J. Biol. Sci. **24**, 1247–1250 (1971)
Inouye, S., Wang, S., Sekizawa, J., Halegoua, S., Inouye, M.: Amino acid sequence for the peptide extension on the prolipoprotein of the *Escherichia coli* outer membrane. Proc. Natl. Acad. Sci. USA **74**, 1004–1008 (1977)
Isles, T.E., Jocelyn, P.C.: The reaction of protein thiol groups with some disulfides. Biochem. J. **88**, 84–88 (1963)
Iwanij, V., Chua, N.-H., Siekevitz, P.: The purification and some properties of ribulosebisphosphate carboxylase and of its subunits from the green alga *Chlamydomonas reinhardtii*. Biochim. Biophys. Acta **358**, 329–340 (1974)
Jacobson, M.F., Baltimore, D.: Morphogenesis of poliovirus. I. Association of the viral RNA with coat protein. J. Mol. Biol. **33**, 369–378 (1968)
Jamieson, J.C., Ashton, F.E.: Studies on acute phase proteins of rat serum. IV. Pathway of secretion of albumin and α_1-acid glycoprotein from liver. Can. J. Biochem. **51**, 1281–1291 (1973)
Jeffay, H., Winzler, R.J.: The metabolism of serum proteins. II. The effect of dietary protein on the turnover of rat serum protein. J. Biol. Chem. **231**, 111–116 (1958)
Jefferson, L.S., Korner, A.: Influence of amino acid supply on ribosomes and protein synthesis of perfused rat liver. Biochem. J. **111**, 703–712 (1969)
John, D.W., Miller, L.L.: Regulation of net biosynthesis of serum albumin and acute phase plasma proteins. Induction of enhanced net synthesis of fibrinogen, α_1-acid glycoprotein, α_2 (acute phase)-globulin, and haptoglobin by amino acids and hormones during perfusion of the isolated normal rat liver. J. Biol. Chem. **244**, 6134–6142 (1969)
Jonckheer, M.H., Karcher, D.M.: Thyroid albumin. I. Isolation and characterization. J. Clin. Endocrinol. Metab. **32**, 7–17 (1971)
Judah, J.D., Gamble, M., Steadman, J.H.: Biosynthesis of serum albumin in rat liver. Evidence for the existence of 'proalbumin'. Biochem. J. **134**, 1083–1091 (1973)
Judah, J.D., Nicholls, M.R.: Role of liver-cell potassium ions in secretion of serum albumin and lipoproteins. Biochem. J. **116**, 663–669 (1970)
Judah, J.D., Nicholls, M.R.: The separation of intracellular serum albumin from rat liver. Biochem. J. **123**, 643–648 (1971a)
Judah, J.D., Nicholls, M.R.: Biosynthesis of rat serum albumin. Biochem. J. **123**, 649–655 (1971b)

Jungblut, P.W.: Biosynthese von Ratten-Serumalbumin. II. Untersuchung der Synthese und Sekretion von Serumalbumin mit der isoliert durchströmten Rattenleber. Biochem. Z. **337**, 285–296 (1963a)

Jungblut, P.W.: Biosynthese von Ratten-Serumalbumin. III. Bildungsmechanismus der Polypeptidkette. Biochem. Z. **337**, 297–302 (1963b)

Kaji, H.: Further studies on the soluble amino acid incorporating system from rat liver. Biochemistry **7**, 3844–3850 (1968)

Kaji, H.: Amino-terminal arginylation of chromosomal proteins by arginyl-tRNA. Biochemistry **15**, 5121–5125 (1976)

Kaji, A., Kaji, H., Novelli, G.D.: Soluble amino acid-incorporating system. II. Soluble nature of the system and the characterization of the radioactive product. J. Biol. Chem. **240**, 1192–1197 (1965)

Kaji, H., Novelli, G.D., Kaji, A.: A soluble amino acid-incorporating system from rat liver. Biochim. Biophys. Acta **76**, 474–477 (1963)

Kallee, E., Lohss, F., Oppermann, W.: Trichloressigsäure-Aceton-Extraktion von Albuminen aus Seren und Antigen-Antikörper-Präzipitaten. Z. Naturforsch. **12b**, 777–783 (1957)

Katz, J., Bonorris, G., Okuyama, S., Sellers, A.L.: Albumin synthesis in perfused liver of normal and nephrotic rats. Am. J. Physiol. **212**, 1255–1260 (1967)

Katz, J., Bonorris, G., Sellers, A.L.: Albumin metabolism in aminonucleoside nephrotic rats. J. Lab. Clin. Med. **62**, 910–934 (1963)

Katz, J., Sellers, A.L., Bonorris, G.: Plasma albumin synthesis in perfused rat liver. In: Stoffwechsel der isoliert perfundierten Leber. Staib, W., Scholz, R. (eds.). 3. Konferenz der Gesellschaft für Biologische Chemie vom 27.–29 April 1967, pp. 100–108. Berlin–Göttingen–Heidelberg: Springer 1968

Kawashima, N., Wildman, S.G.: Fraction I protein. Annu. Rev. Plant Physiol. **21**, 325–358 (1970)

Kellenberger, E., Kellenberger-van der Kamp, C.: On a modification of the gene product P23 according to its use as subunit of either normal capsids of phage T4 or of polyheads. FEBS Lett. **8**, 140–144 (1970)

Keller, G.H., Taylor, J.M.: Effect of hypophysectomy on the synthesis of rat liver albumin. J. Biol. Chem. **251**, 3768–3773 (1976)

Kelman, L., Saunders, S.J., Frith, L., Wicht, S., Corrigal, A.: Effects of dietary protein restriction on albumin synthesis, albumin catabolism. and the plasma aminogram. Am. J. Clin. Nutr. **25**, 1174–1178 (1972a)

Kelman, L., Saunders, S.J., Wicht, S., Frith, L., Corrigal, A., Kirsch, R.E., Terblanche, J.: The effects of amino acids on albumin synthesis by the isolated perfused rat liver. Biochem. J. **129**, 805–809 (1972b)

Kemper, B., Habener, J.F., Ernst, M.D., Potts, J.T., Jr., Rich, A.: Pre-proparathyroid hormone: analysis of radioactive tryptic peptides and amino acid sequence. Biochemistry **15**, 15–19 (1976)

Kemper, B., Habener, J.F., Potts, J.T., Jr., Rich, A.: Proparathyroid hormone: identification of a biosynthetic precursor to parathyroid hormone. Proc. Natl. Acad. Sci. USA **69**, 643–647 (1972)

Kernoff, L.M., Pimstone, B.L., Solomon, J., Brock, J.F.: The effect of hypophysectomy and growth hormone replacement on albumin synthesis and catabolism in the rat. Biochem. J. **124**, 529–535 (1971)

Keston, A.S., Katchen, B.: Incorporation of glycine-2-C^{14} into homologous antibody by rabbit tissue slices. J. Immunol. **76**, 253–258 (1956)

Kiehn, E.D., Holland, J.J.: Synthesis and cleavage of enterovirus polypeptides in mammalian cells. J. Virol. **5**, 358–367 (1970)

King, T.P.: On the sulfhydryl group of human plasma albumin. J. Biol. Chem. **236**, PC5 (1961)

King, T.P., Spencer, M.: Structural studies and organic ligand-binding properties of bovine plasma albumin. J. Biol. Chem. **245**, 6134–6148 (1970)

Kirsch, J.A.W., Wise, R.W., Oliver, I.T.: Post-albumin, a foetal-specific rat plasma protein. Biochem.J. **102**, 763–766 (1967)
Kirsch, R., Frith, L., Black, E., Hoffenberg, R.: Regulation of albumin synthesis and catabolism by alteration of dietary protein. Nature (Lond.) **217**, 578–579 (1968)
Kirsch, R.E., Saunders, S.J., Frith, L., Wicht, S., Kelman, L., Brock, J.F.: Plasma amino acid concentration and the regulation of albumin synthesis. Am. J. Clin. Nutr. **22**, 1559–1562 (1969)
Koga, K., Tamaoki, T.: Developmental changes in the synthesis of α-fetoprotein and albumin in the mouse liver. Cell-free synthesis by membrane-bound polyribosomes. Biochemistry **13**, 3024–3028 (1974)
Korner, A.: Incorporation of radioactive amino acids into serum albumin by isolated rat-liver ribosomes. Biochem. J. **76**, 59P-60P (1960)
Korner, A., Debro, J.R.: Solubility of albumin in alcohol after precipitation by trichloroacetic acid. A simplified procedure for separation of albumin. Nature (Lond.) **178**, 1067 (1956)
Kritzman, M.G., Sukhareva, B.S., Konikuva, A.S.: Sites of incorporation of phenylalanine labelled with carbon-14 into insulin and its chain B *in vitro*. Nature (Lond.) **195**, 600–602 (1962)
Kukral, J.C., Kerth, J.D., Pancner, R.J., Cromer, D.W., Henegar, G.C.: Plasma protein synthesis in the normal dog and after total hepatectomy. Surg. Gynecol. Obstet. **113**, 360–372 (1961)
Kuntz, I.D.: Protein folding. J. Am. Chem. Soc. **94**, 4009–4012 (1972)
Laemmli, U.K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T 4. Nature (Lond.) **227**, 680–685 (1970)
LeBouton, A.V.: Precursor-product relationship between intrahepatic albumin and plasma albumin. Biochem. J. **106**, 503–506 (1968)
Le Marchand, Y., Singh, A., Assimacopoulos-Jeannet, F., Orci, L., Rouiller, C., Jeanrenaud, B.: A role for the microtubular system in the release of very low density lipoproteins by perfused mouse livers. J. Biol. Chem. **248**, 6862–6870 (1973)
Ledford, B.E., Papaconstantinou, J.: Regulation of albumin synthesis in cultured mouse hepatoma cells. Fed. Proc. **32**, 615 (1973)
Ledford, B.E., Warner, R.W., Cochran, R.A.: Albumin synthesis in cultured hepatoma cells – regulation by essential amino acids. Biochim. Biophys. Acta **475**, 90–95 (1977)
Leibowitz, M.J., Soffer, R.L.: Enzymatic modification of proteins. III. Purification and properties of a leucyl, phenylalanyl transfer ribonucleic acid-protein transferase from *Escherichia coli*. J. Biol. Chem. **245**, 2066–2073 (1970)
Leibowitz, M.J., Soffer, R.L.: Enzymatic modification of proteins. VII. Substrate specificity of leucyl, phenylalanyltransfer ribonucleic acid-protein transferase. J. Biol. Chem. **246**, 5207–5212 (1971)
Levine, S.: Solubilization of bovine albumin in nonaqueous media. Arch. Biochem. Biophys. **50**, 515–517 (1954)
Lewis, P.N., Scheraga, H.A.: Predictions of structural homologies in cytochrome c proteins. Arch. Biochem. Biophys. **144**, 576–583 (1971)
Lingrel, J.B., Webster, G.: Serum-albumin synthesis by isolated rat liver microsomes. Biochem. Biophys. Res. Commun. **5**, 57–62 (1961)
Lloyd, E.A., Saunders, S.J., Frith, L.O.C., Wright, J.E.: Albumin synthesis and catabolism following partial hepatectomy in the rat. The effects of amino acids and adrenocortical steroids on albumin synthesis after partial hepatectomy. Biochim. Biophys. Acta **402**, 113–123 (1975)
Lodish, H.F.: Biosynthesis of reticulocyte membrane proteins by membrane-free polyribosomes. Proc. Natl. Acad. Sci. USA **70**, 1526–1530 (1973)
Lodish, H.F.: Translational control of protein synthesis. Annu. Rev. Biochem. **45**, 39–72 (1976)
Lucas-Lenard, J., Lipman, F.: Protein biosynthesis. Annu. Rev. Biochem. **40**, 409–448 (1971)

Madden, S.C., Whipple, G.H.: Plasma proteins: their source, production and utilization. Physiol. Rev. **20**, 194–217 (1940)

Maeno, H., Schreiber, G., Weigand, K., Weinssen, U., Zähringer, J.: Impairment of albumin synthesis in cell-free systems from rat liver. FEBS Lett. **6**, 137–140 (1970)

Maławista, S.E., Weiss, M.C.: Expression of differentiated functions in hepatoma cell hybrids: high frequency of induction of mouse albumin production in rat hepatoma-mouse lymphoblasts hybrids. Proc. Natl. Acad. Sci. USA **71**, 927–931 (1974)

Mancini, G., Carbonara, A.O., Heremans, J.F.: Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry **2**, 235–254 (1965)

Mans, R.J., Novelli, G.D.: A convenient, rapid and sensitive method for measuring the incorporation of radioactive amino acids into protein. Biochem. Biophys. Res. Commun. **3**, 540–543 (1960)

Mans, R.J., Novelli, G.D.: Measurement of the incorporation of radioactive amino acids into protein by a filter-paper disk method. Arch. Biochem. Biophys. **94**, 48–53 (1961)

Margulies, M.M.: Concerning the sites of synthesis of proteins of chloroplast ribosomes and of fraction I protein (ribulose-1,5-diphosphate carboxylase). Biochem. Biophys. Res. Commun. **44**, 539–545 (1971)

Marsh, J.B., Drabkin, D.L.: Metabolic channeling in experimental nephrosis. IV. Net synthesis of plasma albumin by liver slices from normal and nephrotic rats. J. Biol. Chem. **230**, 1073–1081 (1958)

Marsh, J.B., Drabkin, D.L.: Experimental reconstruction of metabolic pattern of lipid nephrosis: key role of hepatic protein synthesis in hyperlipemia. Metabolism **9**, 946–955 (1960)

Marsh, J.B., Drabkin, D.L.: Stimulation of plasma albumin synthesis by rat-liver ribonucleic acid. Biochim. Biophys. Acta **95**, 173–176 (1965)

Marsh, J.B., Drabkin, D.L., Braun, G.A., Parks, J.S.: Factors in the stimulation of protein synthesis by subcellular preparations from rat liver. J. Biol. Chem. **241**, 4168–4174 (1966)

Maurer, R.A., Gorski, J., McKean, D.J.: Partial amino acid sequence of rat pre-prolactin. Biochem. J. **161**, 189–192 (1977)

May, B.K., Elliott, W.H.: Characteristics of extracellular protease formation by *Bacillus subtilis* and its control by amino acid repression. Biochim. Biophys. Acta **157**, 607–615 (1968)

McGown, E., Richardson, A.G., Henderson, L.M., Swan, P.B.: Effect of amino acids on ribosome aggregation and protein synthesis in perfused liver. J. Nutr. **103**, 109–116 (1973)

McLaughlin, C.A., Pitot, H.C.: The effect of various treatments *in vitro* and *in vivo* on the binding of ^{125}I-labelled anti-rat serum albumin Fab' to rat tissue polyribosomes. Biochemistry **15**, 3541–3550 (1976)

McMenamy, R.H.: Association of indole analogues to defatted human serum albumin. Arch. Biochem. Biophys. **103**, 409–417 (1963)

McMenamy, R.H.: Binding of indole analogues to human serum albumin. J. Biol. Chem. **240**, 4235–4243 (1965)

McMenamy, R.H., Oncley, J.L.: The specific binding of L-tryptophan to serum albumin. J. Biol. Chem. **233**, 1436–1447 (1958)

Michael, S.E.: The isolation of albumin from blood serum or plasma by means of organic solvents. Biochem. J. **82**, 212–218 (1962)

Miller, L.L., Bly, C.G., Bale, W.F.: Plasma and tissue proteins produced by non-hepatic rat organs as studied with lysine-ϵ-C^{14}. J. Exp. Med. **99**, 133–153 (1954)

Miller, L.L., Bly, C.G., Watson, M.L., Bale, W.F.: The dominant role of the liver in plasma protein synthesis. A direct study of the isolated perfused rat liver with the aid of lysine-ϵ-C^{14}. J. Exp. Med. **94**, 431–453 (1951)

Millership, A.S.: Synthesis and secretion of bovine serum albumin. M. Sc. Thesis, University of Melbourne, 1977.

Millership, A., Schreiber, G., Christie, B.: Synthesis and secretion of bovine serum albumin. Proc. Aust. Biochem. Soc. **10**, 56 (1977)

Milstein, C., Brownlee, G.G., Harrison, T.M., Mathews, M.B.: A possible precursor of immunoglobulin light chains. Nature (Lond.) **239**, 117–120 (1972)

Moon, K.E., Thompson, E.O.P.: Subunits from reduced and S-carboxymethylated ribulose diphosphate carboxylase (fraction I protein). Aust. J. Biol. Sci. **22**, 463–470 (1969)

Morgan, E.H.: Transferrin and albumin distribution and turnover in the rat. Am. J. Physiol. **211**, 1486–1494 (1966)

Morgan, E.H., Peters, T., Jr.: The biosynthesis of rat serum albumin. V. Effect of protein depletion and refeeding on albumin and transferrin synthesis. J. Biol. Chem. **246**, 3500–3507 (1971a)

Morgan, E.H., Peters, T., Jr.: Intracellular aspects of transferrin synthesis and secretion in the rat. J. Biol. Chem. **246**, 3508–3511 (1971b)

Morgenthaler, J.-J., Mendiola-Morgenthaler, L.: Synthesis of soluble, thylakoid, and envelope membrane proteins by spinach chloroplasts purified from gradients. Arch. Biochem. Biophys. **172**, 51–58 (1976)

Mullins, F., Weissman, S.M., Konen, J.A.: Extraabdominal serum protein synthesis in the rhesus monkey. J. Surg. Res. **6**, 315–321 (1966)

Munro, H.N.: A general survey of mechanisms regulating protein metabolism in mammals. In: Mammalian Protein Metabolism. Munro, H.N. (ed.), Vol. IV, pp. 3–130. New York–London: Academic Press 1970

Mycek, M.J., Clarke, D.D., Neidle, A., Waelsch, H.: Amine incorporation into insulin as catalyzed by transglutaminase. Arch. Biochem. Biophys. **84**, 528–540 (1959)

Nardacci, N.J., Jones, J.P., Hall, A.L., Olson, R.E.: Synthesis of nascent prothrombin and albumin in a heterologous system using rat liver messenger RNA purified on oligo (dT)-cellulose. Biochem. Biophys. Res. Commun. **64**, 51–58 (1975)

Neidle, A., Mycek, M.J., Clarke, D.D., Waelsch, H.: Enzymic exchange of protein amide groups. Arch. Biochem. Biophys. **77**, 227–229 (1958)

Ogata, K.: Protein biosynthesis-translation: albumin biosynthesis. Igakunoaumi **58**, 344–350 (1966)

Ogata, K.: Biosynthesis of serum albumin. Tampakushitsu Kakusan Koso **12**, 1346–1351 (1967)

Ogata, K.: Serum albumin biosynthesis. Taisya **10**, 281–290 (1973)

Ogata, K., Hirokawa, R., Omori, S.: Incorporation of leucine into microsomal albumin by microsomes and pH-5 enzymes from normal rat liver. Biochim. Biophys. Acta **40**, 178–179 (1960)

Ogata, K., Ishikawa, K., Tominaga, H., Watanabe, I., Morita, T., Sugano, H.: Further evidence for the existence of a specific ribonucleic acid in liver ribosomes. Biochim. Biophys. Acta **76**, 630–632 (1963)

Ogata, K., Omori, S., Hirokawa, R., Takahashi, T.: Studies of the Cell Free System for Biosynthesis of Serum Albumin in Liver Cells and Serum γ-Globulin in Immune Spleen Cells. Proc. 5th Int. Congr. Biochem. Moscow, Symp. 2, pp. 183–194, 1961

Ohanian, S.H., Taubman, S.B., Thorbecke, G.J.: Rates of albumin and transferrin synthesis *in vitro* in rat hepatoma-derived H_4II-EC_3 cells. J. Natl. Cancer Inst. **43**, 397–406 (1969)

Oler, A., Lombardi, B.: Further studies on a defect in the intracellular transport and secretion of proteins by the liver of choline-deficient rats. J. Biol. Chem. **245**, 1282–1288 (1970)

Otten, J., Jonckheer, M., Dumont, J.E.: Thyroid albumin. II. *In vitro* synthesis of a thyroid albumin by normal human thyroid tissue. J. Clin. Endocrinol. Metab. **32**, 18–26 (1971)

Ove, P., Coetzee, M.L., Chen, J., Morris, H.P.: Differences in synthesis and degradation of serum proteins in normal and hepatoma-bearing animals. Cancer Res. **32**, 2510–2518 (1972)

Pain, R.H., Robson, B.: Analysis of the code relating sequence to secondary structure in proteins. Nature (Lond.) **227**, 62–63 (1970)

Papaconstantinou, J., Ledford, B.E.: Regulation of albumin synthesis in cultured mouse hepatoma cells. In: The Role of RNA in Reproduction and Development. Niu, M.C., Segal, S.J. (eds.), pp. 43–52. Amsterdam: North-Holland 1973

Patterson, J.E., Geller, D.M.: Bovine microsomal albumin: amino terminal sequence of bovine proalbumin. Biochem. Biophys. Res. Commun. **74**, 1220–1226 (1977)
Peters, T., Jr.: A serum albumin precursor in cytoplasmic particles. J. Biol. Chem. **229**, 659–677 (1957)
Peters, T., Jr.: The isolation of serum albumin from specific precipitates of serum albumin and its rabbit antibodies. J. Am. Chem. Soc. **80**, 2700–2702 (1958)
Peters, T., Jr.: Cytoplasmic particles and serum albumin synthesis. J. Histochem. Cytochem. **7**, 224–234 (1959)
Peters, T., Jr.: The biosynthesis of rat serum albumin. I. Properties of rat albumin and its occurrence in liver cell fractions. J. Biol. Chem. **237**, 1181–1185 (1962a)
Peters, T., Jr.: The biosynthesis of albumin. II. Intracellular phenomena in the secretion of newly formed albumin. J. Biol. Chem. **237**, 1186–1189 (1962b)
Peters, T., Jr.: Serum albumin. Adv. Clin. Chem. **13**, 37–111 (1970)
Peters, T., Jr.: Serum albumin. In: The Plasma Proteins. Structure, Function and Genetic Control. Putman, F.W. (ed.), 2nd ed., Vol. I, pp. 133–181. New York–San Francisco–London: Academic Press 1975.
Peters, T., Jr.: Serum albumin: recent progress in the understanding of its structure and biosynthesis. Clin. Chem. **23**, 5–12 (1977)
Peters, T., Jr., Anfinsen, C.B.: Production of radioactive serum albumin by liver slices. J. Biol. Chem. **182**, 171–179 (1950a)
Peters, T., Jr., Anfinsen, C.B.: Net production of serum albumin by liver slices. J. Biol. Chem. **186**, 805–813 (1950b)
Peters, T., Jr., Danzi, J.T., Ashley, C.A.: Effect of the rate of albumin synthesis on the proportion of hepatocytes containing demonstrable serum albumin. Fed. Proc. **27**, 775 (1968)
Peters, T., Jr., Fleischer, B., Fleischer, S.: The biosynthesis of rat serum albumin. IV. Apparent passage of albumin through the Golgi apparatus during secretion. J. Biol. Chem. **246**, 240–244 (1971)
Peters, T., Jr., Peters, J.C.: The biosynthesis of rat serum albumin. VI. Intracellular transport of albumin and rates of albumin and liver protein synthesis *in vivo* under various physiological conditions. J. Biol. Chem. **247**, 3858–3863 (1972)
Peterson, J.A.: Assay for albumin-messenger-RNA in an *in vitro* protein synthesizing system from wheat germ. Nucl. Acids Res. **3**, 1427–1436 (1976)
Peterson, J.A., Weiss, M.C.: Expression of differentiated functions in hepatoma cell hybrids: induction of mouse albumin production in rat hepatoma-mouse fibroblast hybrids. Proc. Natl. Acad. Sci. USA **69**, 571–575 (1972)
Putnam, F.W.: Thyroxine-binding prealbumin. In: The Plasma Proteins. Structure, Function and Genetic Control. Putman, F.W. (ed.), 2nd ed., Vol. I, pp. 70–72. New York–San Francisco–London: Academic Press 1975
Quinn, P.S., Gamble, M., Judah, J.D.: Biosynthesis of serum albumin in rat liver. Isolation and probable structure of 'proalbumin' from rat liver. Biochem. J. **146**, 389–393 (1975)
Race, J.: The determination of blood-proteins by acid-acetone. Biochem. J. **26**, 1571–1584 (1932)
Rao, K.R., Tarver, H.: Albumin synthesis and release-stimulation with fat rich supernatants and differences between free and bound ribosomes of normal and regenerating rat livers. Fed. Proc. **29**, 919 (1970)
Redman, C.M.: The synthesis of serum proteins on attached rather than free ribosomes of rat liver. Biochem. Biophys. Res. Commun. **31**, 845–850 (1968)
Redman, C.M.: Biosynthesis of serum proteins and ferritin by free and attached ribosomes of rat liver. J. Biol. Chem. **244**, 4308–4315 (1969)
Redman, C.M., Banerjee, D., Howell, K., Palade, G.E.: Colchicine inhibition of plasma protein release from rat hepatocytes. J. Cell Biol. **66**, 42–59 (1975)
Redman, C.M., Cherian, M.G.: The secretory pathways of rat serum glycoproteins and albumin: localization of newly formed proteins within the endoplasmic reticulum. J. Cell Biol. **52**, 231–245 (1972)
Redman, C.M., Grab, D.J., Irukulla, R.: The intracellular pathway of newly formed rat liver catalase. Arch. Biochem. Biophys. **152**, 496–501 (1972)

Richardson, U.I., Tashjian, A.H., Jr., Levine, L.: Establishment of a clonal strain of hepatoma cells which secrete albumin. J. Cell. Biol. **40**, 236–247 (1969)

Roberts, B.E., Paterson, B.M., Sperling, R.: The cell-free synthesis and assembly of viral specific polypeptides into TMV particles. Virology **59**, 307–313 (1974)

Rossing, N.: The normal metabolism of ^{131}I-labelled albumin in man. Clin. Sci. **33**, 593–602 (1967)

Rotermund, H.-M., Schreiber, G., Maeno, H., Weinssen, U., Weigand, K.: The ratio of albumin synthesis to total protein synthesis in normal rat liver, in host liver, and in Morris hepatoma 9121. Cancer Res. **30**, 2139–2146 (1970)

Rothschild, M.A., Oratz, M., Mongelli, J., Schreiber, S.S.: Effects of a short-term fast on albumin synthesis studied *in vivo*, in the perfused liver, and on amino acid incorporation by hepatic microsomes. J. Clin. Invest. **47**, 2591–2599 (1968)

Rothschild, M.A., Oratz, M., Mongelli, J., Schreiber, S.S.: Effect of albumin concentration on albumin synthesis in the perfused liver. Am. J. Physiol. **216**, 1127–1130 (1969)

Rothschild, M.A., Oratz, M., Schreiber, S.S.: Current concepts of albumin metabolism, a review. Gastroenterology **58**, 402–408 (1970)

Rothschild, M.A., Oratz, M., Schreiber, S.S.: Albumin synthesis. (First of two parts). N. Engl. J. Med. **286**, 748–757 (1972a)

Rothschild, M.A., Oratz, M., Schreiber, S.S.: Albumin synthesis. (Second of two parts). N. Engl. J. Med. **286**, 816–820 (1972b)

Rothschild, M.A., Oratz, M., Schreiber, S.S.: Albumin metabolism. Gastroenterology **64**, 324–337 (1973)

Rothschild, M.A., Oratz, M., Schreiber, S.S.: Regulation of albumin metabolism. Annu. Rev. Med. **26**, 91–104 (1975)

Rothschild, M.A., Oratz, M., Wimer, E., Schreiber, S.S.: Studies on albumin synthesis: The effects of dextran and cortisone on albumin metabolism in rabbits studied with albumin-I^{131}. J. Clin. Invest. **40**, 545–554 (1961)

Roy, H., Patterson, R., Jagendorf, A.T.: Identification of the small subunit of ribulose 1,5-bisphosphate carboxylase as a product of wheat leaf cytoplasmic ribosomes. Arch. Biochem. Biophys. **172**, 64–73 (1976)

Russell, J.H., Geller, D.M.: Rat serum albumin biosynthesis: evidence for a precursor. Biochem. Biophys. Res. Commun. **55**, 239–245 (1973)

Russell, J.H., Geller, D.M.: The structure of rat proalbumin. J. Biol. Chem. **250**, 3409–3413 (1975)

Rutner, A.C., Lane, M.D.: Nonidentical subunits of ribulose diphosphate carboxylase. Biochem. Biophys. Res. Commun. **28**, 531–537 (1967)

Samarina, O.P., Zbarskii, I.B., Perevoshchikova, K.A.: The binding of labeled amino acids by protein and nucleic acid preparations. Biokhimiia **25**, 443–451 (1960)

Sandor, G.: Serum Proteins in Health and Disease. London: Chapman and Hall Ltd. 1966

Sandor, G., Sureau, B., Martin, L., Berrod, J., Martin, R.: Hépatite virale et l'origine de l'albumine sérique. C.R. Acad. Sci. (D) (Paris) **265**, 1560–1563 (1967)

Sargent, J.R., Campbell, P.N.: The sequential synthesis of the polypeptide chain of serum albumin by the microsome fraction of rat liver. Biochem. J. **96**, 134–146 (1965)

Sarkar, N.K., Clarke, D.D., Waelsch, H.: An enzymatically catalyzed incorporation of amines into proteins. Biochim. Biophys. Acta **25**, 451–452 (1957)

Sarner, N.Z., Bissell, M.J., DiGirolamo, M., Gorini, L.: Mechanism of excretion of a bacterial proteinase: demonstration of two proteolytic enzymes produced by a *Sarcina* strain (Coccus P). J. Bacteriol. **105**, 1090–1098 (1971)

Schechter, I., Burstein, Y.: Partial sequence of the precursors of immunoglobulin light-chains of different subgroups: evidence that the immunoglobulin variable-region gene is larger than hitherto known. Biochem. Biophys. Res. Commun. **68**, 489–496 (1976a)

Schechter, I., Burstein, Y.: Marked hydrophobicity of the NH_2-terminal extra piece of immunoglobulin light-chain precursors: possible physiological functions of the extra piece. Proc. Natl. Acad. Sci. USA **73**, 3273–3277 (1976b)

Schechter, I., Burstein, Y.: Identification of N-terminal methionine in the precursor of immunoglobulin light chain. Intitiation of translation of messenger ribonucleic acid in plants and animals. Biochem. J. **153**, 543–550 (1976c)

Schreiber, G.: Studien zur Sekretion und Synthese von Serumalbumin in normaler, regenerierender und maligne entarteter Rattenleber. Habilitationsschrift, Freiburg, 1970

Schreiber, G.: Translation of genetic information on the ribosome. Angew. Chemie (Engl.) **10**, 638–651 (1971)

Schreiber, G.: Applications of radioactively labelled antigen-antibody complexes in biochemistry. In: Radioactive Tracers in Microbial Immunology. Kaufmann, B. (ed.), pp. 49–54. Vienna: International Atomic Energy Agency 1972

Schreiber, G., Boutwell, R.K., Potter, V.R., Morris, H.P.: Lack of secretion of serum protein by transplanted rat hepatomas. Cancer Res. **26**, 2357–2361 (1966)

Schreiber, G., Edwards, K., Schreiber, M.: Energy dependence of protein synthesis and secretion in cell suspensions from rat liver. Proc. Aust. Biochem. Soc. **10**, 34 (1977a)

Schreiber, G., Lesch, R., Weinssen, U., Zähringer, J.: The distribution of albumin synthesis throughout the liver lobule. J. Cell Biol. **47**, 285–289 (1970)

Schreiber, G., Rotermund, H.-M., Maeno, H., Weigand, K., Lesch, R.: The proportion of the incorporation of leucine into albumin to that into total protein in rat liver and hepatoma Morris 5123TC. Eur. J. Biochem. **10**, 355–361 (1969)

Schreiber, G., Schreiber, M.: The preparation of single cell suspensions from liver and their use for the study of protein synthesis (Review). Sub-Cell Biochem. **2**, 307–353 (1973)

Schreiber, G., Schreiber, M.: Protein synthesis and excretion in single cell suspensions from liver and Morris hepatoma 5123TC. In: Gene Expression and Carcinogenesis in Cultured Liver. Gerschenson, L.E., Thompson, E.G. (eds.), pp. 46–61. New York–San Francisco–London: Academic Press 1975

Schreiber, G., Urban, J., Dryburgh, H., Bradley, T.R.: The synthesis and secretion of serum albumin in Morris hepatomas 5123TC and 9121. In: Morris Hepatomas: Mechanisms of Regulation. Morris, H.P., Criss, W.E. (eds.). New York: Adv. in Exp. Med. Biol., Plenum Publishing Corp. 1977 b (in press)

Schreiber, G., Urban, J., Edwards, K.: Possible functions of the oligopeptide extension in the albumin precursor. J. Theor. Biol. **60**, 241–245 (1976a)

Schreiber, G., Urban, J., Edwards, K., Dryburgh, H., Inglis, A.S.: Mechanism and regulation of albumin synthesis in liver and hepatomas. Adv. Enzyme Regul. **14**, 163–184 (1976b)

Schreiber, G., Urban, J., Zähringer, J., Reutter, W., Frosch, U.: The secretion of serum protein and the synthesis of albumin and total protein in regenerating rat liver. J. Biol. Chem. **246**, 4531–4538 (1971)

Schreiber, M., Schreiber, G., Kartenbeck, J.: Protein and ribonucleic acid metabolism in single-cell suspensions from Morris hepatoma 5123TC and from normal rat liver. Cancer Res. **34**, 2143–2150 (1974)

Schultze, H.E., Heremans, J.F.: Nature and metabolism of extracellular proteins. In: Molecular Biology of Human Proteins, Vol. I. Amsterdam–London–New York: Elsevier 1966

Schwert, G.W.: Recovery of native bovine serum albumin after precipitation with trichloroacetic acid and solution in organic solvents. J. Am. Chem. Soc. **79**, 139–141 (1957)

Segrest, J.P., Jackson, R.L., Marchesi, V.T., Guyer, R.B., Terry, W.: Red cell membrane glycoprotein: amino acid sequence of an intramembranous region. Biochem. Biophys. Res. Commun. **49**, 964–969 (1972)

Segrest, J.P., Kahane, I., Jackson, R.L., Marchesi, V.T.: Major glycoprotein for the human erythrocyte membrane: evidence for an amphipathic molecular structure. Arch. Biochem. Biophys. **155**, 167–183 (1973)

Shafritz, D.A.: Protein synthesis with messenger ribonucleic acid fractions from membrane-bound and free liver polysomes. Translation characteristics of liver polysomal ribonucleic acids and evidence for albumin production in a messenger-dependent reticulocyte cell-free system. J. Biol. Chem. **249**, 81–88 (1974)

Shapiro, D.J., Taylor, J.M., McKnight, G.S., Palacios, R., Gonzalez, C., Kiely, M.L., Schimke, R.T.: Isolation of hen oviduct ovalbumin and rat liver albumin polysomes by indirect immunoprecipitation. J. Biol. Chem. **249**, 3665–3671 (1974)

Shore, G.C., Tata, J.R.: Two fractions of rough endoplasmic reticulum from rat liver. II. Cytoplasmic messenger RNA's which code for albumin and mitochondrial proteins are distributed differently between the two fractions. J. Cell Biol. **72**, 726–743 (1977)

Smallwood, R.A., Jones, E.A., Craigie, A., Raia, S., Rosenoer, V.M.: The delivery of newly synthesized albumin and fibrinogen to the plasma in dogs. Clin. Sci. **35**, 35–43 (1968)

Soffer, R.L.: The arginine transfer reaction. Biochim. Biophys. Acta **155**, 228–240 (1968)

Soffer, R.L.: Enzymatic modification of proteins. II. Purification and properties of the arginyl transfer ribonucleic acid-protein transferase from rabbit liver cytoplasm. J. Biol. Chem. **245**, 731–737 (1970)

Soffer, R.L.: Enzymatic modification of proteins. V. Protein acceptor specificity in the arginine-transfer reaction. J. Biol. Chem. **246**, 1602–1606 (1971)

Soffer, R.L.: Peptide acceptors in the leucine, phenylalanine transfer reaction. J. Biol. Chem. **248**, 8424–8428 (1973)

Soffer, R.L., Horinishi, H.: Enzymatic modifications of proteins. I. General characteristics of the arginine-transfer reaction in rabbit liver cytoplasm. J. Mol. Biol. **43**, 163–175 (1969)

Soffer, R.L., Horinishi, H., Leibowitz, M.J.: The aminoacyl tRNA-protein transferases. Cold Spring Harbor Symp. Quant. Biol. **34**, 529–533 (1969)

Soffer, R.L., Mendelsohn, N.: Incorporation of arginine by a soluble system from sheep thyroid. Biochem. Biophys. Res. Commun. **23**, 252–258 (1966)

Sonenshein, G.E, Brawerman, G.: Differential translation of rat liver albumin messenger RNA in a wheat germ cell-free system. Biochemistry **16**, 5445–5448 (1977)

Spatz, L., Strittmatter, P.: A form of cytochrome b_5 that contains an additional hydrophobic sequence of 40 amino acid residues. Proc. Natl. Acad. Sci. USA **68**, 1042–1046 (1971)

Spector, A.A.: Fatty acid binding to plasma albumin (Review). J. Lipid Res. **16**, 165–179 (1975)

Steiner, D.F., Cunningham, D., Spigelman, L., Aten, B.: Insulin biosynthesis: evidence for a precursor. Science **157**, 697–700 (1967)

Steiner, D.F., Kemmler, W., Tager, H.S., Peterson, J.D.: Proteolytic processing in the biosynthesis of insulin and other proteins. Fed. Proc. **33**, 2105–2115 (1974)

Steiner, R.F., Roth, J., Robbins, J.: The binding of thyroxine by serum albumin as measured by fluorescence quenching. J. Biol. Chem. **241**, 560–567 (1966)

Sterling, K.: Molecular structure of thyroxine in relation to its binding by human serum albumin. J. Clin. Invest. **43**, 1721–1729 (1964)

Sterling, K., Rosen, P., Tabachnik, M.: Equilibrium dialysis studies of the binding of thyroxine by human serum albumins. J. Clin. Invest. **41**, 1021–1030 (1962)

Sterling, K., Tabachnick, M.: Determination of the binding constants for the interaction of thyroxine and its analogues with human serum albumin. J. Biol. Chem. **236**, 2241–2243 (1961)

Stewart, K.K., Doherty, R.F.: Resolution of DL-tryptophan by affinity chromatography on bovine-serum albumin-agarose columns. Proc. Natl. Acad. Sci. USA **70**, 2850–2852 (1973)

Strauss, A.W., Bennett, C.D., Donohue, A.M., Rodkey, J.A., Alberts, A.W.: Rat liver preproalbumin: Complete amino acid sequence of the pre-piece. J. Biol. Chem. **252**, 6846–6855 (1977)

Strauss, A.W., Donohue, A.M., Bennett, C.D., Rodkey, J.A., Alberts, A.W.: Rat liver preproalbumin: *in vitro* synthesis and partial amino acid sequence. Proc. Natl. Acad. Sci. USA **74**, 1358–1362 (1977b)

Suchanek, G., Kindas-Mügge, I., Kreil, G., Schreier, M.H.: Translation of honeybee promelittin messenger RNA. Formation of a larger product in a mammalian cell-free system. Eur. J. Biochem. **60**, 309–315 (1975)

Szentivanyi, A., Radovich, J., Talmage, D.W.: The separation of free, disulphide-bound and peptide-bound S^{35} radioactivity in serum and tissues. J. Infect. Dis. **109**, 231–237 (1961)

Tabachnick, M.: Thyroxine-protein interactions. I. Binding of thyroxine to human serum albumin and modified albumins. J. Biol. Chem. **239**, 1242–1249 (1964)

Tager, H.S., Steiner, D.F.: Isolation of a glucagon-containing peptide: primary structure of a possible fragment of proglucagon. Proc. Natl. Acad. Sci. USA **70**, 2321–2325 (1973)

Takagi, M., Ogata, K.: Direct evidence for albumin biosynthesis by membrane bound polysomes in rat liver. Biochem. Biophys. Res. Commun. **33**, 55–60 (1968)

Takagi, M., Ogata, K.: Isolation of serum albumin-synthesizing polysomes from rat liver. Biochem. Biophys. Res. Commun. **42**, 125–131 (1971)

Takagi, M., Tanaka, T., Ogata, K.: Evidence for exclusive biosynthesis *in vivo* of serum albumin by bound polysomes of rat liver. J. Biochem. (Tokyo) **65**, 651–653 (1969)

Takagi, M., Tanaka, T., Ogata, K.: Functional differences in protein synthesis between free and bound polysomes of rat liver. Biochim. Biophys. Acta **217**, 148–158 (1970)

Takanami, M.: Labelling of microsomal proteins with radioactive amino acids. Biochim. Biophys. Acta **37**, 556–557 (1960)

Takeda, Y., Reeve, E.B.: Studies of the metabolism and distribution of albumin with autologous I^{131}-albumin in healthy men. J. Lab. Clin. Med. **61**, 183–202 (1963)

Tanaka, Y., Kaji, H.: Incorporation of arginine by soluble extracts of ascites tumor cells and regenerating rat liver. Cancer Res. **34**, 2204–2208 (1974)

Tanaka, S., Scheraga, H.A.: Statistical mechanical treatment of protein conformation. 4. A four-state model for specific-sequence copolymers of amino acids. Macromolecules **9**, 812–833 (1976)

Tarver, H., Reinhardt, W.O.: Methionine labeled with radioactive sulfur as an indicator of protein formation in the hepatectomized dog. J. Biol. Chem. **167**, 395–400 (1947)

Tashjian, A.H., Jr., Bancroft, F.C., Richardson, U.I., Goldlust, M.B., Rommel, F.A., Ofner, P.: Multiple differentiated functions in an unusual clonal strain of hepatoma cells. In Vitro **6**, 32–45 (1970)

Tavill, A.S., Nadkarni, D., Metcalfe, J., Black, E., Hoffenberg, R., Carson, E.R.: Hepatic albumin and urea synthesis. The mathematical modelling of the dynamics of [^{14}C] carbonate-derived guanidine-labelled arginine in the isolated perfused rat liver. Biochem. J. **150**, 495–509 (1975)

Taylor, J.M., Schimke, R.T.: Synthesis of rat liver albumin in a rabbit reticulocyte cell-free protein-synthesizing system. J. Biol. Chem. **248**, 7661–7668 (1973)

Taylor, J.M., Tse, T.P.H.: Isolation of rat liver albumin messenger RNA. J. Biol. Chem. **251**, 7461–7467 (1976)

Thibodeau, S.N., Gagnon, J., Palmiter, R.: Precursor forms of lysozyme and ovomucoid: sequence analysis. Fed. Proc. **36**, 656 (1977)

Tracht, M.E., Tallal, L., Tracht, D.G.: Intrinsic hepatic control of plasma albumin concentration. Life Sci. **6**, 2621–2628 (1967)

Tritsch, G.L., Rathke, C.E., Tritsch, N.E., Weiss, C.M.: Thyroxine binding by human serum albumin. J. Biol. Chem. **236**, 3163–3167 (1961)

Tse, T.P.H., Taylor, J.M.: Translation of albumin messenger RNA in a cell-free protein-synthesizing system derived from wheat germ. J. Biol. Chem. **252**, 1272–1278 (1977)

Urban, J., Chelladurai, M., Millership, A., Schreiber, G.: The kinetics *in vivo* of the synthesis of albumin-like protein and albumin in rats. Eur. J. Biochem. **67**, 477–485 (1976)

Urban, J., Inglis, A.S., Edwards, K., Schreiber, G.: Chemical evidence for the difference between albumins from microsomes and serum and a possible precursor-product relationship. Biochem. Biophys. Res. Commun. **61**, 494–501 (1974a)

Urban, J., Kartenbeck, J., Zimber, P., Timko, J., Lesch, R., Schreiber, G.: Increase of extravascular albumin pool and the intracellular accumulation of vesicles in transplanted Morris hepatoma 9121. Cancer Res. **32**, 1971–1977 (1972)

Urban, J., Schreiber, G.: Biological evidence for a precursor protein of serum albumin. Biochem. Biophys. Res. Commun. **64**, 778–782 (1975)

Urban, J., Zimber, P., Schreiber, G.: Immunoprecipitation is inappropriate for the isolation of radiochemically pure albumin from tissues. Anal. Biochem. **58**, 102–116 (1974b)

Verkleij, A.J., Zwaal, R.F.A., Roelofsen, B., Comfurius, P., Kastelijn, D., van Deenen, L.L.M.: The asymmetric distribution of phospholipids in the human red cell membrane. A combined study using phospholipases and freeze-etch electron microscopy. Biochim. Biophys. Acta **323**, 178–193 (1973)

Volkin, E., Cohn, W.E.: Estimation of nucleic acids. In: Methods of Biochemical Analysis. Glick, D. (ed.), Vol. I, pp. 287–305. New York: Interscience 1954

Wachsmuth, E.D., Jost, J.-P.: Localization of vitellogenin and serum albumin in hepatic parenchymal cells of normal and estradiol-treated immature chickens. Biochim. Biophys. Acta **437**, 454–461 (1976)

Wasserman, K., Joseph, J.D., Mayerson, H.S.: Kinetics of vascular and extravascular protein exchange in unbled and bled dogs. Am. J. Physiol. **184**, 175–182 (1956)

Waterlow, J.C.: The assessment of protein nutrition and metabolism in the whole animal, with special reference to man. In: Mammalian Protein Metabolism. Munro, H.N. (ed.), Vol. III, pp. 325–390. New York–London: Academic Press 1969

Weigand, K.: Die Regulation des Serumalbuminspiegels unter physiologischen und pathologischen Bedingungen. Klin. Wschr. **55**, 295–305 (1977a)

Weigand, K.: Serumprotein-Synthese in isolierten Leberzellen. Fortschr. Med. **95**, 1272–1276 (1977b)

Weigand, K., Müller, M., Urban, J., Schreiber, G.: Intact endoplasmic reticulum and albumin synthesis in rat liver cell suspensions. Exp. Cell Res. **67**, 27–32 (1971)

Weigand, K., Otto, I.: Secretion of serum albumin by enzymatically isolated rat liver cells. FEBS Lett. **46**, 127–129 (1974)

Williams, C.A., Ganoza, M.C., Lipmann, F.: Effect of bacterial infection on the synthesis of serum proteins by a mouse liver cell-free system. Proc. Natl. Acad. Sci. USA **53**, 622–626 (1965)

Wise, R.W., Ballard, F.J., Ezekiel, E.: Developmental changes in the plasma protein pattern of the rat. Comp. Biochem. Physiol. **9**, 23–30 (1963)

Wise, R.W., Oliver, I.T.: Sites of synthesis of plasma proteins in the foetal rat. Biochem. J. **100**, 330–333 (1966)

Yanagida, M.: Isolation by affinity chromatography of a precursor head protein (P23) of bacteriophage T4. J. Mol. Biol. **87**, 317–327 (1974)

Yu, S., Redman, C.: In vitro synthesis of rat pre-proalbumin. Biochem. Biophys. Res. Commun. **76**, 469–476 (1977)

Zähringer, J., Baliga, B.S., Drake, R.L., Munro, H.N.: Distribution of ferritin mRNA and albumin mRNA between free and membrane-bound rat liver polysomes. Biochim. Biophys. Acta **474**, 234–244 (1977)

Zähringer, J., Baliga, B.S., Munro, H.N.: Increased levels of microsomal albumin-mRNA in the liver of nephrotic rats. FEBS Lett. **62**, 322–325 (1976)

Zilversmit, D.B., Entenman, C., Fishler, M.C.: On the calculation of "turnover time" and "turnover rate" from experiments involving the use of labeling agents. J. Gen. Physiol. **26**, 325–331 (1943)

Zimmon, D.S., Oratz, M., Kessler, R., Schreiber, S.S., Rothschild, M.A.: Albumin to ascites: demonstration of a direct pathway bypassing the systemic circulation. J. Clin. Invest. **48**, 2074–2078 (1969)

Addendum

Ratio of Albumin Synthesis to Total Protein Synthesis

Feldhoff et al. (1977) reported that albumin synthesis accounted for 11%–13% of total protein synthesis in normal rat liver in vivo, in perfused liver, and in isolated hepatocytes, confirming previous results by other authors.

Isolation and Properties of Albumin mRNA, Preparation of cDNA for Preproalbumin and Other Precursor Proteins

Several groups purified and studied the mRNA for albumin and transcribed it in vitro into cDNA using reverse transcriptase. *Keller* and *Taylor* (1977) described the substrate and template requirement of the transcription of albumin mRNA. *Strair* et al. (1977) characterized the physical properties of albumin mRNA. Its sedimentation coefficient was found to be 17 *S*; its size corresponded to 5.9×10^5 daltons. It represented 5%–8% of total cytoplasmic polyadenylated RNA. *Yap* and colleagues (1977) prepared albumin [^{3}H] cDNA and used it to determine the distribution of albumin mRNA sequences between membrane-bound and free polyribosomes in liver by molecular hybridization. Of the albumin mRNA sequences in polyribosomes 98% was found in the membrane-bound fraction. *Yang* and *Niu* (1977) isolated total mRNA from the livers of calves, rats, and chickens and injected it into the uterine lumen of immature mice, where it was found not only to program the synthesis of the alien albumins in the uterine epithelial cells, but also to induce the synthesis of mouse albumin.

DNA complementary in sequence to the mRNA for preproparathyroid hormone was synthesized with reverse transcriptase and then translated in a linked transcription-translation system using RNA polymerase and cell-free extract from wheat germ (*Kronenberg* et al., 1977). The product was identified as preproparathyroid hormone by sequencing of its N-terminus and by electrophoretic and immunologic criteria. Complementary DNA was also prepared from mRNA extracted from Langerhans islets or rat pancreas (*Ullrich* et al., 1977) and from a transplantable rat insulinoma (*Efstratiadis* et al., 1977). It was then cloned into bacterial plasmids (*Ullrich* et al., 1977). The four plasmids obtained contained sequences coding for preproinsulin.

Further Reports on Preproteins

The mechanism of the synthesis and secretion of melittin was summarized by *Kreil* and co-workers (1977). The amino acid sequence reported previously for the presegment in precursor lysozyme (Table 7) was confirmed

by *Palmiter* et al. (1977). A putative precursor amylase (approximately 1500 daltons larger than authentic amylase) was obtained when RNA prepared from dog pancreas polysomes or microsomes was translated in cell-free systems from reticulocytes or wheat germ (*MacDonald* et al., 1977). Of the mRNA for amylase 99% was found to be associated with polysomes bound to the endoplasmic reticulum. Precursor proteins containing 25–35 additional N-terminal amino acids were also described for the large and small basic proteins in mouse myelin (*Barbarese* et al., 1977). Preproinsulin from bovine fetus was found to have structural features similar to those of rat preproinsulin (*Lomedico* and co-workers, 1977). It had 23 additional N-terminal amino acid residues preceding the B-chain section of proinsulin and the positions of six of the seven leucine residues found in the presegment of the bovine protein were identical to those in rat preproinsulin.

In contrast to the data for light chains of immunoglobulins the findings of *Bedard* and *Huang* (1977) for the plasmacytoma MOPC-315 seemed to suggest that no precursor proteins might exist for the heavy chains of immunoglobulins. However, other authors detected a precursor protein also for the heavy chain of MOPC-315 mouse immunoglobulin (*Jilka* and *Pestka,* 1977). It possessed the N-terminal extension Met-Lys-Val-Leu-Ser-Leu-Leu-Tyr-Leu-Leu-Thr-Ala-Ile-Pro-His-Ile-Met-Ser.

A precursor segment consisting of Met-X-Thr-Asp-Thr-Leu-Leu-Leu-Trp-Val-Leu-Leu-Leu-Trp-Val-Pro-X- was observed in the cell-free translation of the mRNA coding for the κ-type constant region of immunoglobulin light chains from mouse myeloma MPC-11 (*Burstein* et al., 1977). This sequence does not occur in the variable region of the MPC-11 light chain. The sequence of the presegment to the V region in the MPC-11 light chain precursor is not yet known. However, the sequence of the Cκ presegment listed above shows at least 70% homology with the presegment to the V region of the MOPC-321 light chain precursor. The observation of *Burstein* et al. (1977) can be interpreted in various ways. Two different genes are believed to code for the constant and the variable region of immunoglobulin chains. If only one mRNA chain is formed, as is generally assumed, the DNA region carrying the information for the presegment, which is found at the N-terminus of the constant region, might have been transposed from the end of the DNA segment coding for the variable region to the DNA segment coding for the constant region. However, *Burstein* and colleagues also discuss a "three gene-one polypeptide chain" hypothesis with the DNA region coding for the presegment constituting or being part of a third gene, the "xp gene". This might possibly be involved in regulation of transcription/translation. The discovery of large insertions in the DNA of eucaryotic genes (see for example *Breathnach* et al., 1977), for which no corresponding amino acid sequences are found in the final protein product, adds further possibilities of interpretation.

Precursor forms with N-terminal extensions of the polypeptide chain with clusters of hydrophobic amino acid residues were also reported for further membrane-related proteins, namely two proteins of the outer membrane of *E. coli* (*Halegoua* et al., 1977; *Sekizawa* et al., 1977) and cytochrome P-450 from rabbit liver microsomes (*Haugen* et al., 1977).

Occurrence of Preproteins in Vivo and Processing of the Presegment

The question of whether preproteins actually occur as short-living intermediates in vivo or whether they are artifacts that can be observed only in the cell-free translation of mRNA in systems with low or no proteinase activity is not solved yet. The reaction mechanism of the proteolytic removal of the presegment also remains to be worked out in detail. *Lingappa* et al. (1977) translated RNA from bovine pituitary in a cell-free system from wheat germ with and without added microsomal membranes from canine pancreas or bovine pituitary. When the membranes were present *after* translation, pregrowth hormone and preprolactin was obtained, whereas products of the same size as authentic growth hormone and prolactin were formed when the membranes were present *during* translation. *Lingappa* et al. concluded that the presegments are processed on the nascent polypeptide chains. In contrast to this conclusion *Habener* et al. (1976) had detected preproparathyroid hormone in intact parathyroid cells. The in vivo occurrence of a precursor protein to proinsulin was reported for intact cells in catfish islets (*Albert* et al., 1977) and in isolated rat pancreas islets (*Permutt* and *Routman,* 1977). Poly(A)-containing mRNA from angler fish and sea raven directed the synthesis of preproinsulin in the cell-free system from wheat germ (*Shields* and *Blobel,* 1977). Addition of microsomal membranes from dog pancreas led to the cleavage of the nascent preproinsulin in the correct place, resulting in the synthesis of authentic fish insulins, which were identified by partial sequence analysis. It seems that the mechanism for the intra- and posttranslational processing of secretory proteins is highly conserved during evolution. However, posttranslational proteolytic processing of virus-specific precursor polypeptides which were produced after microinjection of avian myeloblastosis virus took place at a much slower rate in *Xenopus laevis* oocytes than in chick embryo fibroblasts (*Ghysdael* et al., 1977).

A protease removing the presegment from preprolactin or pregrowth hormone was extracted from rough microsomes from dog pancreas (*Jackson* and *Blobel,* 1977). The microsomal extract was active when added during or after translation, provided it had been treated with the detergent sodium deoxycholate. Intact protein synthesis was found to be unnecessary for the proteolytic processing of the precursor for the small subunit of ribulose biphosphate carboxylase in chloroplasts (*Highfield* and *Ellis,*

1978). *Highfield* and *Ellis* proposed an "envelope carrier mechanism" for the transport of the small subunit of the enzyme into the chloroplast.

N-terminal presegments of 18 or 19 residues have also been found in the cell-free produced precursors of the λ-type light chains from the mouse myelomas MOPC-104 E λ_1, RPC-20 λ_1, and MOPC-315 λ_2 (*Burstein* and *Schechter,* 1977). The mature forms of those light chains are blocked at the N-termini by pyrrolid-2-one-5-carboxylic acid. The pyroglutamic acid is formed by cyclization of glutamine after, or concomitantly with, the proteolytic removal of the presegment, yielding the mature *L*-chains.

Anchoring of immunoglobulins in the cell membrane of immature plasma cells had previously been discussed as a possible function for the presegment in the precursor proteins for immunoglobulins (see reference *Schechter* and *Burstein,* 1976b, of main section). However, N-terminal sequence analysis of immunoglobulin light chains isolated from purified plasma membranes of mouse myeloma cells showed that mature *L*-chains rather than *L*-chain precursors were found in the surface membrane of plasmacytoma cells (*Wolf* et al., 1977).

Structure of Proalbumin of Nonmammalian Origin

Proalbumin was isolated from chicken microsomes. Its prosegment was reported to have the structure Arg-Asn-Leu-Gln-Arg-Met-Ala-Arg (*Rosen* and *Geller,* 1977).

Processing of the Prosegment

An extract was prepared from parathyroid gland which preferentially cleaved the Arg-Ala bond joining the prosegment to parathyroid hormone without producing additional cleavages in parathyroid hormone itself (*Habener* et al., 1977). The cleavage was not inhibited by the trypsin inhibitor tosyl-*L*-lysine chloromethyl ketone (TLCK). No fragments of the removed hexapeptide could be detected in the reaction mixture, suggesting rapid further degradation of the hexapeptide in the parathyroid gland. The in vitro convension of proalbumin into albumin was reported to occur during Ca^{2+}-dependent fusion of Golgi vesicles (*Judah* and *Quinn,* 1978). It was inhibited by 50% by 10 μM TLCK.

Note added in proof: Recently, one of the albumin species in a bisalbuminemic family was found to have the N-terminal oligopeptide extension Arg-Gly-Val-Phe-Arg-Gln and was termed *proalbumin Christchurch* (*S. Brennan,* personal communication).

References to the Addendum

Albert, S., Chyn, R., Goldford, M., Permutt, A.: Insulin biosynthesis: evidence for the existence of a precursor to proinsulin in cells. Diabetes **26** (Suppl. 1), 378 (1977)

Barbarese, E., Braun, P.E., Carson, J.H.: Identification of prelarge and presmall basic proteins in mouse myelin and their structural relationship to large and small basic proteins. Proc. Natl. Acad. Sci. USA **74**, 3360–3364 (1977)

Bedard, D.L,, Huang, R.C.C.: Initiation and translation in vitro of mRNA for MOPC 315 immunoglobulin heavy chain and characterization of translation product. J. Biol. Chem. **252**, 2592–2598 (1977)

Breathnach, R., Mandel, J.L., Chambon, P.: Ovalbumin gene is split in chicken DNA. Nature (Lond.) **270**, 314–319 (1977)

Burstein, Y., Schechter, I.: Glutamine as a precursor to N-terminal pyrrolid-2-one-5-carboxylic acid in mouse immunoglobulin λ-type light-chains. Biochem. J. **165**, 347–354 (1977)

Burstein, Y., Zemell, R., Kantor, F., Schechter, I.: Independent expression of the gene coding for the constant domain of immunoglobulin light chain: evidence from sequence analyses of the precursor of the constant region polypeptide. Proc. Natl. Acad. Sci. USA **74**, 3157–3161 (1977)

Efstratiadis, A.; Gilbert, W., Fuller, F., Chick, W.: *In vitro* synthesis of the preproinsulin structural gene. Diabetes **26** (Suppl. 1), 378 (1977)

Feldhoff, R.C., Taylor, J.M., Jefferson, L.S.: Synthesis and secretion of rat albumin *in vivo*, in perfused liver, and in isolated hepatocytes. J. Biol. Chem. **252**, 3611–3616 (1977)

Ghysdael, J., Hubert, E., Trávníček, M., Bolognesi, D.P., Burny, A., Cleuter, Y., Huez, G., Kettmann, R., Marbaix, G., Portetelle, D., Chantrenne, H.: Frog oocytes synthesize and completely process the precursor polypeptide to virion structural proteins after microinjection of avian myeloblastosis virus RNA. Proc. Natl. Acad. Sci. USA **74**, 3230–3234 (1977)

Habener, J.F., Chang, H.T., Potts, J.T., Jr.: Enzymic processing of proparathyroid hormone by cell-free extracts of parathyroid glands. Biochemistry **16**, 3910–3917 (1977)

Habener, J.F., Potts, J.T., Jr., Rich, A.: Pre-proparathyroid hormone. Evidence for an early biosynthetic precursor of proparathyroid hormone. J. Biol. Chem. **251**, 3893–3899 (1976)

Halegoua, S., Sekizawa, J., Inouye, M.: A new form of structural lipoprotein of outer membrane of *Escherichia coli*. J. Biol. Chem. **252**, 2324–2330 (1977)

Haugen, D.A., Armes, L.G., Yasunobu, K.T., Coon, M.J.: Amino-terminal sequence of phenobarbital-inducible cytochrome P-450 from rabbit liver microsomes: similarity to hydrophobic amino-terminal segments of preproteins. Biochem. Biophys. Res. Commun. 77, 967–973 (1977)

Highfield, P.E., Ellis, R.J.: Synthesis and transport of the small subunit of chloroplast ribulose biphosphate carboxylase. Nature (Lond.) **271**, 420–424 (1978)

Jackson, R.C., Blobel, G.: Post-translational cleavage of presecretory proteins with an extract of rough microsomes from dog pancreas containing signal peptidase activity. Proc. Natl. Acad. Sci. USA **74**, 5598–5602 (1977)

Jilka, R.L., Pestka, S.: Amino acid sequence of the precursor region of MOPC-315 mouse immunoglobulin heavy chain. Proc. Natl. Acad. Sci. USA **74**, 5692–5696 (1977)

Judah, J.D., Quinn, P.S.: Calcium ion-dependent vesicle fusion in the conversion of proalbumin to albumin. Nature (Lond.) **271**, 384–385 (1978)

Keller, G.H., Taylor, J.M.: Synthesis of a complementary DNA to rat liver albumin mRNA. Biochem. Biophys. Res. Commun. 77, 328–334 (1977)

Kreil, G., Suchanek, G., Kindås-Mügge, I.: Biosynthesis of a secretory peptide in honeybee venom glands: intermediates detected in vivo and in vitro. Fed. Proc. **36**, 2081–2086 (1977)

Kronenberg, H.M., Roberts, B.E., Habener, J.F., Potts, J.T., Jr., Rich, A.: DNA complementary to parathyroid mRNA directs synthesis of pre-proparathyroid hormone in a linked transcription-translation system. Nature (Lond.) **267**, 804–807 (1977)

Lingappa, V.R., Devillers-Thiery, A., Blobel, G.: Nascent prehormones are intermediates in the biosynthesis of authentic bovine pituitary growth hormone and prolactin. Proc. Natl. Acad. Sci. USA **74**, 2432–2436 (1977)

Lomedico, P.T., Chan, S.J., Steiner, D.F., Saunders, G.F.: Immunological and chemical characterization of bovine preproinsulin. J. Biol. Chem. **252**, 7971–7977 (1977)

Mac Donald, R.J., Przybyla, A.E., Rutter, W.J.: Isolation and *in vitro* translation of the messenger RNA coding for pancreatic amylase. J. Biol. Chem. **252**, 5522–5528 (1977)

Palmiter, R.D., Gagnon, J., Ericsson, L.H., Walsh, K.A.: Precursor of egg white lysozyme. Amino acid sequence of an NH_2-terminal extension. J. Biol. Chem. **252**, 6386–6393 (1977)

Permutt, M.A., Routman, A.: Proinsulin precursors in isolated rat pancreatic islets. Biochem. Biophys. Res. Commun. **78**, 855–862 (1977)

Rosen, A.M., Geller, D.M.: Chicken microsomal albumin: amino terminal sequence of chicken proalbumin. Biochem. Biophys. Res. Commun. **78**, 1060–1066 (1977)

Sekizawa, J., Inouye, S., Halegoua, S., Inouye, M.: Precursors of major outer membrane proteins of *Escherichia coli*. Biochem. Biophys. Res. Commun. **77**, 1126–1133 (1977)

Shields, D., Blobel, G.: Cell-free synthesis of fish preproinsulin, and processing by heterologous mammalian microsomal membranes, Proc. Natl. Acad. Sci. USA **74**, 2059–2063 (1977)

Strair, R.K., Yap, S.H., Shafritz, D.A.: Use of molecular hybridization to purify and analyze albumin messenger RNA from rat liver. Proc. Natl. Acad. Sci. USA **74**, 4346–4350 (1977)

Ullrich, A., Shine, J., Chirgwin, J., Pictet, R., Tischer, E., Rutter, W.J., Goodman, H.M.: Rat insulin genes: construction of plasmids containing the coding sequences. Science **196**, 1313–1319 (1977)

Wolf, O., Zemell, R., Burstein, Y., Schechter, I.: Partial sequence of immunoglobulin light-chain isolated from purified plasma membranes of mouse myeloma cells. Biochem. Biophys. Res. Commun. **78**, 1383–1389 (1977)

Yang, S.-F., Niu, M.C.: Albumin synthesis in mouse uterus in response to liver mRNA. Proc. Natl. Acad. Sci. USA **74**, 1894–1898 (1977)

Yap, S.H., Strair, R.K., Shafritz, D.A.: Distribution of rat liver albumin mRNA membrane-bound and free in polyribosomes as determined by molecular hybridization. Proc. Natl. Acad. Sci. USA **74**, 5397–5401 (1977)

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Gating Currents and Charge Movements in Excitable Membranes

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I. Introduction

Many cells use the cell membrane potential to regulate their physiologic functions. Among the better known examples are: (1) regulation of sodium and potassium permeabilities in nerve and muscle membranes, (2) regulation of transmitter release by the presynaptic terminal and (3) of intracellular Ca^{2+} concentration and contraction in muscle, and, possibly, (4) the regulation of adrenalin secretion by adrenal medulla cells and (5) of insulin secretion by pancreatic cells. As more and more different cell types yield to electrophysiologic analysis, we will no doubt find other instances of regulation by the cell membrane potential. The sequence of events between potential change and physiologic response may be complicated, but one expects that it will always begin with the movement, reorientation, or structural change in the cell membrane of a *voltage sensor,* a molecule which can respond to the cell membrane potential because it or parts of it have a large dipole moment. While moving or twisting inside the membrane under the influence of a change in membrane potential, such a voltage sensor produces an electric current, a *displacement current,* which can be recorded under favorable conditions. Displacement currents probably caused by movement of voltage sensors have been recorded in voltage-clamp experiments on frog skeletal muscle, various nerve fibers (squid axons, frog myelinated nerve, and giant axons from the earthworm *Myxicola*) and some snail nerve cell bodies. The displacement currents in muscle are thought to result from the voltage-sensor for excitation-contraction coupling. Those observed in the various nerve fibers probably represent the structural rearrangements accompanying membrane permeability changes to sodium and are often called "gating currents"; those in snail neurons may be associated with the regulation of the cell membrane permeability to calcium. All of these observations are thought to be related in some form or another to the opening and closing (gating) of *ionic channels,* small ion-permeable holes in the membrane. Among the most thoroughly studied ionic channels are the *sodium and potassium*

channels which enable excitable cells, such as nerve and muscle cells, to propagate impulses. The gating of these channels has been discussed in several excellent recent reviews (*Armstrong,* 1975a,b; *Goldman,* 1976; *Hille,* 1976; *Lüttgau* and *Glitsch,* 1976; *Ulbricht,* 1977; *Meves,* 1977; *Neumcke* et al., 1977) and all these reviews include a discussion of gating currents and related events in nerve and muscle. This article attempts a more general discussion of these currents. It will list and discuss presently available experimental findings on excitable cells, but also review some possibly important findings on artificial systems. It is assumed that the reader is familiar with the mechanism of nerve excitation, as well as some of the newer literature on ionic channels reviewed in the above articles.

A. Asymmetric Displacement Currents in Excitable Membranes: The Basic Finding

Hodgkin und *Huxley* (1952c) pointed out that the steep membrane potential dependence of sodium- and potassium-permeabilities in nerve indicates the presence in the membrane of polar molecules bearing large charges or dipole moments. The idea of a voltage sensor which produces current when it responds to an electric field, is therefore not new. Also recognized more than 10 years ago (*Taylor,* 1965) was the fact that artificial lipid bilayers behave as perfect, and nerve membranes as lossy, capacitors. Nerve membranes must therefore contain polar molecules absent in artificial bilayers made from pure lipid. At that time, *Chandler* and *Meves* (1965) made a first but unsuccessful attempt to record "carrier currents", as they called them, or "gating currents", as we call them today, that is to say, for currents arising in the voltage sensor for the sodium channel.

Charge movements in skeletal muscle. The first observation of currents possibly arising in a voltage sensor is unrelated to sodium channels and was made on frog skeletal muscle by *Schneider* and *Chandler* (1973). They worked in an external medium designed to minimize muscle contraction, as well as the large ionic currents normally possible across the cell membrane of a muscle fiber. When such a fiber is voltage clamped and the membrane potential stepped from near the resting value to some depolarized level (*test pulse,* pulse A in Fig. 1a), one records an outward surge of capacitive current followed by a small, steady ionic leakage and finally a capacitive inward surge on return to the resting potential (Fig. 1b). At first sight, such a record looks uninteresting, since the conductance changes normally resulting in an action potential are prevented here. The signal of interest is contained in the slow portion of the capacitive transient. In order to make it more visible, one corrects for capacitive and leakage

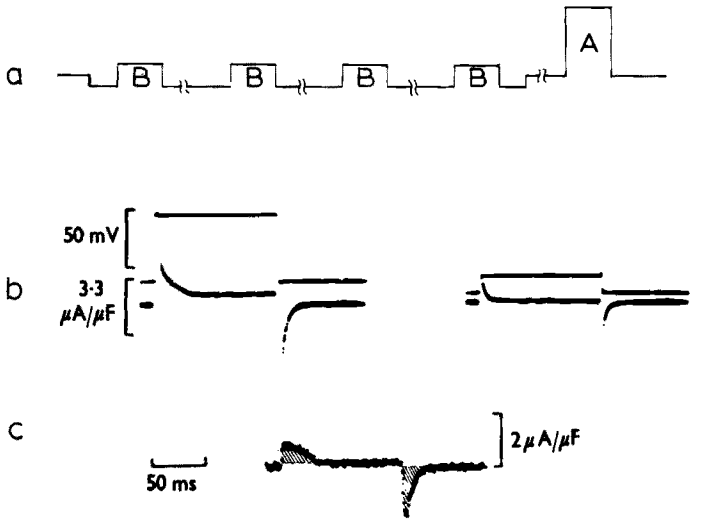

Fig. 1. (a) Pulse protocol for recording charge movements. Control pulses (B) and test pulse (A) have the same duration. (b) Voltage (*top*) and current (*bottom*) during test (*left*) and control pulse (*right*). The solution was made 2.5 times hypertonic in order to inhibit contraction (*Gordon* et al., 1973); it contained 90 mM $(TEA)_2^+SO_4$ and 5 mM Rb_2SO_4 to block K^+ channels, as well as 0.4 μM tetrodotoxin to block sodium channels. (c) Asymmetry current formed by subtracting from the current during the test pulse (A/4B) times the summed currents during four control pulses; A and B are amplitudes of test and control pulses. The sequence drawn in (a) was repeated four times, and (c) is the average of all four runs. The charges carried by the transient portions of the current (*shaded areas*) were 15.9 nC/μF and 16.5 nC/μF, equal within experimental error. Frog muscle, 4°C

admittances as they exist at potentials negative to the resting level. *Schneider* and *Chandler* (1973) did this by recording current during a *control pulse* of exactly the same amplitude and duration as the test pulse, except that it started from a potential so negative that the pulse did not cause membrane depolarization beyond the resting potential. If the membrane behaved linearly, i.e., if it could be represented by any combination of constant capacitors and resistors, then the currents during test and control pulse should be identical, and if one were subtracted from the other, most conveniently with a digital computer, then nothing should be left over. In the experiment of Figure 1, a slightly different procedure was used (*Adrian* and *Almers*, 1976b). Currents from four small control pulses were signal-averaged and stored by a computer. As in *Schneider* and *Chandler*'s experiments, control pulses spanned a potential range more negative than that experienced by the membrane in vivo. The computer then scaled the result by the factor A/4B where A and B are amplitudes of test and control pulse. For a linear membrane, the result of this operation should be identical to the current during the test pulse and, when subtracted from it, should cause perfect cancellation. Instead, there remains

in experiments of this kind a "difference" or *asymmetry current* consisting of an excess outward current during, and an excess inward transient after the pulse (Fig. 1c). Where present, the steady portion ot the excess outward current undoubtedly represents movement of ions across the membrane, and arises because the small residual ionic membrane conductance is not entirely ohmic. The transient portion, however, is now generally agreed to be capacitive, representing slow polarization of the membrane dielectric or some constituent thereof. This point has been discussed extensively, and the first argument raised in its favor (*Schneider* and *Chandler,* 1973) is still the best: Only if these currents are capacitive, i.e., displacement currents, is it easy to understand why the charge carried by the transient (dashed areas in Fig. 1b) is conserved. Whatever charge is carried outward during the pulse returns after the pulse. To the extent that the investigators are successful in correcting for, or much better still, in eliminating voltage-dependent ionic conductance changes, this conservation of charge is always observed, independent of the subtraction procedure used or of amplitude and duration of the test pulse (*Schneider* and *Chandler,* 1973; *Almers* et al., 1975; *Chandler* et al., 1976a; *Adrian* and *Almers,* 1976b). Furthermore, these currents are not the result of peculiarities of cell geometry nor of the complex intracellular membrane systems of a muscle fiber (*Chandler* et al., 1976a; *Adrian* and *Almers,* 1976a). They indicate therefore the presence in the cell membrane of a nonlinear, slowly polarizing (lossy) capacitive element. Clearly, the cell membrane contains polar molecules.

In Figure 2, the charge moved by transients (more precisely, the average charge from transients during and after the pulse) is plotted against the potential during the test pulse. Larger test pulses result in larger *"charge movement"*, but there is saturation at positive potentials. The sigmoidal shape of the curve immediately suggests the presence in the membrane of a finite number of polar molecules that undergo potential-dependent changes of state. At very negative potentials, they are all in one extreme state, at very positive potentials in another, and at intermediate potentials they are distributed between these two or intermediate states. While the curve tells little about the underlying molecular events, it indicates that the molecules concerned are sufficiently potential sensitive to be able to serve as physiologic voltage sensors. A normal action potential starting at − 90 mV and depolarizing the membrane to + 30 mV could, if it lasted long enough, transfer them all from one extreme state into the other. As discussed later, it is thought (*Schneider* and *Chandler,* 1973) that contractile activation derives from them its dependence on the cell membrane potential.

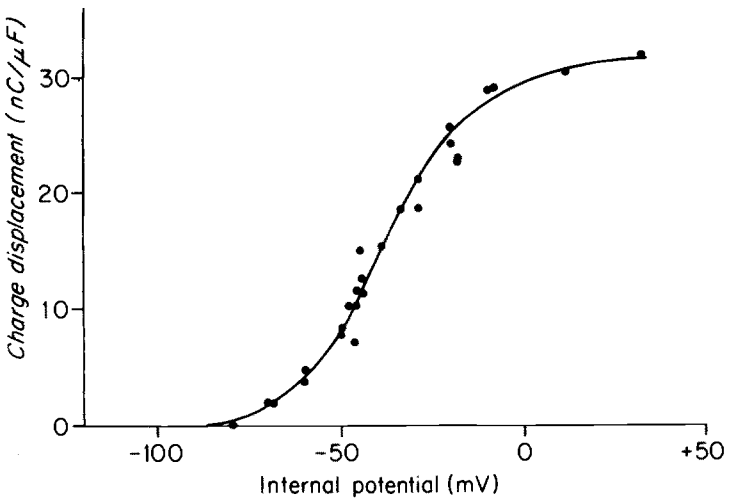

Fig. 2. *Ordinate:* average of *on-* and *off*-charge carried by the transient portion of asymmetry currents as in Figure 1c. *Abscissa:* potential during the test pulse. Curve drawn by eye. The small or zero charge movement near − 100 mV is to some extent an artifact introduced by the subtraction procedure (see *Adrian* and *Almers*, 1976b). Had one taken control pulses around − 150 mV, a test pulse from − 150 mV to 100 mV would have produced a charge displacement of 1–2 nC/μF in this fiber. Frog muscle, 4°C

"Gating currents" in squid axons. Almost simultaneously with *Schneider* and *Chandler* (1973), *Armstrong* and *Bezanilla* (1973) succeeded in recording asymmetry currents in squid giant axons (Fig. 3). As in skeletal muscle, it was necessary to minimize ion movement across the membrane, to use signal averaging for improvement of signal-to-noise ratio, and to correct membrane current during a test pulse for capacitive and leakage admittances at negative potentials. The correction is made either by adding the current during a control pulse of equal amplitude and duration but opposite polarity (*equal-and-opposite* procedure), or by a method similar to that of Figure 1a (*P/4 procedure,* see inset and legend of Fig. 4). The *asymmetric displacement current* transients recorded in this way (Fig. 1a, top) are qualitatively similar to those in muscle, except that they decline about 20 to 100 times faster. As in muscle, the charge carried by the current transients is conserved, although due to kinetic complications (see Sect. IV) this can be demonstrated easily only if pulse duration is short ($\leqslant$ 0.3 ms at 8°C) or the potential from which the test pulse starts very negative ($\leqslant$ − 150 mV). The physical nature of the asymmetry current transients has been discussed (*Armstrong* and *Bezanilla,* 1974) and, for much the same reasons as in muscle, is now generally believed to be capacitive.

Sodium currents through normal sodium channels are shown in the bottom traces of Figure 3 for comparison. They were recorded in the

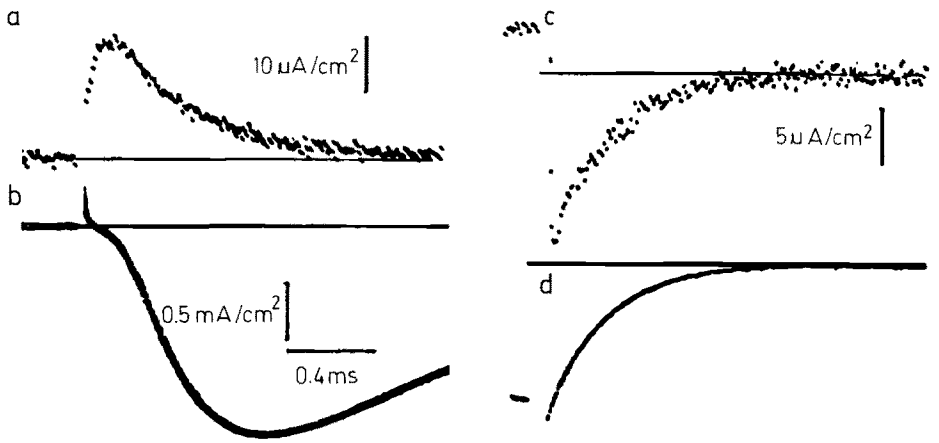

Fig. 3 a–d. *Top* (a, c): asymmetric displacement currents during (a) and after (c) a pulse from – 70 mV to 0 mV. Correction for capacitive and leakage admittance by the equal-and-opposite procedure. Tetrodotoxin was present to block sodium currents. *Bottom* (b, d) sodium currents during and after a similar depolarization. (a, b) from one axon, (c, d) from another. Cs^+ or tetraethylammonium$^+$ was present in the axoplasm to block delayed potassium currents. Squid axon, 2°C. From *Armstrong* and *Bezanilla* (1974)

same axons and at the same potentials as the top traces. It is seen that displacement currents have a time course which seems qualitatively consistent with the suggestion (*Armstrong* and *Bezanilla*, 1973) that they result from the molecular rearrangements attending the opening and closing of sodium channels. Evidence in favor of this view has accumulated (see Sect. IV) and it is now widely believed that the asymmetric displacement currents are what *Hodgkin* and *Huxley* (1952c) predicted should exist, and what *Chandler* and *Meves* (1965) could not find: gating currents for the sodium channel.

Armstrong and *Bezanilla*'s (1973) discovery was soon confirmed by *Keynes* and *Rojas* (1973, 1974) who used the same method [1]. *Keynes* and *Rojas* (1974) studied asymmetric displacement currents over a wide range of potentials, observed saturation of charge movement at positive potentials and gave a first and extensive quantitative description of these currents along similar lines as that provided by *Schneider* and *Chandler* (1973) for the events in muscle. Figure 4, taken from later work of *Bezanilla* and *Armstrong* (1976), shows the dependence of charge displacement on potential. Saturation is not as evident as in *Keynes* and *Rojas*' work (1974),

[1] The signals recorded by *Keynes* and *Rojas* (1974), as well as those recorded subsequently by all other authors, were 5–10 times larger than those published initially by *Armstrong* and *Bezanilla* (1973). In a later paper, *Armstrong* and *Bezanilla* (1974) suggest that signals were small in their first paper due to "inactivation" of "gating currents" during the rapid and repetitive depolarizations they used for signal averaging.

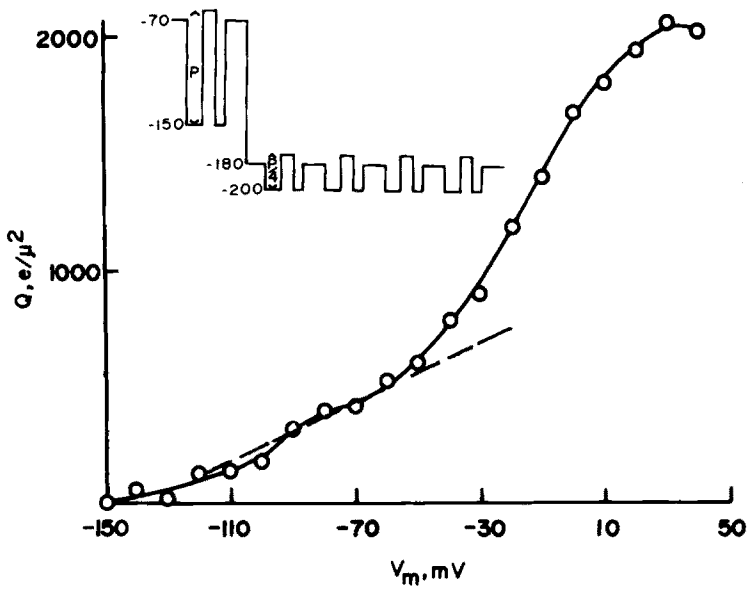

Fig. 4. Dependence of charge displacement on membrane potential during a test pulse, P, in units of electronic charges per membrane area. Correction for capacitive and leakage currents by the P/4 procedure (see *Inset;* the amplitude of the test pulse, P, and the control pulse, P/4, are indicated). The slope of the *dotted line* represents an estimate of the contribution of asymmetric displacement currents to the static membrane capacity at the resting potential. It is 0.1 $\mu F/cm^2$. Modified from *Bezanilla* and *Armstrong* (1975). Squid axon, 8°C

or earlier work by *Bezanilla* and *Armstrong* (1975), probably due to a different subtraction procedure and method of analysis. Although charge displacement depends much less steeply on potential than peak sodium conductance, most of it still falls in the potential range experienced by a healthy nerve fiber during an impulse.

What evidence can be used to link "gating currents" with sodium channels in nerve, or charge movements with the activation of contraction in skeletal muscle? How can we recognize portions related to these processes and separate them from other fractions of displacement currents unrelated to sodium channels and contractile activation? And, most interestingly, what can be learned about these processes by studying displacement currents? These questions will be discussed in later sections (IV and V). Before attempting to answer them,it may be useful to review our present knowledge about the dielectric properties of excitable membranes.

B. Membranes and Macromolecules as Dielectrics

As a first approximation, a cell membrane may be regarded as an insulator separating two conducting aqueous phases, in other words, as the dielectric in a parallel-plate condenser. Like any insulator, a membrane will become polarized when one applies an electric field across it. That is, the electric field produces a redistribution of charge in the membrane, so the surface of the negative side shows an excess of positive charge, and the positive side an excess of negative charge. This redistribution of charge, called polarization, is accompanied by an electric current, a displacement current, whose time integral is finite and equals the excess polarization charge appearing on the two surfaces of the dielectric. Polarization of matter most often occurs due to formation or orientation of *dipoles,* electrically neutral particles carrying some positive charge on one end and an equal amount of negative charge on the other. This dipole character of a particle is expressed as the *dipole moment* which equals $q \cdot d$ where q is the charge excess on each end of the molecule and d the distance between positive and negative end. Dipole moments are measured in debye units (D); one debye is the dipole moment of a rod 0.21 Å long with one electronic charge at either end. Whenever matter is electrically polarized, such dipoles show on average some alignment with the electric field.

Polarization of dielectrics occurs in many different ways. In the case of *electronic polarization,* the electric field produces a slight and reversible displacement of electrons relative to their atomic nuclei, thereby creating aligned dipoles where previously there were none. The dipole moments generated in this way depend, of course, on the field but in practice are never large. All substances show electronic polarization; those showing none other are called *nonpolar.* Examples for nonpolar substances are hydrogen gas and hydrocarbons. A second mechanism, *orientation polarization,* occurs in *polar dielectrics* containing molecules with a permanent dipole moment. These dipoles normally may be randomly oriented but align themselves reversibly in the presence of an electric field to an extent depending on field strength and thermal agitation. Some permanently dipolar molecules with their dipole moments are H_2O (1.85 D), amino acids (glycine and alanine, 12–15 D; *Edsall,* 1941) and protein. Other kinds of polarization can be observed on interfaces between electrolyte solutions and surfaces bearing charge. What concerns us most in this review is what we believe to be the polarization of macromolecules embedded in the membrane and its coupling to simultaneous and subsequent conformational changes.

Dependence of polarization on the electric field. Insofar as polarization involves the movement of matter, it does not occur instantly after a change

in electric field; therefore all dielectrics (except vacuum) show *loss*. A lossy capacitor does not charge instantly under a voltage step and allows current to pass even some time after the voltage has become steady, and if a sinusoidal current is passed through such a condenser, the voltage sinusoid will lag the current by a phase angle less than the ideal 90°. All lossy dielectrics have a nonlossy component, if only because even vacuum has a finite polarizability. Therefore, in the simplest case (Fig. 5) a lossy capacitor can be represented electrically by an ideal component, C_∞, in parallel with the series combination of a resistor and a capacitor of value $(C_0 - C_\infty)$. The resistor is an inextricable part of the dielectric and an expression of, for example, the friction encountered by dipoles when they form or reorient under a change in electric field. For static or slowly varying electric fields, such a condenser will appear perfect, and the charge, Q, stored on it will always be given by the formula for a condenser of constant capacity, the *static capacity* C_0 :

$$Q = C_0 V \tag{1}$$

where V is the applied voltage and C_0 is given by

$$C_0 = \frac{\epsilon \cdot \epsilon_0}{d} A \tag{2}$$

for a parallel-plate condenser. A and d are area and separation of the plates, ϵ the dielectric constant of the insulator, and $\epsilon_0 = 8.8 \times 10^{-14}$ F/cm, the polarizability (or better, the permittivity) of vacuum.

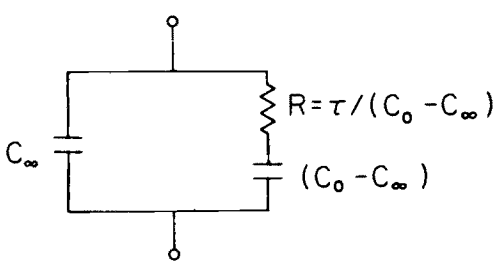

Fig. 5. Electrical representation of a condenser made from a perfectly insulating, but lossy, dielectric

Under a more rapidly changing voltage, such as a sinusoidal field of sufficiently high frequency, fewer and fewer dipoles will manage to form or reorient in time, and *dielectric dispersion* will occur. The apparent capacity of the condenser (more precisely, the apparent dielectric constant since the dimensions of the condenser remain unchanged) will diminish until it reaches the limiting value, C_∞, due to the nonlossy portion of the condenser.

For truly infinite frequencies, C_∞ is given by Eq. (2) with $\epsilon = 1$, i.e., C_∞ is determined by the dielectric constant of vacuum. In the simple dielectric of Figure 5, half of the frequency-dependent portion of the capacity will have disappeared at the characteristic frequency, ν. After a voltage step, the charging of that portion is 67% complete at the time τ where $\tau = 1/(2\pi\nu)$ and is called the dielectric relaxation time. In the frequency range where dispersion occurs, the dielectric absorbs energy from the alternating electric field; if the dispersion occurs at optical frequencies, as in many nonpolar dielectrics, the substance will absorb light at the corresponding wavelength. Outside the dispersion range, the dielectric constant equals the square of the index of refraction. The frequencies at which dispersion occurs depend on the dielectric and the mobility of dipoles in it. As one might expect, electronic polarization is fastest with characteristic dispersion frequencies in and beyond the optical range. Any polarization involving movement of molecularly sized particles is generally much slower; the characteristic dispersion frequency of water, one of the smallest and most mobile polar molecules, is about 10^{10} Hz. Many polar dielectrics have considerably lower characteristic frequencies, depending on the mobility of the dipole. One may learn about the molecules making up a dielectric by investigating the frequency dependence of capacity and dielectric constant under small, sinusoidally alternating electric fields. Such studies have long been an established technique in chemistry and biochemistry (*Takashima* and *Minakata,* 1975). In practice, dielectrics often show more than one dispersion. In that case, C_∞ often refers to the value measured at (finite) frequencies a few hundred times higher than those where the dispersion of interest occurs. In much of the remainder, the terms C_∞ and dielectric loss are used loosely and refer to measurements at frequencies below 1 MHz.

An interesting effect shown by polar dielectrics is that their dielectric constant depends on the static field. Again, we imagine the polarization to have two components, one $(C_0 - C_\infty)$, resulting from orientational, the other C_∞, from electronic polarization of our dielectric. The first will appear lossy compared to the second. In such a dielectric containing a finite number of molecular dipoles, thermal agitation keeps the average dipole orientation virtually (though not precisely) random under small electric fields. Doubling the field approximately doubles the small degree of dipole alignment and therefore doubles polarization as in a perfect dielectric [Eq. (1)]. In the presence of strong electric fields, however, a substantial fraction of all dipoles are aligned and a further increase in field will cause relatively less polarization. Due to this effect, *dielectric saturation,* the capacity will appear field- or voltage-dependent, being largest $(= C_0)$ under small or zero fields and diminishing at the extremes, in the limit to C_∞. The larger the moment of the dipole species, the steeper is

the relation between dielectric "constant" and applied field in polar dielectrics. Dielectric saturation can be observed only under extremely large electric fields. In electrolyte solutions, for example, the electric field directly next to an ion can reach hundreds of kilovolts per cm. As a consequence, the water molecules immediately surrounding an ion are all oriented with respect to the ion and therefore under dielectric saturation. One of the few systems where saturation can be demonstrated directly is in biologic membranes. Examples are shown later (Fig. 12).

II. The Membrane Capacity of Lipid Bilayers

A large percentage of the mass of biologic membrane consists of lipid bilayer which is thought to form the fluid matrix surrounding proteins or other molecules necessary for physiologic events. To electrophysiologists, the lipid bilayer portion always appears electrically in parallel to the physiologically more interesting nonbilayer portion. Which properties of a biologic membrane arise in the bilayer portion, and which in the proteins embedded in the bilayer? The most direct answer comes from studying bilayers formed artificially from pure lipids.

Artificial lipid bilayers are readily formed by the method of *Mueller* et al. (1962) from dispersions of synthetic or biologic lipids, e.g., phospholipids, in straight-chain hydrocarbon solvents, such as decane. They can also be made as virtually solvent-free membranes by bringing together two lipid monolayers spread on an air/water interface (*Montal* and *Mueller*, 1972). Separating two aqueous phases into which electrodes can be inserted, they allow accurate investigation of their electrical properties by voltage- or current-step, charge pulse and sinewave methods. Artificial lipid bilayers free of ionophoretic materials have electrical resistances of 10^7 to 10^9 Ωcm^2, 10^4 to 10^6 times that of most biologic membranes. The static capacity of "solvent-free" artificial membranes is 0.5 to 0.9 $\mu F/cm^2$ depending on composition (*Benz* et al., 1975), a value close to that of biologic membranes. Apart from the fact that artificial bilayers may contain hydrocarbon solvent, they are considered to be similar or identical in structure to the lipid bilayer portion of biologic membranes (*Haydon*, 1970) and their dielectric behavior has been investigated extensively so we may learn about their structure and physicochemical properties. In reviewing some of this work here, the aim is a narrow one, namely, to reconstruct and characterize what is likely to be the contribution of the lipid bilayer portion to the dielectric properties of a biologic membrane.

A. Origin

The structure of lipid bilayers, both artificial and some natural ones, is now known with considerable precision. If one were to cross a lipid bilayer, one would start in the aqueous solution and encounter in succession (i) the aqueous phase immediately adjacent to the bilayer containing, if the lipid is charged, a diffuse double layer of anions and cations, (ii) the layer formed by the polar head groups of the lipid molecules, some 7–10 Å thick from molecular modeling and X-ray diffraction studies. (iii) a nonpolar region occupied by the fatty-acid moiety of the lipid (hydrocarbon core), (iv) and (v) the layers of polar head groups and adjacent aqueous phase on the other side. Each of these have a capacitance and conductance, and in electrical studies of transmembrane properties, all the layers appear electrically in series. Therefore, an electrical representation of the membrane contains, besides the conductances of the various layers in series, a series combination of three capacitors representing (i + v), (ii + iv) and (iii) (*Hanai* et al., 1965). The overall membrane capacity can be no larger than the smallest of these three capacitances.

The smallest capacitor turns out to be the hydrocarbon layer where practically the entire measured bilayer capacity arises. This view is supported by both experiment and theory. (1) Where known, the thickness of the hydrocarbon core agrees well with expectations from the dielectric constant of hydrocarbon and the measured bilayer capacity. *Haydon* (1975) reports that *Fettiplace* obtained $C_m = 0.76\ \mu F/cm^2$ for bilayers made from "solvent-free" lecithin, consistent with an insulating layer of thickness 25 Å and the dielectric constant of bulk hydrocarbon ($\epsilon = 2.14$). From X-ray diffraction, virtually the same value (26 Å) is obtained for the thickness of the hydrocarbon cores of stacked egg lecithin bilayers (*Lecuyer* and *Dervichian,* 1969). (2) Bilayer capacity is inversely proportional to the number of carbons in the lipid hydrocarbon chain, both in solvent-free membranes (*Benz* et al., 1975) and in bilayers containing as solvents straight-chain hydrocarbons of ten carbon atoms or less (*Fettiplace* et al., 1971). This is as expected for a parallel-plate condenser in which the thickness of the dielectric is proportional to the lipid hydrocarbon chain length. (3) For a given lipid hydrocarbon chain, the nature of the polar head group seems to make little difference to the capacity (*Benz* et al., 1975). These findings are strong evidence that the measured electrical capacity arises almost entirely in the hydrocarbon layer. They agree with theoretical expectations that the impedances of polar groups and diffuse double layers should be exceedingly small[2] (*Hanai* et al., 1965;

[2] Experimental suggestions of a small electrical impedance associated with lipid bilayers and in series with the hydrocarbon core have, nevertheless, been obtained by *Coster* and *Smith* (1974). From AC impedance measurements of apparently unprece-

Coster and *Smith,* 1974). Put another way, if a potential is applied across a lipid bilayer, the hydrocarbon core will experience virtually all of it, and polar groups and adjacent aqueous layers nothing. Conductances and capacities of these two layers are so large as to prevent significant charge separation across them under either static or transient conditions.

Many biologic membranes may carry layers of proteins adhering to, but not penetrating into, the lipid bilayer. Since in an aqueous environment the hydrocarbon core is a much better insulator than any other biologic material, such "extrinsic" proteins would experience only a negligible fraction of an applied DC potential change. Only molecules reaching partly or entirely across the bilayer, such as "intrinsic" membrane proteins, fall under the direct influence of DC potential change, and may reorient and produce displacement currents. Extrinsic membrane proteins might be affected *indirectly* by potential changes, perhaps in interaction with intrinsic proteins. Such effects may well be physiologically significant, but they will not produce electrical signals of the kind observed by *Schneider* and *Chandler* (1973), or *Armstrong* and *Bezanilla* (1973).

B. Voltage Dependence

Since the hydrocarbon core should act as a nonpolar dielectric, one would expect its dielectric constant to be practically independent of the electric field across it, be it the magnitude of a static field or the frequency of a sinusoidally alternating field. Nevertheless, in some solvent-containing bilayers the capacity increases when a voltage is applied (*Babakov* et al., 1966; *Andrews* et al., 1970; *Wobschall,* 1972; *White,* 1974). The effect is opposite to what one would expect from dielectric saturation in a polar substance, and is instead a consequence of the compressive force which electric fields exert on dielectrics (electrostriction). An electric field will accumulate positive charges on one side of a bilayer and negative charges on the other. These attract each other with sufficient force to squeeze part of the hydrocarbon solvent, if present, out of the bilayer and into "microlenses". Microlenses are about 1-μm-large lipid-covered droplets of hydrocarbon solvent which float in the lipid bilayer and contribute relatively little to either bilayer area or capacity. Squeezing out the solvent will make the hydrocarbon core thinner so the bilayer capacity will increase.

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dented accuracy, they conclude that egg lecithin bilayers show a small dielectric dispersion between 0.1 and 100 Hz. In this frequency range the bilayer capacity appears to diminish by about 2% from the static value. *Coster* and *Smith* attribute this dispersion to the polar group layer for which they calculate a capacity of 30 μF/cm^2 and a conductance of 2 mS/cm^2 at physiologic salt concentration. These values are within the range predicted theoretically.

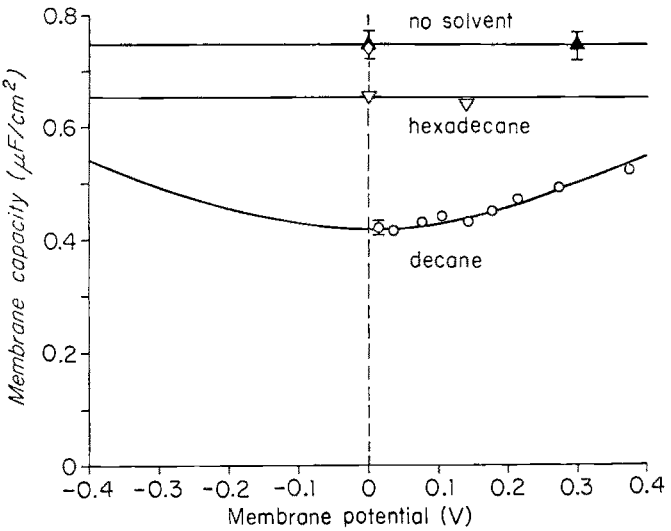

Fig. 6. Voltage dependence of capacity of artificial glycerol monooleate bilayers made with different solvents. ○, ▽ data from *Andrews* et al. (1970); ▲ data from *Benz* et al. (1975); ◇ value obtained by *White* (1974) on a film made with decane solvent and cooled to 10°C. In such a "frozen film", the solvent is thought to be expelled from the bilayer into microlenses. All measurements made in 0.1 *M* NaCl or KCl solution, except the measurement in hexadecane of *Andrews* et al. (1970), ▽, which were made in saturated KCl. Temperatures 20–25°C unless otherwise indicated. Data were obtained at one polarity only but, since the membranes are symmetric, curves were drawn here for both polarities in order to facilitate comparison with Figure 12 showing similar measurements in biologic membranes. Note that the capacity of the solvent-free bilayer is not significantly voltage dependent

Figure 6 shows capacity measurements of *Andrews* et al. (1970), *White* (1974) and *Benz* et al. (1975) on bilayers made from glycerylmonoloeate, an uncharged lipid with a fatty acid chain 18 carbon atoms long. The figure illustrates the dependence of capacity on voltage and hydrocarbon solvent. When the solvent is decane, capacity at low potentials is relatively small because the bilayer contains much solvent and is relatively thick; when a larger potential is applied, electrostriction squeezes out the solvent and the capacity increases within seconds or minutes to a higher value. When the solvent is hexadecane, the zero-field capacity is higher. Since the bilayer probably contains the same number of lipid molecules per unit area as in decane (*Andrews* et al., 1970), it follows that the volume occupied by solvent is much less in this bilayer. Why the bilayers should contain more solvent of short chain length (decane) than of longer chain length (hexadecane) has not been established. (Related to this effect may be the finding that alipathic hydrocarbons of up to 10–12 carbon atoms are powerful general anesthetics, but those of longer chain length are not;

Seeman, 1972). As one may expect, however, for a solvent-poor membrane, the voltage dependence of capacity is now much smaller, if not altogether absent. A further reduction in the bilayer solvent content can apparently be achieved by lowering the temperature below the freezing point of solvent and lipid alkyl chains (*White,* 1974). Finally, membranes made by bringing two lipid monolayers together (*Montal* and *Mueller,* 1972) are thought to be virtually solvent free; their capacity is the largest and shows no significant voltage dependence (*Benz* et al., 1975)[3].

In conclusion, any voltage dependence of static capacity in artificial lipid bilayers is due to electrostrictive squeezing-out of hydrocarbon solvent. In "solvent-free" bilayers, capacity is constant to within 3% over a ± 300 mV range. If significant electrostriction occurs in "solvent-free" biologic membranes, it cannot involve the lipid bilayer portion[4].

C. Frequency Dependence

To the extent that the lipid bilayer capacity arises in a nonpolar hydrocarbon core, one would not expect it to vary with the frequency of sinusoidally alternating fields until optical frequencies are reached. This can be tested with AC admittance measurements (*Hanai* et al., 1964, 1975; *Coster* and *Simons,* 1970; *Takashima* and *Schwan,* 1974b). A bridge circuit is connected to two electrodes, one each in the two aqueous phases separated by a lipid bilayer. Sinusoidally alternating fields are applied and the bridge is balanced by matching the electrical admittance between the two electrodes by a parallel combination of capacitor, C, and resistor, 1/G

[3] Strictly, these measurements apply only to the steady state, and there may be voltage-induced capacity changes which are *transient* and therefore escape detection by conventional bridge measurements. An interesting experiment by *Benz* et al. (1975) rules this out. Applying a 150 mV step of varying duration to bilayers, the authors compared the charges carried by capacitive currents at beginning (*on*) and end (*off*) of the pulse. The charge was measured by fitting exponentials to the capacitive currents and calculating their time integral. Time constants of these exponentials were small compared to the pulse duration and, judging by records from *Montal* and *Mueller* (1972), about 0.1 ms. Any charging current due to a comparatively slower change in capacity will, therefore, not enter into an individual measurement, but instead appear as a difference between *on* and *off* capacities. In a solvent-free bilayer, *on* and *off* capacities stay within 1% of each other during pulses of 1–1000 ms duration. By comparison, a solvent-containing bilayer shows the usual increase in capacity, and *off*-transients are larger than *on*-transients.

[4] *Blatt* (1977) has recently calculated asymmetric displacement currents due to membrane electrostriction. In a lipid bilayer of the compressibility estimated by *Wobschall* (1972) such electrostrictive asymmetric displacement currents could, even in the absence of solvent flow, have one-tenth the amplitude of "gating currents" in squid axons. However, they would flow in the opposite direction and carry 20–200 times less charge.

(Fig. 7a). Figure 8 is from such an experiment (*Hanai* et al., 1964) and shows values of C and G which matched the admittance of the experimental system as a function of frequency. With increasing frequency, C diminishes and G increases, showing all appearances of a dielectric dispersion.

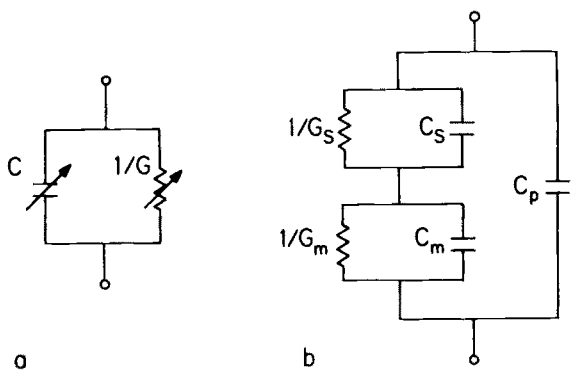

Fig. 7. (a) is the bridge element which is made to match (b) by proper adjustment of C and G. (b) is an electric representation of a lipid bilayer, the experimental chamber and adjacent aqueous phases. Drawn after *Hanai* et al. (1964) but with a different nomenclature. C_m and G_m in (b) correspond to C_f and G_f in Figure 8 of *Hanai* et al. (1964), G_s and C_s to G_m and C_m and C_p to C_s. For explanation of these symbols see text

In order to relate C and G to bilayer conductance and capacity, one must correct for impedances and admittances not arising in the bilayer.

Figure 7b shows an electrical representation of the current paths between the two electrodes in the aqueous phase. The bilayer itself is symbolized by the parallel combination of membrane conductance, G_m, and capacitance, C_m; both parameters may, in principle, vary with frequency. In series with the bilayer is the resistance, $1/G_s$, and parallel capacity, C_s, both arising in electrodes and electrolyte solutions. In practice, C_s and G_m are negligible compared to their parallel elements. The parallel capacitor, C_p, arises from stray fields, e.g., through the partition between the two electrolyte phases which carries the bilayer.

Analysis will show that the dispersion in Figure 8 is at least in part a consequence of the series resistance, $1/G_s$, which, unlike that in Figure 5, is *not* part of the dielectric. As frequency (f) increases, C_m presents less and less of an obstacle to current flow compared to $1/G_s$. The fraction of the voltage experienced by C_m diminishes and, therefore, also the charge withdrawn from or stored on it during each sinusoidal cycle. This appears as a decline in C. At the same time, G increases because the current path, $G_s - C_m$, looks more and more like a pure resistor as f increases, until in the limit C_m becomes effectively a short circuit and the admittance of the entire system attains the value G_s.

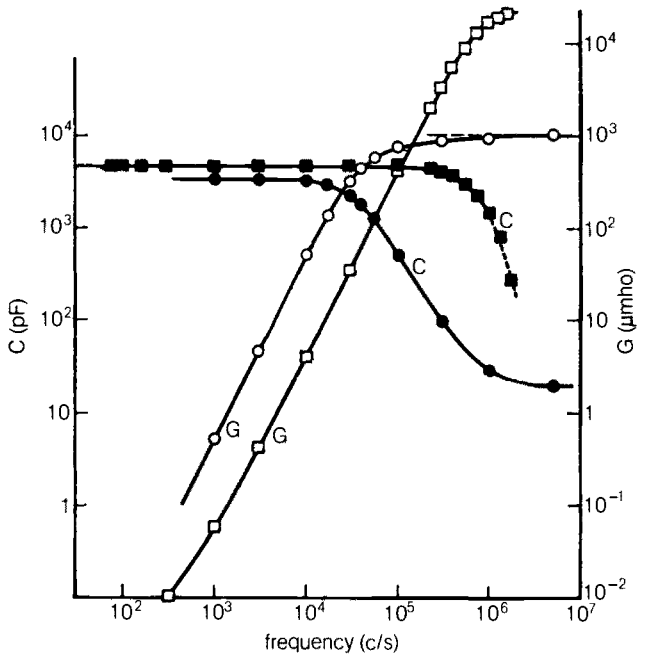

Fig. 8. Frequency dependence of the bridge elements, C and G, needed to match the admittance of a lipid bilayer with series and parallel admittances (see Fig. 7). The bilayer was made from egg lecithin in benzene. *Circles*, 0.1 *M* NaCl; *squares*, saturated NaCl. Modified after *Hanai* et al. (1964). Membrane area about 1–1.5 mm^2

In correcting for series resistance, $1/G_s$, one must analyze circuits a and b in Figure 7, which is easily done for the steady-state under sinusoidal excitation. The admittance of a resistor, 1/G, is then G, and that of a capacitor, C, is $j\omega C$, where $j = \sqrt{-1}$ and $\omega = 2\pi f$ is the frequency of the sinusoidal current through it. The admittances y_a and y_b of circuits a and b are

$$y_a = j\omega C + G \tag{3}$$

$$y_b = j\omega C_p + \frac{G_m + G_s + j\omega\,(C_m + C_s)}{(G_s + j\omega C_s)\,(G_m + j\omega C_m)} \tag{4}$$

These can be separated into real and imaginary parts. When the bridge is at balance, the real parts of y_a and y_b must be equal, and the imaginary parts as well. One obtains two simultaneous equations relating the parameters C and G to the elements in Figure 7b. C_s, C_p, and G_s can be measured at sufficiently high frequencies or in the absence of a lipid bilayer between the two aqueous phases. The two equations can be solved for C_m and G_m (*Coster* and *Simons*, 1970):

$$C_m = \frac{G_s^2 C^* - G^2 C_s + \omega^2 (C_s^2 C^* - C^{*2} C_s)}{(G_s - G)^2 + \omega^2 (C^* - C_s)^2} \tag{5}$$

$$G_m = \frac{G_s^2 G - G^2 G_s + \omega^2 (C_s^2 G - C^{*2} G_s)}{(G_s - G)^2 + \omega^2 (C^* - C_s)^2} \tag{6}$$

where $C^* = C - C_p$. In practice, G_m can be measured accurately only with static fields, since above 1 Hz, G_m is negligible compared to ωC_m. In Figure 9, Eq. (5) was used to obtain C_m from the observations in Figure 8. As anticipated, much of the frequency dependence of the "raw" capacity, C, in Figure 8 does not show up in Figure 9 and must, therefore, have been due to the series resistance rather than reflecting bilayer properties. What frequency dependence remains may well be an erroneous result of limited experimental accuracy. At 300 and 1000 kHz in the experiment of Figure 9 (curve a), only 14% and 5% of the potential applied to the electrodes appear across the film and the remainder across the series impedance.

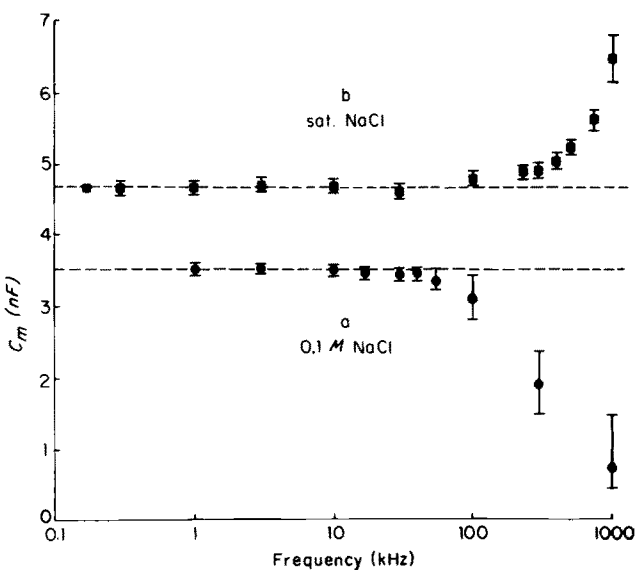

Fig. 9. Frequency dependence of membrane capacity calculated by Eq. (5) from the data in Figure 8. *Tetsuya Hanai* kindly provided these data in numerical form, as well as the values for C_p (20 pF in curve a) and G_s (1.04 mmho and 22.3 mmho in curves a and b, respectively). C_s = 0.68 pF was taken from Table 1 of *Hanai* et al. (1964) and C_p = 0 was assumed for curve b. *Error bars* indicate highest and lowest values obtained for C_m if G_s, C and G are allowed to deviate by 2% from their nominal values. *Dashed lines* indicate static capacitances. The high-frequency deviations of C_m from the static values may well be due to measurement uncertainty

Therefore, even small measurement uncertainties can result in large errors when calculating bilayer parameters at high frequencies. This is illustrated by the error bars which give highest and lowest values obtained by allowing G_s, G and C to deviate by 2% from their nominal values in Figure 8. Curve b in Figure 9 is from an experiment in a medium of higher conductivity, where series impedance is lower. Significant variations in membrane capacity now appear only at ~4 times higher frequencies, as expected if they arose spuriously from the experimental difficulties introduced by the series impedance.

Although the behavior at the highest frequencies is still uncertain, it appears that below 100 kHz and at physiologic salt concentrations C_m does not deviate significantly from its static value (see also *Hanai* et al., 1965). Similar results have been obtained by *Coster* and *Simons* (1970) and *Takashima* and *Schwan* (1974b, oxidized cholesterol membranes). If dielectric loss appears in biologic membranes below 100 kHz, it cannot, therefore, be due to the lipid bilayer portion.

D. Effects of a Lipid-Soluble Ion

The conclusion reached so far is that although the lipid bilayer may make a large contribution to the capacity of biologic membranes, this contribution is relatively simple. Over most frequencies of physiologic interest a pure lipid bilayer behaves as a slightly leaky, but otherwise perfect, parallel-plate condenser. The lipid bilayer portion will, therefore, not contribute to the asymmetric displacement current seen in excitable membranes.

Displacement current transients with strong superficial resemblance to "gating currents" in nerve can, nevertheless, be observed even in artificial lipid bilayers when certain lipid-soluble ions, such as tetraphenylborate ($TPhB^-$), are added to the aqueous phases at submicromolar concentrations (*Ketterer* et al., 1972; *Andersen* and *Fuchs,* 1975). $TPhB^-$ is only slightly water soluble, and, if uncharged, would partition strongly and uniformly into the hydrocarbon core of a bilayer (estimated hydrocarbon/water partition coefficient of 10^6, *Andersen* and *Fuchs,* 1975). Because of its charge, however, $TPhB^-$ is strongly attracted by the aqueous phase with its high dielectric constant. It is thought, therefore, that the ion remains trapped in the two narrow hydrocarbon zones where hydrocarbon borders on the two polar or aqueous phases (*Ketterer* et al., 1972). These zones are "energy wells" for $TPhB^-$ because the potential energy for the ion is lowest there. When a DC field is applied to a $TPhB^-$-doped bilayer, one observes in the steady-state only relatively little current representing $TPhB^-$-transport across the bilayer. However, when the electric field changes, a redistribution of $TPhB^-$ will occur between the two zones

causing a comparatively large current transient which is ionic across the hydrocarbon core and a displacement current across the hydrocarbon/water interface. Since the amount of $TPhB^-$ contained in the two zones is finite, the redistribution current is transient and carries finite charge. Measuring charging transients of this kind, *Andersen* and *Fuchs* could determine the amount of $TPhB^-$ adsorbed, as well as rate and extent of redistribution between the two sides of the membrane after a change in applied potential. This is the only experimental system which is sufficiently well defined for us to understand slow displacement current transients on a molecular level. It is, therefore, worth considering in some detail.

The steady-state occupancies, N_1 and N_2, of the two energy wells may be assumed to be given by the Boltzmann distribution:

$$\frac{N_1}{N_2} = \exp(-zeE_i/kT) \tag{7}$$

where E_i is the potential difference between the two wells, well 1 minus well 2, k and T Boltzmann's constant and absolute temperature, $e = 1.60 \times 10^{-19}$ coulombs is the elementary charge and z the valency of the particle, -1 in this case. Of course, E_i is related to the potential, V, applied to the bilayer. Assuming direct proportionality as a first approximation, we can set $V = \beta E_i$ for a symmetric bilayer, where β may be regarded as an empirical constant. In the absence of a potential, $N_1 = N_2$; after applying a potential, the charge carried by the redistributing ions can be shown from Eq. (7) to be

$$Q = \frac{1}{2} Q_{max} \tanh(\beta eV/2kT) \tag{8}$$

where Q_{max} is the charge on all absorbed TPhB ions. Alternatively, if the potential is held so negative that $N_1 \simeq 0$, then after a step to some more positive value,

$$Q = Q_{max}/[1 + \exp(-\beta eV/kT) \tag{9}$$

which is formally identical to equations used by *Schneider* and *Chandler* (1973), *Keynes* and *Rojas* (1974), and others (*Nonner* et al., 1975) to describe the charge carried by asymmetric displacement currents in various excitable tissues.

Andersen and *Fuchs* (1975) found that Eq. (8) with $\beta = 0.8$ provided an accurate description of their data, which were obtained at relatively low $TPhB^-$ concentrations ($\leqslant 10^{-7}$ M). The value of β, of course, can never be higher than unity and will in general be less. For instance, the two zones where the potential energy for $TPhB^-$ is lowest need not be on

the extreme borders of the hydrocarbon core and, therefore, may not experience between them the entire membrane potential (*Andersen* and *Fuchs*, 1975). Furthermore, and perhaps more importantly, the considerations so far neglect electrostatic interactions between $TPhB^-$ ions. The ions will create boundary potentials in the adsorption zones which in a symmetric membrane will equal each other when $N_1 = N_2$, but otherwise differ in such a way as to diminish the potential difference between the energy minima. The more ions are adsorbed, the more important this effect becomes. As a consequence, the parameter β in Eq. (8) and (9) diminishes when the aqueous $[TPhB]^-$ is increased (*Andersen* et al., 1977) [5]. Qualitatively, the more $TPhB^-$ is in the membrane, the larger the potential V needed to crowd, say, 90% of all the mutually repelling TPhB-ions into the same energy well. In the limit where $[TPhB]^-$ in the aqueous phase is so low that not enough $TPhB^-$ is adsorbed to allow significant electrostatic interaction, $\beta = 0.85$ (*Andersen*, personal communication). At $TPhB^- = 10^{-7}$ M and 1 M NaCl in the aqueous phase, the number of TPhB-ions absorbed in the experiments of *Andersen* et al. (1977) was $2.4 \times 10^4 / \mu m^2$. Moving them all from one energy well into another would represent a charge transfer of 400 nC/cm^2; and from their data and Eq. (9) the parameter β would have a value of about 0.7. At $[TPhB]^- = 3\ \mu m$, total absorption was $10^5 / \mu m^2$ molecules, total charge 1800 nC/cm^2 and β would be only 0.37.

Are sodium channels, like TPhB-ions, affected by electrostatic interaction between each other, or with other polar membrane constituents? A quantitative answer cannot be given before we know the force fields and effective dielectric constants in the environment of the gating particles. All one can say is that the Q_{max} observed in squid nerve and frog muscle is only about 30 nC/cm^2, much less than the values discussed above. In squid giant axons with their 200–500 sodium channels/μm^2 (see Table D-1), it seems, therefore, probable that the effects of electrostatic interaction between sodium channels are negligible. The situation may be different in the node of Ranvier. Assuming a nodal area of 50 μm^2, the work of *Nonner* et al. (1975) on frog myelinated nerve implies values of Q_{max} of 275 nC/cm^2, reaching the range of values discussed above. Values in mammalian nodes may be even higher. *Ritchie* and *Rogart* (1977) report that nodes of Ranvier in rats have about 12,000/μm^2 sodium channels, 5–10 times more than in the frog (see Table D-1). If so, sodium channels with their presumably large gating charge movements

[5] Since the boundary potentials depend on the local intramembrane concentration of $TPhB^-$ and, therefore, on the membrane potential, the simple Eq. (8/9) may no longer be expected to hold. For descriptive purposes, we retain it here as an approximation and consider β as an empirical parameter which represents the membrane potential-sensitivity of $TPhB^-$ distribution, and depends on the amount of $TPhB^-$ adsorbed.

would be in such close proximity that electrostatic interaction between them no longer seems an idle speculation. Such interaction would be relevant for noise analyses where statistical independence of channels is often assumed.

The time course of the $TPhB^-$-current transients is exponential with time constants up to 2 ms at 24°C when $TPhB^- \leqslant 10^{-7}$ (*Andersen* and *Fuchs*, 1975). This figure is 100–1000 times larger than the expected diffusion time across the hydrocarbon core of a neutral molecule similar in size to $TPhB^-$. Clearly, size of the moving particle and viscosity of the hydrocarbon environment are not the only important factors. One would expect, for instance, that $TPhB^-$ ions would encounter on their way through the hydrocarbon core a large energy barrier in overcoming the electrostatic forces pulling them towards the polar phases. By comparison, maximal relaxation time constants for asymmetric displacement current transients are around 0.7 ms for squid axon "gating currents" and 10 ms for frog muscle "charge movement", all recorded at 2–8°C.

III. The Membrane Capacity of Squid Giant Axons

A. Frequency Dependence

The dielectric properties of natural membranes are best discussed by reference to the squid giant axon, where our knowledge is most complete. In a squid giant axon, the axolemma shows relatively little infolding and allows relatively accurate area measurement, as well as uniform electrical polarization. The static or low-frequency differential capacity can be obtained either from the charge deposited on the membrane after a small potential displacement, or from admittance bridge measurements using low-amplitude sinusoidal excitation. All values in Table 1 give differential capacities at the resting potential of around -60 to -70 mV. Agreement among different authors is impressive; the low-frequency capacity is between 1.0 and 1.2 $\mu F/cm^2$. This value does not take into account the possibility of membrane infolding and could, therefore, be slightly too high. Values for skeletal muscle membrane (1–1.2 $\mu F/cm^2$) are included for comparison. When the capacity is measured with high-frequency (70–100 kHz) sinusoids, it diminishes to about half. Squid axolemmal capacity, therefore, shows a frequency dependence over frequencies where pure artificial lipid bilayers show none. The first suggestion of dielectric loss in axon membranes came from the measurements of *Curtis* and *Cole* (1938) who suggested that the membrane behaved as a "constant phase angle dielectric", a complicated and, in most cases, unexplained behavior shown by a number of polar substances (*Cole*, 1965). *Curtis* and *Cole*'s (1938) measurements

Table 1. Membrane capacity of nerve and muscle [a]

Source	Method	Condition	Low-frequency capacity ($\mu F/cm^2$)	High-frequency capacity ($\mu F/cm^2$)
Squid giant axon				
Curtis and *Cole* (1938)	sinewave [b]	normal	1.1 (1 kHz)	–
Hodgkin et al. (1952)	voltage step	normal	1.03 (1 kHz) [c]	–
Taylor (1965, 1977)	sinewave	normal	–	0.5–0.6 (70 kHz)
Takashima and *Schwan* (1974a)	sinewave	TEA^+ inside	1.1–1.2 (0.2 kHz)	0.55 (100 kHz)
Armstrong and *Benzanilla* (1975)	voltage step	no permeant ions inside or outside	1.0 (1.5 ms)	–
Frog Sartorius muscle				
Adrian and *Almers* (1974)	voltage step	K^+-free Ringer outside	1.0–1.2 [d] (1 s)	–
Hodgkin and *Nakajima* (1972a)	measurement of "time constant"	Ringer outside	1.03 [e] (~10 ms)	–

[a] Most measurements were made with sinusoids or by integrating the capacitative charging current during and after a voltage step. Frequency of sinusoid or integration interval given in brackets.
[b] Measurements made with external electrodes.
[c] *Hodgkin* et al. (1952) obtained a value of 0.89 $\mu F/cm^2$ by integrating capacitative charging transients over 50 μs; they estimate that this corresponds to a capacity of 1.03 $\mu F/cm^2$ at 1 kHz.
[d] Obtained from a comparison of electrical and morphologic data. The values in the table represent averages, weighted by membrane areas, of sarcolemma and transverse tubular membrane capacities. From the work of *Mobley* and *Eisenberg* (1975) it can be calculated that the average cylindrical fiber of 80 μm diameter has 1.57 cm^2 of sarcolemma and 4.4 cm^2 of transverse tubular membrane associated with each cm^2 of cylinder surface; *Adrian* and *Almers* (1974) give a membrane capacity of 7.1 $\mu F/cm^2$, *Hodgkin* and *Nakajima* [e] a value of 6.1 $\mu F/cm^2$ of cylinder surface of an 80 μm diameter fiber. The value of *Hodgkin* and *Nakajima* could be too low due to tubule potential decrements and because it is not a true DC measurement.

were made with extracellular electrodes and are, therefore, of limited accuracy in determining axolemmal properties. However, their suggestion of dielectric loss was confirmed with intracellular electrodes by *Hodgkin* et al. (1952) who addressed the problem in passing, 13 years later by *Taylor* and *Chandler* (*Taylor*, 1965, 1977) in the first examination of axolemmal AC impedance with modern methods, and most recently by *Takashima* and *Schwan* (1974a).

Once again, an AC admittance bridge can be used to match the admittance between two electrodes, one inside and one outside the axon, with a parallel combination of resistor and capacitor. After suitable correction for stray capacitances, one can use expressions (5) and (6) to correct for the total impedance $R_s = 1/G_s$ in series with the membrane. This impedance is thought to arise mostly (*Hodgkin* et al., 1952; *Keynes* and *Rojas*, 1976) in the Schwann cell layer surrounding the axon, but to some extent also in the axoplasm and the external solution. R_s is usually regarded as being purely resistive and the same for all parts of the axolemma, an assumption which seems correct for the axoplasm (*Cole*, 1975) and up to 200 kHz probably also for the Schwann cell layer (*Cole*, 1976). Figure 10 is from the work of *Takashima* and *Schwan* (1974a) and shows frequency dependence of capacity in normal axons (curve 1), and after sodium channels were blocked with tetrodotoxin (TTX, curve 2), or potassium channels with tetraethylammonium (TEA^+, curve 3). All curves show a decline of C_m at increasing frequencies, indicating dielectric loss. In absence of TEA^+, C_m seems to diminish also at low frequencies. However, rather than arising in the membrane dielectric, this effect almost certainly results from

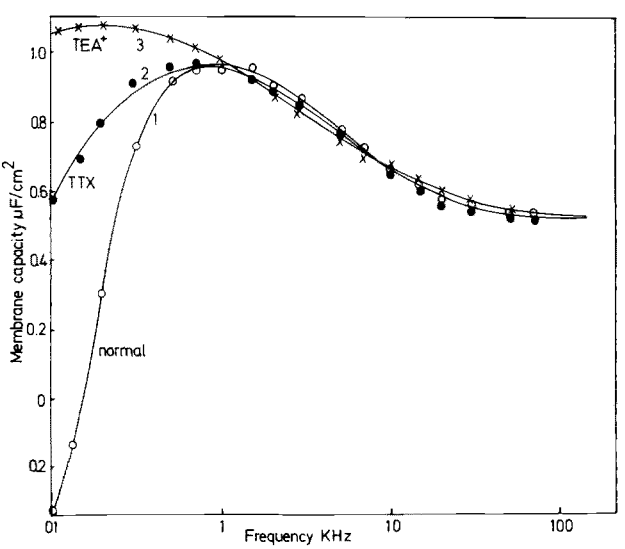

Fig. 10. Frequency dependence of squid axon membrane capacity. Curve 1, 2 axons, control; curve 2, the one axon after external application of approx. 10^{-6} M TTX; curve 3, the other axon after internal application of approx. 100 mM TEA^+. Temperature 20°C. After *Takashima* and *Schwan* (1974a)

voltage- and time-dependent ionic conductance changes in potassium, and, to a lesser extent, sodium channels. At low frequencies, such conductance changes can occur during each sinusoidal cycle and disturb the measurement. When K^+ channels are blocked by TEA^+, this effect is diminished or absent. In the low-frequency region, curve 3 in Figure 10, therefore, represents the membrane dielectric better than the other curves.

Takashima and *Schwan* (1974a) also observed an apparent increase of G_m with frequency which is not shown in Figure 10. However, for much the same reasons as in studies of artificial lipid bilayers, G_m is extremely sensitive to small errors in determining any of the other parameters in Figure 7. It is possible, therefore, that G_m-measurements at high frequencies are not very accurate. On the other hand, a conductance increase is expected because lossy dielectrics appear more and more conductive as frequency approaches and surpasses the characteristic frequency. (The simple dielectric represented in Figure 5, for example, will appear as a capacitor of value C_0 at low frequencies and as an ohmic conductance of value $1/R$ at infinite frequency). Because lossy capacitors have conductive/resistive as well as capacitive properties, they are often represented as complex numbers with a real part (C_m in Fig. 10) and an imaginary part whose magnitude here would equal the membrane conductance minus its DC value. Characterizing the dielectric requires the frequency dependence of both C_m and G_m. First attempts in this direction (*Taylor,* 1965; *Takashima* et al., 1975) suggest that no single dielectric relaxation time can explain the dielectric dispersion in the resting squid axon membrane. There appears to be a spectrum of relaxation times which is hard to decompose into its components by present methods.

If we are correct in our assumption that the Schwann cells act as a spatially constant, pure resistor in series with the axolemma, then the above findings establish that the squid axon membrane as a whole behaves as a lossy dielectric. The delay in membrane birefringence change accompanying potential steps seen by *Cohen* et al. (1971) is probably another indication of slow membrane polarization and, therefore, dielectric loss.

The capacitive current transient under a voltage clamp. Dielectric loss in the squid axon membrane can also be inferred from the capacitive current transient following a step in potential. The first event following a step depolarization is a large capacitive surge of outward current. The current transient can reach hundreds of $\mu A/cm^2$ per mV step displacement (*Hodgkin* et al., 1952) and lasts for 10–50 μs. Both amplitude and time course depend on series resistance and the speed of electronic apparatus. The initial surge presumably includes all loss-free, as well as some lossy polarization (*Fitzhugh* and *Cole,* 1973). In an unperfused axon, it can deposit on the membrane within 50 μs a charge corresponding to 0.89 $\mu F/cm^2$

out of the total 1–1.2 $\mu F/cm^2$ (*Hodgkin* et al., 1952). The initial surge is followed by a "late displacement current" (*Meves*, 1976) which is entirely due to lossy polarization. Figure 11 shows records of late displacement current transients accompanying pairs of equally sized, large hyper- and depolarizing potential steps, starting from potentials between – 70 and – 100 mV, and carrying the membrane to the indicated potentials. The lefthand records were taken from *Keynes* and *Rojas* (1974) on axons perfused with media of high (Fig. 11A) or low (Fig. 11B) conductivity and ionic strength. As *Keynes* and *Rojas* report and Figure 11A, B suggests, increasing the internal ionic strength decreases the late displacement current. It would be interesting to know whether or not this occurs at the expense of the rapid initial surge, which did not photograph. The effect

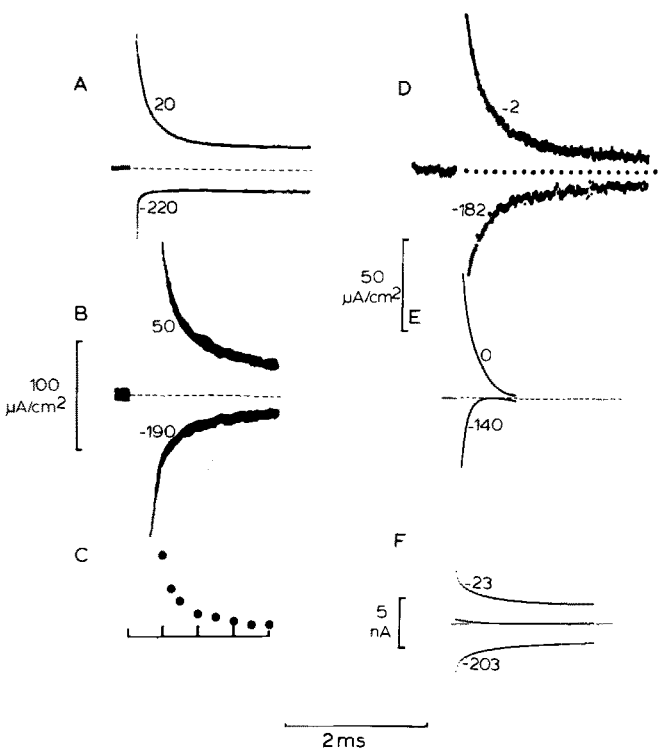

Fig. 11 A–F. Capacity current transients after potential steps. All records from TTX-poisoned nerve fibers at temperatures from 2°C to 6°C. Delayed potassium channels were blocked with external (F) or internal tetraethylammonium, or internal C_s^+. Traces A–F from squid axons, trace E from frog node of Ranvier, obtained by the authors quoted in the text. Traces A, B, D, E, F recorded during equally sized de- and hyperpolarizing steps from holding potentials from – 70 to – 100 mV. Trace C, asymmetry current obtained by adding the inward to the outward transient in B. Notice the small size of the asymmetry current in frog nerve (middle trace in F) compared to the leakage currents

could not be explained by any reasonable change in series resistance. The charge carried by the depolarizing transient exceeds that during hyperpolarization. The "asymmetry current", obtained here as the algebraic sum of the two late displacement transients in Figure 11B, is shown in Figure 11C. Traces on the right are from similar experiments carried out in other laboratories, D (*Meves,* 1976) and E (*Armstrong* and *Bezanilla,* 1974) on squid axons, and F on the node of Ranvier (*Nonner* et al., 1975). The middle trace in F shows the asymmetry current. With regard to squid giant axons, there is much variation between different laboratories which cannot be accounted for by variability of uncompensated series resistances. The records of *Meves* (1976, trace D) at high internal ionic strength resemble those of *Keynes* and *Rojas* (1974) at low ionic strength, whereas the only published record of *Armstrong* and *Bezanilla* (trace E from an axon at relatively low internal ionic strength) resembles the high-ionic strength record of *Keynes* and *Rojas* (1974). In all cases, the asymmetric displacement current or "gating current" is only part of the late displacement current.

B. High-Frequency Capacity and Possible Implications for Membrane Structure

The high-frequency capacity is probably determined to a large extent by the axolemmal lipid bilayer, and one would like to know the capacity of a "solvent-free" bilayer made from axolemmal lipid. This capacity has not yet been measured, but based on chemical composition, one would expect it to be between 0.60 and 0.72 $\mu F/cm^2$ [6]. The high-frequency capacity of squid axolemma is given as 0.55 $\mu F/cm^2$, but this figure could be lower for experimental reasons and because membrane folding may lead to a systemic underestimate of membrane area. It seems reasonable, therefore, to consider that the axolemmal high-frequency capacity may actually be less than that of axolemmal bilayer.

[6] *Zambrano* et al. (1971) have analyzed the lipid composition of squid retinal nerve, a tissue containing axon/Schwann cell membranes in the favorable ratio 5.1 (*Marcus* et al., 1972). 66.7% by weight of the membrane lipids are phospholipids, the rest cholesterol (22%), and free fatty acids (5.2%). Average length of lipid hydrocarbon chains is 17.8 carbon atoms with an average of 1.6 double bonds per chain. A "solvent-free" bilayer made from monounsaturated C_{18}-phosphatidylcholine has a capacity of 0.72 $\mu F/cm^2$ (*Benz* et al., 1975), one made from egg yolk phosphatidylcholine, a mixture of mainly C_{16} and monounsaturated C_{18}-lecithins, has a capacity of 0.76 $\mu F/cm^2$ (*Fettiplace* in *Haydon,* 1975). The effect of cholesterol on the capacity of Montal-Mueller type bilayers is unknown, but a bilayer made with hexadecane and containing egg yolk lecithin and cholesterol in a molar ratio of 2.5:1 has a capacity of 0.59–0.60 $\mu F/cm^2$ (*Fettiplace* et al., 1971), a value which may be taken as a lower limit for a cholesterol-containing, but otherwise solvent-free, axolemmal bilayer.

There are several reasons why this could be so. Firstly, the Schwann cell layer may be a more complicated series impedance than we think it is, perhaps insulating some parts of the axolemma more than others. Besides this possibility, one naturally considers possible effects of membrane proteins. By chemical analysis, vesicular membrane preparations believed to be fragmented sarcolemma from squid giant axons contain 29.5% protein and 70.5% lipid by weight (*Camejo* et al., 1969). These proteins could be electrically in series, or in parallel with the axolemmal bilayer. Some proteins may form a coat covering part or all of the bilayer, as suggested by *Fettiplace* et al. (1971). Such a protein coat could appear as a series impedance at high frequencies without affecting the static capacity. With a capacity of 4.5 $\mu F/cm^2$ and a resistance of 7.7 Ωcm^2, it could diminish a static capacity of 0.72 $\mu F/cm^2$ to an infinite-frequency value of 0.55 $\mu F/cm^2$, the characteristic dispersion frequency being 4 kHz. Indeed the entire frequency dependence of axolemmal capacity could in principle arise in this way. Proteins in series with a lipid bilayer will, of course, not experience static polarization and cannot contribute to asymmetric displacement currents.

Alternatively, an appreciable fraction of the axolemmal area may consist of bilayer-free, proteinous membrane patches. Such membrane-penetrating proteins must of course exist, if only to provide the many known membrane transport functions. They would appear electrically in parallel with the lipid bilayer, and are likely to show dielectric loss, as well as making a large contribution to the static membrane capacity. The structures responsible for asymmetric displacement currents are in this category. Proteinous membrane patches of low capacity at 100 kHz must be much thicker than the lipid bilayer, since the dielectric constant of hydrated proteins at 100 kHz is unlikely to be less than 3–10 [7].

It may be possible to distinguish the dielectric contributions of protein coats and penetrating proteins with AC impedance studies on axons treated with proteo- or lipolytic enzymes. Some first attempts along these lines have been made by *Takashima* et al. (1975). These authors treated squid axons internally with pronase, a mixture of proteolytic enzymes. Pronase treatment (*Armstrong* et al., 1973), sufficient to make sodium channel inactivation incomplete, apparently doubled the frequency-dependent portion of C_m *without altering the high-frequency capacity.* Since pronase might be expected to reach all nonpenetrating membrane proteins, the result argues against an effect of protein coats on high-frequency

[7] Partially hydrated powders of solid protein have dielectric constants in the range 3–10 (*Rosen*, 1963; *Takashima* and *Schwan*, 1965; *Kaufman* and *Bettelheim*, 1971). Unlike protein molecules in aqueous solution, those in solids are unlikely to reorient as a whole when the electric field is changed. In this regard, they may resemble membrane-bound proteins.

capacity, at least with regard to the axoplasmic membrane leaflet. *Takashima* et al. (1975) also reported that treatment with a phospholipase A of unspecified purity and activity approximately doubles both low- and high-frequency capacity. Unfortunately, the value of these results with pronase and phospholipase is diminished by failure of the authors to control average membrane potential during measurements, and to eliminate membrane admittance elements due to time- and voltage-dependent conductance changes. Especially the latter could cause large errors in estimating the low-frequency capacity. The work should be repeated in the presence of external tetrodotoxin and internal tetraethylammonium to block sodium and potassium channels. One may also apply other proteolytic enzymes both inside and out in order to test seriously the hypothesis that protein coats adhering to continuous lipid bilayer affect the high-frequency capacity.

C. Voltage Dependence

The fact that one can record asymmetric displacement currents as in Figures 1 and 3 suggests that the static membrane capacity C_m of excitable membranes is strongly voltage-dependent. In investigating this voltage dependence [8], it is best to measure C_m by integrating the charging current during a voltage step of, ideally, infinitesimal amplitude. The small step is then superimposed on a larger step from the holding potential to the potential at which the capacity is to be measured. As in measurements of charge movements and "gating currents", one must take pains to eliminate the voltage- and time-dependent ionic currents possible across the normal membrane. Measurements of this kind on frog skeletal muscle (*Almers* et al., 1975; *Adrian* and *Almers*, 1976a; *Schneider* and *Chandler*, 1976) and squid giant axons (*Armstrong* and *Bezanilla*, 1975) have given similar results (Fig. 12): C_m rises sharply as the resting membrane is depolarized and falls again at stronger depolarizations, showing a peak at potentials roughly halfway between resting potential and the peak of the action potential. Most of this variation in C_m happens over the physiologic potential range and may, therefore, be related to the movement of voltage sensors associated with ionic channels. The voltage dependence of C_m is opposite to what is found in some lipid bilayers (Fig. 6) or to what would be expected from electrostriction effects. Instead it is of the kind expected

[8] Recently, *Takashima* (1976) has investigated the voltage dependence of squid axon capacity with an AC impedance bridge. At 50 kHz, membrane capacity is constant between − 100 and − 20 mV. Results at lower frequencies are difficult to interpret since a large and unidentified portion of the capacitive dispersions observed are almost certainly due to time- and voltage-dependent conductance changes.

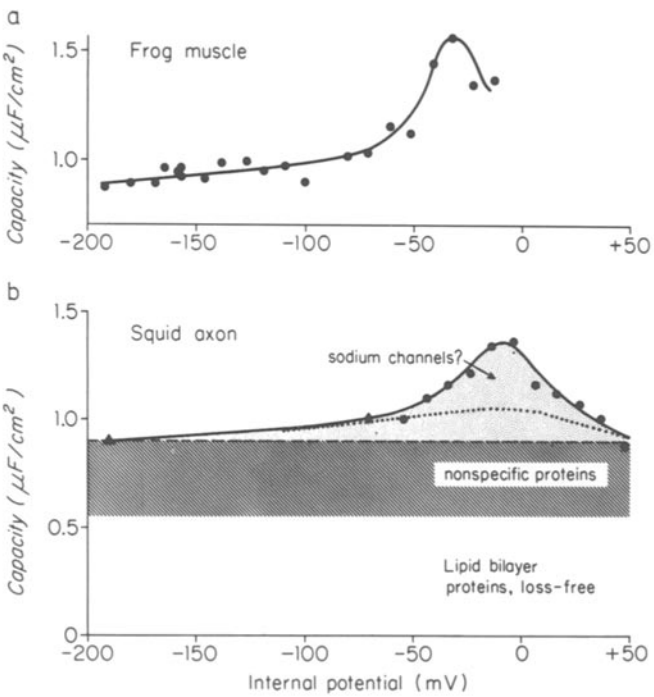

Fig. 12 a and b. Static membrane capacity as a function of membrane potential. (a) modified from *Adrian* and *Almers* (1976a). C_m was measured relative to the value at − 90 mV; the ordinate is obtained by assuming for that potential a value of 1 $\mu F/cm^2$ of total cell membrane area. (b) data from *Armstrong* and Bezanilla (1975). The value at − 70 mV (●) as given in the text of that paper. The value at − 190 mV (▲) was obtained from Figure 4 (*Bezanilla* and *Armstrong*, 1975) as follows. Figure 4 (*dashed line*) implies that during a step from − 80 mV to − 60 mV, the charge carried by displacement currents would be greater by 2.14 nC/cm^2 than that during a step from − 200 to − 180 mV in Figure 4. Therefore, C_m between − 200 and − 180 mV must have been less than at − 70 mV by 2.14 (nC)/20 (mV · cm^2) = 0.107 $\mu F/cm^2$

for a saturable dielectric. Presumably, the molecules giving rise to the peak in Figure 12 are relatively free to move at intermediate depolarizations, but become increasingly immobile as more extreme electric fields drive them into dielectric saturation. Figure 12b also suggests, however, that not all polar membrane constituents suffer dielectric saturation at the potentials explored in Figure 12. Even at − 190 mV, C_m in Figure 12b is much larger than the (probably voltage-independent) high-frequency capacity at the resting potential.

In principle, measurements as in Figure 12 give the same information as integration of "gating current" or charge movement transients gave in Figures 2 and 4. One could obtain from Figure 12b a curve virtually equivalent to that in Figure 4 simply by subtracting a constant from C_m

(*dashed line*) and integrating the remainder with respect to voltage between holding and test potential. Measurements of "gating currents" with large pulses and C_m with small pulses are complementary; the former allows kinetic analysis but the latter has the advantage of not depending on any subtraction of currents.

D. Dielectric Properties of Excitable Membranes

In this somewhat speculative section, I attempt to interpret the dielectric behavior of squid axon membranes, using results on artificial lipid bilayers, as well as the frequency- and voltage-dependence of membrane capacity. Although this section refers specifically to squid giant axons, I believe that it might apply also to frog skeletal muscle or other excitable membranes.

The frequency dependence of capacity shows that C_m consists of lossy and loss-free components. The loss-free portion (white in Fig. 12b) amounts to 0.55 $\mu F/cm^2$ or less. If squid axolemma contains 70.5% lipid by weight (*Camejo* et al., 1969), a large fraction of the axolemma must be lipid bilayer. It seems, therefore, natural to suggest that the 0.55 $\mu F/cm^2$ arises mainly in the lipid bilayer, though loss-free polarization of proteins will also contribute. By analogy with solvent-free artificial lipid bilayers, one may assume that the high-frequency capacity is potential independent. This assumption is tentative since we cannot be sure that the axolemmal bilayer with proteins has precisely the same characteristics as a pure artificial one, but what evidence there is (*Takashima,* 1976; see footnote 7) tends to support it.

The lossy components of C_m (shaded and stippled in Fig. 12b) contribute another 0.45–0.55 $\mu F/cm^2$ at the resting potential. They are, by chemical analysis, most likely due to a mixture of proteins. Some proteins may produce loss by forming an impedance in series with otherwise loss-free membrane constituents (protein coats), others penetrate the membrane, become polarized when a static field is applied across the membrane and behave as genuinely lossy dielectrics. Among the latter proteins are sodium and potassium channels, sodium and calcium pumps, Na^+-Ca exchange proteins, transport proteins for amino acids and sugars, and others. Beyond that, the components contributing to the lossy portion have been characterized neither chemically nor functionally. At potentials negative to the resting potential, it seems unlikely that sodium channels contribute substantially to lossy polarization since they form only a small fraction of all membrane proteins. In particular, the delayed birefringence changes after potential steps, being probable indicators of delayed membrane polarization (*Cohen* et al., 1971), are unlikely to result from the relatively few sodium channels in the membrane.

The potential-dependence of C_m suggests that the lossy portion can be subdivided further into a dielectrically saturable component (stippled in Fig. 12b) and a component (shaded) which persists at all experimentally accessible potentials. The saturable component gives rise to all or most of the gating current and, because it clearly arises in molecules which are exquisitely sensitive to physiologic potential changes in nerve, may have to do largely with the gating of sodium and K^+ channels. The shaded component predominates at potentials negative to the resting potential (– 70 mV). There is no evidence that it is related to the gating of sodium or other ionic channels, and it seems more likely that it comes from a mixture of some or all of the other proteins found in the axolemma. It will, therefore, be called "nonspecific". Though the nonspecific component may not experience strong dielectric saturation at experimentally accessible potentials, it seems possible that at more extreme potentials it too would diminish.

For interpretation of "gating currents", it is important how one distinguishes between saturable and nonspecific polarization. The method used here relies solely on the voltage dependence of total static capacity and forms the basis of all subtraction techniques in measurements of "gating currents" and charge movements. For example, if we interpret the charge displacement in Figure 4 to be exclusively due to gating of sodium channels, then we make the tacit assumption that the potential-dependence of the nonspecific component is given by the dashed line. However, this potential-dependence cannot at present be determined independently and may, for example, just as well be given by the dotted line in Figure 12b (or, in principle, by any other). Unless one has other criteria to distinguish nonspecific membrane polarization from that arising in voltage sensors of ionic channels, one must accept that records of gating currents may be contaminated by nonspecific protein polarization to an extent which is not accurately known. It would be of interest, for example, to know the voltage dependence of capacity in a nerve membrane without sodium channels. Neuroblastoma cell lines may offer an opportunity since mutants with and without sodium channels are available (e.g., *Peacock* et al., 1972) and can be voltage clamped (*Moolenaar* and *Spector*, 1977). Comparing the voltage dependence of C_m in the two mutants may allow one to reconstruct the contribution of sodium channels.

E. Conclusion

Some essential points of the material covered so far can be summarized by considering what happens if we apply a potential step from – 150 to + 50 mV to the axon of Figure 12b. The charge displacement necessary

for this is the integral of C_m with respect to potential between − 150 and + 50 mV, or 212 nC/cm² in total. The lipid bilayer and other portions of the membrane capable of loss-free polarization will absorb 110 nC/cm² instantly. Then there will be a charging transient relaxing with a wide spectrum of time constants centered around, perhaps, a value of 40 μs, 4 kHz being an estimate for the characteristic frequency of the lossy portion of the membrane dielectric at 20° C (*Takashima* and *Schwan*, 1974b). [Whether this value applies at + 50 mV, as well as at − 60 mV as in *Takashima* and *Schwan*'s (1974b) experiment, is, of course, unknown.] The transient is at least partly due to polarization of nonspecific membrane proteins and carries, perhaps, 70 or 87 nC/cm², depending on whether the dotted or the dashed line separates saturable and nonspecific components of C_m. Finally, there will be a "gating current" transient relaxing with a time constant of the order of 60 μs at 8° C (*Bezanilla* and *Armstrong*, 1975). It will carry 32 nC/cm² (dashed line) or 14.5 nC/cm² (dotted line). Polarization due to "gating currents" may amount to only about 1/7 to 1/3 of the total polarization of lossy material.

IV. Gating Currents in Nerve Membranes

This section explores asymmetric displacement currents in nerve and their relation to sodium channels. It begins by examining the arguments in favor of identifying these currents with sodium channel "gating currents". The arguments fall into two groups. In the first, theoretical expectations about gating currents are compared to measurements. In the second, parallel effects of various experimental maneuvers on sodium and asymmetric displacement currents are exploited.

A. Are Asymmetric Displacement Currents "Gating Currents"?

1. Time Course

Even with the most detailed knowledge of the time and voltage dependence of sodium conductance available today, we do not know what kinetics to expect for the "gating currents". A finite but otherwise unknown gating charge movement may be expected to precede any change in conductance. Further, the time courses of gating current and conductance change should overlap because *rate*, as well as amplitude of conductance change, are strongly voltage dependent. The observed asymmetric displacement currents meet both of these not very stringent requirements. The time course of displacement currents, therefore, gives no clue about what fraction of it is associated with gating sodium channels.

Pharmacologic studies of kinetics have also failed to yield firm conclusions. *Armstrong* and *Bezanilla* (1975) have reported that external Zn^{2+} (30 mM) slows both sodium conductance change and displacement current kinetics by a factor of 2 or 3 (P/4 procedure). Replacement of all H_2O wit D_2O, on the other hand, delays the increase in sodium conductance without noticeable effects on displacement currents (*Meves,* 1974), and procaine (37 mM) slows the increase of sodium conductance while reportedly speeding the kinetics of displacement currents (*Keynes* and *Rojas,* 1974; but see also *Kniffki* et al., 1976). Clearly, we do not yet understand how the time courses of sodium and asymmetric displacement currents are related.

2. Maximal Charge and the Number of Sodium Channels

A simple and potentially compelling approach was implicit in several early discussions of "gating currents" (*Hodgkin* and *Huxley,* 1952c; *Armstrong* and *Bezanilla,* 1973; *Keynes* and *Rojas,* 1973, 1974; *Meves,* 1974; *Almers,* 1975). If one knew the charge displacement, q, necessary to open one sodium channel, as well as the number of channels per unit area, N, one could predict the total gating charge due to sodium channels and compare it with measurements of maximal charge displacement, Q_{max}, at saturating depolarizations. Though q is not known with certainty, it seems intuitively that its value should be related to the steepness with which sodium conductance depends on membrane potential. To be more precise, we define

$$K_{Na} = g_{Na}/(g_{Na,max} - g_{Na}) \tag{10}$$

where g_{Na}, the sodium conductance, is assumed to be proportional to the number of open sodium channels, $g_{Na,max}$ being the value with all channels open. K_{Na}, the ratio of open to closed channels, can be looked upon as a voltage-dependent equilibrium constant, and we define $d(\ln K_{Na})/dV$ as the *logarithmic potential sensitivity* of the sodium channel. If the channel were a two-state, open/close system and Boltzmann's principle applied, one would find (*Hodgkin* and *Huxley,* 1952c) that

$$d(\ln K_{Na})/dV = q/kT \tag{11}$$

where V is the membrane potential and k and T Boltzmann's constant and absolute temperature. At potentials so negative that $g_{Na} \ll g_{Na,max}$, g_{Na} then grows exponentially with V:

$$g_{Na} = \text{const} \cdot \exp qV/kT \tag{12}$$

At these potentials, peak sodium conductance grows e-fold within 4 mV [9] (*Hodgkin* and *Huxley*, 1952a; *Chandler* and *Meves*, 1970b), suggesting by Eqs. (11) or (12) that q = 6 elementary charges. Therefore if N is taken as 533 channels/μm^2 (*Levinson* and *Meves*, 1975), one would expect that Q_{max} = 3200 e/μm^2, nearly twice what is observed (1882 e/μm^2, *Keynes* and *Rojas*, 1974; 1600 e/μm^2, *Meves*, 1974). In English squid, therefore, there is barely enough (or not enough) charge movement to account for the estimated number of channels and the assumed q = 6e. One might conclude that what asymmetric charge displacement one *does* observe must be entirely due to the gating of sodium channels.

Besides the accuracy of N, one can question the implausibly simple model on which Eqs (11) and (12) rest, as well as the applicability of Boltzmann's principle to what is probably a field-dependent conformational change of a macromolecule. However, the treatment can be generalized to some degree. Suppose the sodium channel, a macromolecule, is in state a_0 at the resting potential and upon depolarization undergoes a sequence of transitions through various states a_k until it reaches state b_0 and opens:

$$a_0 \underset{}{\overset{q_0}{\rightleftharpoons}} a_1 \overset{q_1}{\rightleftharpoons} \ldots a_k \overset{q_k}{\rightleftharpoons} \ldots a_n \overset{q_n}{\rightleftharpoons} b_0 \tag{13}$$

At each transition $a_k \rightleftharpoons a_{k+1}$, the molecule suffers a change in dipole moment causing a current equivalent to the transfer to q_k electrons across the entire membrane. The charge transfer needed to open one channel is

$$q = q_0 + q_1 + \ldots + q_n \tag{14}$$

Assuming the q_k to be independent of voltage Eq. (12) follows also in this more general case. The logarithmic potential sensitivity now is no longer a constant as in Eq. (11) but grows as the potential becomes more negative, approaching Eq. (11) in the limit. Measuring the logarithmic potential sensitivity at very negative potentials may, therefore, be an approach of some general validity for obtaining a lower limit on q. This applies even if several conducting states exist. Perhaps the most serious reservation about accepting the value $q \geqslant 6e$ from *Hodgkin* and *Huxley*'s data arises from the fact that the treatment applies only at equilibrium and not, strictly speaking, to the peak sodium conductance. It would, therefore, be interesting to reevaluate the limiting logarithmic potential sensitivity of the sodium channel using axons where inactivation

[9] Recently, *Keynes* and *Rojas* (1974) have reported that conductance grows e-fold within "just over 6 mV". However, in absence of sufficient information as to how this value was obtained, *Hodgkin* and *Huxley*'s (1952a) well-documented result is preferred here.

of sodium channels has been prevented or greatly slowed by treatment with pronase (*Armstrong* et al., 1973) or N-bromoacetamide (*Oxford* et al., 1976). Voltage dependence of steady-state sodium conductance in such axons has been studied (*Oxford* and *Yeh*, 1977) and the preliminary report suggests that it is no less steep than that of the parameter, $m^3(V)$, used by *Hodgkin* and *Huxley* (1952c) to describe sodium channel behavior in intact axons. Thus, one may tentatively accept 6e as the lower limit for the gating charge necessary to open a single sodium channel.

Table 2 compares values of Q_{max} and N collected from the literature. The value of Q_{max}/N may be viewed as an estimate of q assuming the entire asymmetry transients to be pure "gating current". Q_{max}/N varies from 3 to 11 elementary charges in squid axons, and is given as 9–17 e in the node of Ranvier. In no case have the measurements necessary to determine N and Q_{max} been carried out on the same fiber, and for this reason alone the large variation in Q_{max}/N is not surprising. In future studies, it may be possible to measure Q_{max} and N on the same axon using the highly radioactive saxitoxin which has recently become available (*Ritchie* et al., 1976). Such measurement may diminish the variability. The values shown in Table 2 are, nevertheless, consistent with the estimated $q \geqslant 6e$. Such agreement, though not very convincing at present, is a potentially powerful argument for identifying asymmetric displacement currents with "gating currents". With $q \geqslant 6e$ and $N = 554$, the observed charge displacement must be entirely due to sodium channel gating. Anyone doubting this would have to explain why the large charge movement expected for gating does *not* form the major part of that actually observed.

3. Block of Gating Currents and Sodium Channels

Perhaps the most direct and convincing information about the physiologic role of asymmetric displacement currents would be obtained by comparing these currents in the same membrane, first with, and then without, sodium channels. Unfortunately, this seems at present impossible, at least in natural excitable membranes. The next best approach is to look for ways to block both sodium channels and asymmetric displacement currents. In such an endeavor, we might expect at the outset that (1) the most interesting treatments will be those that are specific in that they act only on sodium channels, and (2) effects on gating currents will usually be smaller than, and only in exceptional cases equal to, those observed on sodium conductance. [In Eq. (13) on p. 131, for instance, one would need to interfere only with the final transition in order to prevent the channel from opening.] Expectation (2) seems so fully confirmed by experiments that it may be stated as a general rule. Membrane depolarization, local anesthetics, and ultraviolet irradiation all inhibit sodium currents more

Table 2. Maximal charge displacement and number of sodium channels in nerve

	N (μm^{-2})	Q_{max} (electrons/μm^2)	Q_{max}/N (# of electrons per channel)	References
Squid axons				
Loligo pealei (North American)		1200 (1) 2000 (2)		(1) *Armstrong* and *Bezanilla* (1975) (2) *Bezanilla* and *Armstrong* (1976)
L. forbesi or vulgaris (European)	300–600 (3) 554 (4) 220–330* (7)	1600 (5) –1882 (6)	8.6–2.7	(3) *Keynes* et al. (1975) (4) *Levinson* and *Meves* (1975) (5) *Meves* (1974) (6) *Keynes* and *Rojas* (1974) (7) *Conti* et al. (1975)
Node of Ranvier				
Frog sciatic nerve	1300–2000* (8)	17200 (9)	13.2–8.6	(8) *Conti* et al. (1976) (9) *Nonner* et al. (1975)
Rat sciatic nerve	12000 (10)			(10) *Ritchie* and *Rogart* (1977)

N, the number of channels, was obtained from measurement of tetrodotoxin binding except for asterisked values which are from noise analysis. Noise analysis gives the conductance (γ) of single sodium channels; the number of channels is obtained by $\bar{g}_{Na}/\gamma$ or $\bar{g}_{max}/\gamma$ where $\bar{g}_{Na}$ is the maximum sodium conductance obtained by the analysis of *Hodgkin* and *Huxley* (1952c) and g_{max} the largest conductance actually measured. The former is about 50% larger than the latter, hence, the uncertainty in the asterisked values. In the node of Ranvier, a nodal area of 50 μm^2 was assumed.

than displacement currents and, to mention an extreme case, the highly specific drug tetrodotoxin blocks sodium channels entirely without measurable effect on displacement currents (*Armstrong* and *Bezanilla*, 1974).

Effects of Zn^{2+} and other "gating current" blockers. Internally applied Zn^{2+} blocks sodium currents (*Begenisich* and *Lynch*, 1974, 10 mM) and asymmetric displacement currents nearly completely (*Bezanilla* and *Armstrong*, 1974, 10 mM; *Meves*, 1976, 3.3 mM), at least if the equal-and-opposite pulse protocol ist used for subtraction. The effect is reversible, and Zn^{2+} sensitivity has become, perhaps wrongly, a pharmacologic criterion for recognizing sodium channel-related displacement currents (*Meves*, 1976). As far as linking sodium channels and asymmetric displacement currents, the Zn^{2+} effect would be totally conclusive if it were known that at 10 mM concentration, Zn^{2+} interacted only with sodium channels. Unfortunately, Zn^{2+} binds to imidazole groups of many proteins, and even to some amino acids at submillimolar concentrations (*Gurd* and *Wilcox*, 1956). Therefore, Zn^{2+} may bind to and affect the dielectric properties of many membrane proteins. The Zn^{2+} effect on asymmetric displacement currents may be an expression of such nonspecific action having nothing to do with sodium channels.

A clear effect of Zn^{2+} on presumably nonspecific membrane polarization (see Fig. 12) is seen in *Meves*' (1976) experiments where a sizeable "late" displacement current always follows more than 0.1–0.2 ms after a potential step. Only about one-third of the charge carried by this component is due to asymmetric displacement current. Not only blockage of the asymmetric portion, but also halving of the large and presumably nonspecific remainder takes place with 3.3 mM Zn^{2+}. Blockage by Zn^{2+} of both asymmetric displacement current and sodium channel is, therefore, not in itself conclusive evidence that the two are related. It would be useful to have more evidence that both Zn^{2+} effects are related.

Even if this evidence were available, however, it would still be unclear whether or not Zn^{2+} affected nonspecific membrane polarization equally at all potentials. Only if this were known to be true could one use block by internal Zn^{2+} as a pharmacologic criterion for separating nonspecific polarization from gating currents in the way that tetrodotoxin has been used to separate leakage from currents through the sodium channel. Similar considerations may apply to block of sodium channels and asymmetric displacement current by glutaraldehyde (*Meves*, 1974) or ultraviolet irradiation (*Fox* et al., 1976). Treatment with internal pronase also diminishes both gating currents (*Bezanilla* and *Armstrong*, 1976) and sodium currents; due to pronase effects on inactivation (*Armstrong* et al., 1973), it is difficult to decide whether the enzyme affects one more than the other. Regarding effects on dielectric membrane properties, very little

is known about these treatments, and in the absence of evidence to the contrary, one may expect that glutaraldehyde, ultraviolet irradiation, and perhaps also pronase modify many kinds of membrane proteins.

Membrane depolarization. Bezanilla and *Armstrong* (1974) found that membrane depolarization lasting more than about 1 ms reversibly diminishes "gating current", just as it diminishes sodium current. Depolarization is said to cause partial immobilization of the polar membrane constituents responsible for "gating currents". Recovery from this *charge immobilization* occurs with time constants of 4–8 ms at 8°C and – 70 mV (*Armstrong* and *Bezanilla,* 1975). The parallel between charge immobilization and the inactivation of sodium channels seems obvious, and recently *Armstrong* and *Bezanilla* (1977) report that both processes, as well as their reversals, occur with identical time courses over a wide range of membrane potentials. Pronase treatment (*Armstrong* et al., 1973) prevents not only sodium channel inactivation but also the immobilization process (*Bezanilla* and *Armstrong,* 1976). Despite possible reservations about such a nonspecific agent as pronase (*Takashima* et al., 1975), these results provide kinetic and pharmacologic evidence that the immobilizing effect of depolarization is closely related to the inactivation of sodium channels.

Charge immobilization is not predicted by the Hodgkin-Huxley model, and *Rojas* and *Keynes* (1975), *Rojas* (1976) and *Keynes* (1977) have not accepted the finding. They had worked mainly with short pulses and, consequently, did not notice the effect in their experiments. There are two published reasons for their disbelief; one a criticism, the other a seemingly conflicting experimental result. The criticism is implicit in a discussion by *Rojas* (1976), who raised the possibility that the charge deficit at the end of a long depolarizing pulse appears because the subtracted currents at the end of a control pulse are too large, and not because the charge returning at the end of the test pulse is genuinely less than that at the beginning. The experimental result (*Keynes* et al., 1974) was obtained on fibers which were held for minutes at positive potentials, and should have suffered maximal charge immobilization, but still displayed asymmetric displacement currents of similar size and time course as in polarized fibers. Both points have now been convincingly settled. Unlike conventional gating currents, the asymmetric displacement currents in depolarized fibers are not blocked by internal Zn^{2+} (*Meves,* 1976) and have been observed only with equal-and-opposite pulse protocols starting from depolarized potentials. These currents may thus have an origin different from that of the usual "gating currents", and their presence, though interesting, does not conflict with charge immobilization. The criticism of *Rojas* (1975) has been answered by an elegant experiment of *Meves* and *Vogel* (1977a)

which is reproduced in Figure 13. The figure shows the result of subtracting two *off*-transients, one ($I_{off,\ 1\ ms}$) during a brief (1 ms) pulse, the other ($I_{off,\ 10\ ms}$) during a longer (10 ms) but otherwise identical pulse. An inward transient remains after the subtraction, indicating a displacement current deficit which must have developed during the last 9 ms of the longer pulse.

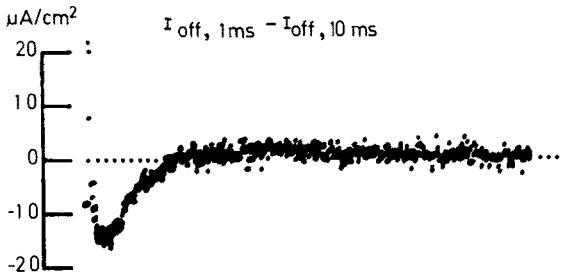

Fig. 13. Immobilization of gating charge by depolarization. The trace is the difference between *off*-transients following pulses from − 70 to + 20 mV lasting 1 and 10 ms. The inward current shows that *off*-charge after the 10-ms pulse was less than after the 1-ms pulse. Ionic channels blocked by TTX and TEA^+. Squid axon, 9°C. From *Meves* and *Vogel* (1977a)

Charge immobilization has now been observed in squid giant axons (*Bezanilla* and *Armstrong*, 1976; *Meves* and *Vogel*, 1977a; *Cahalan* and *Almers*, in preparation) and in the node of Ranvier (*Neumcke* et al., 1976; *Nonner* et al., as communicated by *Rojas*, 1976). The finding may be regarded as well established, even though it does not fit into the Hodgkin-Huxley model. However, the kinetic correlation between sodium channel inactivation and charge immobilization is still disputed. *Meves* and *Vogel* (1977a) report that sodium channel inactivation at + 20 mV occurs three times faster than the immobilization process. It seems extremely important that agreement be reached on the matter. The close kinetic and pharmacologic parallelism between the two processes reported by *Armstrong* and *Bezanilla* is the strongest evidence yet for linking displacement currents with sodium channels. If it is confirmed, at least one-half or two-thirds of the asymmetric displacement current in squid axons must be gating current, since up to one-half (*Meves* and *Vogel*, 1977a; *Armstrong* and *Bezanilla*, 1977, their Fig. 10) or two-thirds (*Bezanilla* and *Armstrong*, 1976) of the charge carried by these currents is immobilized, the extent of immobilization depending somewhat on pulse protocol. In the node of Ranvier, nearly two-thirds of the asymmetric charge displacement is subject to immobilization (*Rojas*, 1976).

Long-lasting membrane depolarization. Depolarizing the membrane for periods of 1 min or more produces an inactivation or block of sodium channels which differs from that described by *Hodgkin* and *Huxley* (1952c) in that recovery takes minutes instead of milliseconds. Asymmetric displacement currents are also blocked almost completely under that condition (*Armstrong* and *Bezanilla,* 1974; *Meves* and *Vogel,* 1977b) and there is a suggestion that after repolarization they recover at about the same rate as sodium currents. This again might suggest that the two effects are linked, but the point cannot be pressed as there is no quantitative comparison of time courses. It is interesting that K^+ channels also show slow inactivation (*Ehrenstein* and *Gilbert,* 1966), as do asymmetric displacement currents in frog skeletal muscle (*Chandler* et al., 1976b; *Adrian* and *Almers,* 1976b) although they are for the most part unrelated to sodium channels. Membrane depolarization lasting minutes, therefore, appears to affect many different membrane constituents. It is not clear whether such depolarizations also affect displacement currents at potentials outside the range where gating of sodium channels is thought to occur. Figure 4 of *Meves* (1976) suggests that they do, but no such effect is apparent in Figure 5 of the same paper. *Rudy* (1976) reports an effect probably related to that of long-lasting depolarization. In *Myxicola* axons, both sodium and asymmetric displacement currents are diminished by repetitive stimulation.

4. Contamination of Gating Currents and Its Dependence on Pulse Protocol

Among the arguments for identifying a large portion of the asymmetric displacement currents with sodium channel gating, two are particularly compelling. (1) Given the potential sensitivity of sodium conductance and the number of sodium channels, "gating currents" are expected on theoretical grounds to carry a charge of similar magnitude as the observed asymmetric displacement currents. (2) Relatively brief membrane depolarization inactivates sodium channels and immobilizes the molecular components giving rise to asymmetric displacement currents. The pharmacologic and kinetic similarities between the two processes strongly suggest that they are linked and, therefore, that asymmetric displacement currents are closely related to the events which open and close sodium channels. Taken together, these arguments provide strong support for a view which is no longer seriously challenged: that in nerve a large part of the observed asymmetric displacement currents are "gating currents" in that they arise from the molecular rearrangements necessary for the opening and closing of sodium channels. Theoretically expected contributions from other potential-dependent membrane components, such as potassium channels, are discussed later; they are probably small and have not yet been identified.

This may be sufficient to allow many useful qualitative studies of gating currents. Before one can go much further, however, one will need to know precisely which portion of the asymmetric displacement currents are gating currents. So far, our methods for recording these currents rely on a subtraction, and ultimately on the fact that displacement current transients at different potentials will carry different charge and relax with different kinetics. Unless sodium channels are the only source of this membrane nonlinearity, all gating current records will be contaminated by polarization of unrelated, nonspecific polar membrane constituents. It is not yet possible to study these in isolation. However, after block of gating currents in their usual form by permament membrane depolarization or treatment with internal Zn^{2+}, one can still record asymmetric displacement currents if one adds currents during equal-but-opposite pulses starting from potentials positive to -30 mV (*Keynes* et al., 1974; *Meves*, 1974, 1976). These displacement currents in depolarized fibers have no known physiologic significance, but their presence suggests that even without sodium channels, the squid axon membrane would behave as a dielectric which is neither linear nor loss-free. Thus one expects that gating currents will in general be contaminated. Two kinds of contamination are discussed here; *charge contamination* and *kinetic contamination.*

Charge contamination is expected whenever nonspecific membrane proteins contribute to the variation of static capacity with voltage. The effect is illustrated in the hypothetical example of Figure 14. The figure shows as a function of voltage the static membrane capacity (solid line), as well as the (in reality unknown) polarizability of the membrane in the absence of sodium channels (dotted line). The charge displacement caused by a pulse from V_1 to V_2 is given by the integral of the curve with respect to voltage between V_1 and V_2. The stippled area is assumed to represent the charge carried by gating currents during a pulse from -70 to $+30$ mV, namely, 16.5 nC/cm^2, and Figure 14 explores how accurately this charge would be obtained with the various pulse protocols given next to each graph. In Figure 14a the P/4 procedure is used with a control pulse from -190 to -165 mV which causes the charge displacement given by the dashed area. After four times this charge is subtracted from the charge displacement during the test pulse there remains an asymmetric charge displacement of 27.4 nC/cm^2 which exceeds the gating charge by 67%, namely, the amount contained in the white section beneath the dotted curve. In Figure 14b the control pulse is taken at less negative potentials, and the asymmetric charge displacement is 19.6 nC/cm^2, exceeding the gating charge by only 20%. Charge contamination is less in this example. In Figure 14c the equal-and-opposite procedure is used, giving an asymmetric displacement charge of 22.7 nC/cm^2, 38% too much.

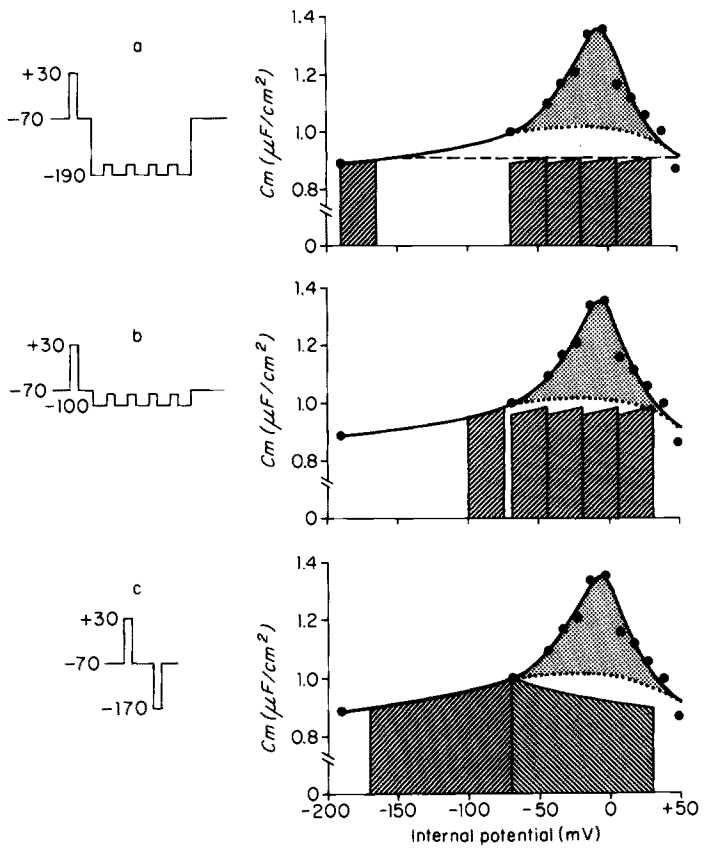

Fig. 14 a–c. Charge contamination with three pulse protocols as shown on the left of the figure. Graphs on the right show three capacity/voltage curves: total capacity (*solid*, taken from Fig. 12) and possible hypothetical curves in the absence of sodium channels (*dotted* and *dashed*). *Shaded areas* to the left of − 70 mV represents the charge carried by one control pulse. The *blank* and *stippled areas* under the solid curve between − 80 and + 30 mV represents the charge carried by displacement current. The more negative the average potential during the control pulse, the larger the asymmetry charge; it is unknown, however, whether the additonal charge becoming visible in this way is due to gating currents

It must be emphasized that the voltage-dependence of nonspecific polarizability is unknown. If it were described by the horizontal dashed line instead, then the gating charge displacement carried by the 100 mV test pulse would be 26.4 nC/cm^2. The pulse protocol of Figure 14a would then give a correct answer, wehreas b and c would give asymmetric charge displacements which are too low. [*Some* charge displacement arising in sodium channels during pulses negative to − 70 mV seems likely because *Armstrong* and *Bezanilla* (1974) find that sodium channel opening is delayed when a hyperpolarization precedes the depolarizing pulse.]

The example shows that the more negative the average potential during the control pulse, the larger the asymmetric charge displacement. Very negative control pulses will ensure that virtually the entire gating current survives the subtraction, but they will also tend to increase charge contamination. Less negative control pulses will minimize contamination but may "hide" part of the gating current and severely distort the relationship between gating charge and membrane potential (see *Adrian* and *Almers*, 1976b). It is at present difficult to decide upon an optimal control pulse regime, and the many different protocols used in the literature may have contributed their share to the variability of published results.

Kinetic contamination is expected if the kinetics of nonspecific membrane polarization show potential-dependence. It can occur even if there is no voltage dependence of static membrane capacity other than that due to sodium channel gating. To illustrate the effect, Figure 15 shows capacitive transients and asymmetric displacement currents in a hypothetical membrane without sodium channels and with a constant static capacity. It is merely assumed that the relaxation time constants vary with membrane potential, being 40 μs at potentials negative to -150 mV and otherwise 60 μs. In this example, there is an asymmetric displacement current during, but not after the pulse; the time integral during the pulse is zero. Such effects, when superimposed on gating currents, could distort their kinetics even though they may not alter the charge displacement. Again, the danger of kinetic contamination is largest when control and test pulses span widely different voltage ranges. There is no documented example of

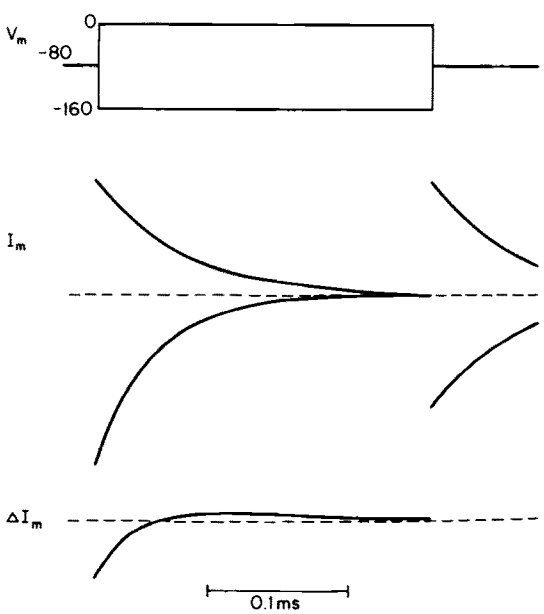

Fig. 15. Kinetic contamination of gating currents. *Top*, voltage; *middle*, membrane currents in a hypothetical membrane without sodium channels; *bottom*, asymmetry current

kinetic contamination; however, the effect would be hard to detect unless one has prior knowledge of the gating-current kinetics.

5. Conclusions

Although there is good reason to believe that a large portion of the asymmetric displacement currents in squid giant axons is gating current, it appears equally likely that some of it is not. We expect the contamination of gating-current records to depend on pulse protocol as well as on other unknown factors. However, in assessing the "purity" of gating currents, the best one can do at present is to take the portion which is resistant to, say, a 10-ms depolarization, as an upper limit for the contribution of nonspecific polar membrane constituents. On this basis, the degree of contamination varies appreciably from axon to axon and also between the two groups of investigators who have studied charge immobilization (*Armstrong* and *Bezanilla,* 1977; *Meves* and *Vogel,* 1977a). The possibility of varying degrees of contamination must be taken into account when comparing seemingly discordant results. A definitive and complete description of the relationship between gating currents and sodium-channel gating will depend on obtaining uncontaminated records of gating currents, or, at least, on being able to correct for existing contamination. Unfortunately, study of gating currents in isolation may not be possible until we learn to isolate sodium channels from excitable tissues and incorporate them into artificial lipid bilayers.

B. Gating Currents and Sodium Channels

This section explores some of the conclusions that would follow if two-thirds or all of the observed asymmetric displacement current were gating current of the sodium channel.

1. Time Courses of Sodium and Gating Currents

In *Hodgkin* and *Huxley*'s (1952c) model, sodium conductance, g_{Na}, is desribed by

$$g_{Na} = m^3 h \cdot \bar{g}_{Na} \tag{15}$$

where $\bar{g}_{Na}$ is the conductance expected when all channels are open, and m and h are voltage-dependent parameters which adjust with first-order kinetics to changes in membrane potential. The parameter m rapidly increases, and h slowly decreases upon depolarization. In a physical inter-

pretation of the Hodgkin-Huxley model, one imagines that there are two gates on each sodium channel. The first, sometimes called "m" or "activation gate", responds quickly, opening during depolarization and closing upon repolarization. The second, called "inactivation-" or "h-gate", responds more slowly, closing upon depolarization and opening again after repolarization. Operating in series, the two gates produce the transient increase in sodium conductance observed after depolarization. Activation and inactivation can be separated kinetically and pharmacologically (e.g., by treatment with intracellular pronase). In this section we will concentrate on the activation process as it appears in studies with brief (< 1 ms) depolarization.

In the most popular physical interpretation of *Hodgkin* and *Huxley*'s description of the activation process, it is imagined that a sodium channel becomes conducting after three subunits, the "m-particles", have made independent first-order transitions from the resting to the activating position, and closes as soon as the first m-particle has reverted to the resting position. Since in this model the sodium channel derives its voltage dependence entirely from that of the m-particles, all gating current should be due to them. Gating and sodium currents seen upon depolarization (see Fig. 3) seem qualitatively consistent with this idea; much gating charge moves before the first channels open, perhaps due to movement of the first two m-particles. However, the model does not correctly describe the events following repolarization. The model predicts that a channel should close as soon as the first m-particle has reverted to the resting position, but continue to generate gating current until all three m-particles have returned. Gating current should, therefore, last approximately three times longer than the sodium current "tail". Instead, both decline at almost exactly the same rate in Figure 3. Also, gating currents often cannot be described by single exponentials, and depend in time course and amplitude on factors other than the potential at which they are measured (*Armstrong* and *Bezanilla*, 1974; *Keynes* and *Rojas*, 1974; *Meves*, 1974).

Keynes and *Rojas* (1976) have recently reinvestigated the properties of sodium and gating currents and have concluded that they are consistent with the Hodgkin-Huxley model. However, some of their own data (their Table 4) conflict with this conclusion: While gating currents outlast sodium currents after repolarization to very negative potentials, those recorded at more physiologic potentials behave as in *Armstrong* and *Bezanilla*'s work (1974). The matter was taken up again by *Neumcke* et al. (1976). In a careful investigation of gating currents and sodium currents in the node of Ranvier, they showed convincingly that for any fixed exponent of (m) in Eq. (15), there is no first-order parameter which will describe both gating currents and sodium conductance changes in the manner demanded by the equation. Equation (15) fails both kinetically

and in the description of the steady state (see also *Meves,* 1974). Not even the sodium conductance data on their own (*Frankenhaeuser* and *Hodgkin,* 1957; *Armstrong* and *Bezanilla,* 1974; *Neumcke* et al., 1976) are always consistent with a constant exponent in Eq. (15). Therefore, one of the first definite insights to emerge from the study of gating currents is a negative one: Any literal physical interpretation of the Hodgkin-Huxley equations for sodium conductance is now ruled out. The sodium channel cannot derive its voltage dependence solely from three, or any other constant number of, independent subunits obeying first-order kinetics. Remembering that the Hodgkin-Huxley equations were never intended to be more than a mathematically convenient formalism designed for reconstructing action potentials, it now seems that theoretical considerations based on a literal physical interpretation of these equations are unlikely to be helpful.

While there is no evidence for independent gating subunits, the delayed increase in sodium conductance after a step depolarization indicates at once that the channel molecule must undergo a sequence of transitions before it can open. The portion of the gating current preceding the sodium current in Figure 3 (left) undoubtedly arises from some or all of these preliminary transitions. Upon repolarization, this sequence of transitions presumably is driven backwards. The identical time course of gating and sodium currents at -70 mV suggests that the channel-closing transition at that potential is the rate-limiting step for the backward reaction.

No other conclusions can be reached at present regarding the gating of sodium channels during and after brief depolarizations. Early studies gave the misleading impression that within experimental error, gating current transients can be fitted by single exponential functions. The discouraging implication of such a conclusion might be that our methods are insufficient to reveal the complexities one might expect from the kinetics of sodium conductance changes. As methods have improved, however, it became clear that the time course of gating current transients is far from featureless. Some of these features are reviewed below.

On-transient (following step depolarization). (1) The transient shows an initial rising phase extending over more than 50–100 μs. All data and discussions regarding this effect are due to *Bezanilla* and *Armstrong* (1975) since other authors could not record the initial time course reliably. The rising phase is most pronounced if control pulses start from relatively positive potentials, a fact which led to the early suggestion (*Armstrong* and *Bezanilla,* 1973, 1974) that a rapid component of gating current flows during the control pulse. However, the rising phase is seen to some extent even when control pulses are restricted to the most negative potential ranges which are experimentally practicable. The origin and significance of the rising phase are unknown. (2) At very positive (> 20 mV) potentials,

transients show a slow and a rapid phase. The effect has been observed on squid axons with the P/4 procedure (*Bezanilla* and *Armstrong,* 1975), as well as on the node of Ranvier with the equal/opposite method (*Neumcke* et al., 1976). It may be associated with the transition of sodium channels from one open state into another (see later). *Keynes* and *Rojas* (1974, 1976) and *Meves* and *Vogel* (1977a) report no evidence of two components in the *on*-transient.

Off-transient. Off-transients cannot be described by single exponentials (*Meves* and *Vogel,* 1977a) and vary in their time course with duration and amplitude of the preceding depolarization (see Fig. 16). Some of these effects are undoubtedly related to the charge immobilization occurring during maintained depolarization. In addition, *off*-transients, like *on*-transients, often show a rising phase (Fig. 16).

The complicated time course of gating currents promises to provide much information about sodium channels in the future. As methods improve it may become possible to make quantitative comparisons of sodium conductance changes and gating currents. This can be done on the same axon, as *Keynes* and *Rojas* (1976) and *Neumcke* et al. (1976) have shown; measurements of sodium conductance in the absence of TTX can be followed by measurements of gating currents in the presence of the drug. It will be important to record the entire gating current transient, and to avoid discarding information during the first 20–100 μs after a potential step, as was done in all early studies. Data acquisition problems caused by the rapid capacitive charging transient can be overcome most conveniently by subtracting it out at an early stage using an analogue "transient generator" (*Armstrong* and *Bezanilla,* 1975).

2. Immobilization of Gating Charge and Its Relation to Inactivation of Sodium Channels

The following discussion concerns the second voltage-dependent gating process, namely the depolarization-induced closing (inactivation) of sodium channels (*Hodgkin* and *Huxley,* 1952b). Once again, a literal interpretation of the Hodgkin-Huxley formalism is the most convenient starting point although the model does not account for all sodium conductance inactivation data (*Goldman* and *Schauf,* 1972; *Chiu,* 1976). Since inactivation is just as steeply potential-dependent as the activation process, one might expect there to be gating currents associated with the inactivation gate carrying as much charge as those associated with activation. These displacement currents may be small at physiologic potentials since inactivation is a comparatively slow process. At extreme potentials, however, inactivation can proceed or be reversed within milliseconds, and the

currents generated by the hypothetical "inactivation gate" should be large enough to measure. Even so, displacement currents of time course similar to inactivation have never been observed. Therefore, it seems reasonable to suggest, as *Armstrong* and *Bezanilla* (1977) have done, that there are no separate "inactivation gating currents", at least not in the presence of TTX where all relevant measurements have been carried out.

Instead, maintained depolarization immobilizes the gating mechanism as shown in Figures 13 and 16. The longer the depolarization, the smaller are the gating currents and the charge carried by them after repolarization. Up to two-third of the total charge can be immobilized in this way (*Bezanilla* and *Armstrong*, 1976). When repolarized, the fiber recovers from immobilization within some 10 ms at − 70 mV (*Armstrong* and *Bezanilla*, 1975). As soon as the gating machinery is freed from the immobilizing influence, it is thought to return to the resting configuration, carrying its full complement of charge. At − 70 mV and the usual low temperatures, this happens very slowly, so the delayed charge movement does not generate currents large enough to be visible. At − 150 mV, however, the rate of recovery is so rapid that remobilized gating units returning to rest can generate a slow but prominent transient inward current (*Armstrong* and *Bezanilla*, 1977). I will call this slow transient the "remobilization transient" because its time course is thought to be determined by the rate at which gating units escape from the immobilizing influence. Charge movements during and after a long depolarization are thought to be about equal if the remobilization transient is included in the measurement.

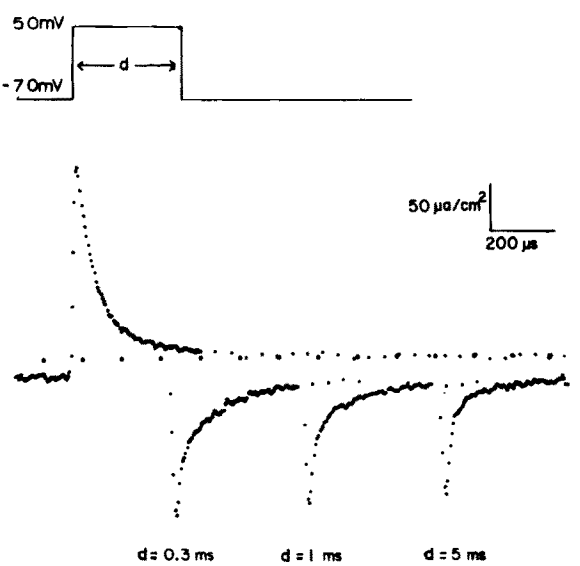

Fig. 16. Effect of pulse duration on the *off*-gating current transient. Test pulses as shown in the inset; pulse duration is given beneath each *off*-transient. Subtraction pulses were four times smaller and started at − 170 mV. Ionic channels blocked with TTX and internal cesium. Squid axon, 8°C. After *Bezanilla* and *Armstrong* (1975)

According to *Armstrong* and *Bezanilla* (1977), there are many parallels between charge immobilization and inactivation. (1) At positive internal potentials, the time courses of the two processes coincide. (2) At − 70 mV, recovery from the two effects occurs with the same time course. (3) At − 150 mV, the remobilization current decays with a time constant similar to recovery from inactivation at that potential. (4) When the duration of a depolarizing pulse is varied, the charge carried by the remobilization current increases with pulse duration at a rate similar to that of inactivation during the pulse. (5) The steady-state potential-dependences of charge immobilization and inactivation approximately coincide if one corrects for the immobilization-resistant portion of the charge movements. (6) Treatment with internal pronase prevents both inactivation and charge immobilization. These results provide strong evidence that inactivation and immobilization are related. They indicate that at any moment in time the amount of immobilized charge is proportional to the number of inactivated sodium channels. Based on these findings, *Armstrong* and *Bezanilla* (1977) have proposed the following sequence of events for inactivation of sodium channels and their recovery:

Depolarization. (1) Sodium channels undergo a sequence of three transitions through closed states x_5 to x_2 until, after a fourth transition, they reach the first open state, x_1. These transitions are accompanied by dipole moment changes generating the fast component of *on*-gating current. This first open state cannot inactivate. (2) The channel undergoes a fifth transition to a second conducting ("wide open") state, $x_1 y$; this produces the slow component of *on*-gating current [10]. (3) The inner channel opening is now large enough to accomodate a blocking particle waiting near-by. The blocking particle inserts itself into the inner channel mouth, plugging it up and producing the nonconducting inactivated state, $x_1 yz$. Transitions between $x_1 y$ and the blocked state $x_1 yz$ are thought not to be measurably voltage dependent because no gating current has been found which could be associated with this transition. (4) Presence of the blocking particle in the pore "stabilizes" the channel in the inactivated state $x_1 yz$. In *Armstrong* and *Bezanilla*'s (1977) model, the particle is positively charged, and stabilization occurs by electrostatic interaction between particle and gating machinery. If the charge move-

[10] The concept of this second open state $x_1 y$ is new. In particular, state $x_1 y$ is not identical to the inactivation-resistant open state observed at extremely positive potentials by *Chandler* and *Meves* (1970b) and *Bezanilla* and *Armstrong* (1977). If this inactivation-resistant state is included, the total number of open states in *Armstrong* and *Bezanilla*'s (1977) model would be three. There are so far no known features of gating current which are associated with the extreme state postulated by *Chandler* and *Meves* (1970b).

ment accompanying the transition from rest to state $x_1 y$ is visualized as being due to the movement of an "equivalent gating dipole", it is easy to imagine how such a gating dipole, having turned its negative end towards the axoplasm, could create a more favorable environment for a positively charged blocking particle.

Repolarization. (1) Equilibrium now strongly favors the resting state. The channel begins to close and, in closing half-ways, generates the immobilization-resistant component of *off*-gating current. (2) Complete recovery cannot occur, however, as long as the blocking particle is stuck inside the channel and has stabilized it in the depolarized configuration. In other words, electrostatic interaction between blocking particle and channel has erected a large energy barrier in the reaction path to the resting configuration. "Immobilization" has occurred. Return to rest is so slow that the accompanying gating currents, though carrying normal charge, are too small to see. "Remobilization" gating current can be seen easily only at very negative potentials. (3) Having adjusted itself slowly to the resting potential, the equivalent gating dipole has turned its positive end towards the axoplasm, and the inner channel mouth is no longer a stable position for the blocking particle. Dissociation occurs and the channel can quickly complete its return to rest.

Some previously unexplained findings are qualitatively consistent with the model. (1) Inactivation of sodium channels proceeds with a delay (*Bezanilla* and *Armstrong,* 1977), which may result because inactivation cannot occur before the channel reaches state $x_1 y$. (2) The rate of inactivation seems to reach a limiting, maximal value at positive potentials (squid axon: *Hodgkin* and *Huxley,* 1952; frog node of Ranvier and frog muscle: *Hille* and *Campbell,* 1976). This would be expected if the voltage-independent entry of a blocking particle into the channel became rate limiting. (3) After a depolarization too brief to produce substantial inactivation, closing of channels at the resting potential depends on pulse duration, being slower after the longer pulse (*Hodgkin* and *Frankenhaeuser,* 1957). This might be expected if a channel in state $x_1 y$ must transform into the other open state x_1 before it can close.

In the future, *Armstrong* and *Bezanilla*'s (1977) model should be tested both experimentally (*Meves* and *Vogel,* 1977a, were unable to confirm the temporal correlation between inactivation and charge immobilization) and theoretically (a quantitative test of the model has not yet been made). The complicated effect of calcium on activation and inactivation (*Frankenhaeuser* and *Hodgkin,* 1957) might well be particularly interesting in future studies of sodium channel gating.

3. Pharmacologic Findings

Much has been learned from pharmacologic studies of sodium currrents, and similar investigations of gating currents will be no less rewarding. At present, however, the accuracy with which gating currents can be recorded is limited, and subtle pharmacologic effects are likely to escape detection. Therefore, interesting early attempts to investigate the effects of pH (*Rojas,* 1975) and calcium (*Keynes* and *Rojas,* 1974) sould be repeated with improved methods. The insecticide DDT slows the closing of open sodium channels (*Hille,* 1968) without striking effects on gating currents (*Dubois* and *Bergman,* 1977). Perhaps the clearest results have been obtained with some sodium channel blockers, and these are reviewed below.

Lack of effect of tetrodotoxin and related compounds. Tetrodotoxin (TTX), saxitoxin (STX), and maculotoxin (MTX) are cationic compounds with molecular weights less than 540 which block sodium channels at nanomolar (STX, TTX) or less than micromolar (MTX, *Gage* et al., 1976) concentrations. None of them have noticeable effects on gating currents (TTX, 0.3 μM: *Armstrong* and *Bezanilla,* 1974; MTX, 0.02 mM: *Armstrong* et al., as cited by *Gage* et al., 1976; STX, 0.3 μM: *Keynes* and *Rojas,* 1974). Both TTX and STX bear a (positively charged) guanidinium group which is thought to insert itself into the outer channel mouth (*Hille,* 1975), thereby blocking ion flow through the channel. Maculotoxin is now thought to be identical to tetrodotoxin (*Sheumack* et al., 1978). Within experimental error, the otherwise extremely potential-sensitive gating portion of the sodium channel evidently fails to notice the insertion of these molecules with their positive charge. The qualification "within experimental error" is necessary because the potential dependence of gating currents is very much less steep than that of sodium conductance. Small changes in gating currents can accompany large effects on sodium conductance and may have gone unnoticed in the experiments of *Keynes* and *Rojas* (1974) and *Armstrong* and *Bezanilla* (1974).

Just as the gating machinery seems to remain unaware of the presence of TTX in the outer channel mouth, the TTX molecule apparently remains unaware of the state of the gating machinery. In frog skeletal muscle, membrane depolarization from -80 to -15 mV, enough to activate and inactivate virtually all sodium channels (*Adrian* et al., 1970), changes the dissociation constant between TTX and its receptor by less than 50% (*Almers* and *Levinson,* 1975). Similarly, *Ulbricht* and *Wagner* (1975) show that between -50 and -90 mV block of sodium channels is independent of the potential at which a node of Ranvier is held. Together, these findings establish a remarkable degree of molecular independence between the "gating portion" of the sodium channel and that which binds TTX.

Local anesthetics and sodium channels. Recent work suggests that local anesthetics block sodium channels at a common site inside the channel (*Strichartz,* 1973; *Courtney,* 1975; *Hille,* 1977a,b; *Schwarz* et al., 1977) which is accessible either from the axoplasm (*Strichartz,* 1973) or from a nonpolar compartment, perhaps the lipid bilayer, which will accept substances to the extent that they are lipid soluble (*Hille,* 1977b). The first experiments with local anesthetics were carried out by *Keynes* and *Rojas* (1974) who reported reduction of gating current size to one-third their normal value by 37 mM procaine. In later studies (*Almers* and *Cahalan,* 1977; and in preparation), it proved convenient to be able to alter the fraction of anesthetic-blocked channels rapidly and reversibly. This is possible with an internally applied, membrane-impermeant quaternary lidocaine derivative, QX-314, whose block of sodium conductance appears to be voltage-dependent. Block at 1 mM internal concentration is not very marked in a resting axon, but is strongly enhanced after a train of depolarizing pulses. Sodium currents can be diminished tenfold in this manner. Figure 17 shows gating currents before and after a train of depolarizing pulses which normally (a) has no effect, but in presence of QX-314 reduced peak sodium conductance about tenfold (not shown). Gating charge is reduced about twofold (b); the effect is reversible.

So far, the effect is reminiscent of *Armstrong* and *Bezanilla*'s model for sodium channel inactivation; entry of a blocking particle into the axoplasmic end of the channel immobilizes gating charge movement. However, here the similarity ends. Figure 17c shows the same experiment in the presence of 0.4 μM external TTX which, according to *Armstrong* and *Bezanilla* (1977), does not affect inactivation-related charge immobilization or the recovery from this effect. Again, QX-314 is present internally. Now gating charge displacement is *always* half the normal size, regardless of whether or not a train of depolarizing pulses is given. The system, therefore, behaves as if in the presence of TTX the QX-314 is permanently present in the channel. Curiously, TTX^+ in the outer channel mouth makes the local anesthetic binding site more favorable for the positively charged QX-314. The effect may be related to the finding that external sodium antagonizes block by QX-314 (*Almers* and *Cahalan,* in preparation; *Strichartz,* unpublished) and by other compounds (e.g., strychnine; *Shapiro,* 1977a,b) as if they could remove the drug from its receptor inside the sodium channel. TTX^+ could enhance the binding of QX-314 by blocking entry of external sodium ions (*Almers* and *Cahalan,* 1977).

4. Theoretical Considerations

This section provides a discussion of the most frequently used mathematical expression for quantitative descriptions of gating currents. Some of

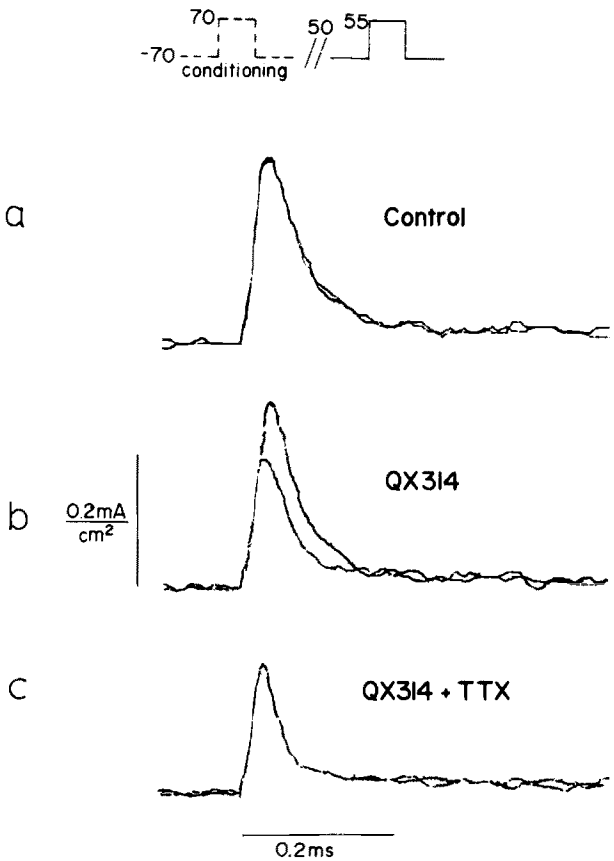

Fig. 17 a–c. Effect of internal QX-314 (1 mM) on gating current. (a) control in an external medium containing 29 mM sodium; (b) after applying 1 mM internal QX-314 to the same axon; (c) with QX-314 plus 4 x 10^{-7} M external TTX. Each panel shows two superimposed traces, one before, the other after conditioning the membrane with a train of 50 conditioning pulses to + 70 mV. Such conditioning has no effect in a normal axon (a) but produces "use-dependent inhibition" of sodium currents (not shown) and gating currents (b). Reversal of the effect takes tens of seconds. In presence of external TTX *and* internal QX-314, gating currents are always inhibited (c). Test pulse went from − 70 to 55 mV, the reversal potential for the sodium channel in this fiber. Subtraction pulses went from − 110 to − 79 mV. K^+-free, C_S^+-containing internal solution. Squid axon, 16°C. From *Almers* and *Cahalan* (in preparation)

the treatment given here follows that of *Schwarz* (1977). In addition, this section will explore some features of the potential-dependence of multistate ionic channels in terms of the charge transfer caused by gating currents.

The two-state, constant-dipole model. Starting with the most simple gating mechanism, we imagine a population of macromolecules which can exist in two states a and b, depending on the membrane potential. If the

macromolecule is an ionic channel, state a may be the closed and b the open conformation:

$$a \underset{}{\overset{\Delta\mu}{\rightleftharpoons}} b \tag{16}$$

We imagine the macromolecule to be so firmly embedded in an electrically incompressible lipid bilayer that it does not change its overall direction in the membrane; i.e., the extracellular end of the molecule will always remain extracellular, and the intracellular end always intracellular (*Schwarz,* 1977). This assumption is untested but most likely correct; inside-out transition of phospholipid molecules takes minutes to hours (e.g., *Kornberg* and *McConnell,* 1971), much longer than the times with which we are concerned, and it is difficult to see how intramembrane macromolecules, such as lipoproteins, could undergo such "flip-flop" transitions any faster. Instead we imagine that the macromolecule may change its overall dipole moment. Let $\Delta\mu$ be the component of this dipole moment change in the direction of the electric field, that is, normal to the membrane. If we define a (potential-dependent) equilibrium constant $K = A/B$ where A and B are the occupancies of states a and b, then, in general, K is a function of the Gibbs free-energy change experienced by the system during the transition. Holding pressure and temperature constant, we are interested only in the dependence of K on membrane potential. It follows from thermodynamic principles (e.g., *Schwarz,* 1967) that

$$\frac{d \ln K}{dE} = \frac{\Delta\mu\,(E)}{kT} \tag{17}$$

where E is the electric field experienced by the macromolecule and k, T have their usual significance. Usually, dipole moments are measured in Debye units, but in our case the dipole moment cannot be measured at all, if only because the distance δ over which the membrane potential V drops, and, therefore, E are unknown. Instead it is convenient to define

$$\Delta\bar{\mu}\,(V) = \frac{\Delta\mu\,(E)}{\delta} \tag{18}$$

$\Delta\bar{\mu}$ can be looked upon as the charge carried on each end of an equivalent dipole extending across the distance over which the membrane potential drops. If we measure $\Delta\bar{\mu}$ in units of charge carried across the membrane when one macromolecule makes the transition from a to b, Eq. (17) can be rewritten

$$\frac{d \ln K}{dV} = \frac{\Delta\bar{\mu}\,(V)}{kT} \tag{19}$$

In Eq. (19), $\Delta\bar{\mu}$ depends on V, and this dependence includes all interactions between the macromolecule and its environment, the transmembrane profile of the electric field and its voltage dependence, as well as other unknown factors (*Stevens*, 1977). For the present, we ignore these complications and assume for no reason other than simplicity that over the voltage range of interest, $\Delta\bar{\mu}$ (V) = constant = q. Then Eq. (19) can be integrated and one obtains

$$K = \frac{A}{B} = \exp\left[q\,(V - \bar{V})/kT\right] \tag{20}$$

where $\bar{V}$ is the potential where K = 1. This equation is formally equivalent to the Boltzmann relation and follows from first principles if the dipole moment change is assumed constant. If A and B are the fraction of molecules in states a and b, one can show from Eq. (20) and from A + B = 1 that

$$B = \frac{1}{1 + \exp\left[-q\,(V - \bar{V})/kT\right]} \tag{21}$$

With regards to asymmetric displacement currents, one can simulate the experiment in Figures 1 and 2 by taking q positive and holding the membrane potential so negative that B = 0. Since the amount of charge transferred per transition is assumed constant, the total charge transfer is given by $Q/Q_{max} = B$; here Q_{max}, the maximum charge at saturation, equals q times the number of macromolecules, N, per unit area. Eq. (21) can, therefore, be used to describe the potential dependence of charge displacement, as in the case of lipid soluble ions [Eq. (15)].

Various authors have derived identical expressions picturing permanent dipoles which flip-flop between two positions (*Meves*, 1974; *Almers* et al., 1975) or charged particles which are trapped in the membrane and redistribute themselves between the two sides (*Schneider* and *Chandler*, 1973; *Keynes* and *Rojas*, 1974). All models assume that the system has only two states and that the dipole moment change is constant. In all these models, the transition should obey first-order kinetics and asymmetric displacement currents should relax as single exponentials whose time constants depend only on the membrane potential. While the two-state, constant-dipole model provides a useful starting point for quantitative analysis, it probably does not apply to any of the excitable membranes studied so far. Equation (21) often does not provide a good fit, asymmetric displacement currents often do not follow single exponentials (*Armstrong* and *Bezanilla*, 1975; *Meves* and *Vogel*, 1977a), and their time course depends on past voltage history besides the membrane potential at which they are measured (*Keynes* and *Rojas*, 1974; *Meves*, 1974). Other models which can be explored quantitatively picture continuously (*Meves*, 1974) or discontinu-

ously (*Chiu,* 1977) rotating dipoles, or continuous electrodiffusion of charged particles across the membrane (*Neumcke* et al., 1977). Although it is often convenient to speak in terms of "equivalent gating dipoles" or the movement of charged particles, and although such models may, as mathematical equivalents, fit experimental data artibrarily well, they do not seem very realistic. When constructing models, it may ultimately seem preferable to cast them in the form of what most likely happens on a molecular level: Dipole moment changes in a macromolecule whose overall orientation in the membrane does not change.

More general two-state models. If one rejects the constant-dipole assumption, one may consider expanding $\Delta\bar{\mu}$ (V) as a polynomial:

$$\Delta\bar{\mu} = c_1 + c_2 V + c_3 V^2 + \ldots \tag{22}$$

where c_0, c_1, c_2, etc., are constants. Now Eq. (19) can be integrated. If the above series converges, as it will for sufficiently small values of V, one can approximate

$$\Delta\bar{\mu} \simeq c_1 + c_2 V \tag{23}$$

and obtain after integration of Eq. (19)

$$K = \exp\left[(c_0 + c_1 V + \frac{c_2}{2} V^2)/kT\right] \tag{24}$$

Equation (23) has been called the Debye low-field limit for a polarizable dipole (*Stevens,* 1977). Physically realizable dipoles may increase but never decrease their moment when the electric field is increased, implying that c_1, c_2 in Eq. (23) must be positive. In our case, however, one is dealing with *changes* in dipole moment, and thus the coefficients c_0, c_1 and c_2 can in principle have either sign. *Hill* and *Chen* (1972) found that Eqs. (23) and (24) provided an accurate description for the voltage dependence of the Hodgkin-Huxley parameters m, n, and h, and discuss the physical significance of the coefficients c_1 and c_2. However, since m, n, and h have no known physical meaning and the reaction scheme of channel gating is altogether unknown, such a theoretical finding is at present of little help. We do not know whether the coefficients c_2, c_3 ... in Eq. (22) are other than negligible for the gating of ionic channels over experimentally accessible voltage ranges.

Reaction rates. For a description of reaction rates α and β in the scheme Eq. (16)

$$a \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} b \tag{25}$$

we follow Eyring rate theory and assume an intermediate "transition state" x:

$$a \underset{\alpha'}{\overset{\alpha}{\rightleftharpoons}} x \underset{\beta}{\overset{\beta'}{\rightleftharpoons}} b \tag{26}$$

where α' and β' are so fast that they are never rate limiting. Since α and β are voltage-dependent, we assume that the macromolecule undergoes the dipole moment changes $\Delta\bar{\mu}_\alpha{}^\dagger$ and $-\Delta\bar{\mu}_\beta{}^\dagger$ when state x is formed from a or b respectively. The dipole moment change appearing as a gating current will generally be the sum, $\Delta\bar{\mu} = \Delta\bar{\mu}_\alpha{}^\dagger + \Delta\bar{\mu}_\beta{}^\dagger$, since by assumption, the transition state is too short-lived to ever be populated appreciably.

In scheme Eq. (25), the rates α, β will be determined by the (negligible) occupancy X of state x, and X can be looked upon as being related to A and B by the "equilibrium constants"

$$\begin{aligned} K^\dagger_{a,x} &= \frac{A}{X} \\ K^\dagger_{x,b} &= \frac{X}{B} \end{aligned} \tag{27}$$

where these $K^\dagger$'s are related to the $\Delta\bar{\mu}^\dagger$'s by equations of the form (19). If the $\Delta\bar{\mu}^\dagger$'s are constant, then α and β increase or diminish exponentially with potential:

$$\begin{aligned} \alpha &= C_\alpha \exp\left(\Delta\bar{\mu}^\dagger_\alpha V/kT\right) \\ \beta &= C_\beta \exp\left(-\Delta\bar{\mu}^\dagger_\beta V/kT\right) \end{aligned} \tag{28}$$

These equations may apply to the cholinergic channel at the neuromuscular junction of *Rana pipiens* (*Magleby* and *Stevens*, 1972) but probably not to many other examples. If the reaction sequence is known, failure to obey Eq. (28) would imply that the "equivalent dipoles" $\Delta\bar{\mu}^\dagger$ are voltage-dependent, i.e., polarizable (see, e.g., *Levitan* and *Palti*, 1975). The physical meaning of a polarizable dipole moment change associated with the formation of a hypothetical transition state is at present unclear.

Sequential models. Here we will show that certain observed deviations of conductance-voltage curves from the constant-dipole, two-state Eqs. (20) and (21) are expected from a sequential gating reaction. We will also explore the possibility of obtaining a lower limit on the gating charge per channel from the logarithmic potential sensitivity of a membrane conductance. We repeat here Eq. (13),

$$a_0 \underset{}{\overset{q_0}{\rightleftharpoons}} a_1 \underset{}{\overset{q_1}{\rightleftharpoons}} a_2 \cdots a_i \underset{}{\overset{q_i}{\rightleftharpoons}} \cdots a_n \underset{}{\overset{q_n}{\rightleftharpoons}} b_0$$

as a reaction sequence for channel opening or closing. The resting state is denoted by a_0, the single and final open state by b_0 and intermediate states by a_i. Each transition is accompanied by a constant change in dipole moment equivalent to transferring a charge q_i through the entire potential drop across the membrane. (In the Hodgkin-Huxley model, b_0 would be the state of the sodium channel with all three m-particles in the activating position, a_2 that with two, a_1 with one and a_0 the resting state with none.) Let the occupancy of a state a_i be A_i and that of state b_0, B_0. Then by the constant-dipole Eq. (20)

$$\frac{A_i}{A_{i+1}} = \exp w_i$$

where w_i is the reduced work of moving the charge q_i across the membrane:

$$w_i = q_i (V - \bar{V}_i)/kT$$

The fractional occupancy B_0 of the final state b_0 can then be calculated as

$$B_0 = \frac{\exp (w_0 + w_1 + \ldots + w_n)}{1 + \exp w_0 + \exp (w_0 + w_1) + \ldots + \exp (w_0 + w_1 + \ldots + w_n)} \quad (29)$$

In general, B_0 will depend on all the parameters q_i and $\bar{V}_i$. However, for the limiting case where V becomes sufficiently negative, the exponential terms in the denominator of Eq. (29) become negligible and the denominator becomes unity:

$$B_0 = \exp (w_0 + w_1 + \ldots + w_n) \quad V \to -\infty$$

Factoring out terms of the form $\exp (-\bar{V}_i q_i/kT)$, and combining them into a constant C

$$B_0 = C \exp [(q_0 + q_1 + \ldots + q_n)\, V/kT] \quad V \to -\infty \quad (30)$$

Since the total charge transfer q which accompanies the transition from a_0 to b_0 is $q = q_0 + q_1 + \ldots + q_n$, it appears that

$$B_0 = C \exp qV/kT \quad V \to -\infty \quad (31)$$

Equation (31) is identical to Eq. (12) except for a constant. At very negative potentials, the voltage dependence of B_0 is determined in its steepness only by the overall dipole moment change occurring during the transition

(here equivalent to the charge movement q), and not by the number or nature of intermediate states.

Turning next to the logarithmic potential sensitivity, we define an apparent equilibrium constant K between the sum of all closed states and the open state, namely, $K = B_0/(1 - B_0)$. From Eq. (29), it can be shown that

(32)

$$\frac{d \ln K}{dV} = \frac{q}{kT}\left(\frac{1 + \sum_{2}^{n} \frac{q_i}{q} \exp w_1 + \sum_{3}^{n} \frac{q_i}{q} \exp (w_1 + w_2) + \ldots + \frac{q_n}{q} \exp \sum_{1}^{n-1} w_i}{1 + \exp w_1 + \exp (w_1 + w_2) + \ldots + \exp \sum_{1}^{n-1} w_i}\right)$$

Again, for $V \to -\infty$, Eq. (32) reduces to Eq. (11). Remembering that $\Sigma q_i = q$, however, it is clear that generally the expression in brackets in Eq. (32) will be less than unity. Consequently, the logarithmic potential sensitivity in a sequential system will usually be less than in a two-state system, the two being equal only in the limit $V \to -\infty$. Figure 18 compares three ionic channels in order to illustrate how a multistep reaction affects the steady-state voltage dependence. Figure 18a shows data on the sodium channel from *Hodgkin* and *Huxley* (1952a). Assuming peak sodium conductance to be proportional to the number of open sodium channels, we set $B_0 = g/g_{max}$. Given a limiting logarithmic potential sensitivity corresponding to six elementary charges per channel, a two-state model predicts the straight line, whereas the data appear to trace out a curve whose slope is less at all but the most negative potentials, as expected from the kinetically established fact that the opening of sodium channels is a sequential reaction. Figure 18b shows similar data for the potassium channel of frog skeletal muscle (*Stanfield*, 1975), also a multistate channel, whose limiting logarithmic potential sensitivity (solid line) is equivalent to more than eight elementary charges per channel (*Almers*, 1976). Included for comparison (Fig. 18c) are data obtained by *Noble* and *Tsien* (1971) on an inwardly rectifying potassium channel in sheep Purkinje fibers, whose slow time and voltage dependence is the basis for pacemaker activity in this cardiac tissue. The voltage dependence appears to follow first-order kinetics, so the underlying gating mechanism, the s-system, may be an open-close, two-state system. If the kinetic parameter s (see *Noble* and *Tsien*, 1968, for method of measurement) is regarded as the fraction of open channels and plotted as in the other two systems, one would expect a straight line, that is, a constant logarithmic voltage sensitivity. The data in Figure 18c suggest that this may be so, although the agreement would be more convincing if the data extended to smaller values of s_∞.

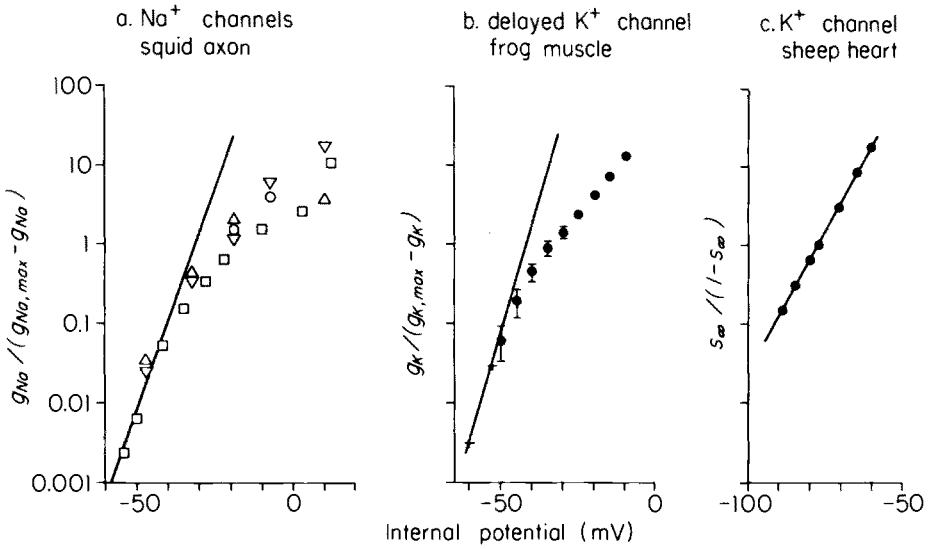

Fig. 18 a–c. Steady-state potential-sensitivity and membrane potential. (a) Data of *Hodgkin* and *Huxley* (1952a); different symbols from different fibers. g_{Na} is the peak sodium conductance during a given depolarization. If the average single channel conductance is independent of potential, the ordinate gives the ratio open/closed sodium channels. The slope of the curve – an e-fold increase per 4 mV at the most negative potentials (*solid line*) – is the logarithmic potential sensitivity. (b) A similar analysis for potassium channels of frog muscle (data from *Stanfield*, 1975), g_K is steady-state potassium conductance. The limiting logarithmic potential sensitivity (*solid line*, e-fold in 3 mV) was measured over the range given by the *crosses* (*Almers*, 1976). (c) A similar analysis for the slow gating mechanism of a cardiac potassium channel (g_{K2} of *Noble* and *Tsien*, 1968). The parameter s_∞ can be viewed as giving the fraction of open channels. This channel is a two-state system on the basis of its kinetic behavior, and behaves in accordance with Eq. (11) with q = 4.5 electronic charges. Data from *Tsien* and *Noble* (1969)

As a whole, these results confirm the notion that multistate channels, but not two-state channels, seem to lose some of their voltage-sensitivity at potentials where a substantial fraction of them is conducting.

It must be emphasized that estimates of the limiting logarithmic potential sensitivity always give a lower limit for q. (1) Exploration of more negative potentials may always reveal greater potential sensitivity. (2) State b_0 in scheme Eq. (13) may not be the first conducting state in the sequence. The conductance due to conducting precursors of b_0 will develop with a voltage sensitivity less than corresponding to a transfer of charge q. Apart from these reservations and the nonequilibrium nature of the peak sodium conductance, the treatment depends only on fairly general assumptions and suggests that at potentials close to the resting potential, the overall dipole moment change associated with the opening of a sodium channel in squid axons is equivalent to $q \geqslant 6e$. It seems possible that at

more positive potentials the channel molecule becomes dielectrically polarized into a state where this dipole moment change is less. However, this hypothetical polarization of the channel molecule would itself contribute to gating current, so $q \geqslant 6e$ will probably remain a valid lower limit.

5. A Design Consideration for Gating Mechanisms

Given the amplitude of the action potential, the range of membrane potentials available to excitable cells is only about 120 mV. Therefore, the exquisite potential sensitivity of sodium and potassium channels, or other voltage dependent processes, such as contractile activation, is not surprising. As discussed above, it cannot be obtained without a large dipole moment change in the gating- or voltage-sensing mechanism. Such dipole moment changes have their price: They tend to increase the membrane capacity (see Fig. 12) and constitute a load on the impulse propagation mechanism. As *Hodgkin* (1975) suggested, and *Adrian* (1975) confirmed by calculation, the capacitive load caused by the sodium channel gating mechanism is already so large that inserting more sodium channels into the axon membrane would fail to speed impulse propagation or could even slow the process. Although adding more sodium channels increases sodium conductance, this advantage is outweighed by the additional capacitive load caused by the additional gating mechanism. As a corollary, an excessively large gating charge movement, be it associated with sodium channels or with other gating mechanisms fast enough to respond to the action potentials would be a distinct disadvantage as far as the nerve impulse is concerned [11]. One may expect that as a principle of economy, the equivalent dipole moment change (or charge movement) per gating unit should be the minimum necessary to achieve a required voltage sensitivity. The most "economical" gating mechanism would be the two-state model with constant, maximal logarithmic voltage sensitivity.

The sodium channel does not reach this theoretical ideal. Its multistate nature, causing the delayed opening of sodium channels, may have other biologic advantages but also results in a loss of voltage sensitivity at medium and strong depolarizations (see Fig. 18a). However, at excitation threshold where a steep steady-state potential dependence seems most important, the voltage sensitivity corresponds to a charge movement of about six electrons/channel; this is probably not much less than, or equal

[11] As pointed out by *Armstrong* and *Bezanilla* (1975), any superfluous polarizability of excitable membranes would be disadvantageous, and one may expect that evolution will have selected against it. It is probably no accident that at frequencies important for the rising phase of an impulse, the background capacity of squid axolemma, lipid bilayer plus nonspecific proteins, is at most 50% higher than expected for a bilayer made from pure axolemmal lipid (see Sect. III).

to, the total gating charge per channel estimated by other means (Table 2). Near threshold the axon, therefore, utilizes almost the entire dipole moment change experienced by a sodium channel on its way from rest to activity. As another possible indication of the economy of sodium channel gating, the inactivation process is said to derive part or all of its voltage dependence from the activation process, and needs no charge movement of its own. It seems, therefore, that in designing the sodium channel, nature has avoided redundant charge movement.

Why have gating currents for potassium channels not been seen? Even during depolarizations long and large enough to activate more than half of all K^+ channels, there is no conspicuous sign of such currents (see, e.g., Figure 3 of *Bezanilla* and *Armstrong,* 1975). Most likely, K^+-channel gating currents are very small. In analogy with the sodium channel, the limiting logarithmic voltage sensitivity suggests that each K^+ channel generates a charge movement of not much more than eight electronic charges on its way from rest to activity (*Almers,* 1976; data on frog muscle). The number of potassium channels in squid axons, though not accurately known, is given as $60–70/\mu m^2$ (*Armstrong,* 1966, 1969; *Conti* et al., 1975). This would give a maximal K^+-channel gating charge movement of 500–600 electrons/μm^2, half to one-quarter that due to sodium channels. Since K^+ channels open and close roughly 10 times more slowly than sodium channels (*Hodgkin* and *Huxley,* 1952c), the actual K^+-channel gating current amplitude (charge/time) may be only 0.05–0.03 times that of sodium channels at a given potential, difficult to see with present methods (see, however, *Armstrong* and *Bezanilla,* 1977). In a sense, our failure to see K^+-channel gating current tends to support the notion that nature avoids redundant gating charge movement.

However, an alternative reason for this negative finding is difficult to rule out and has to do with the impossibility of observing small gating currents while ions flow through open channels. Whereas sodium channels blocked by TTX appear to generate unaltered gating currents, TEA^+-blocked potassium channels may not. If in an attempt to avoid TEA^+ one eliminates permeant ions inside and out, one finds that replacement of all internal K^+ with Na^+ (*Chandler* and *Meves,* 1970a) or tetramethylammonium$^+$ (*Armstrong* and *Almers,* unpublished) irreversibly denatures or removes potassium channels. K^+-channel gating currents may have escaped detection because no method has yet been found to maintain the gating mechanism of K^+ channels intact, while at the same time preventing ion flow through them.

V. Charge Movements in the Membrane of Skeletal Muscle

If in nerve we are only beginning to understand asymmetric displacement currents and their physiologic role, this section should start with the observation that the state of knowledge is even less advanced in skeletal muscle. All difficulties encountered in nerve – identifying a physiologic role, complicated effects of pulse protocol, various kinds of contamination of displacement currents transients – also apply in skeletal muscle. We know a priori that more than one physical process will contribute to displacement currents in muscle. Furthermore, while in nerve we can observe asymmetric displacement currents and their physiologic consequence (sodium conductance changes) in the same molecule (the sodium channel), we are much less fortunate in muscle. Even if present hypotheses are correct and displacement currents in muscle arise in the voltage sensor for a Ca^{2+}-permeable ionic channel in the sarcoplasmic reticulum, we can in general observe only distant effects of this Ca^{2+}-channel, such as contraction. Finally, the asymmetrical displacement currents observed in muscle may be greatly distorted versions of what one might observe under uniform membrane polarization. This difficulty arises from the complicated geometry of muscle cell membrane.

A. Geometry, Capacitance and Passive Properties of the Muscle Cell Membrane

The cell membrane of a skeletal muscle fiber is highly invaginated. There are folds, many 0.1 μm large, vesiclelike infoldings (caveoli), and, most importantly, the transverse tubular system (T system). The T system is a network of branching tubules, each 500 Å in diameter, and allows the action potential to spread from the surface into the center of the fiber. The tubule lumen is most likely filled with bulk extracellular fluid since molecules as large as 110 Å in diameter, such as ferritin, have ready access to it. Historically, morphologic and electrical measurements have been referred to the smooth surface of a circular cylinder as wide as the muscle fiber. In an average cylindrical fiber of 80 μm diameter, the tubule membranes then have an area of 4.4, and the surface membrane or sarcolemma an area of 1.8 cm^2/cm^2 cylinder surface (*Peachey*, 1965; *Mobley* and *Eisenberg*, 1975). The total static membrane capacity of 7 $\mu F/cm^2$ cylinder surface reflects this geometry and results from an average static capacity of 1 – 1.2 $\mu F/cm^2$ membrane area.

In electrical measurements, the tubule lumen acts as a resistance in series with the tubule membranes, which is "distributed" in that its magnitude depends on the position of the tubule inside the fiber. Therefore,

even if one has achieved uniform polarization of the surface membrane, radially uniform polarization of the T system is often impossible. In fact, with present methods we can measure the membrane potential only across the surface membrane; that in the T system must be inferred indirectly and on the basis of models. In general, electrical signals generated across the surface membrane will suffer decrement and distortion as they spread into the T system, just as they would spreading along a leaky cable. The same is true for electrical signals in the T system spreading to the surface membrane. "Cable effects" of this kind are particularly important for rapidly changing voltage signals. Any electrical event in the T system can at present be recorded only with relatively low time resolution.

As a consequence of cell geometry, the electrical admittance between inside and outside the cell shows what one might call a pronounced dispersion in the frequency range 0.2–5 kHz. The inside/outside admittance has been investigated extensively with various excitation waveforms (sine-waves, frequencies up to 2–10 kHz: *Falk* and *Fatt,* 1964; *Schneider,* 1970; *Valdiosera* et al., 1974; voltage steps: *Chandler* and *Schneider,* 1976; current steps and action potentials: *Hodgkin* and *Nakajima,* 1972a,b). Throughout, sarcolemma and tubular membranes were assumed to behave as leaky, but otherwise perfect, capacitors. All studies then indicated a resistance in series with the tubular membranes, whose magnitude and distribution are now (*Mathias* et al., 1977) thought to be consistent with it arising in a space of extracellular resistivity and the morphologic dimensions of the tubule lumen. Decreasing the conductivity of the extracellular fluid increases the series resistance proportionately (*Valdiosera* et al., 1974).

In view of the squid axon data and the voltage dependence of static capacity, one may expect the membrane to show dielectric loss. This does not invalidate the basic and qualitative conclusion concerning the origin of the resistance in series with the tubular membranes. On the other hand, the presence of the large and distributed series resistance makes an exploration of dielectric loss virtually impossible at any other than the lowest frequencies (< 200 Hz). For higher frequencies, one cannot in general determine with the required accuracy which portion of the "series" resistance arises as dielectric loss *in* the membrane, and which in the electrical access *to* the membrane.

Uniform cell membrane polarization, though difficult under transient conditions, can be achieved in the steady state if permeant ions are removed and the membrane resistance is sufficiently high. Membrane conductances as low as 20–50 μmho/cm^2 of cylinder surface (or 3–7 μmho/cm^2 of membrane area) can be obtained (*Adrian* and *Almers,* 1974). Given the lumen resistance obtained by AC admittance analysis, or calculated from morphology, the T system is expected in that case to experience DC sarcolemmal potential changes uniformly and without decrement.

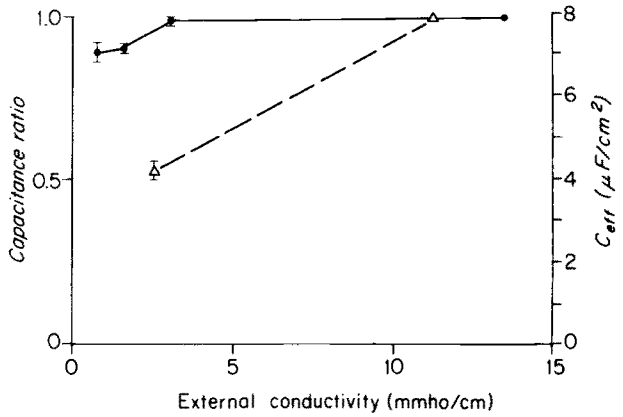

Fig. 19. Effective static membrane capacity, C_{eff}, as a function of external conductivity and membrane conductance. *Left ordinate,* C_{eff} as fraction of maximum; *right ordinate,* absolute values of C_{eff} referred to unit cylinder surface. *Triangles:* Cl^--containing solutions; the low-conductivity solution also contained 12 mM of the highly permeant K^+, so the membrane conductance, 700 μmho/cm^2 cylinder surface, was relatively high. The low value of C_{eff} indicates substantial potential decrement in the transverse tubules. *Black dots* in media containing the relatively impermeant ions Rb^+, SO_4^{2-}, Na^+, and Ca^{2+}. Membrane conductance 20–50 μmho/cm^2 of cylinder surface. C_{eff} now shows little or no decline at low condictivities, indicating the absence of potential decrements throughout. The value of C_{eff} in the high-conductivity, chloride-containing medium (membrane conductance 300 μmho per cm^2 cylinder surface) was the same as that in the SO_4^{2-}-containing fluids, indicating that in this condition, too, potential decrements were practically negligible. *Error bars* refer to left ordinate and give standard error of mean. Modified from *Adrian* and *Almers* (1974). Frog muscle, 20–25°C

An experimental demonstration of this fact is shown in Figure 19, where the effective static membrane capacity per unit cylinder surface, C_{eff}, is plotted as a function of external fluid conductivity. If C_{eff} is defined as the charge supplied to the cell membrane divided by the sarcolemmal potential displacement, it is easy to see that C_{eff} should depend on the extent of potential decrements in the tubules. Under uniform cell membrane polarization, C_{eff} should equal the product of total cell membrane area (sarcolemma plus tubules) times the average specific capacity. If, on the other hand, a sarcolemmal voltage step suffers steady-state attenuation in the tubules, the charge stored on tubular membranes, and thus C_{eff}, will be less. For instance, low conductivity in the tubule lumen should enhance tubule potential decrements, and this in turn should diminish C_{eff}. The triangles in Figure 19 show this effect; they represent experiments in solutions where membrane conductance is appreciable. The black dots show the same experiment in media maintaining a low membrane conductance (3–7 μmho/cm^2 membrane area) throughout. Lowering the conductivity fourfold now fails to decrease C_{eff}; the apparent decrease at even lower conductivities is probably an artifact caused by

microelectrode impalement leaks. Theory shows that had there been substantial potential decrements at the high external conductivity, lowering external (and probably tubule lumen) conductivity should have made decrements substantially worse. Instead, potential decrements in the tubules must have been equal over a wide range of external, and presumably tubule lumen, conductivities. The only way this can happen is for potential decrements to be negligible throughout. Therefore, if membrane conductance is low enough, adequate potential uniformity in the steady-state can readily be achieved.

The voltage dependence of static membrane capacity (Fig. 12) was measured under such conditions (see legend for details). It indicates that, as in squid giant axons, there are polar intramembrane molecules with sufficient equivalent dipole moment to experience dielectric saturation over the physiologic potential range. In analogy with the squid axon results, there may in additon be many other lossy membrane constituents which do not suffer dielectric saturation over experimentally accessible potentials and contribute to the membrane capacity throughout.

The depolarization-induced capacity increase in Figure 12 was observed earlier (*Schneider* and *Chandler*, 1973; *Adrian* and *Almers*, 1974) and reported most recently by *Schneider* and *Chandler*[12] (1976). These authors also describe experiments where they varied the (potassium) conductance of the tubule membrane by changing the membrane potential. Increasing this conductance produced DC potential decrements in the tubules, and, therefore, a fall in C_{eff}. The authors used these measurements to calculate the membrane conductance of the transverse tubules. In the present context, their work is important mainly because it provides an elegant example of how an apparent effect of membrane potential on static capacity can result from nonuniform cell membrane polarization. Their result should be borne in mind by all those investigating asymmetric displacement currents in systems where membrane polarization is likely to be nonuniform, be it due to cell membrane invaginations, the presence of dendrites and other cell processes, or in tissues composed of electrically connected cells.

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[12] *Schneider* and *Chandler* (1976) worked in an isotonic medium almost identical in composition to Ringer's fluid and did not observe the gradual fall in capacity evident in Figure 12 at potentials more negative than − 100 mV. The effect has appeared in many other experiments (*Chandler* et al., 1976a, their Fig. 9; *Adrian* and *Almers*, 1976a,b, all in hypertonic solution; *Adrian* et al., 1976, their Fig. 17b; *Almers*, 1976, his Fig. 10, all in isotonic solutions), and the reasons for its absence in *Schneider* and *Chandler*'s experiment is not clear.

B. Description of Asymmetric Displacement Currents

Asymmetric displacement currents in skeletal muscle decline 20–100 times slower than in nerve. Since it is possible that part or all of these currents flow across the transverse tubular membrane, one asks immediately to what extent the kinetics are affected by tubular delays. *Chandler* et al. (1976a) have treated the problem quantitatively, using a simplified, "lumped" representation of sarcolemma and transverse tubular system (*Falk* and *Fatt*, 1964). Their calculations show that tubular series resistances of the magnitude observed in hypertonic solutions can cause apparent slowing of the relaxation time constant by several milliseconds. For example, if sodium channel gating currents of time constant 0.5 ms flow in the transverse tubules, they could appear with an apparent time constant of up to 5 ms in experimental recordings. Tubular delays could, therefore, cause significant distortion. However, some of the longest relaxation times observed – from 10–50 ms – significantly exceed any expected tubular delay; at least some of the asymmetric displacement currents are, therefore, very much slower than sodium channel gating currents in nerve.

The kinetics and physiologic significance of asymmetric displacement currents in muscle are not well understood, and tubular delays will make their analysis difficult. Nevertheless, the data in the literature are sufficiently well documented to allow compiling a catalogue of observed kinetic behavior. The following discussion emphasizes results obtained with depolarizing test pulses approximately to the "transition potential", defined as the potential where asymmetric charge displacement is about half-maximal. Relaxation times there are longest, and distortions due to tubular delays should be smallest.

Figure 20 shows asymmetric displacement currents recorded during and after depolarizations to potentials about 10 mV more negative than the transition potential. The two top traces show transients recorded in isotonic media from normal fibers (a) or after block of contraction by 2 mM tetracaine (b). Very similar traces are also recorded after contraction block in hypertonic solutions (c–e), except that transients sometimes are somewhat slower (e.g., the *off*-transient in d), perhaps because hypertonicity tends to increase the resistance in series with the tubular membranes (*Valdiosera* et al., 1974)[13]. More variable and perhaps more inter-

[13] *Valdiosera* et al. (1974) report a nearly sevenfold increase in the tubule lumen resistivity ($1/G_L$) They referred the resistance in series with the tubule membrane to the osmotically diminished, rather than the physiologic diameter. However, there is no evidence that the geometric current path changes in hypertonic fluids. In order to reach an axial tubule, current probably will still have to flow through the lumen of the same tubules as before. Therefore, it seems more appropriate to refer T system variables to the physiologic fiber diameter throughout. If this is done, the data of *Valdiosera* et al. (1974) indicate an only three- to fourfold increase in the tubule lumen resistivity, $1/G_L$.

esting results are recorded during depolarizations to, or beyond, the transition potential as in Figure 21. Traces in Figures 20 and 21 marked with the same letter are from the same fiber. These stronger depolarizations would activate the contraction mechanism and open delayed potassium channels in a normal fiber. In trace a of Figure 21, contraction was only narrowly avoided by keeping the pulse short, and current through the delayed K^+-channels obscures the later part of the transient. K^+ currents are not evident in the other traces due to the presence of tetracaine (which shifts activation of K^+-channels to more positive potentials) or the K^+-channel blocker TEA^+ (c–e), and contraction was blocked in traces b–e by pharmacologic or osmotic means. The relatively rapid transients in Figure 20 are still visible in Figure 21 at the beginning of traces a, b, and e. In addition, a slower component appears in many traces (d, e), sometimes as a distinct "hump" (trace e). The hump in trace e could not have arisen from the subtraction procedure because it was visible even during a single depolarizing test pulse and before any subtraction (see, e.g., Fig. 1 of *Almers* et al., 1975). At more positive potentials, humps and slow phases merge into the initial transient.

Slow phases, though not generally of the magnitude observed in hypertonic fluids, are often seen also in isotonic media (Fig. 21b) when contraction is blocked with 2 mM tetracaine. A slow phase might have appeared even in trace a had conditions allowed longer pulses and had the delayed K^+-channel been blocked. While the initial rapid transient becomes more visible when control pulses are taken at extremely negative potentials, the slow phase does not (*Almers,* 1976, isotonic media containing tetracaine; *Adrian* and *Almers,* unpublished results in hypertonic fluids). It seems that the molecules responsible for the slow phase are relatively more potential sensitive, being in a state of dielectric saturation already at relatively moderate (– 100 mV) potentials. The same is true for humps (Fig. 21). Humps have so far only been observed in moderately hypertonic, chloride-free media (*Almers,* 1975; *Almers* et al., 1975; *Adrian* and *Peres,* personal communication) and never under the more extreme hypertonic conditions used by *Chandler* et al.,1976a) nor in tetracaine-poisoned or formaldehyde-treated muscles in isotonic fluids (*Almers* and *Best,* 1976; *Almers,* 1976). The significance of slow phases and humps are unknown, although their steep potential dependence suggests that they could be physiologically important.

All the kinetic effects discussed here are subject to distortion by tubular delays. For instance, one may consider (*Chandler* et al., 1976a) that rapid and slow phases represent the same event, and that their kinetic differences arise only because the sarcolemmal portion is recorded faithfully and the tubular portion with a delay. This seems unlikely, however; slow phases and humps are often too slow to be explicable solely by tubular delays. It seems that the kinetics of charge movements during depolarizations to, or beyond, the transition potential are genuinely complicated.

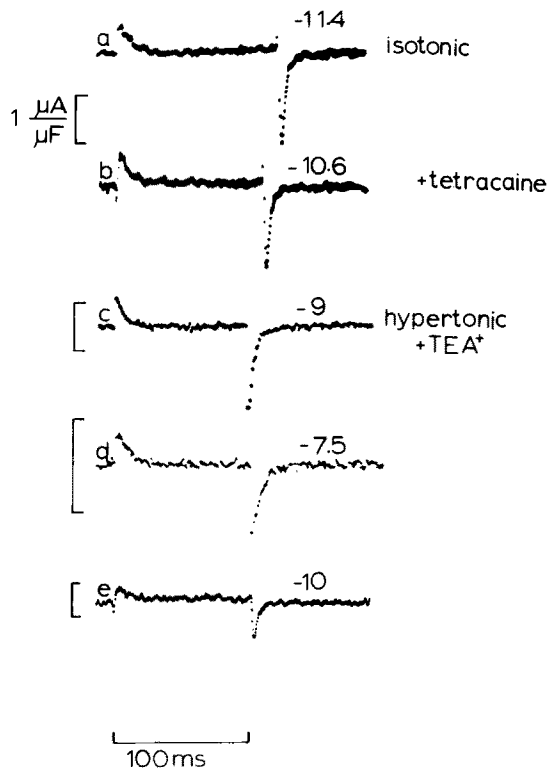

Fig. 20 a–e. Asymmetric displacement currents during test depolarizations which fall short of the transition potential by the amounts indicated in mV. Experimental conditions follow. (a, b) holding potential − 100 mV, control pulses between − 100 and − 90 mV. Isotonic solution containing 180 mM Na_2SO_4 and 5 mM Rb_2SO_4, plus Ca^{2+} and buffer (pH 7.2). (a) in the absence, (b) in the presence of 2 mM tetracaine. Transition potentials − 26.6 mV in (a) and − 27.4 mV in (b); two fibers from the same muscle (*Almers*, 1976). (c, d) from *Chandler* et al. (1976a) in a strongly hypertonic Ringer's fluid containing TEA^+ in place of Na^+ and 5 mM Rb^+ in place of K^+. Holding potentials − 100 mV (c) and − 90 mV (d), transition potentials − 41 mV (c) and − 54.5 mV (d), control pulses from − 150 to − 100 mV (c) and from − 169 to − 140 mV (d). The resistance in series with the tubule membrane was less in (c) than in (d). (e) from *Adrian* and *Almers* (unpublished), solution and pulse protovol as in (a) except that TEA^+ replaced Na^+ and the medium was moderately hypertonic. Transition potential − 40 mV. TTX present throughout (0.4–1 μM); frog muscle, 1–5°C

C. Asymmetric Displacement Currents and Ionic Channels in the Muscle Cell Membrane

Given the results on nerve, there are experimental and theoretical reasons to expect that gating currents in sodium and potassium channels will contribute to asymmetric displacement currents in muscle.

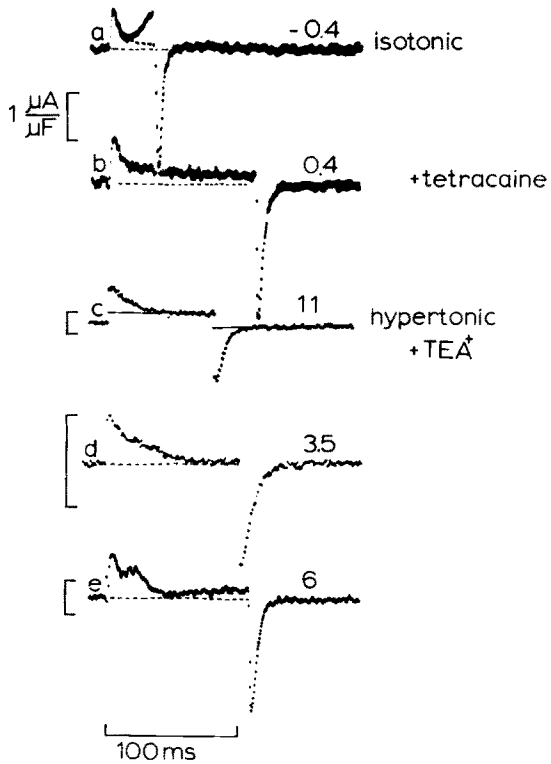

Fig. 21 a–e. Asymmetric displacement currents during test depolarizations going slightly bexond the transition potential by the amounts indicated. (a) from the same fiber as in Figure 20a, b from the same experiment as Figure 20b, etc., and under precisely the same experimental conditions and pulse protocols. An exception is trace (c) where the holding potential was − 80 mV and the control pulse went from − 130 to − 80 mV. Baselines (*dashed*) or steady levels (*solid*) are indicated. *Dashed line* in (a) indicates possible time course of displacement current underneath a developing outward current through the K^+ channel. Note pronounced hump in trace (e). Frog muscle; details as in Figure 20

Sodium channels. Asymmetric displacement currents in muscle are too slow to be concerned solely with sodium channel gating. Based on the number of sodium channels in frog muscle and the probable gating charge per channel, Table 3 shows that sodium channel gating currents should contribute at most one-tenth to one-third of the total observed charge displacement (*Almers* and *Levinson,* 1975). The figures given are upper limits in that they depend on assuming the entire asymmetric charge displacement of squid nerve to arise in sodium channels. Clearly, there are too few sodium channels to account for all the asymmetric charge displacement in muscle.

Under a step depolarization, sodium channel gating current would appear as an initial "spike" superimposed on the slower portions of asym-

Table 3. Expected contributions of gating currents to charge movements in frog skeletal muscle

	Number (μm^{-2} of cylinder surface)	q (electronic charges)	Q_{max} (nC/μF)	Reference
Na^+ channels	380 (1)	3–13 (2)	26–113	(1) *Almers* and *Levinson* (1975) (2) Table 2
K^+ channels	10–20 (3)	8–10 (4)	0.2–0.5	(3,4) *Almers* (1977)
Total observed			25 (5) 29 (6) 32 (7)	(5) *Chandler* et al. (1976a) (6) *Almers* and *Best* (1976) (7) *Adrian* and *Almers* (1976b)

Q_{max}, maximal expected gating charge; q, gating charge per channel. (1) From tetrodotoxin binding; a similar result was obtained also by others (*Jaimovitch* et al., 1976; *Ritchie* and *Rogart*, 1976). (3) Obtained by dividing the maximal K^+ channel in isotonic media by the estimated conductance for a single K^+ channel (4 pmho); noise analysis on frog myelinated nerve by *Begenisich* and *Stevens* (1975). (4) From measurements of limiting logarithmic potential sensitivity. (5, 7) Hypertonic media. (6) Tetracaine-poisoned isotonic medium. Membrane capacity was assumed to be 7 $\mu F/cm^2$ of cylinder surface.

metric displacement current; even if Q_{max} for sodium channels is only one-tenth of the total, the gating current amplitude should be as large as or larger than that of the observed currents. Sodium channel gating currents should, therefore, in principle be large enough to see in frog skeletal muscle, and may have been seen by *Hille* and *Campbell* (1976). Their failure to appear prominently in all other studies so far is probably due to (1) the relatively slow microelectrode voltage-clamp techniques usually employed and, (2) the possibility that more than half of all sodium channels reside in the transverse tubules (*Jaimovitch* et al., 1976) where a step sarcolemmal potential change can reach them only after a delay. Both factors would tend to prolong the gating current transient and diminish its amplitude. Sodium channel gating currents may contribute significantly, though not predominantly, to the relatively rapid outward transients in Figures 20 and 21.

Potassium channels. On kinetic grounds, an association between delayed K^+ channels and asymmetric displacement currents may seem likely. However, unless the gating charge of a single potassium channel exceeds that of a sodium channel more than tenfold, there are far too few K^+ channels in muscle membrane to contribute appreciably to the total charge observed (Table 3). Present evidence (failure of K^+ gating currents to appear in nerve, *Chandler* et al., 1976b, and *Armstrong* and *Bezanilla*, 1977; the

limiting logarithmic potential sensitivity of K^+ channels in muscle, *Almers*, 1976) does not indicate that a K^+ channel requires substantially more gating charge than a sodium channel. There is other evidence against K^+ channels as major contributors to the observed asymmetric displacement currents. (1) *Chandler* et al. (1976b) found that the sudden withdrawal of glycerol from glycerol-loaded fibers (*Eisenberg* and *Eisenberg*, 1968) strongly reduced asymmetric displacement current but probably not the number of functional K^+ channels. This statement relies on the reasonable assumption that K^+ outward currents at extreme depolarizations are proportional to the internal K^+ activity, as they are in squid giant axons (*Chandler* et al., 1965). (2) *Almers* (1976) has studied the effects of a local anesthetic, tetracaine, on potassium and asymmetric displacement currents. The drug (2 mM) was found to slow the opening of K^+ channels fourfold, as well as shift their voltage dependence to 24 mV more positive potentials. Despite these large effects on the gating of potassium channels, effects of the drug on asymmetric displacement currents were minimal (see, e.g., Figs. 20 and 21). Tetraethylammonium ions, another agent known to slow K^+ conductance changes (*Stanfield*, 1970), is also without measurable effects on the displacement currents (*Almers*, 1976). Presently published evidence, therefore, unanimously suggests that the contribution of delayed K^+ channels to the observed displacement currents is negligible [14].

Since sodium and potassium channels are unlikely to be major contributors to displacement currents in muscle, one turns next to contractile activation, one of the most steeply potential-sensitive processes known.

D. Excitation-Contraction Coupling

The contractile proteins in a muscle fiber will contract and develop force when the myoplasmic Ca^{2+} concentration, $[Ca]_i^{2+}$, is sufficiently high. $[Ca]_i^{2+}$ is controlled by a system of intracellular vesicles, the sarcoplasmic reticulum (called SR in the remainder). The SR occupies roughly 10% of the fiber volume and its membrane area is about 100 times larger than the smooth surface of a cylindrical muscle fiber (*Peachey*, 1965). Most of this membrane area is densely populated with a protein of 110,000 molecular

[14] *Adrian* and *Peres* (1977) have recently pointed out that the hump sometimes seen in moderately hypertonic media in the presence of TEA^+ resembles the time derivative of K^+ conductance changes in the absence of TEA^+, and have suggested that it may, therefore, represent a portion of the K^+ gating current. There is at present little evidence for this provocative suggestion. However, the fact that it has been made illustrates that the case against a significant contribution from K^+-channels is not universally accepted.

weight, a Ca^{2+} pump. The pump captures Ca ions in the myoplasm and transfers them into the lumen of the SR. Due to the pump, the Ca content of the lumen is high – about 10^{-2} M – and $[Ca]^{2+}$ in the myoplasm at rest below 10^{-7} M. During activity, the SR releases Ca^{2+}, probably via a Ca^{2+}-permeable ionic channel in the SR membrane. We know virtually nothing about this hypothetical *Ca^{2+} channel* other than what might be inferred from the fact that muscle contraction is regulated by the (tubular) membrane potential (*Hodgkin* and *Horowicz,* 1960; *Adrian* et al., 1969; *Heistracher* and *Hunt,* 1969a,b). Depolarization causes contraction and, presumably, Ca^{2+} release, and repolarization stops both processes. This strongly suggests that depolarization (repolarization) causes the Ca^{2+} channel to open (close).

In order for the membrane potential in the T-system to influence an ionic channel in the SR, the two membrane systems must interact. On morphologic grounds, the most likely site for this to happen is where SR and T-system meet in close apposition and form a structure called "triad" (for the anatomy of the triad, see *Franzini-Armstrong,* 1970). The nature of this interaction is entirely unclear, but one might expect that it begins with the movement or structural change of a voltage sensor in the tubule membrane. *Schneider* and *Chandler* (1973) and *Chandler* et al. (1976b) have suggested that most of the asymmetric displacement current in skeletal muscle arises in this voltage sensor. More specifically, they suggested that the triadic gap between T-system and SR is bridged by long macromolecules, which sense the tubule membrane potential with one end and with the other open or close Ca^{2+} permeable channels in the neighboring SR. The remainder of this section will discuss the evidence linking asymmetric displacement currents in muscle – or charge movements, as *Chandler* et al. (1976a,b) call them – with Ca^{2+} release from the SR. Once again, two strategies will be explored: (1) correlations between block of charge movements and block of muscle contraction, (2) correlations between charge movements and the gating of Ca^{2+} channels.

E. Block of Charge Movement and Muscle Contraction

Agents and experimental maneuvers of interest here are those that block contraction by blocking the intracellular Ca^{2+} release induced by cell membrane depolarization. They are glycerol treatment, long-lasting membrane depolarization, and treatment with some local anesthetics.

Glycerol treatment. Sudden glycerol withdrawal from glycerol-loaded muscles disrupts the transverse tubular system, or at least its continuity with the extracellular space (*Eisenberg* and *Eisenberg,* 1968; *Franzini-Armstrong*

et al., 1973). The technique seemed suitable for deciding whether charge movements occur across sarcolemma or T-system membranes. In the experiments of *Chandler* et al. (1976b), glycerol treatment reduced the effective static capacity by about one-third, consistent with a disruption or disconnection of about 40% of the T-system. Maximal charge movement was reduced by about two-thirds, so glycerol treatment is more effective in producing block of charge movement than in disconnecting transverse tubules. Thus, the one cannot be solely a consequence of the other. While this finding is disappointing in that the treatment cannot be used to localize charge movements, it does have a pharmacologic parallel: The muscle twitch is also more sensitive to glycerol treatment than could be explained by tubule disruption alone (*Dulhunty* and *Gage,* 1973). Perhaps glycerol treatment has a more pronounced effect on the SR-tubule junction than on the tubule-sarcolemmal junction, thereby interfering with Ca^{2+}-release in response to tubule membrane depolarization.

Long-lasting depolarization. Like sodium channel gating currents, charge movements are reversibly blocked by long-lasting depolarization [15]. At − 20 mV, block is from 70% (*Adrian* and *Almers,* 1976b, their Fig. 13) to 100% complete (*Chandler* et al., 1976b; *Adrian* and *Almers,* 1976b, their Fig. 9). The time course of the effect suggests first-order kinetics with a time constant of about 20 s at − 21 mV and 1°−2°C; reversal at − 80 mV occurs with a time constant of about 40 s (*Chandler* et al., 1976b) or 100 s (*Adrian* et al., 1976) depending on pulse protocol. Muscle's contrac-

[15] Although conventional charge movement disappears under maintained depolarization, asymmetric displacement currents can still be recorded in depolarized fibers if control pulses are taken at positive potentials (*Adrian* and *Almers* in *Almers,* 1975). Furthermore, the static membrane capacity remains voltage dependent (*Adrian* and *Almers,* 1976a; *Schneider* and *Chandler,* 1976). Charge movements in depolarized fibers – called "charge 2" by *Adrian* and *Almers* (1976b) and *Adrian* et al. (1976) – are similar in size to the conventional charge movements (charge 1) in polarized fibers. However, the voltage dependence of charge 2 is less steep, the charge/voltage curve varying over the entire range of experimentally accessible potentials. Most likely, this second type of charge movement occurs independently of the holding potential in both polarized and depolarized fibers (*Adrian* et al., 1976). Since the voltage dependence of relaxation rates and equilibrium parameters is relatively slight, charge 2 does not contribute prominently to asymmetric displacement currents recorded in the conventional way. When control pulses are taken at sufficiently negative potentials, proportionally about the same displacements of charge 2 will occur during test and control pulses, and little or no charge 2 will remain after the subtraction (*Almers,* 1975).

Charge 2 may be responsible for the gradual decline in static capacity observed in Figure 12 (top) at very negative potentials. We do not know whether charge 2 in skeletal muscle is related to the asymmetric displacement currents recorded from depolarized squid giant axons under similar conditions (*Keynes* et al., 1974). It seems possible that both in squid and skeletal muscle, these depolarization-resistant asymmetric displacement currents arise in nonspecific membrane proteins.

tile response to cell mebrane depolarization is also blocked by maintained depolarization (*Hodgkin* and *Horowicz*, 1960). A muscle fiber will respond with a "K^+ contracture" to membrane depolarization by K^+-rich external media, and then relax spontaneously. After relaxation is complete, the Ca^{2+} release mechanism is inactivated (refractory), and no other contraction can be elicited by changes in membrane potential unless the fiber is allowed to recover (reprime) at the resting potential. The final time constant of relaxation in K^+-rich media, possibly the "inactivation time constant" of the release mechanism, is about 10 s at 0 mV; half-time of recovery is about 1 min at -80 to -90 mV (3°C, *Caputo*, 1976a,b). Both are of the same order of magnitude as the time constants of loss and reappearance of charge movement.

Tetracaine. The local anesthetic tetracaine inhibits the contractile response to cell membrane depolarization (*Lüttgau* and *Oetliker*, 1968). Further investigation of this effect (*Almers* and *Best*, 1976) has shown that in "skinned" fibers where the sarcolemma has been removed mechanically, this inhibition is observed only when contraction depends on calcium release from internal stores as, for example, during exposure to the calcium-releasing agent, caffeine. When Ca^{2+} is applied directly to the contractile proteins, tetracaine (2 mM) diminishes neither the force developed by a skinned fiber nor its sensitivity to Ca^{2+}. Therefore, the drug blocks contraction in intact fibers by inhibiting Ca^{2+} release from the sarcoplasmic reticulum. Tetracaine effects on the strength-duration curves for contractile activation (Fig. 22) are consistent with the drug blocking 82% and 96% of all release sites at 0.5 and 2 mM concentration (see later). Despite this large effect on Ca^{2+} release, tetracaine has little or no effect on charge movements (*Almers* and *Best*, 1976; see Figs. 20, 21 and Table 3) other than possibly eliminating the hump sometimes observed in moderately hypertonic media. It may shift the charge-voltage dependence to 4 mV more positive potentials, but does not, within an experimental error of perhaps ± 30%, affect any other parameter of charge movements.

Conclusion. Attempts to obtain parallel block of contraction (Ca^{2+} release) and charge movements have given results which are consistent with, but do not prove, a relationship between these two events. In the first two examples, it is not clear whether the contraction-blocking effects of glycerol treatment and membrane depolarization are related to their effects on charge movements. Glycerol treatment has many poorly understood effects, and long-lasting membrane depolarization shows a general tendency to eventually inhibit the processes which it transiently activates. On the other hand, the failure of tetracaine to block charge movements is not necessarily evidence aginst their involvement in regulating Ca^{2+} release nor

even against a direct linkage (*Chandler* et al., 1976b) between the molecules giving rise to charge movements and the hypothetical Ca^{2+} channel in the SR. For example, tetrodotoxin blocks ion flow through the sodium channel without appreciably disturbing the "gating currents" arising in that channel.

F. Attempts to Correlate Charge Movements with the Gating of a Hypothetic Ca^{2+} Channel in the SR

Cell membrane depolarization and contractile response are only the beginning and end of a long chain of events, with many steps intervening. This fact is one of the most serious difficulties in the study of excitation-contraction coupling. Ideally, one would like to have a measurement related to the number of open Ca^{2+} channels in the SR membrane, just as in nerve membrane one has the sodium conductance as a measure of the number of open sodium channels. However, electrical observation of events in the SR membrane is at present impossible. Optical signals possibly related to SR membrane potential changes (*Bezanilla* and *Horowicz*, 1975; *Oetliker* et al., 1975) may become useful in the future and are now under intensive investigation (*Baylor* and *Oetliker*, 1977a,b,c; *Kovacs* and *Schneider*, 1977). Further interesting information may be obtained by using the Ca^{2+}-activated, bioluminescent protein aequorin (*Taylor* et al., 1975) or certain dyes, such as arsenazo-III (*DiPolo* et al., 1976) as indicators of myoplasmic Ca^{2+} concentration (*Miledi* et al., 1977). Future results with these new optical methods are anxiously awaited. So far, however, almost all information on the regulation of Ca^{2+} release by the cell membrane potential has come from monitoring contraction, that is, from using the contractile proteins as an indicator for Ca^{2+} release. The most promising experimental technique is that of *Heistracher* and *Hunt* (1969a) and *Bezanilla* et al. (1972) who voltage-clamped short muscle fibers and simultaneously recorded tension. However, some of the best analyzed results so far have been obtained by using voltage-clamp techniques for mapping the dependence of contraction threshold on membrane potential. This highly indirect approach was introduced by *Adrian* et al. (1969). In order to outline the many assumptions one needs to make before conclusions can be reached, it is convenient to begin by describing an experiment which is interpreted only later.

Strength-duration curves. In experiments such as in Figure 22, a muscle fiber is voltage-clamped at -100 mV, close to the normal resting potential, and observed under a microscope. Periodically, depolarizing pulses are applied, whose duration (abscissa) and amplitude (ordinate) are adjusted

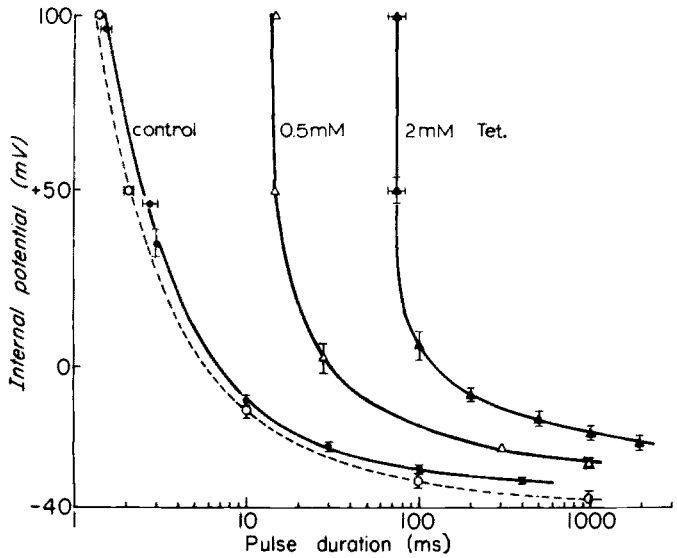

Fig. 22. Strength-duration curves for contractile activation. External medium contained 90 mM Na_2SO_4, 5 mM Rb_2SO_4, as well as Ca^{2+}, buffer at pH 7.0 and TTX to block sodium channels. The ionic strength of this solution was twice that of Ringer's fluid, hence the abnormally positive rheobasic threshold of − 35 to − 38 mV. *Open symbols* from one muscle, *closed symbols* from another. Tetracaine (Tet) present as indicated. Data from *Almers* and *Best* (1976), and unpublished material. Holding potential − 100 mV. Frog muscle, 5°C

to produce a threshold contaction. All experimental points in Figure 22 define pulses which were just strong enough and long enough to give a just visible flicker of contraction under the microscope. Together, they define strength-duration curves of contractile activation.

In interpreting strength-duration curves, we assume that (1) a threshold contraction will result whenever the myoplasmic Ca^{2+} concentration has risen to a certain value which is unknown but constant.

Two parameters of these curves are of particular interest: the threshold potential for activation with long (0.1−1 s) pulses, called *rheobase*, and the threshold duration of a strong depolarization to 50 or 100 mV, called *minimum stimulus duration* (abbreviated *msd*). At rheobase, Ca^{2+} uptake by and release from the SR must be equal; that is, the number of open Ca^{2+} channels is just large enough to balance the activity of the Ca^{2+} pump. If a treatment inhibited or inactivated Ca^{2+} channels more (less) than the pump, one might expect the rheobase to shift in the negative (positive) direction. At positive potentials, Ca^{2+} release will ordinarily exceed uptake, and if the msd gives an estimate of the time needed by the SR to release a threshold amount of Ca^{2+} into the myoplasm, then block or inactivation of some Ca^{2+} channels in the SR might show up as a lengthening of the msd. In the presence of tetracaine (0.5 mM and 2 mM in Fig. 22), the msd

is greatly lengthened suggesting strong inhibition of Ca^{2+} release; the abnormally positive rheobase indicates that the drug inhibits Ca^{2+} release more strongly than Ca^{2+} uptake. Strength-duration curves are readily measured in the presence of other pharmacologic agents. Such studies (*Almers,* 1977) show that among local anesthetics, tetracaine and dibucaine (not shown) are extreme in their ability to block Ca^{2+} release. Procaine lengthens the msd while making the rheobase more negative; the drug probably inhibits uptake more than release. Lidocaine makes the rheobase more negative without lengthening the msd, most likely the drug inhibits Ca^{2+} uptake only.

In all further discussions, we will make additional assumptions: (2) The SR Ca^{2+} pump is not directly influenced by the cell membrane potential. Its activity is determined only by the myoplasmic Ca^{2+} concentration. (3) Ca^{2+} release by an open Ca^{2+} channel is constant over the times and conditions of concern here.

With these untested but plausible assumptions, rheobase and msd can be used to obtain information about the relative number of open Ca^{2+} channels. Unfortunately, kinetic and equlibrium information about the Ca^{2+} channel gating system are inextricably mixed in the curves of Fig. 22, and it is convenient to consider next a slightly different kind of experiment.

An attempt to determine the dependence of Ca^{2+} release on the cell membrane potential [16]. In the experiment of Figure 23, the fiber is kept chronically at a potential (− 21 mV) producing complete mechanical refractoriness, and presumably complete inactivation of all Ca^{2+} channels.

Periodically, the membrane is stepped to − 120 mV for a time (t) (ordinate) and then depolarized to a variable potential V (abscissa). During repolarization, some Ca^{2+} channels recover from inactivation, and the stronger the following depolarization, the more of them open. If the number open reaches a certain value, Ca^{2+} escape through them will balance (or slightly outweigh) uptake by the pump, and, since the potential (V) is maintained for 2–10 s, a contraction will eventually occur. All points in Figure 23 may, therefore, be assumed to define pulse patterns where the number of Ca^{2+} channels opened by the final depolarization reached an unknown, but constant, value. By increasing the recovery interval (t), we increase the pool from which open channels can be recruited, and by increasing the final (depolarized) potential, one can increase the fraction of recovered channels which will open. On this basis, one expects (and finds) that the stronger the final depolarization, the shorter the recovery period

[16] In the interest of clarity, the description of *Adrian* et al's (1976) work given here is a great deal less cautious than the original paper. *Adrian* et al. (1976) should not be held responsible for the present overinterpretation of their experiments.

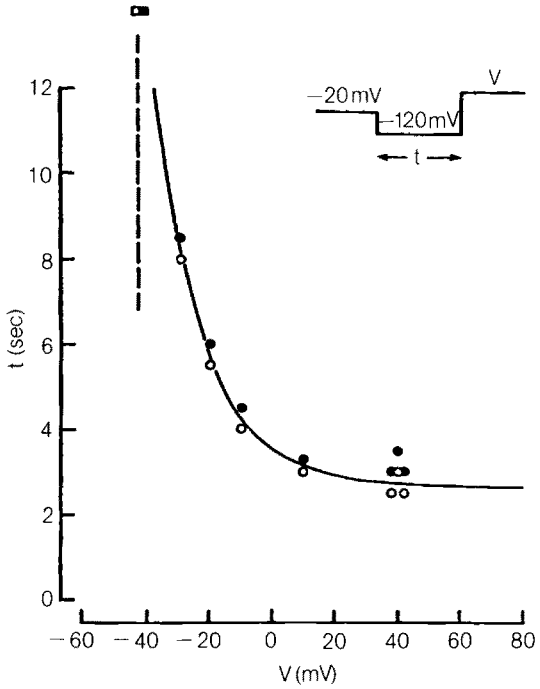

Fig. 23. Recovery interval (t) (*ordinate*) necessary to give a just-visible contraction during depolarization to the potential (V) (*abscissa*). For pulse pattern see inset. Pulses represented by *filled symbols* did, those by *open circles* did not, give a contraction. External medium identical to that of Figure 22 except that 90 mM Na_2SO_4 was replaced with 50 mM $(TEA)_2^+SO_4$ and 40 mM Rb_2SO_4. *Vertical dahed line* represents the rheobasic contraction threshold in the fully recovered fiber. Resting potential − 27 mV. Frog muscle, 4.1–4.4°C. From *Adrian* et al. (1976)

required for contraction, until a minimum value is reached (about 3 s in Fig. 23) below which contraction is impossible regardless of the final depolarization. The curve becomes horizontal, indicating that *the voltage dependence of contractile activation saturates at positive potentials.* Under our assumption, depolarization to 20–40 mV must open *all* recovered Ca^{2+} channels, since otherwise one should, by stronger final depolarization, be able to obtain contraction with shorter recovery intervals.

The steady-state voltage dependence of Ca^{2+} channels could be partially reconstructed from the experiment if one knew the time course with which Ca^{2+} channels recover from maintained depolarization. *Adrian* et al. (1976), therefore, made an additional assumption:

(4) Recovery of Ca^{2+} channels from inactivation proceeds linearly with time.

With this assumption, the *fraction* of recovered Ca^{2+} channels opened at the potential (V) (the total *number* of open channels at threshold being constant) should be proportional to the reciprocal of the recovery interval. For example, after 6 s recovery in Figure 23, one should have recovered twice as many Ca^{2+} channels as after 3 s; contraction threshold at − 20 mV then indicates that half of all recovered channels opened at that potential.

Figure 24 shows the average "steady-state activation curve" for Ca^{2+} channels obtained in this way (curve A). Also shown (B) is the curve

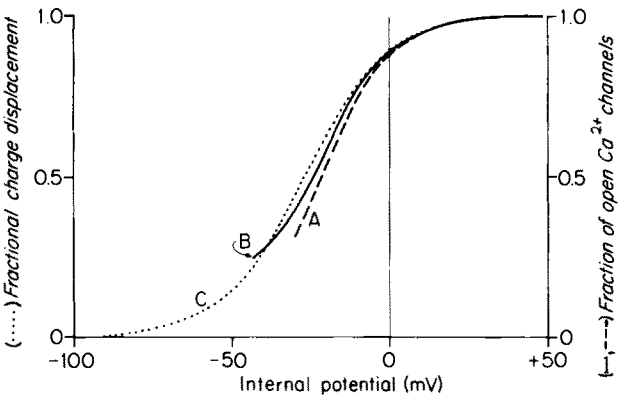

Fig. 24. Steady-state voltage dependence of threshold activation obtained from experiments as in Figure 23. Curve A (*dashed*) gives 1/t normalized so 1/t = 1 at extreme positive potentials. The curve is, on average, the best least-squares fit of

$$1/t = F(V)$$

to the data of *Adrian* et al. (1976), where

$$F(V) = 1/1 + \exp[(\overline{V} - V)/\kappa]$$

and $\overline{V} = -21.5$ mV and $\kappa = 10.9$ mV (*Adrian* et al., 1976). This equation is formally identical to the two-state, constant dipole equation [Eq. (21)]. Curve B (*solid*) plots the function G (V) obtained from curve A by

$$G(V) = \frac{1 - \exp(-\phi)}{1 - \exp[-\phi/F(V)]}$$

where $\phi = 0.25$. With the assumptions outlined in the text, these curves give the fraction of open Ca^{2+} channels if recovery from inactivation proceeds linearly (curve A) or exponentially (curve B); $\phi = 0.25$ is the fraction of Ca^{2+} channels open at rheobase in a fully recovered fiber; rheobase was assumed to be at -43 mV for the calculation of G (V). With a mean minimum recovery time of 3.4 s and $\phi = 0.25$, the time constant of recovery becomes 13.7 s, in reasonable agreement with the recovery of charge movement at -80 mV (*Chandler* et al., 1976b). Note that G (V) and F (V) can be determined from contraction thresholds only over the range 0.25–1.00. The data of *Adrian* et al. allow F (V) and G (V) to be reconstructed only at potentials positive to -30 mV, so there is no experimental basis for curve B between -43 mV and -30 mV.

Included for comparison is the average charge/voltage curve (C, *dotted*) obtained in a medium virtually identical to that of Figure 22 containing 2 mM tetracaine (*Almers* and *Best*, 1976, their Table 2). Frog muscle, 1–5°C

obtained from the same data if time course of recovery is assumed to be exponential with time-constant 13.7 s, a perhaps more reasonable assumption (*Adrian* et al., 1976). The curve is somewhat flatter and shifted to the left. A sigmoid recovery time course (see, e.g., *Heistracher* and *Hunt*, 1969a,b) would have given a curve which is steeper and shifted to the right. Curve C, for comparison, shows the average charge-voltage curve obtained (*Almers* and *Best*, 1976) in a tetracaine-poisoned but otherwise nearly identical medium. It may be concluded (*Adrian* et al., 1976) that

within experimental uncertainties, the charge/voltage distribution curve and the steady-state activation curve for the SR-Ca^{2+} channel are similar in shape and position on the abscissa. If both curves coincided, it would follow that each aliquot of charge transfer opens a constant number of Ca^{2+} channels, a behavior very different from that of sodium channels in the cell membrane. *Adrian* et al. (1976) also measured charge movements in partially reprimed fibers; in one case, both the charge/voltage curve and part of the Ca^{2+} channel activation curves were measured on the same fiber. Within the large experimental uncertainties, the two curves were found to coincide.

Further interpretation of strength-duration curves. Among the conclusions reached in the foregoing discussion, the saturation of voltage-dependence of contraction in severely inactivated fibers relies on the fewest assumptions. Yet the strength-duration curve of a normal fiber ("control" in Fig. 22) shows no evidence of saturation. The more extreme the depolarization, the less the duration of a threshold pulse. This feature is not a consequence of tubular delays (*Costantin,* 1974), and must therefore reflect properties of the Ca^{2+} channel gating system. In explaining this effect, one considers that the amount of Ca^{2+} released due to a depolarizing pulse can depend not only on the steady-state relation between cell membrane potential and the number of open Ca^{2+} channels, but also on the (potential-dependent) rate with which Ca^{2+} channels open and close. Even if cell membrane depolarizations to both 50 and 100 mV could eventually open all Ca^{2+} channels, the threshold pulse duration at 100 mV may still be less than at 50 mV simply because Ca^{2+} channels at 100 mV open more quickly.

On the other hand, under conditions where the msd is long compared to the response time of Ca^{2+} channels, the strength-duration curve at positive potentials may be expected to reflect only the steady-state properties of the Ca^{2+} channel gating system. This is most likely the case when a Ca^{2+} release inhibitor, such as tetracaine, is present. Whereas in a normal fiber, contraction can be elicited by 1–3 ms depolarizations, it appears that whatever Ca^{2+} channels remain intact in tetracaine must remain open for at least 16 ms (0.5 mM) or 73 ms (2 mM) to release a threshold amount of Ca^{2+} into the myoplasm. The strength-duration curves are now vertical at positive potentials, showing the expected saturation of the voltage dependence of contractile activation. Comparison of strength-duration curves with and without tetracaine therefore suggests that 2 ms is insufficient, but 16 ms ample time for all Ca^{2+} channels to open under a depolarization to positive potentials.

The kinetics of charge movement (tetracaine-poisoned fibers) fall within these constraints. In Figure 25, kinetic and steady-state charge

movement parameters were used in a partial reconstruction of the strength-duration curves. It was assumed that the number of open Ca^{2+} channels is at all times proportional to the amount of charge in the depolarized (active) configuration. The maximal Ca^{2+} release rate (or number of functional channels) was assumed to be reduced 5.6-fold (o.5 mM) or 25-fold (2 mM) in the presence of tetracaine. The calculated curves reproduce the main feature of the observation, namely, that a vertical strength-duration curve results when the msd is long (as it is in tetracaine-poisoned fibers) but not when Ca^{2+} release is as rapid as in normal fibers. The treatment fails at negative potentials, perhaps in part because the activity of the Ca^{2+} pump has been neglected.

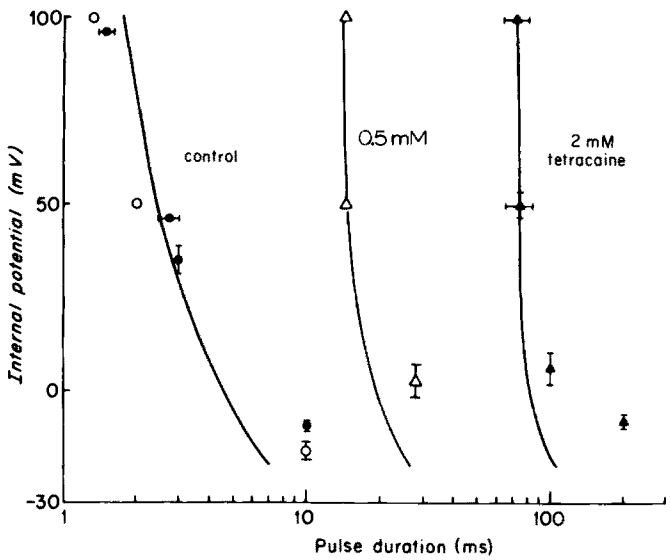

Fig. 25. Partial reconstruction of the data in Figure 22 using charge movement parameters obtained in a solution identical to that of Figure 22 except that TEA^+ replaced Na^+ and 2 mM tetracaine were added. Neither TEA^+ nor tetracaine have large effects on charge movements (*Almers*, 1976). Method of reconstruction and parameters of charge movement are the same as in Figure 11 of *Almers* and *Best* (1976). It was assumed that 0.5 mM tetracaine blocked 82% and 2 mM tetracaine 96% of all release sites. From *Almers* and *Best* (1976) and unpublished material. Frog muscle, 5°C

Conclusion. The gating of Ca^{2+} release, as reflected by measurements of contraction thresholds, is consistent with an instantaneous, one-to-one correspondence between charge displacement and open Ca^{2+} channels. However, this conclusion is based on many untested assumptions, and measurement uncertainties are large. The correlations established here are far less tight than those established by *Keynes* and *Rojas* (1976) between

sodium channel gating currents and the behavior of the hypothetical m-particle of the Hodgkin-Huxley model. Yet on the basis of additional gating current measurements, it is now clear that gating currents are irrevocably inconsistent with m particles. Our considerations on muscle do not even remotely approach, in accuracy or detail, those which were required in squid axons to first establish and then rule out the m-particle model for sodium channel gating.

G. Possible Implications of Charge Movements for Excitation-Contraction Coupling

The evidence for a role of charge movements in regulating intracellular Ca^{2+} release and contraction rests on the following:

1. Charge movements are large and steeply potential dependent. Except for a relatively small portion, they seem to be related to neither sodium nor potassium channels. The only other steeply potential-dependent process of major physiologic importance is contraction.

2. Some treatments inhibit charge movements, as well as the contractile response to cell membrane depolarization.

3. On the basis of contraction threshold measurements, it appears that the voltage and time dependence of a hypothetical Ca^{2+} channel in the SR correlates well, or even coincides, with the voltage and time dependence of charge movements.

The above evidence is suggestive, but not conclusive. For instance, it seems possible that only a portion of the observed displacement currents have to do with contraction, such as the hump in Figure 21 or the so far poorly defined, relatively slow and steeply potential-dependent fraction of charge movements. It would clearly be helpful to decompose charge movements into well-characterized components, using voltage dependence and pharmacologic properties as criteria. The localization of charge movements (T system vs. sarcolemma) in particular is unclear at present and could be investigated, for example, by creating steady-state potential differences between sarcolemma and T system (see *Adrian* and *Almers*, 1974; *Schneider* and *Chandler*, 1976) and taking advantage of the steep potential dependence of rate of charge recovery from maintained depolarization (*Adrian* et al., 1976). The point is important, because any component of charge movement residing in the sarcolemma would at once be unlikely to participate in the regulation of Ca^{2+} release. Studies along these lines will be difficult, but it is hard to see how further progress in the field can be made without them. Useful recent attempts at separating charge movements into components have been made by *Adrian* and *Peres* (1977).

It may be of interest to list some implications of charge movements for the mechanism of excitation-contracting coupling.

1. An upper limit on the number of voltage-sensing units for intracellular Ca^{2+} release. If the charge/voltage curve (e.g., Fig. 2) is fitted with the two-state, constant-dipole equation [Eq. (21)], one obtains a logarithmic voltage sensitivity corresponding to three (*Schneider* and *Chandler,* 1973; *Chandler* et al., 1976b) or two (*Adrian* and *Almers,* 1976a; *Almers* and *Best,* 1976; *Almers,* 1976) electronic charges per voltage-sensing unit. These figures are lower limits, since the steady-state characteristics for charge displacement is often more steeply potential-dependent at negative than at positive potentials. Dividing the total charge observed at saturating depolarizations, 30 nC/μF, by the minimum charge per voltage-sensing unit, one obtains 6.25×10^{10} to 9.4×10^{10} per μF of static capacity as an upper limit for the number of voltage sensors. If there are about 0.6 cm^2 of tubule membrane associated with each μF of total cell membrane capacity, voltage-sensing units are distributed in the T-system membrane at a density of no more than 1000–1500/μm^2. As pointed out by *Schneider* and *Chandler* (1973), this number is similar to the number of electron-dense bridges between tubule and SR membranes, 500–1000/μm^2 of tubule membrane (*Franzini-Armstrong,* 1970). The similarity has led *Schneider* and *Chandler* (1973) to suggest a role of these "bridges" in excitation-contraction coupling. With 1000 voltage sensors/μm^2, the rate of Ca^{2+} release from the SR during contractile activation would be between 2000 and 100,000 Ca ions per second and voltage sensor (*Almers,* 1975; *Almers* et al., 1975; *Chandler* et al., 1976b). Since ion transit rates through known ion-permeable pores can exceed this number 10- to 1000-fold, one would probably need only one Ca^{2+} channel per voltage sensor, perhaps in the immediate neighborhood of a T-system-SR-bridge, in order to satisfy the calcium requirements for contractile activation in muscle.

2. Other conclusions which would follow if charge movements regulate contraction. (a) Under maintained cell membrane depolarization, inactivation of Ca^{2+} channels in the SR, like that of sodium channels in nerve, is accompanied by immobilization of the voltage sensor. (b) It is possible to block the Ca^{2+} channel in the SR, as with tetracaine, without measurably interfering with the voltage sensor for that channel.

VI. Other Systems

Besides in axons and muscle fibers, asymmetric displacement currents have been recorded in snail nerve cell bodies (*Adams* and *Gage,* 1976; *Krishtal* and *Pidoplichko,* 1976; *Kostyuk* et al., 1977). Asymmetric displacement currents in *Helix pomatia* neurones recorded at 25°C decline about ten

times faster than those in muscle at 2–5°C; it seems possible that, if investigated at the same temperature, their kinetics in the two tissues would be similar. *Kostyuk* et al. (1977) have suggested that the asymmetric displacement currents in *H. pomatia* neurones are gating currents for a Ca^{2+} channel in the cell membrane, since internal fluoride (100–200 mM) blocks both the Ca^{2+} channel and the asymmetric displacement currents.

Asymmetric displacement currents have so far been found in all cells where they have been looked for. It seems safe to predict that attempts to record them will be made in many other cells. These attempts will probably succeed. Capacitive transients during equal-and-opposite pulses will never completely cancel, if only because biologic membranes are seldom that perfect. Although much may ultimately be learned from studying asymmetric displacement currents in all kinds of tissues, considerable experimental effort may be necessary before a physiologic significance of such currents is established.

So far, the discussion has centered on probable intramembrane dipole moment changes due to cell membrane potential changes. For completeness, one should refer to a case where such dipole moment changes are caused by other energy sources. In photoreceptors of vertebrate retinae, bright light flashes produce brief transmembrane currents; these are thought to be displacement currents caused by photoisomerization of a membrane protein, rhodopsin. They cause the "early receptor potential" which has been recorded extracellularly (*Brown* and *Murakami*, 1964) and intracellularly (*Murakami* and *Pak*, 1970; *Hodgkin* and *O'Bryan*, 1977). The early receptor potential is probably no more than a byproduct in the visual process, and any information contained in it is most likely discarded by the CNS during further processing. Nevertheless, the effect has been investigated fairly extensively (*Cone* and *Pak*, 1971) and is of interest as a signal which can be recorded in intact cones and is correlated with some of the first events in the visual process.

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References

Adams, D.J., Gage, P.W.: Gating currents associated with sodium and calcium currents in an Aplysia neuron. Science **192**, 783–784 (1975)

Adrian, R.H.: Conduction velocity and gating current in the squid giant axon. Proc. Roy. Soc. Lond. (Biol.) **189**, 81–86 (1975)

Adrian, R.H., Almers, W.: Membrane capacity measurements of frog skeletal muscle in media of low ion content. J. Physiol. (Lond.) **237**, 573–604 (1974)

Adrian, R.H., Almers, W.: The voltage dependence of membrane capacity. J. Physiol. (Lond.) **254**, 317–338 (1976a)

Adrian, R.H., Almers, W.: Charge movement in the membrane of striated muscle. J. Physiol. (Lond.) **254**, 339–360 (1976b)

Adrian, R.H., Chandler, W.K., Hodgkin, A.L.: The kinetics of mechanical activation in frog muscle. J. Physiol. (Lond.) **204**, 207–230 (1969)

Adrian, R.H., Chandler, W.K., Hodgkin, A.L.: Voltage clamp experiments in striated muscle fibres. J. Physiol. (Lond.) **208**, 607–644 (1970)

Adrian, R.H., Chandler, W.K., Rakowski, R.F.: Charge movement and mechanical re-priming in skeletal muscle. J. Physiol. (Lond.) **254**, 361–388 (1976)

Adrian, R.H., Peres, A.R.: A gating signal for the potassium channel? Nature (Lond.) **267**, 800–804 (1977)

Almers, W.: Observations on intramembrane charge movements in skeletal muscle. Philos. Trans. Roy. Soc. Lond. (Biol. Sci.) **270**, 507–513 (1975)

Almers, W.: Differential effects of tetracaine on delayed potassium channels and displacement currents in frog skeletal muscle. J. Physiol. (Lond.) **262**, 613–637 (1976)

Almers, W.: Local anesthetics and excitation-contraction coupling in skeletal muscle: Effects on a Ca^{++}-channel. Biophys. J. **18**, 355–357 (1977)

Almers, W., Adrian, R.H., Levinson, S.R.: Some dielectric properties of muscle membrane and their possible importance for excitation-contraction coupling. Ann. N.Y. Acad. Sci. **264**, 278–292 (1975)

Almers, W., Best, P.M.: Effects of tetracaine on displacement currents and contraction in frog skeletal muscle. J. Physiol. (Lond.) **262**, 583–611 (1976)

Almers, W., Cahalan, M.D.: Interaction between a local anesthetic, the sodium channel gates and tetrodotoxin. Biophys. J. **17**, 205a (1977)

Almers, W., Levinson, S.R.: Tetrodotoxin binding to normal and depolarized frog muscle and the conductance of a single sodium channel. J. Physiol. (Lond.) **247**, 483–509 (1975)

Andersen, O.S., Feldbert, S., Nakadomari, H., Levy, S., McLaughlin, S.: Electrostatic potentials associated with the absorption of tetraphenylborate into lipid bilayer membranes. In: Ion Transport Across Membranes – the Proceedings of a Joint US-USSR Conference. Tosteson, D.C., Ovchinnikov, Y.A., Latorre, R. (eds.). New York: Raven Press 1977 (in press)

Andersen, O.S., Fuchs, M.: Potential energy barriers to ion transport within lipid bilayers. Studies with tetraphenylborate. Biophys. J. **15**, 795–830 (1975)

Andrews, D.M., Manev, E.D., Haydon, D.A.: Composition and energy relationships for some thin lipid films, and the chain conformation in monolayers at liquid-liquid interfaces. Spec. Disc. Faraday Soc. **1**, 46–56 (1970)

Armstrong, C.M.: Time course of TEA^+-induced anomalous rectification in squid axons. J. Gen. Physiol. **50**, 491–503 (1966)

Armstrong, C.M.: Inactivation of the potassium conductance and related phenomena caused by quarternary ammonium ion injection in squid axons. J. Gen. Physiol. **54**, 553–575 (1969)

Armstrong, C.M.: Ionic pores, gates, and gating currents. Q. Rev. Biophys. **7**, 179–210 (1975a)

Armstrong, C.M.: K pores of nerve and muscle membranes. In: Membranes: A Series of Advances 3. Eisenman, G. (ed.). New York: Marcel Dekker 1975b, pp. 325–358

Armstrong, C.M., Bezanilla, F.: Currents related to movement of the gating particles of the sodium channels. Nature (Lond.) **242**, 459–461 (1973)

Armstrong, C.M., Bezanilla, F.: Charge movement associated with the opening and closing of the activation gates of the Na channels. J. Gen. Physiol. **63**, 533–552 (1974)

Armstrong, C.M., Bezanilla, F.: Currents associated with the ionic gating structures in nerve membrane. Ann. N.Y. Acad. Sci. **264**, 265–277 (1975)

Armstrong, C.M., Bezanilla, F.: Inactivation of the sodium channel. II. Gating current experiments. J. Gen. Physiol. **70**, 567–590 (1977)

Armstrong, C.M., Bezanilla, F., Rojas, E.: Destruction of sodium conductance inactivation in squid axons perfused with pronase. J. Gen. Physiol. **62**, 375–391 (1973)

Babakov, A.V., Ermishkin, L.N., Liberman, E.A.: Influence of electric field on the capacity of phospholipid membranes. Nature (Lond.) **210**, 953–955 (1966)

Baylor, S.M., Oetliker, H.: A large birefringence signal preceding contraction in single twitch fibres of the frog. J. Physiol. (Lond.) **264**, 141–162 (1977a)

Baylor, S.M., Oetliker, H.: The optical properties of birefringence signals from single muscle fibres. J. Physiol. (Lond.) **264**, 163–198 (1977b)

Baylor, S.M., Oetliker, H.: Birefringence changes from surface and T-system membranes of frog single muscle fibres. J. Physiol. (Lond.) **264**, 199–213 (1977c)

Begenisich, T., Lynch, C.: Effects of internal divalent cations on voltage-clamped squid axons. J. Gen. Physiol. **63**, 675–689 (1974)

Begenesich, T., Stevens, C.F.: How many conductance states do potassium channels have? Biophys. J. **15**, 834–846 (1975)

Benz, R., Fröhlich, O., Läuger, P., Montal, M.: Electrical capacity of black lipid films and of lipid bilayers made from monolayers. Biochem. Biophys. Acta **394**, 323–334 (1975)

Bezanilla, F., Armstrong, C.M.: Gating currents of the sodium channels: three ways to block them. Science **183**, 753–754 (1974)

Bezanilla, F., Armstrong, C.M.: Kinetic properties and inactivation of the gating currents of sodium channels in squid axon. Philos. Trans. Roy. Soc. Lond. (Biol. Sci.) **270**, 449–458 (1975)

Bezanilla, F., Armstrong, C.M.: Properties of the sodium channel gating current. Cold Spring Harbor Symp. Quant. Biol. **XL**, 297–304 (1976)

Bezanilla, F., Armstrong, C.M.: Inactivation of the sodium channel. I. Sodium current experiments. J. Gen. Physiol. **70**, 549–566 (1977)

Bezanilla, F., Caputo, C., Horowicz, P.: Voltage activation of contraction in single fibers of frog striated muscle. J. Physiol. Soc. Jap. **34**, 1 (1972)

Bezanilla, F., Horowicz, P.: Fluorescence intensity changes associated with contractile activation in frog muscle stained with nile blue A. J. Physiol. (Lond.) **246**, 709–735 (1975)

Blatt, F.J.: Gating currents. The role of nonlinear capacitive currents of electrostrictive origin. Biophys. J. **18**, 43–52 (1977)

Brown, K.T., Murakami, M.: A new receptor potential of the monkey retina with no detectable latency. Nature (Lond.) **201**, 626–628 (1964)

Camejo, G., Villegas, G.M., Barnola, F.V., Villegas, R.: Characterization of two different membrane fractions isolated from the first stellar nerve of the squid Dosidicus gigas. Biochem. Biophys. Acta **193**, 247–259 (1969)

Caputo, C.: The effect of low temperature on the excitation-contraction coupling phenomena of frog single muscle fibres. J. Physiol. (Lond.) **223**, 461–482 (1976a)

Caputo, C.: The time course of potassium contractures of single muscle fibres. J. Physiol. (Lond.) **223**, 483–505 (1976b)

Chandler, W.K., Hodgkin, A.L., Meves, H.: The effect of changing the internal solution on sodium inactivation and related phenomena in giant axons. J. Physiol. (Lond.) **180**, 821–836 (1965)

Chandler, W.K., Meves, H.: Voltage clamp experiments on internally perfused giant axons. J. Physiol.(Lond.) **180**, 788–820 (1965)

Chandler, W.K., Meves, H.: Sodium and potassium currents in squid axons perfused with fluoride solutions. J. Physiol. (Lond.) **211**, 623–652 (1970a)
Chandler, W.K., Meves, H.: Evidence for two types of sodium conductance in axons perfused with sodium fluoride solution. J. Physiol. (Lond.) **211**, 653–678 (1970b)
Chandler, W.K., Rakowski, R.F., Schneider, M.F.: A nonlinear voltage dependent charge movement in frog skeletal muscle. J. Physiol. (Lond.) **254**, 245–283 (1976a)
Chandler, W.K., Rakowski, R.F., Schneider, M.F.: Effects of glycerol treatment and maintained depolarization on charge movement in skeletal muscle. J. Physiol. (Lond.) **254**, 285–316 (1976b)
Chandler, W.K., Schneider, M.F.: Time-course of potential spread along a skeletal muscle fiber under voltage clamp. J. Gen. Physiol. **67**, 165–184 (1976)
Chiu, S.Y.: Inactivation of sodium channels: second-order kinetics in myelinated nerve. J. Physiol. (Lond.) **273**, 573–596 (1978)
Cohen, L.B., Hille, B., Keynes, R.D., Landowne, D., Rojas, E.: Analysis of the potential-dependent changes in optical retardation in the squid giant axon. J. Physiol. (Lond.) **218**, 205–234 (1971)
Cole, R.H.: Relaxation processes in dielectrics. J. Cell Comp. Physiol. **66**, 13–20 (1965)
Cole, K.S.: Resistivity of axoplasm. I. Resistivity of extruded squid axoplasm. J. Gen. Physiol. **66**, 133–138 (1975)
Cole, K.S.: Electrical properties of the squid axon sheath. Biophys. J. **16**, 137–142 (1976)
Cone, R.A., Pak, W.L.: The early receptor potential. In: Handbook of Sensory Physiology. Loewenstein, W.R. (ed.), Vol. I, pp. 345–365. Berlin–Heidelberg–New York: Springer 1971
Conti, F., deFelice, L.J., Wanke, E.: Potassium and sodium current noise in the membrane of the squid giant axon. J. Physiol. (Lond.) **248**, 45–82 (1975)
Conti, F., Hille, B., Neumcke, B., Nonner, W., Stampfli, R.: Measurement of the conductance of the sodium channel from current fluctuations at the node of Ranvier. J. Physio. (Lond.) **262**, 699–727 (1976)
Costantin, L.L.: Contractile activation in frog skeletal muscle. J. Gen. Physiol. **63**, 657–674 (1974)
Coster, H.G.L., Simons, R.: Anomalous dielectric dispersion in bimolecular lipid membranes. Biochem. Biophys. Acta **203**, 17–27 (1970)
Coster, H.G.L., Smith, J.R.: The molecular organisation of bimolecular lipid membranes – A study of the low frequency Maxwell-Wagner impedance dispersion. Biochem. Biophys. Acta **373**, 151–164 (1974)
Courtney, K.R.: Mechanism of frequency-dependent inhibition of sodium currents in frog myelinated nerve by the lidocaine derivative GEA 968. J. Pharmacol. Exp. Ther. **195**, 225–236 (1975)
Curtis, H.J., Cole, K.S.: Transverse electric impedance of the squid giant axon. J. Gen. Physiol. **21**, 757–765 (1938)
DiPolo, R., Requena, J., Brinley, F.J., Jr., Mullins, L.J., Scarpa, A., Tiffert, T.: Ionized calcium concentrations in squid axons. J. Gen. Physiol. **67**, 433–467 (1976)
Dubois, J.M., Bergmann, C.: Asymmetrical currents and sodium currents in Ranvier nodes exposed to DDT. Nature (Lond.) **266**, 741–742 (1977)
Dulhunty, A.F., Gage, P.W.: Differential effects of glycerol treatment on membrane capacity and excitation-contraction coupling in toad sartorius fibres. J. Physiol. (Lond.) **234**, 373–408 (1973)
Edsall, J.T.: Dielectric constants and dipole moments of dipolar ions. In: Proteins, Amino Acids and Peptides. Cohn, E.J., Edsall, J.T. (eds.). New York: Reinhold Publishing Co. 1941
Ehrenstein, G., Gilbert, D.L.: Slow changes of potassium permeability in the squid giant axon. Biophys. J. **6**, 553–566 (1966)
Eisenberg, B., Eisenberg, R.S.: Selective disruption of the sarcotubular system in frog sartorius muscle. J. Cell. Biol. **39**, 451–467 (1968)

Falk, G., Fatt, P.: Linear electrical properties of striated muscle fibres observed with intracellular electrodes. Proc. Roy. Doc. Lond. (Biol.) **160**, 69–123 (1964)

Fettiplace, R., Andrews, D.M., Haydon, D.A.: The thickness, composition and structure of some lipid bilayers and natural membranes. J. Membrane Biol. **5**, 277–296 (1971)

Fitzhugh, R., Cole, K.S.: Voltage- and current clamp transients with membrane dielectric loss. Biophys. J. **13**, 1125–1140 (1973)

Fox, J.M., Neumcke, B., Nonner, W., Stämpfli, R.: Block of gating currents by ultraviolet radiation in the membrane of myelinated nerve. Pflügers Arch. **364**, 143–145 (1976)

Frankenhaeuser, B., Hodgkin, A.L.: The action of calcium on the electrical properties of squid axons. J. Physiol. (Lond.) **137**, 218–244 (1957)

Franzini-Armstrong, C.: Studies of the triad. I. Structure of the junction in frog twitch fibers. J. Cell Biol. **47**, 488–499 (1970)

Franzini-Armstrong, C., Venosa, R.A., Horowicz, P.: Morphology and accessibility of the 'transverse' tubular system in frog sartorius muscle after glycerol treatment. J. Membrane Biol. **14**, 197–212 (1973)

Gage, P.W., Moore, J.W., Westerfield, M.: An octopus toxin, maculotoxin, selectively blocks sodium current in squid axons. J. Physiol. (Lond.) **259**, 427–443 (1976)

Goldman, L.: Kinetics of channel gating in excitable membranes. Q. Rev. Biophys. **9**, 491–526 (1976)

Goldman, L., Schauf, C.L.: Quantitative description of sodium and potassium currents and computed action potentials in *Myxicola* giant axons. J. Gen. Physiol. **59**, 659–675 (1973)

Gordon, A.M., Godt, R.E., Donaldson, S.K.B., Harris, C.E.: Tension in skinned frog muscle fibers in solutions of varying ionic strength and neutral salt composition. J. Gen. Physiol. **62**, 550–574 (1973)

Gurd, R.N., Wilcox, E.: Complex formation between metallic cations and proteins, peptides, and amino acids. In: Advances in Protein Chemistry. Anson, M.L., Bailey, K., Edsall, J.T. (eds.), Vol. XI. New York: Academic Press 1956

Hanai, T., Haydon, D.A., Taylor, J.: An investigation by electrical methods of lecithin-in-hydrocarbon films in aqueous solutions. Proc. Roy. Soc. Lond. A **281**, 377–391 (1964)

Hanai, T., Haydon, D.A., Taylor, J.: Polar group orientation and the electrical properties of lecithin bimolecular leaflets. J. Theor. Biol. **9**, 278–296 (1965)

Haydon, D.A.: The organization and permeability of artificial lipid membranes. In: Membranes and Ion Transport. Bittar, E. (ed.), Vol. I. New York: Wiley & Sons 1970

Haydon, D.A.: Functions of the lipid in bilayer permeability. Ann. N.Y. Acad. Sci. **264**, 2–16 (1975)

Heistracher, P., Hunt, C.C.: The relation of membrane changes to contraction in twitch muscle fibres. J. Physiol. (Lond.) **201**, 589–611 (1969a)

Heistracher, P., Hunt, C.C.: Contractile repriming in snake muscle fibres. J. Physiol. (Lond.) **201**, 589–611 (1969b)

Hill, T.L., Chen, Y.: On the theory of ion transport across the nerve membrane. VI. Free energy change and activation free energies of conformational change. Proc. Natl. Acad. Sci. USA **69**, 1723–1726 (1972)

Hille, B.: Pharmacological modicications of the sodium channels of frog nerve. J. Gen. Physiol. **51**, 199–219 (1968)

Hille, B.: The receptor for tetrodotoxin and saxitoxin. A structural hypothesis. Biophys. J. **15**, 615–619 (1975)

Hille, B.: Gating in sodium channels in nerve. Annu. Rev. Physiol. **38**, 139–152 (1976)

Hille, B.: The pH-dependent rate of local anesthetics on the node of Ranvier. J. Gen. Physiol. **69**, 475–496 (1977a)

Hille, B.: Local anesthetics' hydrophilic and hydrophobic pathways for the drug-receptor reaction. J. Gen. Physiol. **69**, 497–515 (1977b)

Hille, B., Campbell, D.T.: An improved vaseline gap voltage clamp for skeletal muscle fibers. J. Gen. Physiol. **67**, 265–293 (1976)

Hodgkin, A.L.: The optimum density of sodium channels in an unmyelinated nerve. Philos. Trans. Roy. Soc. Lond. (Biol. Sci.) **270**, 297–300 (1975)

Hodgkin, A.L., Horowicz, P.: Potassium contractures in single muscle fibres. J. Physiol. (Lond.) **153**, 386–403 (1960)

Hodgkin, A.L., Huxley, A.F.: Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. J. Physiol. (Lond.) **117**, 449–472 (1952a)

Hodgkin, A.L., Huxley, A.F.: The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo*. J. Physiol. (Lond.) **116**, 497–506 (1952b)

Hodgkin, A.L., Huxley, A.F.: A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol. (Lond.) **117**, 500–544 (1952c)

Hodgkin, A.L., Huxley, A.F., Katz, B.: Measurement of current-voltage relations in the membrane of the giant axon of *Loligo*. J. Physiol. (Lond.) **116**, 424–448 (1952)

Hodgkin, A.L., Nakajima, S.: The effects of diameter on the electrical constants of frog skeletal muscle fibres. J. Physiol. (Lond.) **221**, 105–120 (1972a)

Hodgkin, A.L., Nakajima, S.: Analysis of the membrane capacity in frog muscle. J. Physiol. (Lond.) **221**, 121–136 (1972b)

Hodgkin, A.L., O'Bryan, P.M.: Internal recording of the early receptor potential in turtle cones. J. Physiol. (Lond.) **267**, 736–766 (1977)

Jaimovitch, E., Venosa, R.A., Shrager, P., Horowicz, P.: Density and distribution of tetrodotoxin receptors in normal and detubulated frog sartorius muscle. J. Gen. Physiol. **67**, 399–416 (1976)

Kaufman, L.J., Bettelheim, F.A.: Effect of water sorption on the dielectric behavior of calcium chondroitin-4-sulfate. J. Polym. Sci. (A 2) **9**, 917–926 (1971)

Ketterer, B., Neumcke, B., Läuger, P.: Transport mechanism of hydrophobic ions through lipid bilayer membranes. J. Membrane Biol. **5**, 225–245 (1972)

Keynes, R.D.: The molecular organisation of the sodium channels in nerve. In: Biochemistry of Membrane Transport, FEBS-Symposium No. 42. Semenza, G., Carafoli, E. (eds.), pp. 442–448. Berlin–Heidelberg–New York: Springer 1977

Keynes, R.D., Bezanilla, F., Rojas, E., Taylor, R.E.: The rate of action of tetrodotoxin on sodium conductance in the squid giant axon. Philos. Trans. Roy. Soc. Lond. (Biol. Sci.) **270**, 365–375 (1975)

Keynes, R.D., Rojas, E.: Characteristics of the sodium gating current in the squid giant axon. J. Physiol. (Lond.) **233**, 28–30P (1973)

Keynes, R.D., Rojas, E.: Kinetics and steady state properties of the charged system controlling sodium conductance in the squid giant axon. J. Physiol. (Lond.) **239**, 393–434 (1974)

Keynes, R.D., Rojas, E.: The temporal and steady-state relationships between activation of the sodium conductance and movement of the gating particles in the squid giant axon. J. Physiol. (Lond.) **255**, 157–189 (1976)

Keynes, R.D., Rojas, E., Rudy, B.: Demonstration of a first-order voltage-dependent transition of the sodium activation gates. J. Physiol. (Lond.) **239**, 100–101P (1974)

Kniffki, K.D., Koppenhöfer, E., Vogel, W.: Effects of procaine on gating and sodium currents at the nodal membrane. Pflügers Arch. **365**, 33 (1976)

Kornberg, R.D., McConnell, H.M.: Inside-outside transitions of phospholipids in vesicle membranes. Biochemistry **10**, 1110–1120 (1971)

Kostyuk, P.G., Khristal, O.A., Pidoplichko, V.I.: Asymmetrical displacement currents in nerve cell membrane and effect of internal fluoride. Nature (Lond.) **267**, 70–72 (1977)

Khrishtal, O.A., Pidoplichko, V.I.: Displacement currents connected with the activation of gating mechanism of calcium channels in nerve cell membrane. Dokl. Akad. Nauk SSSR **231**, 1248–1251 (1976)

Kovacs, L., Schneider, M.F.: Increased optical transparency associated with excitation contraction coupling in voltage-clamped cut skeletal muscle fibres. Nature (Lond.)

Lecuyer, H., Dervichian, D.G.: Structure of aqueous mixtures of lecithin and cholesterol. J. Mol. Biol. **45**, 39–57 (1969)
Levinson, S.R., Meves, H.: The binding of tritiated tetrodotoxin to squid giant axons. Philos. Trans. Roy. Soc. Lond. (Biol. Sci.) **270**, 349–352 (1975)
Levitan, E., Palti, Y.: Dipole moment, enthalpy, and entropy changes of Hodgkin-Huxley type kinetic units. Biophys. J. **15**, 239–251 (1975)
Lüttgau, H.C., Glitsch, H.: Membrane physiology of nerve and muscle fibres. Fortschr. Zool. **24**, 1–132 (1976)
Lüttgau, H.C., Oetliker, H.: The action of caffeine on the activation of the contractile mechanism in striated muscle fibres. J. Physiol. (Lond.) **194**, 51–74 (1968)
Magleby, K.L., Stevens, C.F.: A quantitative description of end-plate currents. J. Physiol. (Lond.) **223**, 173–197 (1972)
Marcus, D., Canessa-Fischer, M., Zampighi, G., Fischer, S.: The molecular organization of nerve membranes. VI. The separation of axolemma from Schwann cell membranes of giant and retinal squid axons by density gradient centrifugation. J. Membrane Biol. **9**, 209–228 (1972)
Mathias, R.T., Eisenberg, R.S., Valdiosera, R.: Electrical properties of frog skeletal muscle fibers interpreted with a mesh model of the tubular system. Biophys. J. **17**, 57–93 (1977)
Meves, H.: The effect of holding potential on the asymmetry currents in squid giant axons. J. Physiol. (Lond.) **243**, 847–867 (1974)
Meves, H.: The effect of zinc on the late displacement current in squid giant axons. J. Physiol. (Lond.) **254**, 787–801 (1976)
Meves, H.: Intramembrane charge movement in squid giant nerve fibres. In: The Behaviour of Ions in Macromolecular and Biological Systems. Colston Papers No. 29, Bristol: Scientechnica Publishers 1977
Meves, H., Vogel, W.: Inactivation of the asymmetrical displacement current in giant axons of *Loligo forbesi*. J. Physiol. (Lond.) **267**, 377–393 (1977a)
Meves, H., Vogel, W.: Slow recovery of sodium current and gating current from inactivation. J. Physiol. (Lond.) **267**, 395–410 (1977b)
Miledi, R., Parker, I., Schalow, G.: Measurement of calcium transients in frog muscle by the use of arsenazo III. Proc. Roy. Soc. Lond. (Biol. Sci.) **198**, 201–210 (1977)
Mobley, B.A., Eisenberg, B.R.: Sizes of components in frog skeletal muscle measured by methods of stereology. J. Gen. Physiol. **66**, 31–45 (1975)
Montal, M., Mueller, P.: Formation of bimolecular membranes from lipid monolayers and a study of their electrical properties. Proc. Natl. Acad. Sci. USA **69**, 3561–3566 (1972)
Moolenaar, W.H., Spector, I.: Membrane currents examined under voltage clamp in cultured neuroblastoma cells. Science **196**, 331–333 (1977)
Mueller, P., Rudin, D.O., Tien, H.T., Wescott, W.C.: Reconstitution of cell membrane structure in vitro and its transformation into an excitable system. Nature (Lond.) **194**, 979–980 (1962)
Murakami, M., Pak, W.L.: Intracellularly recorded early receptor potential of the vertebrate photoreceptors. Vision Res. **10**, 965–975 (1970)
Neumcke, B., Nonner, W., Stämpfli, R.: Asymmetrical displacement current and its relation with the activation of sodium current in the membrane of frog myelinated nerve. Pflügers Arch. **363**, 193–203 (1976)
Neumcke, B., Nonner, W., Stämpfli, R.: Gating currents in excitable membranes. In: M.T.P. International Review of Science, Series 2, Biochemistry Series, Vol. II (in press) 1977
Noble, D., Tsien, R.W.: The kinetics and rectifier properties of the slow potassium current in cardiac Purkinje fibres. J. Physiol. (Lond.) **195**, 185–214 (1968)
Nonner, W., Conti, F., Hille, B., Neumcke, B., Stämpfli, R.: Current noise and the conductance of single sodium channels. Pflügers Arch. **362**, 27 (1976)
Nonner, W., Rojas, E., Stämpfli, R.: Gating currents in the node of Ranvier: voltage and time dependence. Philos. Trans. Roy. Soc. Lond. (Biol. Sci.) **270**, 483–492 (1975)

Oetliker, H., Baylor, S.M., Chandler, W.K.: Simultaneous changes in fluorescence and optical retardation in single muscle fibres during activity. Nature (Lond.) **257**, 693–696 (1975)

Oxford, G.S., Yeh, J.Z.: Observations on sodium channel gating in squid axons internally perfused with pronase or N-bromoacetamide. Biophys. J. **17**, 207a (1977)

Oxford, G.S., Wu, C.H., Narahashi, T.: Removal of sodium channel inactivation in squid axon membranes by N-bromoacetamide. Biophys. J. **16** (2, Pt. 2), 187a (1976)

Peachey, L.D.: The sarcoplasmic reticulum and transverse tubules of the frog's sartorius. J. Cell. Biol. **25**, 209–231 (1965)

Peacock, J., Minna, J., Nelson, P., Nirenberg, M.: Use of aminopterin in selecting electrically active neuroblastoma cells. Exp. Cell Res. **73**, 367–377 (1972)

Ritchie, J.M., Rogart, R.B.: The binding of labelled saxotoxin to normal and denervated muscle. J. Physiol. (Lond.) **263**, 129–130 (1976)

Ritchie, J.M., Rogart, R.B.: Density of sodium channels in mammalian myelinated nerve fibers and nature of axonal membrane under the myelin sheath. Proc. Natl. Acad. Sci. USA **74**, 211–215 (1977)

Ritchie, J.M., Rogart, R.B., Strichartz, G.R.: A new method for labelling saxitoxin and its binding to non-myelinated fibres of the rabbit vagus, lobster walking leg, and garfish olfactory nerve. J. Physiol. (Lond.) **261**, 477–494 (1976)

Rojas, E.: Gating mechanisms for the activation of sodium conductance in nerve membranes. Cold Spring Harbor Symp. Quant. Biol. **40**, 305–320 (1975)

Rojas, E., Keynes, R.D.: On the relation between displacement currents and activation of the sodium conductance in squid giant axons. Philos. Trans. Roy. Soc. Lond. (Biol. Sci.) **270**, 459–482 (1975)

Rosen, D.: Dielectric properties of protein powders with adsorbed water. Trans. Faraday Soc. **59**, 2178–2191 (1963)

Rudy, B.: Sodium gating currents in *Myxicola* giant axons. Proc. Roy. Soc. Lond. (Biol.) **193**, 469–475 (1976)

Schneider, M.F.: Linear electrical properties of the transverse tubules and surface membrane of skeletal muscle fibers. J. Gen. Physiol. **56**, 640–671 (1970)

Schneider, M.F., Chandler, W.K.: Voltage-dependent charge movement in skeletal muscle: a possible step in excitation-contraction coupling. Nature (Lond.) **242**, 244–246 (1973)

Schneider, M.F., Chandler, W.K.: Effects of membrane potential on the capacitance of skeletal muscle fibres. J. Gen. Physiol. **67**, 125–163 (1976)

Schwarz, G.: On dielectric relaxation due to chemical rate processes. J. Phys. Chem. **71**, 4021–4030 (1967)

Schwarz, G.: Electric field effects on macromolecules and the mechanism of voltage-dependent processes in biological membranes. In: The Neurosciences: Fourth Study Program, Schmitt, F.O., Worden, F.A. (eds.) (in press)

Schwarz, W., Palade, P.T., Hille, B.: Local anesthetics: Effects of pH on use-dependent block of Na channels in frog muscle. Biophys. J. **20**, 343–368 (1977)

Seeman, P.: The membrane actions of anesthetics and tranquilizers. Pharmac. Rev. **24**, 583–655 (1972)

Sheumack, D.D., Howden, M.E.H., Spence, I., Quinn, R.J.: Maculotoxin: a neurotoxin from the venom glands of the octopus Hapalochlaena maculosa identified as tetrodotoxin. Science **199**, 188–189 (1978)

Stanfield, P.R.: The effect of the tetraethylammonium ion on the delayed currents of frog skeletal muscle. J. Physiol. (Lond.) **209**, 209–229 (1970)

Stanfield, P.R.: The effect of zinc ions on the gating of the delayed potassium conductance of frog sartorius muscle. J. Physiol. (Lond.) **251**, 711–735 (1975)

Stevens, C.F.: Interactions between intrinsic membrane protein and electric field. Biophys. J. **17**, 264a (1977)

Strichartz, G.R.: The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine. J. Gen. Physiol. **62**, 37–57 (1973)

Takashima, S.: Membrane capacity of squid giant axon during hyper- and depolarizations. J. Membrane Biol. **27**, 21–39 (1976)

Takashima, S., Minakata, A.: Dielectric behavior of biological macromolecules. In: Digest of Dielectric Literature. Vaughn, W. (ed.), National Research Council: National Academy of Sciences USA 1975 (in press)

Takashima, S., Schwan, H.P.: Dielectric dispersion of crystalline powders of amino acids, peptides and proteins. J. Phys. Chem. **69**, 4176–4182 (1965)

Takashima, S., Schwan, H.P.: Passive electrical properties of squid axon membrane. J. Membrane Biol. **17**, 51–68 (1974a)

Takashima, S., Schwan, H.P.: Dielectric relaxation in lipid bilayer membranes. In: Liquid Crystals and Ordered Fluids. Johnson, J.F., Porter, R.S. (eds.). New York: Plenum Press 1974b

Takashima, S., Yantorno, R., Pal, N.C.: Electrical properties of squid axon membrane. II. Effect of partial degradation by phospholipase A and pronase on electrical characteristics. Biochem. Biophys. Acta **401**, 15–27 (1975)

Taylor, R.E.: Impedance of the squid axon membrane. J. Cell. Physiol. **66** (Pt. II), 21–25 (1965)

Taylor, R.E.: Electrical impedance of excitable membranes. Ann. N.Y. Acad. Sci. (in press) (1977)

Taylor, S.R., Rüdel, R., Blinks, J.R.: Calcium transients in amphibian muscle. Fed. Proc. **34**, 1379–1381 (1975)

Tsien, R.W., Noble, D.: A transition state theory approach to the kinetics of conductance changes in excitable membranes. J. Membrane Biol. **2**, 248–273 (1969)

Ulbricht, W.: Ionic channels and gating currents in excitable membranes. Ann. Rev. Biophys. Bioeng. (in press) (1977)

Ulbricht, W., Wagner, H.-W.: The influence of pH on equilibrium effects of tetrodotoxin on myelinated nerve fibres of *Rana esculenta*. J. Physiol. (Lond.) **252**, 159–184 (1975)

Valdiosera, R., Clausen, C., Eisenberg, R.S.: Impedance of frog skeletal muscle fibers in various solutions. J. Gen. Physiol. **63**, 460–491 (1974)

White, S.H.: Temperature-dependent structural changes in planar bilayer membranes: solvent "freeze-out". Biochem. Biophys. Acta **356**, 8–16 (1974)

Wobschall, D.: Voltage dependence of bilayer membrane capacity. J. Colloid Interface Sci. **40**, 417–423 (1972)

Zambrano, F., Cellino, M., Canessa-Fischer, M.: The molecular organization of nerve membranes. IV. The lipid composition of plasma membranes from squid retinal axons. J. Membrane Biol. **6**, 289–303 (1971)

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COLLINS, J.: Gene Cloning with Small Plasmids. Curr. Top. Microbiol. Immunol. 78, 121–170 (1977)

HAUSEN, H. ZUR: Human Papillomaviruses and Their Possible Role in Squamous Cell Carcinomas. Curr. Top. Microbiol. Immunol. 78, 1–30 (1977)

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JELINKOVA, J.: Group B Streptococci in the Human Population. Curr. Top. Microbiol. Immunol. 76, 127–165 (1977)

KANO, K., MILGROM, F.: Heterophile Antigens and Antibodies in Medicine. Curr. Top. Microbiol. Immunol. 77, 43–70 (1977)

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NASH, H.A.: Integration and Excision of Bacteriophage Lambda. Curr. Top. Microbiol. Immunol. 78, 171–200 (1977)

PLOTKIN, S.A.: Perinatally Acquired Viral Infections. Curr. Top. Microbiol. Immunol. 78, 111–120 (1977)

PRINGLE, C.R.: Enucleation as a Technique in the Study of Virus-Host Interaction. Curr. Top. Microbiol. Immunol. 76, 49–82 (1977)

RAWLS, W.E., BACCHETTI, S., GRAHAM, F.L.: Relation of Herpes Simplex Viruses to Human Malignancies. Curr. Top. Microbiol. Immunol. 77, 71–96 (1977)

RICHTER, D., ISONO, K.: The Mechanism of Protein Synthesis. Initiation, Elongation and Termination in Translation of Genetic Messages. Curr. Top. Microbiol. Immunol. 76, 83–125 (1977)

SKALKA, A.M.: DNA-Replication – Bacteriophage Lambda. Curr. Top. Microbiol. Immunol. 78, 201–238 (1977)

STORZ, J., SPEARS, P.: Chlamydiales: Properties, Cycle of Development and Effect on Eukaryotic Host Cells. Curr. Top. Microbiol. Immunol. 76, 167–214 (1977)

WENGLER, G.: Structure and Function of the Genome of Viruses Containing Single-Stranded RNA as Genetic Material: The Concept of Transcription and Translation Helices and the Classification of these Viruses into Six Groups. Curr. Top. Microbiol. Immunol. 78, 239–248 (1977)

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DEUTICKE, B.: Properties and Structural Basis of Simple Diffusion. Pathways in the Erythrocyte Membrane. Rev. Physiol. Biochem. Pharmacol. 78, 1–98 (1977)

ELLENDORFF, F.: Evaluation of Extrahypothalamic Control of Reproductive Physiology. Rev. Physiol. Biochem. Pharmacol. 76, 103–128 (1976)

GRUNEWALD, W.A., SOWA, W.: Capillary Structures and O_2 Supply to Tissue. Rev. Physiol. Biochem. Pharmacol. 77, 149–200 (1977)

HILZ, H., STONE, P.: Poly (ADP-Ribose) and ADP-Ribosylation of Proteins. Rev. Physiol. Biochem. Pharmacol. 76, 1–58 (1976)

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SACHS, G.: H^+ Transport by a Non-Electrogenic Gastric ATPase as a Model for Acid Secretion. Rev. Physiol. Biochem. Pharmacol. 79, 133–162 (1977)

SATO, K.: The Physiology, Pharmacology and Biochemistry of the Eccrine Sweat Gland. Rev. Physiol. Biochem. Pharmacol. 79, 51–132 (1977)

STARKE, K.: Regulation of Noradrenaline Release by Presynaptic Receptor Systems. Rev. Physiol. Biochem. Pharmacol. 77, 1–124 (1977)

WARD, P.A., BECKER, E.L.: Biology of Leukotaxis. Rev. Physiol. Biochem. Pharmacol. 77, 125–148 (1977)

WUTTKE, W.: Neuroendocrine Mechanisms in Reproductive Physiology. Rev. Physiol. Biochem. Pharmacol. 76, 59–102 (1976)

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