# ELECTROLYTES AND EXCITABLE TISSUES[1,2]

By Juan A. Izquierdo and Iván Izquierdo[3]

*Cátedra de Farmacología Experimental, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina*



An effort has been made to avoid those topics covered by the two previous reviews under the title Electolyte and Mineral Metabolism in this *Annual Review* (1, 2). This was facilitated by the personal taste of the reviewers. Emphasis has been placed here on problems of electrolyte metabolism at excitable tissues. It will be noted throughout that under each heading, some aspects will be found that deviate somewhat from the heading. Headings are used in this review as rather broad guidelines. We apologize for the exclusion of many valuable references and of much valuable material within the articles mentioned, all for the sake of conciseness.

## Cardiac Glycosides, Active Cation Transport, and Inotropism

Since the original observation by Schatzmann that strophanthin-K prevents the active transport of $Na^+$ and $K^+$ in red cells (3), a considerable amount of work has been done extending this finding to other glycosides and tissues, including, of course, the heart (4–20, 35). The mechanism by which these ionic effects of cardiac glycosides are produced is most probably through inhibition of the $Na^+$-, $K^+$-, and $Mg^{2+}$-dependent ATPase system (20–39). This enzyme system appears to be almost universal in promoting active $Na^+$ efflux and $K^+$ influx of living cells (20, 28, 30–32, 38), although recently the alga *Ulva lactuca* was found to have a $K^+$ influx much larger than the $Na^+$ efflux and to lack a ouabain-sensitive $Na^+$-$K^+$-dependent ATPase (40).

The ATPase system on which cardiac glycosides are supposed to act is probably a combination of a phosphokinase plus a phosphatase (30–33, 41–45). There are possibly diverse ATPase systems in living cells, but there is evidence that points to a localization of the $Na^+$-, $K^+$-, and $Mg^{2+}$-dependent one, which is inhibited by ouabain, on a membrane-microsomal fraction (39, 42, 46). There is evidence both for (42, 43) and against (41) correlation of $K^+$-dependent *p*-nitrophenyl-phosphatase with ouabain-sensitive ATPase activity. It is possible that not all authors deal with the same enzyme prep-

[1] The survey of the literature pertaining to this review was concluded in March 1966.

[2] The following abbreviations will be used: ATPase (adenosine triphosphatase); e.p.p. (end plate potential); m.e.p.p. (miniature end plate potential).

[3] Present address: Cátedra de Farmacología, Instituto de Ciencias Químicas, Ciudad Universitaria, Córdoba, Cba., Argentina.

arations, as the tissues and extraction procedures differ (41, 42, 43). Purified, highly specific $Na^+$- and $K^+$-dependent ATPase preparations have been obtained. Perhaps some of the earlier work using less purified preparations, contaminated to some extent by other ATPases, $Na^+$- and $K^+$-independent, might need revision (39, 46). Using procedures by which membranous and microsomal fractions could be separated, $Na^+$-$K^+$-dependent ATPase activity was found in the former (41).

Cardiac glycosides exert their action on the second, $K^+$-dependent, phosphatase step of the ATPase reaction [(33); see also (44, 45)]. There seems to be an electrogenic $Na^+$ pump sensitive to ouabain in papillary muscle (15).

A point worthy of discussion is whether the effect of cardiac glycosides on $Na^+$ and $K^+$ movements bears any relation to their cardiotonic action (20). No definite answer can be given at present, although there are some indications that it might not be so, and others that it might. The structural requirements for glycosides to exert both sets of effects are analogous (20). Chemically different *Erythrophloeum* alkaloids exert cardiotonic and ATPase effects akin to those of glycosides (25, 47, 48), and their effect on ATPase can be antagonized by $K^+$, as can that of glycosides (25). Erythrophleine and ouabain have similar effects on membrane potential and $K^+$, $Na^+$, and $Cl^-$ concentrations in frog sartorius muscle (49). The diverse sensitivity of hearts of several species to cardiotonic activity of strophanthin is roughly correlated with their red cell ATPase sensitivity to this glycoside (37). The toad heart shows seasonal variations in the membrane-microsomal $Na^+$-$K^+$-$Mg^{2+}$-dependent ATPase activity (50), which might correlate with seasonal variations in the sensitivity of these hearts to glycosides. At high concentrations or doses of cardiac glycosides which produce toxic effects on the heart, there is increased $(Na^+)_i$ and decreased $(K^+)_i$ [see (20)], and the toxic effects of digitalis can be antagonized by $K^+$ (51).

However, there is considerable doubt as to whether the cellular $Na^+$ and $K^+$ concentrations of the heart are modified at all at glycoside concentrations which bring about increased inotropism rather than standstill [(5–8); see also (20)]. Some investigators have found that at low ($10^{-8}$ *M* or less) concentrations of ouabain, the $Na^+$- and $K^+$-dependent ATPase is stimulated (19, 25, 52), or the $Na^+$ transport is enhanced (16) under certain experimental conditions and in certain preparations. The "therapeutic" concentration of ouabain in human beings has been calculated to be around $10^{-8}$ *M* (53). The stimulatory effect on ATPase has not been found by some authors, however (54).

A point to be considered is whether $Na^+$ or $K^+$ plays any role in the cardiac action potential, its coupling with contraction mechanisms, and contraction mechanisms themselves. The inotropic effect of glycosides (20) precedes any change in the action potential that these might produce (20, 55, 56). Recently it has been shown that the peculiar heart action potential con-

sists of a $Na^+$-conductance and of a $Ca^{2+}$-conductance phase, coinciding with the initial rise and the plateau (57, 58); the $Na^+$-phase can be blocked by tetrodotoxin and the $Ca^{2+}$-phase by $Mn^{2+}$ (58). Under normal conditions, the magnitude of cardiac contractions is roughly dependent on the magnitude of the plateau phase (59), although once both are inhibited by acetylcholine, $Ca^{2+}$ restores the contractile capability independently of the duration of the action potential (60), and wide action potentials but no contractions may occur in $Ca^{2+}$-free tyrode (59). It is not known at present whether in all species, circumstances, or preparations the plateau phase and the contraction are dependent on $Ca^{2+}$, or whether it might be substituted by other divalent ions, such as for example $Mg^{2+}$ [see (59)]. $Zn^{2+}$ depresses cardiac muscle (62, 63, 64) but it may restore contractility in the absence of $Ca^{2+}$, as do other divalent cations (61).

That the effect of cardiac glycosides on inotropism is sensitive to external $Ca^{2+}$ has been known since the days of Clark (65) and Loewi (66). Since, in some species, the time that glycosides take to reach a maximal inotropic effect is shorter, the higher the predrug heart rate (67), and since each beat is associated with an inward $Na^+$ and $Ca^{2+}$ movement (58), and since heart work is associated sometimes with $K^+$ loss (68–70), any enhancing effect of glycosides on $Ca^{2+}$ entry, $Na^+$ entry, or $K^+$ loss may be causally related to the inotropic action. Indeed, cardiac glycosides increase the $K^+$ loss of working hearts (71). Reasons were given above to doubt somewhat the association of the actions on $Na^+$ and $K^+$ transport and inotropism. A further reason might be that certain $Na^+$-$K^+$-dependent ATPase preparations from red cell membranes seem to induce $Na^+$ retention rather than loss (72). Some reasons will be given below which might favor the idea of a correlation between the cardiotonic action and some effect on $Ca^{2+}$ movement of cardiac glycosides.

Ouabain increases $Ca^{2+}$-uptake by isolated rabbit atria at the same time that an inotropic effect is being produced (73). Digitalis-like compounds possibly increase $Ca^{2+}$ at the site of excitation-contraction coupling (74). Lock (75), using a hen auricle preparation (76), found that temperature affects similarly the inotropic effect of ouabain and that of $Ca^{2+}$ added to the bath in excess of the normal $Ca^{2+}$ concentration; indeed, the slopes of dose-response curves for both ouabain and added $Ca^{2+}$ increase to the same extent with increased temperature. Several authors have found that the inotropic effect of cardiac glycosides is accompanied by an increased exchange of tissue $Ca^{2+}$ (73, 77). Ouabain induces no increase of $^{45}Ca^{2+}$ entry in quiescent rabbit atria but it increases it enormously if these are made to beat (78).

While all these observations seem to support the hypothesis that cardiac glycosides exert their inotropic effect through a $Ca^{2+}$ mechanism, there are several rather obscure points. First, the original observation by Wilbrandt et al. (67) on predrug heart rate and inotropic effect of glycosides has since been extended to some species, but was shown not to apply to rabbit, guinea pig (79), and hen (75) myocardia, all of which show increased ino-

tropism by glycosides even if these are applied to the quiescent preparations. Then, there is no agreement between the various authors as to what definite effect the glycosides have on $(Ca^{2+})_i$ when in "therapeutic" concentrations [see (20)]. However, it is possible that the cardiotonic action of these substances does not depend as much on total $(Ca^{2+})_i$ as on how much $Ca^{2+}$ goes into the vesicles whose $Ca^{2+}$ uptake seems to regulate "relaxing factor" activity (20). Only myocardium containing the so-called "relaxing factor" seems able to respond to ouabain (80). The above mentioned findings by Lock (75) on the effects of temperature on dose-response curves of ouabain and of added $Ca^{2+}$ deserve also a cautious comment, which he has made himself: why does temperature affect the response to added $Ca^{2+}$, but not in the same way as those of substracted $Ca^{2+}$, that is to say, of lower than normal $(Ca^{2+})_o$?

Other than ionic effects of cardiac glycosides may or may not be related to their inotropic action. Direct effects on contractile proteins have been postulated [see (20, 93)]. Very low ($10^{-10}$ to $10^{-9}$ *M*) ouabain concentrations increase the production of $CO_2$ from glucose and pyruvate of *in vitro* dog myocardium (81); this effect is caused neither by catecholamine (82) nor by acetylcholine release (83); what relation, causal or other, this might have with the inotropic effect is not known. Effects of ouabain on tissue respiration, with special reference to the brain rather than the heart, and to phosphate turnover, may be found in a recent article by Rose (84).

Frog hearts may beat and conduct action potentials for long periods in nonionic media consisting of 0.112 *M* sucrose (85–87). Preliminary observations by Roth and the authors have shown that ouabain added to the perfusing fluid in this kind of preparation may produce cardiac standstill. We have not been able to ascertain, however, whether the perfusing fluid had been completely nonelectrolytic, or simply a low ionic strength solution. Indeed, hearts of other species have been traditionally known to cease beating altogether in nonelectrolyte media; rabbit hearts stop beating below critical $(Ca^{2+})_o$ levels (88), and in these animals a delicate $(Ca^{2+})_o$ and $(K^{2+})_o$ interrelation exists in order to maintain their excitability and contractility (89). Brady (90) has questioned some aspects of the experiments of hearts functioning in sucrose solutions, and he and Tan (91) have found that frog atrial trabeculae can only beat and perform if the extracellular ionic concentration remains above 2 per cent of normal. This is in clear contradiction with the findings by Van der Kloot & Dane (87) that hearts beating under sucrose solutions have no demonstrable $Na^+$ space, or with Singh's statements (92) that frog stomach muscle or heart loses all $Na^+$ in one hour when washed with 0.112 *M* sucrose. Indeed, Singh & Singh (86) recently came to the truly remarkable conclusion that action potentials are not due to ionic mechanisms, based on the assumption that they could not be due to $Na^+$ influx as they can occur in $Na^+$-free media, or to anion efflux as they are not abolished by increasing the extracellular concentration of various anions so as to exceed

the intracellular ones. One wonders whether the electrolyte washout by sucrose is so absolute as to sweep away the ionic atmosphere presumably attached to the fixed charges of the membranes, or whether ($Na^+$) measurements can be so precise as to be within 2 per cent error.

For references older than 1963 that are not mentioned here, the reader is referred to the excellent review by Glynn (20). Useful references on the effect of digitalis on the heart are also to be found in the review on cardiac metabolism by Bing (93). Good reviews on active transport and $Na^+$-$K^+$-dependent ATPase have appeared (30, 31, 33, 38). As space does not allow us to be more explicit on the role of $Ca^{2+}$ in excitation-contraction coupling, the following relevant recent papers are recommended (59, 90, 94–100).

## Electrolyte Mechanisms in Liberation of Transmitter Substances

The role of $Ca^{2+}$ in transmitter release is now widely accepted [see (101)]. In nerve-muscle mammalian preparations, where acetylcholine is the transmitter, the release of this agent starts 0.5 msec after the presynaptic spike (102), and "the only known intermediate reaction is that the entry of $Ca^{2+}$ is by all means necessary" (103). In adrenal medullary granule preparations, as low a $(Ca^{2+})_o$ as 0.2 m*M* produces a detectable increase in catecholamine release (104). This concentration is equal to the one which produces a detectable increase in m.e.p.p. frequency in the cholinergic nerve-muscle preparation (105), and higher than that found for earlier, more crude adrenal granule preparations [see (104)].

The posterior lobes of rats release vasopressin when $(K^+)_o$ is high (106, 107), or when electrically stimulated (108); the latter also applies to guinea pig posterior lobes (109). The release is inhibited by $Mg^{2+}$ and $Na^+$, and abolished by omitting $Ca^{2+}$ from the bathing fluid; it increases when $Ca^{2+}$ is increased, and is accompanied by a $^{45}Ca^{2+}$ influx (106, 107, 110). The interpretation afforded for these findings is that, as presumably happens in cholinergic nerve endings, $Ca^{2+}$ enters into neurosecretory terminals upon arrival of spikes therein (107, 108, 110).

Low blood $Ca^{2+}$ blocks monosynaptic transmission in the cat spinal cord; restoration of blood $Ca^{2+}$ levels to normal results in recovery (111). $Mg^{2+}$ suppresses a number of spinal cord potentials thought to reflect various types of synaptic transmission. It has the same effect on the potential found on one ventral root upon stimulation of an adjacent ventral root which is considered to reflect perhaps electrotonically transmitted depolarization (113) between adjacent motoneurons (112). This latter potential is suppressed by $Mg^{2+}$ more slowly than the others, and in this particular case $Ca^{2+}$ increases this suppression rather than reversing it as is the case with the other potentials (113).

Recent work has been done on the relation between $Na^+$, $Ca^{2+}$, and trans-

mitter release by cholinergic nerve endings. When $(Ca^{2+})_o$ is low, e.p.p.'s are reduced in size. Lowering $(Na^+)_o$ then restores e.p.p.'s to their original dimensions (114). When $(Ca^{2+})_o$ is normal, lowering $(Na^+)_o$ does not increase e.p.p. quantal content (115), but it does increase m.e.p.p. frequency (116). This effect of low $(Na^+)_o$ seems rather similar to the well-known effect of high $(K^+)_o$. It has been suggested that $Na^+$ and $Ca^{2+}$ compete for membrane sites involved in transmitter release (114) on the basis of the generally accepted competition of these ions on cardiac contraction [(117); see also (20)]. In this respect, the findings of Gage & Quastel (116) do not agree with the quantitative aspects of this postulated competition (two $Na^+$ ions versus one $Ca^{2+}$ ion). They found that lowering $(Na^+)_o$ from 100 to 70 per cent was more effective on m.e.p.p. frequency than lowering it from 70 to 40 per cent; this decrease of $(Na^+)_o$ from 100 to 70 per cent of normal was approximately as effective as a fourfold rather than a twofold increase of $Ca^{2+}$.

The effect of increasing $(K^+)_o$ on transmitter release has recently been reinvestigated (118–120). Increased $(K^+)_o$ results in increased m.e.p.p. frequency, a result generally ascribed to presynaptic depolarization [(121); see also (101)]. Gage & Quastel (118) have produced rather strong evidence that it is not correct simply to convert $(K^+)_o$ to presynaptic depolarization in terms of the Nernst equation, as they found that the $(K^+)_o$ effect on m.e.p.p. frequency continued for some time after the effect on membrane potential had reached a maximum. Parsons et al. (119) found that by increasing $(K^+)_o$ from 5 to 15 m$M$, the rate of transmitter depletion after a tetanus is increased, doubling the absolute number of quanta per impulse. On the basis of their results, they postulated two direct effects of $K^+$ on transmitter release: an increase in the probability of transmitter release per impulse, and an increase in the number of quanta in the so-called "readily releasable store."

## Glia and the Extracellular Space of Nervous Tissue

The extracellular space around nerve cells and their processes appears in electron micrographs as if it consisted only of 100 to 200 Å clefts, and there is no certainty from the pictures that these are occupied by fluids or by a cementing or other solid substance. Were it a fluid phase, the volume of the "true" extracellular space in the brain would be about 5 per cent (122). However, a variety of other measurements have given values of about 15 to 25 per cent: electrical resistance (123), $^{24}Na^+$, and inulin spaces (124–126), etc. An obvious suggestion from the comparison of electron micrographs and these measurements is that some cellular compartment accounts for this extra space in addition to the 5 per cent allowed by the clefts; indirect evidence points to the glial cells as being such a compartment [see (125–127)]. The question immediately arises whether glial cells are rich in $Na^+$ and relatively poor in $K^+$, which is what the ionic theory of electrogenesis would require of an extracellular space (128–130). Such electrolyte determinations have been

carried out just recently by Villegas et al. in squid giant axon Schwann cells ("peripheral glial cells"), and compared with the values found for the axons (131). Schwann cells were indeed found to be richer in $Na^+$ (312 m$M$/1) than the axons (52 m$M$), but they also contain a considerable amount of $K^+$ (220 versus 335 m$M$/1 in axons) and a rather similar $Cl^-$ concentration. These values by Villegas et al. may be compared with those found by Brinley for squid axons (132). However, the data by Villegas et al. do not settle the controversy of which is the extracellular space, the glia or the clefts. As they stated themselves, they have been unable to show whether the high $Na^+$ of Schwann cells is largely bound or not, and they mentioned the well-known fact that phosphate esters, polyphosphate nucleic acids, and proteins inside the cell may all bind alkali metal ions.

Toad brains immersed in isotonic solution take up water, and, after fixation, their glial but not their nerve cells appear swollen in electron micrographs (127). However, hydration and dehydration *in vivo* by perfusion with hypotonic or hypertonic solutions of rabbit brains causes changes in their $Na^+$ and $Cl^-$, but not in their $K^+$, which suggests that the changes occur in an extracellular compartment (133). It remains, of course, to be seen whether cerebral glial cells are rich in free $Na^+$ or not. Mice were similarly hydrated and dehydrated, and the cerebellar cortex was prepared for electron microscopy by way of a freeze-substitution method which is thought to preserve water distribution better than the more usual immersion or perfusion fixation techniques (134, 135). No morphological changes suggestive of shrinking or swelling were found in glial and nerve cells of the hydrated or dehydrated cerebellar cortex when compared with controls (133). These results by Van Harreveld et al. are apparently in conflict with those by De Robertis and his group (127), which were obtained at a time when fixation for electron microscopy was much cruder than it is now. It may be added that, on $OsO_4$ fixation, *Limnea* eggs become completely permeable to all ions in a few seconds (136). Anyway, the results on toad brain by the De Robertis group were obtained *in vitro* (127), so probably they cannot be too directly compared with those obtained *in vivo* by Van Harreveld et al. (133).

A number of physiologists have been reluctant to accept the idea that the glial cells function as an extracellular space. They base this reluctancy on evidence such as that by Frankenhaeuser & Hodgkin (137), who, by careful calculation, concluded that a space no larger than 270 Å, or perhaps even less, around squid axons, was needed in order to account for some aftereffects of spikes. Green (138) has advanced a coherent hypothesis for the peculiar seizure susceptibility of the hippocampus, based on a necessarily reduced size of the local extracellular space. Nicholls & Kuffler (139) have furnished rather direct evidence that in the leech central nervous system glial cells do not act as extracellular space for the neurons. These glial cells are rich in $K^+$, behave as $K^+$ electrodes at high $(K^+)_o$ much in the same way as their neurons do, and their resting potential does not vary appreciably during the

movement of $Na^+$, sucrose, or choline through the nervous system, although the neuronal activity is correspondingly affected, and they presumably do not act as active transporters of such substances to nerve cells. Upon surgical removal of most of the surrounding glial cells, leech neurons continue to function normally (139). It might be argued that the findings of Nicholls & Kuffler (139) apply only to the leech and not necessarily to other species. The same type of argument might be used for the findings of Zadunaisky et al. (124–126) in the isolated toad brain, for those by Gerschenfeld et al. in the same type of preparation (127), or for those by Lasansky & Wald in the toad retina, where ferrocyanide space is in the glial cells (140). Recent morphological evidence (141, 142) shows the leech nervous system to be no different from those of other phyla in regard to the presence of narrow intercellular clefts.

It might be interesting to try to repeat Nicholls & Kuffler's experiments using an isolated toad or frog spinal cord preparation, where the composition of a bathing fluid can be changed, and then try to locate the tips of intracellular microelectrodes with a procedure such as the one recently described by Thomas & Wilson (143). It is well known to anybody who has used intracellular microelectrodes in vertebrate preparations that many high resting potentials are found that do not respond to intracellular, antidromic, or orthodromic stimulation; one wonders how many of those resting potentials belong to glial cells.

A truly ambitious hypothesis on the role of glia as part of a "potassium-mediated neuronal-glial-neuronal impulse transmission system" has been proposed, which includes explanations of memory processes and of spreading depression (144). Some kind of metabolic rather than ionic connection between glia and neurons has been envisaged for a long time, and recently resuggested on experimental grounds [see (145, 146)].

## Tetrodotoxin

The puffer-fish toxin, tetrodotoxin, abolishes action potentials at low ($10^{-7}$ to $10^{-8}$ g/ml) concentrations in excitable cells in which the initial inward current of spikes is carried by $Na^+$. It has been found to do so in frog skeletal muscle fibers (147–149), in lobster axons (150), in eel electroplaques (151), and in frog heart (58, 152). It does not block the initial conductance component of spikes when this is caused by increased $Ca^{2+}$ rather than $Na^+$ permeability, as in guinea pig taenia coli (153) or crustacean muscle fibers (152, 154). Tetrodotoxin does not block the plateau of frog heart action potentials, which is due presumably to $Ca^{2+}$ permeability (58). It does not block the repolarization component of spikes, as measured under voltage clamp, in squid giant axons (155) or eel electroplaques (156); in eel electroplaques this component is $K^+$ inactivation (151). In vertebrate motor nerve-muscle preparations, tetrodotoxin abolishes the axonal spike but not the end

plate sensitivity to acetylcholine or the m.e.p.p.'s (147, 148, 157–159). It does not affect generator potentials of crustacean stretch receptors (160), nor endocochlear or cochlear microphonic potentials (161). The firing of crustacean stretch receptors is, however, blocked by this substance (160), and the cochlear potentials are blocked by tetraethylammonium (161).

All these data suggest a very specific action of tetrodotoxin on the $Na^+$ conductance change of $Na^+$ spikes, as it does not block $Ca^{2+}$ spikes, $K^+$ activation or inactivation leading to repolarization, or even the $Na^+$ conductance change of e.p.p.'s or m.e.p.p.'s. However, Tasaki and his group (162, 163) have been able to obtain excitation of squid giant axons in $Na^+$-free media, and in such cases, tetrodotoxin also blocked the initial inward current regardless of which cations (other than $Na^+$) were responsible for it. This suggested to them that either the carrier which has been postulated to transport $Na^+$ inward during the spike rising phase is not $Na^+$-specific, or that tetrodotoxin blocks nonspecifically whatever mechanism is responsible for the production of the inward current (162). At least the latter explanation is not in agreement with the findings in crustacean muscle fibers (152, 154) where the inward current cannot be carried by $Na^+$ at all, but by $Ca^{2+}$ instead (or other divalent cations), and is not blocked by tetrodotoxin.

It should be noted here that procaine abolishes $Na^+$ spikes as does tetrodotoxin (152, 164, 165), but it affects $K^+$ conductance as well (164, 165).

Tetrodotoxin seems to be more effective in blocking $Na^+$ conductance (or is it the initial inward current of normally $Na^+$ spikes?) when applied to the outer than to the inner surface of squid axon membranes [(166); see also (155)]. In the hands of Moore (166), a concentration which was effective when applied externally, was ineffective when perfused internally. Applied by Nakamura et al. (155), it was in general effective at either side at somewhat higher concentrations than those used by Moore. The possible lower effectiveness of tetrodotoxin when perfused internally might be due to some possible binding by residual axoplasm, or by the $Na^+$ permeability being controlled better by the outer surface of the membrane [(167); see also last section of this review].

### Internal Perfusion with Abnormal Salt Solutions

The introduction of methods to perfuse squid giant axons (168–170), and the recently described (171, 172) barnacle giant muscle fibers (173, 174), have allowed various groups of workers to experiment with changes in the intracellular ionic environment of these excitable tissues that depart considerably from the normal axoplasm.

The lowering of $(Ca^{2+})_i$ by its binding with $SO_4^{2-}$, EDTA, EGTA, or citrate induces the production of spikes in barnacle muscle fibers in response to depolarization; these muscle fibers do not normally fire (173). The critical

$(Ca^{2+})_i$ for excitation and contraction in these fibers has recently been established (175). The changes in the overshoot of such spikes follow the Nernst equation up to close to 100 m$M$ $(Ca^{2+})_o$. It should be noted that increases in $(Ca^{2+})_o$ shift firing levels toward positivity. Changes in $(Na^+)_o$ do not affect the spikes; replacement of $(Ca^{2+})_o$ by $Sr^{2+}$ or $Ba^{2+}$ results in restoration of abolished spikes, and then the overshoot follows $(Sr^{2+})_o$ or $(Ba^{2+})_o$ changes, although largely departing from the Nernst equation when $Ba^{2+}$ is used (173). As the spike overshoot depends on $(K^+)_i$ as well as on $Ca^{2+}$ (174), the effect of $(Ba^{2+})_o$ is thought to be due to a depression of $K^+$ permeability. Indeed, barnacle spikes under $(Ba^{2+})_o$ instead of $Ca^{2+}$ are heart-like (173), as are squid axon spikes (176). This also applies to squid axon spikes (174) under tetraethylammonium.

The factors involved in obtaining plateau spikes with low $(K^+)_i$ (174, 177–179), or when $K^+$ permeability is blocked, be it by internally injected tetraethylammonium (176, 180) or external $Ba^{2+}$ (173), are, however, possibly complex. One of these factors is no doubt $K^+$. Adelman et al. (179) measured $Na^+$ conductance against time in internally perfused squid axons. Spike plateaux were found to be predominantly determined by a "slow time-variant $Na^+$ conductance," whose activation by $(K^+)_i$ would lead to repolarization, as the lower the $(K^+)_i$ the longer the plateaux. However, even in the absence of $(K^+)_i$ and $(K^+)_o$ the plateaux did eventually terminate, so other factors than $K^+$ must also be involved.

In the squid giant axon, dilution of $(K^+)_i$ with nonelectrolytes "shifts the threshold and inactivation curves in the direction of a more positive internal potential" (169, 177, 178, 181–186). Chandler et al. (183, 184) produced quite clear evidence in favor of the view that these effects are due to a decrease in the ionic strength of the perfusing solution, rather than to the decrease of $(K^+)_i$. They found that dilution of $(K^+)_i$ with choline or NaCl lead to no change in the inactivation and threshold curves, whereas dilution with sucrose gave a shift of +20 mV. Hodgkin and his group developed an explanation for these findings, based on the potential of the membrane itself, rather than on the potential difference between the inner and outer solutions (178, 184–186). $Na^+$ permeability would depend on this actual membrane potential, which in turn would depend on the fixed charges of the membrane ($PO_4^{3-}$ and $COO^-$ groups), and on the ionic atmosphere near the inner surface (the one presumably not neutralized by divalent cations, $Ca^{2+}$ or $Mg^{2+}$) (184–186).

The various, already described, results by Hagiwara and his collaborators (152, 173–175) and those by Hodgkin and his colleagues on changes in $Na^+$ and spike overshoot (181, 185–188) seem to agree very well with the now classical Hodgkin and Huxley theory of spike production. The fact that the initial inward current of barnacle muscle spikes would be carried by $Ca^{2+}$ instead of $Na^+$ should be taken as an extension, rather than a contradiction to

the ionic theory. The results obtained by Tasaki and his group (162, 163, 189–196) suggested to them instead a departure from such a theory. Contrary to Baker et al. (181), who found that the potential at the peak of the spike over-shoot followed an equation coherent with the $Na^+$ theory, and that it never exceeded $E_{Na}$, and to Hagiwara & Naka's similar results with $Ca^{2+}$ or $Sr^{2+}$ instead of $Na^+$ (173), Tasaki et al. consistently found overshoots which varied little, and not according to the Nernst equation, over a wide range of $(Na^+)_i$ (191, 193). Tasaki et al. also discovered resting and overshoot potentials which varied differently from the Nernst equation over wide ranges of $(K^+)_i$ (191), excitation of squid axons in the absence of external $Na^+$ when this was replaced by a variety of organic and inorganic cations (162, 163, 194–196), and diverse effects of anions on excitability (162, 163, 194–196) which have in part been confirmed by others (197). The ability of anions and cations to maintain excitability followed the lyotropic number of these ions (162, 163, 194–197). The earlier Tasaki & Takenaka experiments (189, 191, 192) on high overshoot upon very low $(Na^+)_o/(Na^+)_i$ ratio were questioned by Hodgkin and his collaborators (185–187), but were later repeated and confirmed by Tasaki et al. (193), using technical modifications close to those recommended by Hodgkin & Chandler (185, 187), albeit on a different squid species than the one they had previously used.

Tasaki and his colleagues have proposed a theory for the production of resting and action potentials which diverges from the classical ionic one. The Tasaki theory prefers to consider the membrane as a cation exchanger, and uses what they term a "macromolecular approach," based on colloid chemistry and other physicochemical considerations, to account for what they call "the two stable-states of the membrane," the resting and the active one (162 163, 191, 194–196). The "two stable-state" concept is based on the already mentioned finding of plateau spikes when $K^+$ permeability (or perhaps, the repolarization component) is blocked [see (176)] or $(K^+)_i$ is low (174, 179). It is impossible to describe the Tasaki theory in detail here, but for such description and for pertinent references, the reader is referred to the most recent articles on this theory (162, 163, 195).

Cation-exchange theorists postulate the possibility of membrane structural changes during the action potential (198). The compatibility or not of the ionic theory with the concept of the membrane as a cation exchanger will perhaps be clarified in the future.

The bibliography on intracellular perfusion of excitable tissues is rapidly expanding. Only a few glimpses of certain aspects have been included here. For those interested, the Miami Symposium is recommended (199), as well as the brief but compact recent review article by Hodgkin (186). Some references as well as useful comments will be found in the 1964 and 1965 articles on "Nervous function at the cellular level" in the *Annual Review of Physiology* (200, 201).

## ELECTROGENIC SODIUM PUMPS

If the net active outward movement of $Na^+$ from a living cell is not counterbalanced by positive charges ($K^+$) going in (202, 208, 212, 213), there would be an electrogenic $Na^+$ pump in that cell, tending to increase the negative resting potential. There is now evidence for such pumps in different tissues (12, 13, 203–214). Such a pump should be stimulated by increasing $(Na^+)_i$. Indeed, upon injecting $Na^+$ into squid giant axons with a microsyringe, a small hyperpolarizing effect was noted by Hodgkin & Keynes (203). By allowing $Na^+$ to flow out of a large intracellular NaCl electrode, a large hyperpolarizing effect was found by Kerkut & Thomas in snail neurons (212). An electrogenic $Na^+$ pump has been shown to account for the post-tetanic hyperpolarization of crayfish stretch-receptor neurons where, however, the shorter-lasting hyperpolarizing after-potential which follows one or a few spikes is due to $K^+$ conductance, as in many other cells (214).

Ito & Oshima (209) found that, upon the intracellular injection of $Cs^+$, $Li^+$, or $Na^+$, cat motoneurons became reversibly depolarized, and their resting potential recovered on extrusion of these cations. Depolarization increases $Na^+$ extrusion from motoneurons, but the rate of this extrusion is independent of the resting potential level (210, 211). Intracellular $N_3Na$ injections do not always depolarize motoneurons (211); however, an increase in extracellular $N_3Na$ to muscle fibers does have this effect (13). The intracellular azide injection, acting presumably as a metabolic inhibitor, depresses the $Na^+$ pump of motoneurons (211); extracellular azide depolarizes and thus increases the $Na^+$ extrusion from muscle fibers (13). This diversity of action of $N_3Na$ on $Na^+$ efflux, depending on which side of the membrane it acts, is perhaps not too easy to explain. The increased $Na^+$ efflux of muscle fibers when depolarized by $N_3Na$ (13) or by high $(K^+)_o$ (12) is blocked by strophanthin, which indicates that it is probably not simple diffusional $Na^+$ efflux, but rather a metabolic one (12, 13). Metabolic inhibitors, such as the already mentioned intracellular $N_3Na$, strophanthin, fluoroacetate, iodoacetate, etc., depress the various electrogenic $Na^+$ pumps (12, 13, 211, 212).

There have lately been many observations, besides those on Na extrusion, on the effect of intracellular injections of cations into mammalian motoneurons (101, 209–211, 215–217). The results are too numerous to be analyzed here in detail and the interested reader is referred to the original papers. We would only like to summarize that resting membranes seem highly permeable to $Rb^+$ and $K^+$, less so to $Cs^+$, and even less to $Li^+$ and $Na^+$ (209). In the active membrane, $Li^+$ would pass through $Na^+$ channels, $Rb^+$ through $K^+$ channels, and $Cs^+$ through neither (217). The results of $Rb^+$ and $K^+$ correlate partly with those found by Adrian in striated muscle, where the pathway that allows large outward $K^+$ currents permits $Rb^+$ movements, but the one that allows large inward $K^+$ currents does not (218). It might be added that $Li^+$ fails to replace $Na^+$ in mammalian sympathetic ganglionic responses to electrical stimulation, potassium, and acetylcholine (219).

It must be kept in mind that the concepts of channels or pathways that allow the passage of certain but not of other ions have been altogether discarded and replaced by others based on the lyotropic number of ions by Tasaki et al. (162, 163, 195, 196).

## Possible Functional Differences Between Inner and Outer Side of Excitable Membranes

Phosphatidyl-serine might provide negatively charged sites in membranes for cation exchange (220, 221). Phosphatidyl-serine monolayers effectively act as cation exchangers; in such layers, $Ca^{2+}$ adsorption depends on the concentration of other cations which can compete with it, $La^{3+}$ in particular being a strong competitor (222). This fact had been suspected from electron micrographs using $La^{3+}$, in which it densely stained the outer side of nerve membranes [see (167, 222)], which is presumably normally occupied by divalent cations (184–186). This would also explain why $La^{3+}$ reversibly blocks excitable membranes [see refs. in (222)]. By autoradiography, Shimamoto found that when squid axons are immersed in seawater containing $^{45}Ca^{2+}$, this goes mainly to the surface layer of the axon [cited in (191)]. The idea of phosphatidyl-serine providing polar groups for cation exchange is consistent with current concepts of membrane chemical structure.

The participation of gangliosides in the structure and function of living membranes, including especially those of nerves, has also been repeatedly suggested (223–225). There is recent convincing evidence that a sialic acid conjugate (sialo-lipid or -peptide) might participate in a $K^+$-dependent phosphatase reaction which might well be the second step of $Na^+$-$K^+$-$Mg^{2+}$-dependent ATPase activity (226). In this reaction, "the $COO^-$ group of sialic acid conjugate . . . would act as a cation-receptor (carrier or exchanger) in the active transport of cations" across the membrane of liver cells (226).

One seemingly logical a priori idea would be that $Na^+$ permeability should be controlled by the outer and $K^+$ permeability by the inner side of excitable membranes (167). In favor of such an idea are the experiments which show that tetrodotoxin is more active when in the external than in the internal solution bathing squid axon membranes (166, 232, 233), and that tetraethylammonium and similar compounds block $K^+$ permeability from the inside and not as much from the outside [(176, 209); see also (167)]. Against this idea are the facts that $Na^+$ conductance can be regulated by the ionic strength of the internal solution (178, 183–186), the alleged nonspecificity of tetrodotoxin action (162, 163), the data by Hagiwara & Naka on external $Ba^{2+}$ blocking $K^+$ permeability (173), and perhaps the old evidence by Shanes (227) that veratrine, acting presumably from the outside, increases the $K^+$ leakage per spike. External procaine abolishes $Na^+$ and $K^+$ conductance alike (164, 165). Some data by Hagiwara et al. (174, 228) are of interest in this connection. They found that in internally cannulated barnacle giant muscle fibers, when $(K^+)_i$ was changed below and up to the normal value of

150–200 m$M$, the resting potential suffered modifications according to the Nersnt equation for changes in $K^+$ or $Cl^-$ on either or both sides of the membrane; above 200 m$M(K^+)_i$ the resting potential became less sensitive to $(K^+)_i$, and it even decreased when $(K^+)_i$ was above 300 m$M$. Narahashi (229) found that in perfused squid axons the membrane behaves symmetrically to $K^+$, in the sense that membrane resistance was highest with low $K^+$ on both sides, lowest with high $K^+$ on both sides, and intermediate with high $K^+$ on one side and low $K^+$ on the other. This symmetry did not extend to delayed rectification, however, as it occurred in the normal direction only when $(K^+)_i$ was high and $(K^+)_o$ was low. Delayed rectification decreased with depolarization; with great hyperpolarization, anomalous rectification took place independently of the $K^+$ gradient across the membrane (229).

Diverse proteases and lipases cause no damage to excitability when applied externally (230), but block the action potential when applied to the interior of squid giant axons. Other further comments on the asymmetry of the membrane will be found in (167, 229).

## Addendum

Since this manuscript went to press, some references have appeared which significantly modify or add to some reviewed facts.

*Glia and extracellular space of nervous tissue.*—The existence of a "true" extracellular space in the frog brain, permeable to particles as large as $ThO_2$, was demonstrated; this space includes synaptic clefts (231).

*Tetrodotoxin.*—Further data on the diverse effectiveness of this substance on either side of squid axon membranes have been produced by Moore et al. (232, 233): external perfusion with $10^{-7}$ $M$ or less blocks inward $Na^+$ current and resulting spikes, whereas internal perfusion with $10^{-6}$ $M$ does not (232). Also, tetrodotoxin blocks inward $Li^+$ flow through the $Na^+$ channel (233); this points toward an effect of tetrodotoxin on the (normally Na)-inward ion carrier system. It might do this by getting lodged in the "gate of the $Na^+$ channel" (whatever that is) by its guanidinium group (232). A thorough review on tetrodotoxin and saxitoxin has appeared recently, covering from folkloric to chemical and pharmacologic aspects (234).

*Inner versus outer side of excitable membranes.*—$UO_2^{2+}$, which has high affinity for phospholipids, sensitizes frog sartorius end plates to carbaminoylcholine; this is prevented by high $Ca^{2+}$ (235). This is taken as an indication that postjunctional membrane receptors are $PO_4$-containing macromolecules (235), presumably on the outer surface.

## LITERATURE CITED


1. Myers, H. M., and Hendershot, L. C., *Ann. Rev. Pharmacol.*, **3,** 307–34 (1963)
2. Welt, L. G., Sachs, J. R., and Gitelman, H. J., *Ann. Rev. Pharmacol.*, **6,** 77–88 (1966)
3. Schatzmann, H. J., *Helv. Physiol. Pharmacol. Acta*, **11,** 346–54 (1953)
4. Grupp, G., and Charles, A., *J. Pharmacol. Exptl. Therap.*, **143,** 356–65 (1964)
5. Klaus, W., Kuschinsky, G., and Lüllmann, H., *Arch. Exptl. Pathol. Pharmakol.*, **242,** 480–96 (1962)
6. Rayner, B., and Weatherall, M., *Brit. J. Pharmacol.*, **12,** 371–81 (1957)
7. Tuttle, R. S., Witt, P. N., and Farah, A., *J. Pharmacol. Exptl. Therap.*, **133,** 281–87 (1961)
8. Tuttle, R. S., Witt, P. N., and Farah, A., *J. Pharmacol. Expil. Therap.*, **137,** 24–30 (1962)
9. Widdas, W. F., *Proc. Intern. Pharmacol. Meeting, 1st, Stockholm, 1961*, **3,** 239–44 (1962)
10. Vogel, G., *Arch. Exptl. Pathol. Pharmakol.*, **246,** 70–71, (1963)
11. Whittam, R., and Willis, J. S., *J. Physiol. (London)*, **168,** 158–77 (1963)
12. Horowicz, P., and Gerber, C. J., *J. Gen. Physiol.*, **48,** 489–514 (1965)
13. Horowicz, P., and Gerber, C. J., *J. Gen. Physiol.*, **48,** 515–25 (1965)
14. Goerke, J., and Page, E., *J. Gen. Physiol.*, **48,** 933–48 (1965)
15. Page, E., and Storm, S. R., *J. Gen. Physiol.*, **48,** 957–72 (1965)
16. McClane, T. K., *J. Pharmacol. Exptl. Therap.*, **148,** 106–10 (1965)
17. Schneyer, L. H., and Schneyer, C. A., *Am. J. Physiol.*, **209,** 111–18 (1965)
18. Levy, J. V., and Richards, V., *Proc. Soc. Exptl. Biol. Med.*, **118,** 501–2, (1965)
19. Palmer, R. F., Lasseter, K. C., and Melvin, S. L., *Arch. Biochem. Biophys.*, **113,** 629–33 (1966)
20. Glynn, I. M., *Pharmacol. Rev.*, **16,** 381–407 (1964)
21. Bonting, S. L., Caravaggio, L. L., and Hawkins, N. M., *Arch. Biochem. Biophys.*, **98,** 413–19 (1962)
22. Dunham, E. T., and Glynn, I. M., *J. Physiol. (London)*, **156,** 274–93 (1961)
23. Bonting, S. L., and Caravaggio, L. L., *Arch. Biochem. Biophys.*, **101,** 37–46 (1963)
24. Bonting, S. L., Caravaggio, L. L., and Hawkins, N. M., *Arch. Biochem. Biophys.*, **101,** 47–55 (1963)
25. Bonting, S. L., Hawkins, N. M., and Canady, M. R., *Biochem. Pharmacol.*, **13,** 13–22 (1964)
26. Kinsolving, C. R., Post, R. L., and Beaver, D. L., *Biochim. Biophys. Acta*, **62,** 85–93 (1963)
27. Schatzmann, H. J., *Helv. Physiol. Pharmacol. Acta*, **21,** C60–C63 (1963)
28. Charnock, J. S., and Post, R. L., *Nature*, **199,** 910–11 (1963)
29. Yoshida, H., and Fujisawa, H., *Biochim. Biophys. Acta*, **60,** 443–44 (1962)
30. Skou, J. C., *Physiol. Rev.*, **45,** 596–617 (1965)
31. Whittam, R., in *The Cellular Functions of Membrane Transport*, 139–54 (Hoffmann, J. F., Ed., Prentice-Hall, Englewood Cliffs, N. J., 1964)
32. Charnock, J. S., Rosenthal, A. S., and Post, R. L., *Australian J. Exptl. Biol. Med. Sci.*, **41,** 675 (1963)
32. Opit, L. J., and Charnock, J. S., *Nature*, **208,** 471–74 (1965)
34. Glynn, I. M., *J. Physiol. (London)*, **169,** 452–65 (1963)
35. Hokin, M. R., *Biochim. Biophys. Acta*, **77,** 108–20 (1963)
36. Repke, K., and Portius, H. J., *Experientia*, **19,** 452–58 (1963)
37. Repke, K., *Klin. Wochschr.*, **42,** 157–65 (1964)
38. Csáky, T. Z., *Ann. Rev. Physiol.*, **27,** 415–50 (1965)
39. Tashima, Y., Nakao, T., Nagano, K., Mizuno, N., and Nakao, M., *Biochim. Biophys. Acta*, **117,** 54–62 (1966)
40. Bonting, S. L., and Caravaggio, L. L., *Biochim. Biophys. Acta*, **112,** 519–23 (1966)
41. Albers, R. W., Rodríguez de Lores Arnaiz, G., and De Robertis, E., *Proc. Natl. Acad. Sci. U.S.*, **53,** 557–64 (1965)
42. Fujita, M., Nakao, T., Tashima, Y., Mizuno, N., Nagano, K., and Nakao, M., *Biochim. Biophys. Acta*, **117,** 42–53 (1966)
43. Emmelot, P., and Bos, C. J., *Biochim. Biophys. Acta Previews*, **5,** 9 (1966)

44. Post, R. L., Sen, A. K., and Rosenthal, A. S., *J. Biol. Chem.*, **240,** 1437–45 (1965)
45. Ahmed, K., and Judah, J. D., *Biochim. Biophys. Acta*, **104,** 112–20 (1965)
46. Germain, M., and Proulx, P., *Biochem. Pharmacol.*, **14,** 1815–19 (1965)
47. Kahn, J. B., Jr., *Federation Proc.*, **21,** 149 (1962)
48. Kahn, J. B., Jr., *Proc. Soc. Exptl. Biol. Med.*, **110,** 412–14 (1962)
49. Corrie, W. S., and Bonting, S. L., *Biochim. Biophys. Acta Previews*, **5,** 31 (1966)
50. Kennedy, K. E., and Nayler, W. G., *Comp. Biochem. Physiol.*, **16,** 175–80 (1965)
51. Müller, P. von, *Cardiologia*, **42,** 176–88 (1963)
52. Repke, K., *Proc. Intern. Pharmacol. Meeting, 1st, Stockholm, 1961*, **3,** 47–73 (1962)
53. Okita, G. T., Talio, P. J., Curry, J. H., Jr., Smith, F. D., Jr., and Geiling, E. M. K., *J. Pharmacol. Exptl. Therap.*, **115,** 371–79 (1955)
54. Kinsolving, C. R., Post, R. L., and Beaver, D. L., *J. Cell. Comp. Physiol.*, **62,** 85–93 (1963)
55. Kassebaum, D. G., *J. Pharmacol. Exptl. Therap.*, **140,** 329–38 (1963)
56. Dudel, J., and Trautwein, W., *Arch. Exptl. Pathol. Pharmakol.*, **232,** 393–407 (1958)
57. Orkand, R. K., and Niedergerke, R., *Science*, **146,** 1176–77 (1964)
58. Hagiwara, S., and Nakajima, S., *Science*, **149,** 1254–55 (1965)
59. Fleckenstein, A., in *The Cellular Functions of Membrane Transport* (Hoffmann, J. F., Ed., Prentice-Hall, Englewood Cliffs, N. J., 71–93, 1964)
60. Reuter, H., *Experientia*, **22,** 39–40 (1966)
61. Frank, G. B., *J. Physiol. (London)*, **163,** 254–68 (1962)
62. Harnack, E., *Arch. Exptl. Pathol. Pharmakol.*, **3,** 44 (1875) (Cited in Ref. 64)
63. Nayler, W. G., and Anderson, J. E., *Am. J. Physiol.*, **209,** 17–21 (1965)
64. Ciofalo, F. R., and Thomas, L. J., Jr., *J. Gen. Physiol.*, **48,** 825–39 (1965)
65. Clark, A. J., *Proc. Roy. Soc. Med.*, **5,** 181–97 (1912)
66. Loewi, O., *Arch. Exptl. Pathol. Pharmakol.*, **82,** 131–58 (1917)
67. Wilbrandt, W., Brawand, K., and Witt, P. N., *Arch. Exptl. Pathol., Pharmakol.*, **219,** 397–407 (1953)
68. Cingolani, H. E., Dutrey, D. E., and Serur, J., *Acta Physiol. Latinoam.*, **14,** 19–23 (1964)
69. Sybers, R. G., Sybers, H. D., Helmer, P. R., and Murphy, Q. R., *Am. J. Physiol.*, **209,** 699–701 (1965)
70. Cingolani, H. E., Blesa, E. S., González, N. C., and deLew, M. F., *Am. J. Physiol.*, **210,** 181–85 (1966)
71. Areskog, N. H., Arturson, G., and Grotte, G., *Biochem. Pharmacol.*, **14,** 783–87 (1965)
72. Walz, F. G., Jr., and Chan, P. C., *Arch. Biochem. Biophys.*, **113,** 569–74 (1966)
73. Govier, W. C., and Holland, W. C., *J. Pharmacol. Exptl. Therap.*, **148,** 284–89 (1965)
74. Walker, J. M. G., and Weatherall, M., *Brit. J. Pharmacol.*, **23,** 66–79 (1964)
75. Lock, J. A., *Brit. J. Pharmacol*, **25,** 557–65 (1965)
76. Lock, J. A., *Brit. J. Pharmacol.*, **21,** 393–401 (1963)
77. Keeton, W. F., and Briggs, A. H., *Proc. Soc. Exptl. Biol. Med.*, **118,** 1127–29 (1965)
78. Sekul, A. A., and Holland, W. C., *Am. J. Physiol.*, **199,** 457–59 (1960)
79. Moran, N. C., *Proc. Intern. Pharmacol. Meeting, 1st, Stockholm, 1961*, **3,** 251–57 (1962)
80. Lee, K. S., *J. Pharmacol. Exptl. Therap.*, **132,** 149–55 (1961)
81. Yusuf, S. M., and Gans, J. H., *Arch. Intern. Pharmacodyn.*, **160,** 188–95 (1966)
82. Yusuf, S. M., and Gans, J. H., *Arch. Intern. Pharmacodyn.*, **160,** 196–203 (1966)
83. Yusuf, S. M., and Gans, J. H., *Arch. Intern. Pharmacodyn.*, **160,** 204–10 (1966)
84. Rose, S. P. R., *Biochem. Pharmacol.*, **14,** 589–601 (1965)
85. Singh, I., *Am. J. Physiol.*, **203,** 422–24 (1962)
86. Singh, I., and Singh, S. I., *Experientia*, **22,** 165 (1966)
87. Van der Kloot, W. G., and Dane, B., *J. Gen. Physiol.*, **48,** 199–224 (1964)
88. Lee, Y. C. P., Richman, H. G., and

Visscher, M. B., *Am. J. Physiol.*, **120,** 493–98 (1966)
89. Lee, Y. C. P., Richman, H. G., and Visscher, M. B., *Am. J. Physiol.*, **210,** 499–504 (1966)
90. Brady, A. J., *Ann. Rev. Physiol.*, **26,** 341–56 (1964)
91. Brady, A. J., and Tan, S. T., *J. Gen. Physiol.*, **49,** 781–91 (1966)
92. Singh, I., and Raju, N. V., *Experientia*, **21,** 77–78 (1965)
93. Bing, R. J., *Physiol. Rev.*, **45,** 171–213 (1965)
94. Sandow, A., *Pharmacol. Rev.*, **17,** 265–320 (1965)
95. Carsten, M. E., and Mommaerts, W. F. H. M., *J. Gen. Physiol.*, **48,** 183–97 (1964)
96. Briggs, F. N., and Fleischman, M., *J. Gen. Physiol.*, **49,** 131–49 (1965)
97. Schuel, M., Lorand, L., Schuel, R., and Anderson, N. G., *J. Gen. Physiol.*, **48,** 737–52 (1965)
98. Lee, K. S., *Nature*, **207,** 85–86 (1965)
99. Winegrad, S., *J. Gen. Physiol.*, **48,** 997–1002 (1965)
100. Bianchi, C. P., and Bolton, T. C., *J. Pharmacol. Exptl. Therap.*, **151,** 456–63 (1966)
101. Eccles, J. C., *The Physiology of Synapses* (Springer, Berlin, 316 pp., 1964)
102. Katz, B., and Miledi, R., *Proc. Roy. Soc. (London), Ser. B*, **151,** 483–95 (1965)
103. Katz, B., and Miledi, R., *Proc. Roy. Soc. (London), Ser. B*, **161,** 496–503 (1965)
104. Oka, M., Ohuchi, T., Yoshida, H., and Imaizumi, R., *Life Sci.*, **5,** 433–38 (1966)
105. Hubbard, J. I., *J. Physiol. (London)*, **159,** 507–17 (1961)
106. Douglas, W. W., *Nature*, **197,** 81–82 (1963)
107. Douglas, W. W., and Poisner, A. M., *J. Physiol. (London)*, **172,** 1–18 (1964)
108. Douglas, W. W., and Poisner, A. M., *J. Physiol. (London)*, **172,** 19–30 (1964)
109. Haller, E., Takabatake, Y., and Sachs, H., *Federation Proc.*, **23,** 205 (1964)
110. Mikiten, T. M., and Douglas, W. W., *Nature*, **207,** 302 (1965)
111. Décima, E. E., and Green, J. D. (Personal communication, 1965)
112. Stensaas, L. J. (Personal communication, 1965)
113. Grinnell, A. D., *J. Physiol. (London)*, **182,** 612–48 (1966)
114. Kelly, J. S., *Nature*, **205,** 296–97 (1965)
115. Del Castillo, J., and Katz, B., *J. Physiol. (London)*, **128,** 396–411 (1955)
116. Gage, P. W., and Quastel, D. M. J., *Nature*, **206,** 1047–48 (1965)
117. Lüttgau, H. C., and Niedergerke, R., *J. Physiol. (London)*, **143,** 486–505 (1958)
118. Gage, P. W., and Quastel, D. M. J., *Nature*, **206,** 625–26 (1965)
119. Parsons, R. L., Hoffmann, W. W., and Feigen, G. E., *Nature*, **208,** 590–91 (1965)
120. Elmqvist, D., *Acta Physiol. Scand.*, **64,** 340–44 (1965)
121. Liley, A. W., *J. Physiol. (London)*, **134,** 427–43 (1956)
122. Horstmann, E., and Meves, H., *Z. Zellforsch. Mikroskop. Anat.*, **49,** 569–604 (1959)
123. Van Harreveld, A., and Ochs, S., *Am. J. Physiol.*, **187,** 180–92 (1956)
124. Zadunaisky, J. A., and Curran, P. F., *Am. J. Physiol.*, **205,** 949–56 (1963)
125. Zadunaisky, J. A., Wald, F., and De Robertis, E. D. P., *Exptl. Neurol.*, **8,** 290–309 (1963)
126. Zadunaisky, J. A., Wald, F., and De Robertis, E. D. P., in *Progress in Brain Research*, **15,** 196–218 (De Robertis, E. D. P., and Carrea, R., Eds., Elsevier, Amsterdam, 1965)
127. Gerschenfeld, H. M., Wald, F., Zadunaisky, J. A., and De Robertis, E. D. P., *Neurology*, **9,** 412–25 (1959)
128. Sjöstrand, F., in *Modern Scientific Aspects of Neurology*, 158 (Cummings, J. N., Ed., Arnold Ltd., London, 1960) (Cited in Ref. 131)
129. Katzman, R., *Neurology*, **11,** 27–36 (1961)
130. Koch, A., Ranck, J. B., and Newan, B. L., *Exptl. Neurol.*, **6,** 186–200 (1962)
131. Villegas, J., Villegas, L., and Villegas, R., *J. Gen. Physiol.*, **49,** 1–7 (1965)
132. Brinley, F. J., Jr., *J. Neurophysiol.*, **28,** 742–72 (1965)
133. Van Harreveld, A., Collewijn, H., and Malhotra, S. K., *Am. J. Physiol.*, **210,** 251–56 (1966)
134. Van Harreveld, A., and Crowell, J., *Anat. Record*, **149,** 381–85 (1964)

135. Van Harreveld, A., Crowell, J., and Malhotra, S. K., *J. Cell Biol.*, **25,** 117–37 (1965)
136. Elbers, P. F., *Biochim. Biophys. Acta,* **112,** 318–29 (1966)
137. Frankenhaeuser, B., and Hodgkin, A. L., *J. Physiol. (London),* **131,** 341–76 (1956)
138. Green, J. D., *Physiol. Rev.*, **44,** 561–608 (1964)
139. Nicholls, J. G., and Kuffler, S. W., *J. Neurophysiol.*, **27,** 645–71 (1964)
140. Lasansky, A., and Wald, F., *J. Cell Biol.*, **15,** 463–79 (1962)
141. Gray, E. G., and Guillery, R. W., *Z. Zellforsch. Mikroskop. Anat.*, **60,** 826–49 (1963)
142. Coggeshall, R. E., and Fawcett, D. W., *J. Neurophysiol.*, **27,** 229–89 (1964)
143. Thomas, R. C., and Wilson, V. J., *Science,* **151,** 1538–39 (1966)
144. Hertz, L., *Nature,* **206,** 1091–95 (1965)
145. Hydén, H., and Lange, P., *Science,* **149,** 654–56 (1965)
146. Hamberger, A., Hydén, H., and Lange, P., *Science,* **151,** 1394–95 (1966)
147. Furukawa, T., Sasaoka, T., and Hosoya, Y., *Japan. J. Physiol.*, **9,** 143 (1959) (cited in Ref. 58)
148. Narahashi, T., Deguchi, T., Urakawa, N., and Ohkubo, Y., *Am. J. Physiol.*, **198,** 934–38 (1960)
149. Nakajima, S., Iwasaki, S., and Obata, K., *J. Gen. Physiol.*, **46,** 97–115 (1962)
150. Narahashi, T., Moore, J. W., and Scott, W. R., *J. Gen. Physiol.*, **47,** 965–74 (1964)
151. Nakamura, Y., Nakajima, S., and Grundfest, H., *Science,* **146,** 266–68 (1964)
152. Hagiwara, S., and Nakajima, S., *J. Gen. Physiol.*, **49,** 793–806 (1966)
153. Nonomura, Y., Hotta, Y., and Ohashi, H., *Science,* **152,** 97–99 (1966)
154. Ozeki, M., and Grundfest, H., *Federation Proc.*, **24,** 648 (1965)
155. Nakamura, Y., Nakajima, S., and Grundfest, H., *J. Gen. Physiol.*, **48,** 985–96 (1965)
156. Nakamura, Y., Nakajima, S., and Grundfest, H., *Biol. Bull.*, **127,** 382 (1964) (Cited in Ref. 155)
157. Moshar, H. S., Fuhrman, F. A., Buchwald, H. D., and Fischer, H. G., *Science,* **144,** 1100–10 (1964)
158. Katz, B., and Miledi, R., *Nature,* **207,** 1097–98 (1965)
159. Elmqvist, D., and Feldman, D. S., *Acta Physiol. Scand.*, **64,** 475–76 (1965)
160. Loewenstein, W. R., Terzuolo, C. A., and Washizu, Y., *Science,* **142,** 1180–81 (1963)
161. Katsuki, Y., Yanagisawa, K., and Kanzaki, J., *Science,* **151,** 1544–55 (1966)
162. Tasaki, I., and Singer, I., *Ann. N.Y. Acad. Sci.* (In press, 1966)
163. Tasaki, I., Singer, I., and Watanabe, A. (Submitted for publication, 1966)
164. Shanes, A. M., Freygang, W. H., Grundfest, H., and Amatniek, E., *J. Gen. Physiol.*, **42,** 793–802 (1959)
165. Taylor, R. E., *Am. J. Physiol.*, **196,** 1071–78 (1959)
166. Moore, J. W., *J. Gen. Physiol.*, **48** (5) (Part 2), 11–18 (1965)
167. Brinley, F. J., Jr., *J. Gen. Physiol.*, **48**(5) (Part 2), 41–48 (1965)
168. Baker, P. F., Hodgkin, A. L., and Shaw, T. I., *Nature,* **190,** 885–87 (1961)
169. Baker, P. F., Hodgkin, A. L., and Shaw, T. I., *J. Physiol. (London),* **164,** 330–54 (1962)
170. Oikawa, T., Spyropoulos, C. S., Tasaki, I., and Teorell, T., *Acta Physiol. Scand.*, **52,** 195–96 (1961)
171. Hoyle, G., and Smyth, T., Jr., *Science,* **139,** 49–50 (1963)
172. Hoyle, G., and Smyth, T., Jr., *Comp. Biochem. Physiol.*, **10,** 291–314 (1963)
173. Hagiwara, S., and Naka, K. I., *J. Gen. Physiol.*, **48,** 141–62 (1964)
174. Hagiwara, S., Chichibu, S., and Naka, K. I., *J. Gen. Physiol.*, **48,** 163–79 (1964)
175. Hagiwara, S., and Nakajima, S., *J. Gen. Physiol.*, **49,** 807–18 (1966)
176. Tasaki, I., *J. Physiol. (London),* **148,** 306–31 (1959)
177. Narahashi, T., *J. Physiol. (London),* **169,** 91–115 (1963)
178. Baker, P. F., Hodgkin, A. L., and Meves, H., *J. Physiol. (London),* **170,** 541–60 (1964)
179. Adelman, W. J., Jr., Dyro, F. M., and Senft, J., *J. Gen. Physiol.*, **48**(5) (Part 2), 1–10 (1965)
180. Armstrong, C. M., and Binstock, L., *J. Gen. Physiol.*, **48,** 859–72 (1965)
181. Baker, P. F., Hodgkin, A. L., and Shaw, T. I., *J. Physiol. (London),* **164,** 355–74 (1962)

182. Moore, J. W., Narahashi, T., and Ulbricht, W., *J. Physiol. (London)*, **172**, 163–73 (1964)
183. Chandler, W. K., and Meves, H., *Arch. Ges. Physiol.*, **281**, 25–26 (1964)
184. Chandler, W. K., Hodgkin, A. L., and Meves, H., *J. Physiol. (London)*, **180**, 821–36 (1965)
185. Hodgkin, A. L., and Chandler, W. K., *J. Gen. Physiol.*, **48**(5) (Part 2), 27–30 (1965)
186. Hodgkin, A. L., *Intern. Congr. Physiol. Sci., 23rd Tokyo, 1965, Excerpta Med.*, Intern. Congr. Ser., No. 87, 515–20 (1965)
187. Chandler, W. K., and Hodgkin, A. L., *J. Physiol. (London)*, **181**, 594–611 (1965)
188. Chandler, W. K., and Meves, H., *J. Physiol. (London)*, **180**, 788–820 (1965)
189. Tasaki, I., and Takenaka, T., *Proc. Natl. Acad. Sci. U. S.*, 619–26 (1963)
190. Tasaki, I., and Takenaka, T., *Proc. Natl. Acad. Sci., U. S.*, **52**, 804–10 (1964)
191. Tasaki, I., and Takenaka, T., in *The Cellular Functions of Membrane Transport*, 95–111 (Hoffmann, J. F., Ed., Prentice-Hall, Englewood Cliffs, N. J., 1964)
192. Tasaki, I., and Luxoro, M., *Science*, **145**, 1313–15 (1964)
193. Tasaki, I., Luxoro, M., and Ruarte, A., *Science*, **150**, 899–901 (1965)
194. Tasaki, I., Singer, I., and Watanabe, A., *Proc. Natl. Acad. Sci. U. S.*, **54**, 763–69 (1965)
195. Tasaki, I., Singer, I., and Takenaka, T., *J. Gen. Physiol.*, **48**, 1095–1123 (1965)
196. Tasaki, I., and Singer, I., *J. Cellular Comp. Physiol.*, **66**, Part II, Suppl. 2, 1–9 (1965)
197. Adelman, W. J., Jr., Dyro, F. M., and Senft, J., *Science*, **151**, 1392–94 (1966)
198. Spyropoulos, C. S., *Brain Res.*, **1**, 105–8 (1966)
199. Cole, K. S., Chairman; Mullins, L. J., Ed., *J. Gen. Physiol.*, **48** (Part 2), 92 pp. (1965)
200. Bennett, M. V. L., *Ann. Rev. Physiol.*, **26**, 289–340 (1964)
201. Strumwasser, F., *Ann. Rev. Physiol.*, **27**, 451–76 (1965)
202. Garrahan, P. J., and Glynn, I. M., *Nature*, **207**, 1098–99 (1965)
203. Hodgkin, A. L., and Keynes, R. D., *J. Physiol. (London)*, **131**, 592–616 (1956)
204. Kernan, R. P., *Nature*, **193**, 986–87 (1962)
205. Keynes, R. D., and Rybova, R., *J. Physiol. (London)*, **168**, 58P (1963)
206. Mullins, L. J., and Noda, K., *J. Gen. Physiol.*, **47**, 117–32 (1963)
207. Adrian, R. H., and Slayman, C. L., *J. Physiol. (London)*, **175**, 49P–50P (1964)
208. Frumento, A. S., *Science*, **147**, 1442–43 (1965)
209. Ito, M., and Oshima, T., *Proc. Roy. Soc. (London), Ser. B*, **161**, 92–108 (1965)
210. Ito, M., and Oshima, T., *Proc. Roy. Soc. (London), Ser. B*, **161**, 109–31 (1965)
211. Ito, M., and Oshima, T., *Proc. Roy. Soc. (London), Ser. B*, **161**, 132–41 (1965)
212. Kerkut, G. A., and Thomas, R. C., *Comp. Biochem. Physiol.*, **14**, 165–83 (1965)
213. Mullins, L. J., and Awad, M. Z., *J. Gen. Physiol.*, **49**, 761–75 (1965)
214. Nakajima, S., and Takahashi, K., *Nature*, **209**, 1220–21 (1966)
215. Eccles, J. C., Eccles, R. M., and Ito, M., *Proc. Roy. Soc. (London), Ser. B*, **160**, 181–96 (1964)
216. Eccles, J. C., Eccles, R. M., and Ito, M., *Proc. Roy. Soc. (London), Ser. B*, **160**, 197–210 (1964)
217. Araki, T., Ito, M., Kostyuk, P. G., Oscarsson, O., and Oshima, T., *Proc. Roy. Soc. (London), Ser. B*, **162**, 319–32 (1965)
218. Adrian, R. H., in *The Cellular Functions of Membrane Transport*, 55–70 (Hoffmann, J. F., Ed., Prentice-Hall, Englewood Cliffs, N. J., 1964)
219. Pappano, A. J., and Volle, R. L., *Science*, **152**, 97–99 (1966)
220. Nash, H. A., and Tobias, J. M., *Proc. Natl. Acad. Sci. U. S.*, **51**, 476–80 (1964)
221. Rojas, E., and Tobias, J. M., *Biochim. Biophys. Acta*, **94**, 394–404 (1965)
222. Rojas, E., Lettvin, J. Y., and Pickard, W. F., *Nature*, **209**, 886–87 (1966)
223. Johnston, P. V., and Roots, B. I., *Nature*, **205**, 778–80 (1965)
224. Woolley, D. W., and Gommi, B. W., *Nature*, **202**, 1074–75 (1964)

225. Woolley, D. W. and Gommi, B. W., *Proc. Natl. Acad. Sci. U. S.*, **53**, 959–63 (1965)
226. Emmelot, P., and Bos, C. J., *Biochim. Biophys. Acta*, **115**, 244–47 (1966)
227. Shanes, A. M., *J. Gen. Physiol.*, **34**, 795–807 (1951)
228. Hagiwara, S., *J. Gen. Physiol.*, **48**(5) (Part 2), 55–57 (1965)
229. Narahashi, T., *J. Gen. Physiol.*, **48**(5) (Part 2), 19–25 (1965)
230. Narahashi, T., and Tobias, J. M., *Am. J. Physiol.*, **207**, 1441–46 (1964)
231. Villegas, G. M., and Fernández, J., *Exptl. Neurol.*, **15**, 18–36 (1966)
232. Narahashi, T., Anderson, N. C., and Moore, J. W., *Science*, **153**, 765–67 (1966)
233. Moore, J. W., Anderson, N. C., and, Narahashi, T., *Federation Proc.*, **25**, 569 (1966)
234. Kao, C. Y., *Pharmacol. Rev.*, **18**, 997–1049 (1966)
235. Liu, J. H., and Nastuk, W. L., *Federation Proc.*, **25**, 570 (1966)

# CONTENTS