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Bioenergetics of Nerve Excitation

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Abstract

The process of action potential production is analyzed in relation to the problem of energy transduction in the nerve. Describing the conditions required for the maintenance of excitability, the indispensability of divalent cations and the dispensability of univalent cations in the external medium are emphasized. Univalent cations with a strong tendency toward hydration enhance the action potential amplitude when added to the external Ca-salt solution. Experimental facts are described in consonance with the macromolecular interpretation of nerve excitation which postulates a transition of the negatively charged membrane macromolecules from a hydrophobic (resting) state to a hydrophilic (excited) state. Thermodynamic implications are discussed in relation to changes in enthalpy and volume accompanied by action potential production. Difficulties associated with analyses of excitation processes on a molecular basis are stressed.

Introduction

From a physicochemical and biochemical point of view, nervous tissue is peculiar, difficult material to investigate. The physicochemical processes underlying production of an action potential occur within an extremely thin membrane structure and progress at a disturbingly high rate. Most of the chemists' standard tools are totally inadequate to follow such rapid processes involving such a limited quantity of chemical substance in labile, "living" tissue. Only electronic devices employed by communication engineers have had the sensitivity and the rapidity to respond to the signs of physicochemical events taking place in the nerve membrane. For this reason, "axonology" has developed almost as a branch of applied electronic engineering.

In spite of this past and present trend, many "axonologists" are keenly aware of the fact that precise measurements of electrical

quantities alone do not yield meaningful information about what is happening in the nerve membrane. It is now widely recognized that the goal and the destiny of "axonology" is toward harmony and amalgamation with the branch of science known as molecular biology. New instruments and techniques introduced in recent years to study excitation processes may be regarded as products of a painstaking struggle to achieve this goal.

In this article, we make an attempt to review the experimental findings obtained by using these new instruments and techniques. We clarify, in the first place, the conditions which internal and external media have to satisfy in order to maintain excitability. Then, we describe the experimental data concerning ion fluxes across the membrane. Next, we discuss our present day knowledge about the thermochemical and metabolic processes in axons. Attempts will be made, whenever possible, to point out thermodynamic implications of the experimental findings described.

Conditions Necessary for Maintenance of Excitability

Soon after Hodgkin and Katz[1] confirmed and extended Overton's[2] finding demonstrating the importance of the role played by sodium ion in nerve excitation, many electrophysiologists believed that the presence of Na^+ in the external medium (or the existence of an electrochemical potential gradient for Na^+) was an indispensable factor for the maintenance of axon excitability. Later, however, Lorente de No and his associates[3] found that the ability of frog nerve fibers to develop action potentials could be maintained in a medium containing no Na^+, namely after replacing Na^+ in the medium with guanidinium and other univalent cations (cf. Hille,[4] for more recent data).

Figure 1 shows two examples of observations demonstrating the dispensability of Na^+ in squid giant axons.[5] The axons were internally perfused with a Rb-salt solution and were immersed in a medium containing either hydrazinium or guanidinium chloride. There was neither Na^+ nor K^+ on either side of the membrane. It is important to note, however, that a calcium-salt was present in the external medium in addition to the salt of nitrogenous univalent cations. It is seen in these records that the all-or-none action potentials evoked were accompanied by a simultaneous reduction in the membrane impedance. The voltage clamp technique applied to axons under these conditions yielded an N-shaped curve which was qualitatively similar to those obtained with Na^+ (and Ca^{2+}) externally and K^+ internally.

An extensive study was made concerning the ability of polyatomic univalent cations in the external medium to enhance the action

potential amplitude. Ammonium-ions can, to some extent, replace Na^+ in the external medium and maintain axon excitability. When one H-atom on NH_4^+ was replaced with CH_3, the Na-substituting capability of the resultant cation was decreased considerably. With two or more H-atoms substituted with nonpolar groups, such as methyl or ethyl, the resultant cation was no longer capable of replacing Na^+. When, however, the H-atoms were replaced with polar groups, such as amino or hydroxyl, the resultant cation was capable of

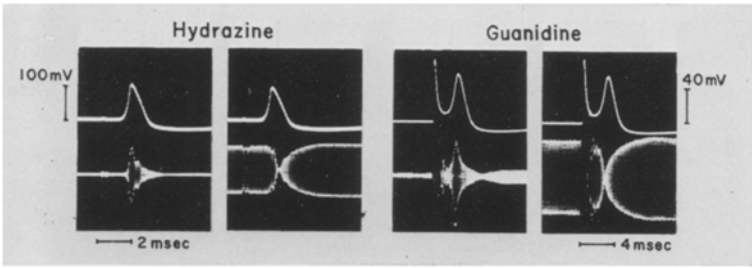

Figure 1. Impedance and potential variation recorded from squid giant axons immersed in sodium-free external media. In each record, potential variations are indicated by upper trace; simultaneous impedance variations are indicated by lower trace. In each pair of records, the record at left was obtained when the impedance bridge was balanced initially for the resting state. Record at right was obtained when the bridge was unbalanced in the resting state, so that best balance could be obtained in the excited state. *Left.* The external medium contained 0·3 M hydrazinium chloride and 0·2 M $CaCl_2$; the internal perfusion solution contained 0·1 M RbF. *Right.* The external medium contained 0·1 M guanidinium chloride, 0·2 M TMA-Cl and 0·2 M $CaCl_2$; the internal solution contained 0·1 M RbF. (From I. Tasaki, I. Singer and A. Watanabe, *Amer. J. Physiol.*, **211** (1966) 746.)

sustaining large action potentials. These findings can be summarized in the following manner:

$$CH_3 < H < OH \sim NH_2$$

where the inequality signs describe the relative magnitude of the amplitude-augmenting effect on the action potential.[6]

Soon after the dispensability of Na^+ in squid giant axons was established, there was another step forward in our understanding of the ionic requirement of the axon membrane.[7,8] It was found that, under intracellular perfusion with a dilute solution of favorable salts, large action potentials could be obtained in the complete absence of univalent cation in the external medium. The first demonstration of action potentials of this type was made under maintained internal perfusion with a solution containing a mixture of CsF and Cs-phosphate at pH 7·3. The external medium contained only $CaCl_2$ and a trace of Tris-buffer. (Glycerol or sucrose was added to the media on both sides of the membrane to maintain the tonicity.)

The Cs-salts used in those initial experiments were not the only

favorable internal electrolytes.[9] Tetramethylammonium (TMA) tetraethylammonium (TEA), guanidium, aminoguanidinium, Na^+, Li^+, etc., were found to be usable instead of Cs^+ in this type of experiment. The action potentials observed with the salt of a univalent cation internally and the salt of a divalent cation externally are often called "bi-ionic action potentials". The amplitude of a bi-ionic action potential was found to vary with the chemical species of the internal cation. Among the nitrogenous cations mentioned above, TMA and TEA gave rise to very large bi-ionic action potentials. The salt of guanidinium or aminoguanidinium ion were found to produce all-or-none, but small action potentials. In general, the cations that are favorable as Na-substitutes (externally) gave rise to small bi-ionic

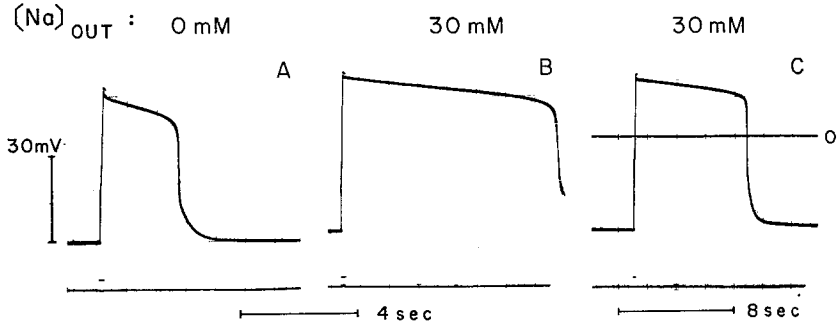

Figure 2. Resting and action potentials recorded from an axon internally perfused with a 30 mM sodium solution. The external media contained 100 mM $CaCl_2$ for all the records. The external sodium concentrations were varied as indicated. The internal anion was 1:1 mixture of fluoride and phosphate in this case. Stimuli used were 100 msec in duration and approximately 1·5 $\mu a/cm^2$ in intensity (indicated by the lower trace). Note that record C was taken at a slower sweep speed. In record C, the potential recorded when the internal recording electrode was withdrawn and placed in the external medium was superposed on the action potential trace. Axon diameter: approximately 450 μ. 20°C. (From A. Watanabe, I. Tasaki and L. Lerman, *Proc. Nat. Acad. Sci.*, **58** (1967) 2246.)

action potentials, and vice versa. In other words, the sequence of substituted ammonium-ions in affecting the action potential amplitude determined by internal application is roughly opposite to that established by external application. The significance of these findings will be clarified later.

The amplitude of a bi-ionic action potential is affected to some extent by the divalent cation concentration in the external medium, a higher concentration leading to a larger amplitude. Addition of the salt of Na^+ or its substitutes increases the amplitude also. In the external medium, Sr^{2+} or Ba^{2+} (but not Mg^{2+}) can be used to sustain bi-ionic action potentials. It is important to note that in the internal medium the divalent cation concentration has to be kept at an extremely low level, for the axon membrane would otherwise be irreversibly damaged. Figure 2 shows an example of the action poten-

tial records obtained under continuous intracellular perfusion with a dilute solution of a Na-salt.

We may summarize the experimental facts described above in the following manner. To maintain the ability of a squid giant axon to develop all-or-none action potentials, the presence of the salt of a divalent cation (Ca, Sr, or Ba) externally and of the salt of a univalent cation internally is required. Addition to the external medium of the salt of a univalent cation with a strong tendency toward hydration increases the action potential amplitude. Univalent cations with nonpolar (hydrophobic) side-groups are more favorable as the internal cations than those with polar side-groups. (Information concerning the effects of different anions may be found elsewhere.[10])

Ion Fluxes Across Axon Membrane

The electric resistance (or impedance) of the axon membrane is known to fall drastically when the membrane develops an action potential.[11] Reflecting this situation, there is, during an action potential, an enormous increase in the fluxes of cations across the membranes; this can be demonstrated readily by the use of the radio-tracer technique.[12,13] Unfortunately, the time resolution of the radio-tracer technique is quite limited. Furthermore, for ion species which have finite concentrations on both sides of the membrane, estimation of the (net) ion flux is difficult (see Kedem and Essig[14]).

A squid giant axon immersed in artificial sea water remains highly excitable under continuous intracellular perfusion with a K-phosphate solution. The influx of Na^+ can be traced without ambiguity, under these conditions, by labelling the external Na-ions with Na^{22} or Na^{24}. Complete removal of the K^+ in the artificial sea water changes neither the resting potential nor the action potential of the axon significantly. The efflux of K^+ can then be traced without ambiguity by labelling the internal perfusion fluid with K^{42}. Because of the poor time resolution of the radio-tracer technique, however, no information can be obtained by this technique concerning the time course of the fluxes during different phases of an action potential.

Figure 3 in reference 15 shows an example of the results obtained with radioactive K^+. The average level of K-efflux during one sampling period (5 min) is seen to show a definite increase when the axon was stimulated repetitively at a rate of 25/sec. The extra K-efflux was roughly doubled when the frequency of stimulation was doubled. From these measurements, the amount of K-ion transferred across the membrane associated with production of an action potential was estimated to be about 15 pmole/cm^2 per nerve impulse.[15] This value is not very different from those obtained by previous investigators (see below).

Transport of one species of cation across the membrane should be accompanied by transfer of net electric charge. The amount of 15 pmole carries approximately $1{\cdot}5 \times 10^{-6}$ coulomb which would alter the potential difference across the membrane capacity of 1 $\mu F/cm^2$ by 1·5 V. Actually, such charge transfer does not take place, because this K-efflux is accompanied by simultaneous influx of Na^+. The total amount of Na^+ transferred during one action potential is known to be very close to the value for K^+. (Note, however, that previous ion-flux measurements were always complicated by the presence of the non-radioactive counterpart of the labelled species on both sides of the axon membrane.) There is also a drastic increase in Ca-influx during excitation;[16, 7] but the total charge transferred by Ca^{2+} is far smaller than the value mentioned above.

Had the axon membrane no capacitative properties, the axon interior would be expected to satisfy the conditions of electroneutrality. The apparent capacity of the membrane is about 1 $\mu F/cm^2$; and this capacity is charged at the peak of the action potential to about 0·1 v above its resting potential level. This process involves transfer of charge of about 10^{-7} coulomb/cm^2, or about 1 pmole/cm^2 of univalent cations. This quantity is far smaller than (and is equal to only 7% of) the amount of univalent cation transported across the membrane during an action potential. It follows from these considerations that the deviation from the condition of electroneutrality of the axon interior is less than about 7% and, consequently, that the fluxes of Na- and K-ions are roughly equal in magnitude and opposite in sign during the entire course of an action potential. It is important to note that this requirement is independent of the difference in intramembrane mobility between the two cations involved.

It is of some interest to consider, in passing, the amount of free energy dissipated as the result of cation interdiffusion during an action potential. We denote the difference in chemical potential of ion species i across the membrane by $\Delta\mu_i$ and the quantity of this species transferred across the membrane by n_i. Then, the free energy dissipated during one complete cycle of an action potential is given by the sum of products $n_i \Delta\mu_i$ for all the ion species involved. Under the conditions of internal perfusion mentioned above, n's are known; but $\Delta\mu$'s are either positive or negative infinity. Since ions are moving downhill along their chemical potential gradient, this dissipation of free energy is very large. Obviously, this energy derives from the difference in the ion concentrations maintained by perfusion. Similarly, there is a very large free energy dissipation associated with production of a bi-ionic action potential.

In an intact axon immersed in normal sea water $\Delta\mu$'s are finite. It is difficult, however, to estimate $\Delta\mu$'s and n's accurately under these

conditions. Assuming that the net flux of Na^+ and K^+ to be about 5 pmole/cm^2 per impulse and the Na- and K-ion activity ratio across the membrane to be 10:1 and 1:10, respectively, the amount of free energy dissipated (in the form of entropy production) is found to be roughly 3×10^{-9} cal/cm^2 per impulse. [The free energy ($\frac{1}{2}CV^2$) lost (or gained) by charging or discharging the membrane capacity (C) when the voltage (V) involved is ± 50 mV is about 10 times less than the value stated above.] The contribution of the divalent cation fluxes to energy dissipation is also considered to be very small. The difference in ion activities (concentrations) in this case is maintained by metabolism (see below).

Macromolecular Interpretation of Nerve Excitation

The electric resistance of the membrane of an unperfused axon is known to fall, at the peak of excitation, to about 1/200 of its value in the resting state.[11] Under bi-ionic conditions,[9] the ratio of the membrane resistance at the peak of an action potential to that at rest is known to be 1/20. This large fall indicates that there is a drastic change in the structure of the membrane during excitation.

Most biological macromolecules have their isoelectric points in the acidic range of pH; therefore, at physiological pH, they are negatively charged. There is ample evidence to suppose that the macromolecules of which the axon membrane is composed are negatively charged[6,7] and, as the consequence, that the membrane exhibits properties of a cation-exchanger membrane.[17] In inanimate ion-exchanger membranes, the membrane conductance varies directly with its water content.[18,19] It is therefore highly probable that, during nerve excitation, the membrane macromolecules undergo a drastic conformational change which leads to an increase in the membrane water content.[6]

The possibility that the membrane macromolecules may undergo a conformational change during excitation presents a new problem concerning energy transduction in axons. When the membrane is thrown into a state with a low electric resistance, the cations start to flow rapidly in the direction of negative concentration gradient. The observed increase in cation fluxes during excitation is then nothing more than a reflection of high cation mobilities in the low resistance state of the membrane; and, the direction of ion flow is determined simply by the distribution of the cation species chosen by the experimenter (see e.g., Fig. 2) or adopted by nature. Thus, this line of argument leads us to believe that radio-tracer measurements as well as resistance measurements tell us something about the low resistance (i.e., excited) state of the membrane but yield no information about how such a state is produced by a stimulus. Thus, a different

approach has to be adopted to elucidate the mechanism whereby the membrane macromolecules are transformed from their "resting" conformation to their "excited" conformation.

Many electrophysiologists visualize the axon membrane as consisting of "ion-pores" which can be opened or closed by application of an electric field.[20, 21, 4] This popular viewpoint is vividly illustrated in a figure published by Baker in *Scientific American*.[21] In the resting state, the membrane is assumed to possess pores suited for transport of only K^+. On application of an electric field, a new type of pores, capable of transporting Na^+, are opened. Then, after a certain delay, these Na-pores are automatically closed and more K-pores are opened. Obviously, this scheme is a pictorial representation of the equivalent circuit theory of Hodgkin and Huxley.[22] One shortcoming of this scheme is that it does not answer the question of how ion-pores with such sizes and complex time-dependent characteristics can be constructed with proteins and phospholipids.

An alternative approach to the problem of nerve excitation is to rely on the knowledge about the behavior of various bio-colloids and macromolecules. The axon membrane, consisting of negatively charged macromolecules, is immersed in a medium usually containing the salts of divalent and univalent cations. If the charge density is high, the electroselectivity of the membrane (see Helfferich[23]) is expected to selectively bring divalent cations to the negatively charged sites. There is good evidence that the charge density in the squid axon membrane is much higher than the anion concentration in the external medium.[6] In physical chemistry, many instances are known[24–26] in which synthetic polyacids make a hydrophobic complex with Ca^{2+} or Sr^{2+} but not with Na^+ or K^+. It is also known that a cation exchanger with a rigid crystalline structure sometimes undergoes a phase-transition when one kind of counter ion is replaced with another.[27, 28] Since the axon membrane has a rigid, crystalline structure,[29] it is possible that the membrane macromolecules undergo some kind of phase-transition when the chemical composition of the external medium is varied. A series of experiments carried out on internally perfused axons[30] indicate that such a transition actually occurs (Fig. 3).

An axon, immersed in a $CaCl_2$ solution, was internally perfused with a dilute Cs-salt solution. The axon in this circumstance is capable of responding to electric stimuli with all-or-none action potentials; but no electric stimulus was delivered during the following observation. When a small amount of KCl solution was added to the external $CaCl_2$ solution, no significant change in the membrane potential was observed. When the external K^+ concentration was raised to 10 mequiv/l, a large, abrupt rise in the membrane (intracellular) poten-

tial was observed after a long delay. This abrupt potential rise was accompanied by a simultaneous fall in the membrane resistance. Following production of an abrupt potential rise, a further continuous increase in the external KCl concentration changed the membrane potential continuously. A decrease in the KCl concentration or an increase in $CaCl_2$ concentration frequently produced an abrupt fall in the membrane potential.

This phenomenon of abrupt depolarization was first described by Osterhout and Hill[31] who analyzed the effect of KCl on single plant cells of Nitella. These investigators regarded this potential jump as generation of an action potential with an infinitely long depolarization period. We recognize striking similarities between this phenomenon

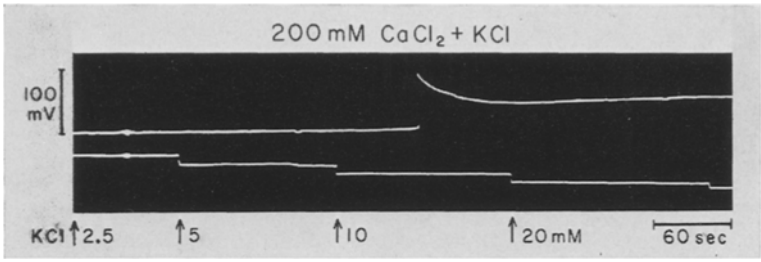

Figure 3. Abrupt depolarization of squid axon membrane induced by external application of KCl (upper oscillograph trace). The KCl concentration in the rapidly flowing external fluid medium was raised by a factor of 2 at the times marked by the lower oscillograph trace. The axon was perfused internally with a CsF perfusion fluid. No electric stimuli were delivered to the axon during the experiment. The potential jump produced by 10 mM KCl was 83 mV initially, and was followed by a gradual potential fall of approximately 40 mV. 16°C. (From I. Tasaki, T. Tekenaka and S. Yamagishi, *Amer. J. Physiol.*, **215** (1968) 152.)

and the phenomenon of phase-transition in various synthetic and natural macromolecules.[25,26,32] As one might expect from those *in vitro* experiments, abrupt depolarization in axons with prolonged action potentials can be produced not only with the salt of K^+ but also with those of Rb^+, Cs^+, or Na^+. The existence of a hysteresis loop has been demonstrated in axons undergoing abrupt depolarization and repolarization.[30]

In axons which develop action potentials of a short duration, it is practically impossible to raise the external K^+-concentration uniformly during the time involved in production of an action potential. This is the reason why abrupt depolarization has not been observed in intact axons.

The natural termination of the action potential (repolarization), in a manner opposite to depolarization, might be characterized as an exchange of Ca^{2+} for K^+. An experiment on repolarization similar to K^+-depolarization would be to raise the external Ca^{2+}-concentration during the action potential, which is also clearly impossible in axons which develop action potentials of short duration. In unperfused

axons immersed in normal sea water, there is an enormous increase in cation fluxes during excitation. This increase is expected to bring about a gradual change in the ionic composition (including Ca^{2+}) in and on both sides of the axon membrane. There seems little doubt that the rapidity of the potential fall from the peak of an action potential is directly related to the rate at which the ionic composition changes during excitation.[6]

Thermochemical Studies of Axons

In inanimate cation-exchangers, exchange of K^+ or Na^+ for Ca^{2+} is known to be exothermic; the change in enthalpy associated with such exchange is of the order of 2·5 kcal per equivalent of exchanger.[33–35] In consonance with the notion that a cation-exchanger process is operative in axons, the initiation of an action potential in axons is known to be exothermic.[36, 37] The macromolecular interpretation of nerve excitation mentioned above demands that the termination of an action potential involves exchange of divalent cations for univalent cations. In agreement with this interpretation, the process of action potential termination is endothermic.[36, 37]

The positive initial heat produced in the rabbit vagal nerve[37] is about 24 μcal/g. With an estimated membrane area of 6000 cm^2/g, the heat production in the membrane is roughly 0·004 μcal/cm^2. One explanation is that all of this heat derives from ion-exchange processes. If exchange of 2 Na^+ for Ca^{2+} at the negatively charged membrane sites is taken as generating 2·5 kcal/equiv, then the density of the sites in the membrane is roughly $1{\cdot}6 \times 10^{-12}$ equiv/cm^2 or 10^{12} sites/cm^2. A somewhat larger estimate for the number of sites was obtained from measurements of hydrophobic probe binding to squid axons (unpublished). Estimation based on the effect of tetrodotoxin[38] is very different; this discrepancy may be resolved by assuming that TTX affects only trigger sites for a cooperative process in the axon membrane (see below).

LeChatelier-Braun's law states that an endothermic process is promoted by a rise in temperature. Hence, it is expected that exchange of Ca^{2+} for univalent cations in the axon membrane is encouraged by a temperature rise. The fact that a brief heat pulse (as well as a Ca-pulse) applied during the plateau of a prolonged action potential can bring about abrupt repolarization[39] is therefore in agreement with the macromolecular interpretation. It is well-known that the action potential duration is reduced by a rise in temperature; this fact is also consistent with the notion that repolarization of the axon membrane is an endothermic process.

The fact that the process of action potential termination is endothermic poses an interesting thermodynamic problem. Since the free energy of the system (G) must not increase during a spontaneously

progressing process, there must be a simultaneous increase in the entropy ($\Delta S > 0$) large enough to overcome the increase in the enthalpy ($\Delta H > 0$). (Note that $\Delta G = \Delta H - T\Delta S \geqslant 0$, where T is the absolute temperature.) It was surmised that the expected increase in entropy is associated with rearrangement of water molecules. In the excited state of the axon, water molecules are tightly fixed around the negatively charged sites of the membrane macromolecules; when this "special hydration region" (see Oosawa[40]) in the membrane is destroyed by invasion of divalent cations, the entropy of the system is expected to increase.

The notion that water molecules are involved in nerve excitation received experimental support when the physicochemical properties of favorable univalent cations in- and outside the axon were examined. As we have discussed already, cations of a hydrophilic nature (Na^+, Li^+, hydrazinium, guanidinium, etc.) are capable of sustaining large action potentials when applied externally. In the internal solution, polyatomic cations with hydrophobic side-groups (TMA, TEA, choline, etc.) are favorable. These findings can readily be explained on the assumption that nerve excitation involves a transition of the membrane from a hydrophobic (resting) state to a hydrophilic (excited) state. Additional support of this notion was obtained from the studies of changes in fluorescent light emitted by a "hydrophobic probe". In axons injected with 2-*p*-toluidinylnaphthalene-*b*-sulfonate (TNS), it was found that the wave-length of emission maximum shortens and the quantum yield falls when an action potential is produced.[29] These changes are typical of the effects associated with a rise in the polarity (dielectric constant) of the microenvironment of TNS molecules.

The density of water molecules in the "special hydration region" is higher than that in ordinary water.[40] Hence, a small decrease in the total volume of a nerve (including the surrounding medium) is expected to occur when an action potential is produced and restoration of the volume to take place on termination of an action potential. Although no direct detection of a volume change has been made, the existence of such a volume change has been inferred from studies of the effect of a high hydrostatic pressure on frog myelinated nerve fibers and squid axons.[41] It was found that high pressure increased the action potential duration reversibly. The effect of heavy water on excitation processes[42, 43] also indicates the importance of water in the process of action potential production.

From the thermodynamic considerations stated above, it may be argued that the hydrophobic-hydrophilic transition in the axon membrane exhibits many characteristics of a phase-transition of the first-order. In a true first order phase-transition, the change in the total

free energy (ΔG) is zero; in the axon membrane the change in the free energy associated with rapid, reversible transition between the two membrane states (ΔG) is undoubtedly very small as compared with the changes in enthalpy (ΔH) and in entropy ($T\Delta S$).

In this connection, it is important to note that the energy delivered to the membrane by a brief electric stimulus is extremely small. In threshold stimulation, an action potential is produced when the potential difference across the axon membrane is altered by 12–25 mV. Since kT/e (where k is the Boltzmann constant and e is the electronic charge) is approximately 25 mV, and since the thermal energy is $\frac{1}{2}\ kT$ per degree of freedom, the energy delivered by a stimulus is, by itself, insufficient to drastically alter the thermal motion of the ions, water or of side-groups of macromolecules in the membrane. It is, by itself, far smaller than the value needed to break the weakest chemical bonds such as H-bonds. (Note that $\frac{1}{2}\ kT$ corresponds to about 0·3 kcal/mole.) We introduce at this point the concept of thermodynamic instability (see e.g., Guggenheim,[44] p. 37). The system consisting of the axon membrane and the two contiguous aqueous phases is stable against very small perturbations;[6] but, the system is so close to an unstable state that large perturbations are not required to trigger a large change in the system cooperatively. The nature of the forces involved in this cooperative change of the axon membrane is not well understood at present. It has been pointed out, however, that both short-range forces (action between neighboring sites in the membrane) and long-range forces (mediated by electric currents) are operative in the axon membrane.[6]

Active Transport and Metabolism

It is generally believed that active transport and metabolism in the nerve are only indirectly related to the process of action potential production. In order to maintain excitability, it is essential that the normal structure of the membrane macromolecules be kept unaltered and the chemical composition of the axon interior be maintained within a certain limit. Particularly, a rise in the internal Ca^{2+} concentration is detrimental to the maintenance of excitability. A rise in the internal Na^{+} concentration tends also to diminish the action potential amplitude. Active transport and metabolism is regarded as playing a role of maintaining a high K^{+}, a low Na^{+} and a low Ca^{2+} concentration in the axon interior.

Investigations of active transport in axons rely almost exclusively on measurements of radio-tracers of the ion species involved. Radio-tracer fluxes across biological membranes have been analyzed on the basis of thermodynamics of irreversible processes.[14, 45] From this stand-

point, it is in general very difficult to decide whether an observed tracer flux is taking place through the active transport channel or through the passive channel.[45] Partly because of this difficulty and partly because the unfamiliarity of the present authors with the vast literature dealing with this subject, the problem of active transport and metabolism is treated in this article only superficially.

It seems clear from extensive studies of cation transport in squid giant axons[46] that there are two distinct processes influencing the efflux of the radio-tracer (and hence the cold species) of Na^+. The first process is considered to account for 50–90% of the Na-efflux in normal axons. The Na-efflux by this pump is coupled to the K-influx and the required energy derives from the metabolism of high energy phosphate compounds, particularly from ATP.[47,48] This pump is inhibited by cyanide which blocks ATP synthesis and by ouabain which works in an unknown fashion (without seemingly interfering with metabolism). Many biologists believe that this type of Na-pump can be electrogenic, that is to say, it alters the membrane potential when the K-influx and Na-efflux are not strictly equal (see e.g., Rapoport[49]). The second kind of Na-efflux process involves a Na–Ca exchange.[46] The Na-efflux by this process is independent of the external K^+ concentration and ouabain, but is dependent on the external Ca^{2+} concentration. This pump can also be blocked by cyanide. Recent studies[50] indicate that this process in reverse, Ca-efflux coupled to Na-influx, is present in nerve; this is the only pump found so far in nerve which deals with Ca-extrusion.

The metabolic activity of nervous tissues has been studied using squid axons,[51,52] crab nerves,[53] mammalian non-myelinated nerve fibers,[54] sympathetic ganglia,[55] and crayfish stretch receptor neurons.[56] The conclusions drawn from these studies are that, in the presence of oxygen, glucose is metabolized through glycolysis, the Kerbs cycle and oxidative phosphorylation and the end product is ATP. Repetitive stimulation is known to enhance metabolism of the nerve.

Current Trend of Research Activities

Most of the current research can be divided into the following four categories: (1) precise measurements of various electrical properties of the axon membrane;[57,58] (2) measurements of non-electrical phenomena during nerve excitation including changes in turbidity,[59] birefringence,[60] and extrinsic fluorescence;[29] (3) experimental studies of model membranes;[61,62] and (4) theoretical studies of excitation processes on a mathematical, physical or physicochemical basis.[63–68] It is evident that most investigators are interested in elucidating the excitation process on a molecular basis.

Theoretical approaches are expected to be quite valuable, but are (and always will be) limited by the quality and quantity of the experimental results available. Model membrane studies have been exciting because the underlying molecular events are relatively well defined and amenable to analysis; their limitation lies in the uncertainty as to the similarity between model and real membranes. Further analyses of electrical properties are expected to reveal shortcomings of previous experimental data. It is our view that studies of non-electrical properties of the axon membrane should lead to deeper insights into the molecular events in the nerve membrane. With this new information, our understanding of excitation processes may reach in the near future a level far beyond what has been described in this article.

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