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51
Recovery Processes and Metabolism of Nerve

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INTRODUCTION

THE prime function of peripheral nerve is the conduction of impulses. In biological terms, the nerve impulse is the means of transmission of an item of information from one point in an organism to another. In terms of physics and chemistry, the impulse is a spatially propagating, transient disturbance in the state of a complex reaction system; it might be detected by, or it might be described in terms of, any of those conceivably measurable changes which are a part of it or to which it gives rise. In thermodynamic terms, the impulse is a dissipative process in which the free energy of the nerve and its environment is decreased, principally by a flow of ions down gradients of their electrochemical potential. In this discussion, attention is focused upon processes by which resting nerve maintains itself in a steady state, ready to function, and upon processes which serve to restore nerve to its resting state after it has conducted impulses. A central question is how energy-yielding processes may be coupled to those requiring energy. In an approach to a more specific formulation of this question, the discussion emphasizes correlations between changes in rate of oxidative metabolism and electrochemical manifestations of ionic movement.

In resting nerve there are at least two processes which may be presumed to require energy. These are the maintenance of structural integrity and the maintenance of the ionic-distribution characteristic of the resting state. About the former very little can be said. Perhaps the cell bodies from which the excised nerve fibers have been severed are the primary site of synthetic reactions underlying such maintenance. During activity and recovery therefrom energy demand may be increased in two ways: there must be an acceleration of ion transport processes in order to reverse the exchange of ions which occurs during the passage of impulses and, secondly, there may be a dissipation of chemical energy associated with the permeability cycle that allows the ionic exchange to take place.

Insofar as nerve at rest is in a steady state and following activity returns to the same steady state, the over-all process of impulse conduction involves no net external work and results only in the conversion of chemical energy to heat.

The following discussion indicates that oxidative metabolism in nerve can serve as an adequate energy source. Brink[1] has recently reviewed the evidence that the biochemistry of peripheral nerve of the frog resembles that of other animal cells; the nerve appears to contain and utilize the Meyerhof-Embden pathway of carbohydrate breakdown, the Krebs tricarboxylic acid cycle, and the cytochrome chain of enzymes. The energy turnover appears to be mediated by the usual system of phosphate compounds (adenosine phosphates and creatine phosphate) which are replenished principally by oxidative phosphorylation carried out by mitochondria. In what follows, it will be assumed (1) that any processes which rely upon metabolic energy do so by chemical reactions coupled to the breakdown of adenosine triphosphate, or its equivalent, and thereby increase the intracellular level of phosphate acceptor; (2) that the kinetics of oxygen uptake by nerve are some reflection or measure of the changes in concentration of phosphate acceptors at the mitochondria. A particularly dramatic experiment directly supporting the first of these assumptions is that in which Caldwell and Keynes[2] injected ATP into a metabolically poisoned squid axon and observed a partial restoration of the rate of sodium extrusion.

Firstly, the kinetics of the increases in oxygen utilization associated with the conduction of impulses is described and compared with the heat measurements of Hill, and then it is shown how the kinetics are modified under circumstances in which rates of ion transport have presumably been affected. Secondly, data on the ionic fluxes across the nerve surface are examined and the estimated energy requirements of transport processes are compared with energy available from oxidative metabolism. Thirdly, some observations are described of prolonged positive afterpotential (post-tetanic hyperpolarization) which appears to be closely related to ionic transport processes.

Most of the observations which follow come from experiments on the excised sciatic nerve of the frog. This preparation has some advantages over the giant axon of the squid in that its metabolic and electrical properties remain more nearly constant over an experimental period of ten to fifteen hours. It has, however, the disadvantage of being a bundle of fibers of several different types and there are complications arising from the existence of an appreciable extracellular space. The minimum environment required by frog nerve to maintain function and a satisfactory steady state at 20°C is a balanced bathing solution containing sodium, potassium and calcium salts, and dissolved oxygen. It should be emphasized that many or most of the electrical and metabolic properties of nerves appear to be basically similar from one species of animal to another, and similar also, in fact, to the properties of other excitable tissues such as skeletal and cardiac muscle.

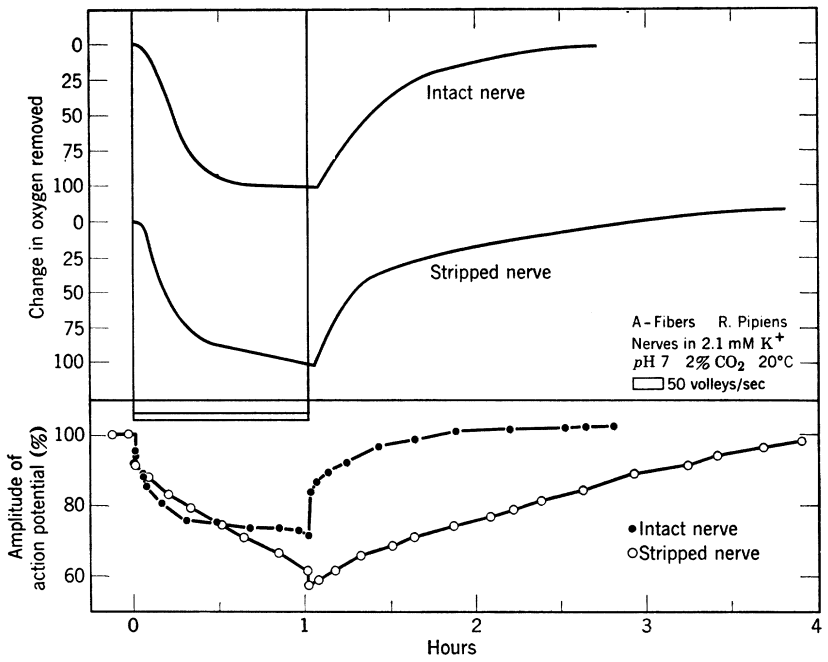

FIG. 1. (Upper) Relative time courses of the increases in oxygen uptake resulting from activity in intact frog sciatic nerve (perineurium not removed) and in stripped nerve (perineurium removed). Measurements made in a flow respirometer[3] with a polarized oxygen cathode. Polarographic current is a linear measure of dissolved oxygen remaining in solution after it has flowed past the nerve, a downward deflection in the trace, as shown, indicating an increase in oxygen uptake. (In all tetani in this and the following figures, only type A fibers stimulated.) (Lower) Time courses of changes in amplitudes of compound action potentials of the two nerves, measured during tetanus and occasionally by test shocks during recovery.

OXIDATIVE METABOLISM AND HEAT PRODUCTION

The oxygen uptake by resting frog nerve is usually 30 to 40 mm³/g(wet)/hr or about 1.5 μmoles/g(wet)/hr. When a nerve is tetanized, its rate of oxygen uptake increases and approaches a new steady level in about 30 min, closely following an exponential time course with a time constant of 5 to 8 min, Fig. 1, upper curve.[3] The amplitude of the increase is greater the higher the frequency of tetanus, at low frequencies, and approaches a maximum limiting value (of about 1 μmole/g (wet)/hr) at about 100 volleys/sec,[4] as shown in Fig. 2. At the end of tetanus, the rate of uptake declines slowly toward the resting value along an exponential whose time constant is usually 15 to 25 min. [After tetani at frequencies lower than about 10/sec, recoveries may be more rapid than this (see Fig. 11 in an article by Connelly *et al.*[5]).]

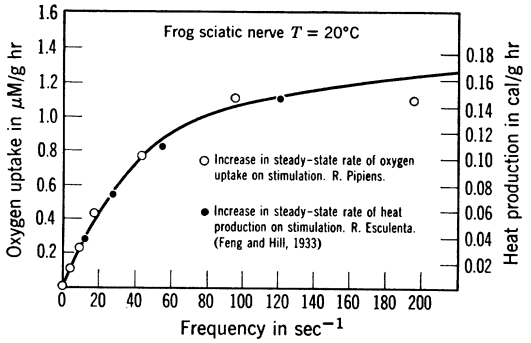

FIG. 2. Oxygen uptake and heat output for active nerve in relation to frequency of conducted impulses. Perineuria not removed. Ordinate scale symbol μM means micromole [from F. Brink, D. W. Bronk, F. D. Carlson, and C. M. Connelly, Cold Spring Harbor Symposia **17**, 53 (1952)].

The over-all increases in heat production by tetanized frog nerve show kinetics similar to those of the increases in oxygen consumption.[6] The quantitative comparison of steady-state heat rate and oxygen consumption as a function of frequency is shown in Fig. 2. The ratio of the right-hand to the left-hand scale is within 15% of the accepted value of the calorific equivalent of oxygen, 5 cal/cc. The agreement is quite satisfactory, in view of the difference in species.

A detailed analysis of the heat production of nerve during short tetani reveals the presence of a component whose onset and termination are abrupt and correspond to the beginning and end of the tetanus.[7] This "initial heat," only a few percent of the total heat associated with the tetanus, appears to have no counterpart in the time course of the increase in oxygen uptake, the curve of which rises linearly from the beginning of the tetanus.[4] In recent elegant work, Hill and his coworkers[8] have resolved the initial heat associated with a single volley in crab nerve into a positive phase (heat production) followed by a smaller negative phase (heat absorption). These phases may result from the heats of dilution or mixing of the sodium and potassium ions exchanged during the impulse, but other events of the permeability cycle have not been excluded as contributing causes.

The second curve of Fig. 1 illustrates the time course of increase in rate of oxygen uptake by a nerve trunk from which the perineurium, or connective-tissue sheath, has been removed. The striking difference between this and the upper curve is that the recovery is characterized by two components, the first lasting no more than about 30 min and the second extending over several hours.

The lower part of Fig. 1 shows the accompanying changes in the amplitudes of the compound action potentials of the two nerves, the stripped nerve showing much less rapid recovery than the unstripped nerve. The possibility that these differences in the metabolic and electrical behavior of stripped and intact nerve might result principally from differences in the extracellular level of potassium ions was tested by the series of experiments illustrated in Figs. 3 and 4. These compare the kinetics of the changes in oxygen uptake and action potentials, respectively, of stripped nerves bathed in solutions containing different concentrations of potassium ion. The three lower curves of Fig. 3 possess fast components of about the same relative magnitude and time course whereas the slow components of these curves show gradation from effectively no recovery in K-free solution, to a slow almost linear recovery in 2.1 mM K^+, to a somewhat more rapid curvature characteristic of an exponential of about a 40-min time constant, in 5 mM K^+. In the top curve (8.5 mM K^+), the over-all recovery is even more rapid than any observed in intact nerve (in 2.1 mM K^+), with an exponential time constant of only 11 min instead of 15 to 25. It is not possible to distinguish two phases of recovery in this case and it is not clear whether the slower phase observed in the lower curves has been speeded up to merge with the rapid phase or whether it has decreased in magnitude to zero. In Fig. 4, one sees that the lower the concentration of potassium in the bathing solution, the more rapid is the decline in height of the action potential during tetanus and the less rapid is its recovery. Recovery of action potential is incomplete in K-free solution, as the recovery of oxygen uptake was seen to be. In 8.5 mM K^+, however, not only does the action potential not decline in amplitude during tetanus but it shows about 13% increase in amplitude during the post-tetanic period.

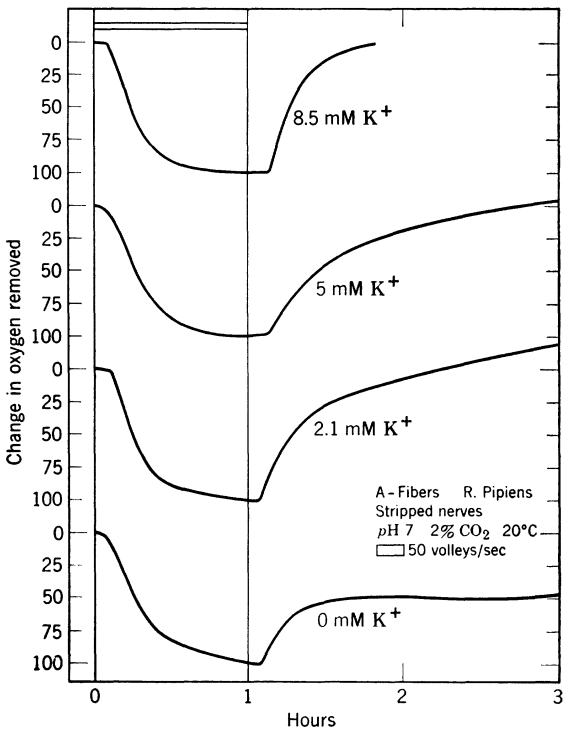

FIG. 3. Relative time courses of the increase in oxygen uptake by stripped nerve bathed in Ringer's solutions containing different concentrations of K^+. Ordinate as described for Fig. 1.

A possible key to the interpretation of these observations is the finding of Hodgkin and Keynes[9] (see also Hodgkin's Croonian Lecture[10] for an extended discussion of the movements of ions in giant nerve fibers) that the efflux of sodium from *Sepia* fibers is increased in a solution containing more potassium than does sea water and is depressed by K-free solution to one-third or one-

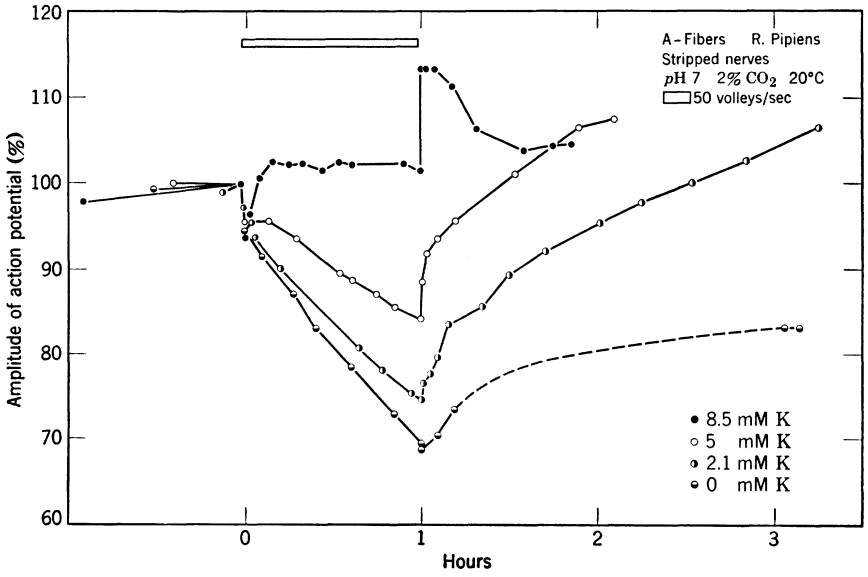

FIG. 4. Time courses of changes in amplitudes of compound action potentials of the four nerves of Fig. 3.

quarter of the efflux into sea water. If, in frog nerve, the rate of sodium extrusion is similarly dependent upon the level of potassium in the external solution, the slow component of recovery respiration may be interpreted as reflecting the energy demand of the transport machinery. The fast component, on the other hand, might result either from cessation at the end of tetanus of energy demand by the excitability cycle, or from a rapid reduction in rate of ion transport, at the end of tetanus, because of relaxation by diffusion of the internal ionic gradients maintained near the nerve surface by the entrance of sodium and by the exit of potassium during each impulse. The following considerations suggest that additional factors may affect the kinetics of the fast component.

The principal locus of ionic flow during an impulse in a myelinated fiber appears to be across the membrane at the node.[11,12] If ionic recovery takes place principally across the nodal membrane, the question arises as to whether or not phosphate compounds participating in this recovery are rephosphorylated by mitochondria close to the node or by those farther down the internodal space. Thus, longitudinal diffusion of phosphate acceptors in the internode may determine in part the kinetics of changes in rate of oxygen uptake. These considerations apply, as well, to the kinetics of the rising phase of uptake that occurs during the tetanus. The fact that the time constant of this rising phase in intact nerve is as long as it is (5 to 8 min) and does not vary markedly with tetanus frequency[4] is consistent with the idea that the kinetics of this phase are restricted in part by configurational parameters.

The differences in the kinetics of the changes in oxygen uptake and in the action potentials of intact and stripped nerves in 2.1 mM K^+ Ringer's appear, then, to be due to restrictions imposed by the perineurium on the diffusion of ions between the extracellular space and the bathing solution. The differences arise from the secondary effects of a changing ionic composition around the fibers of the intact nerve, especially as regards the concentration of potassium.

A most important and revealing experiment is that of measuring the respiration of nerve bathed by a solution in which lithium has replaced sodium ion. It is known that lithium can substitute for sodium in the conduction process. As soon as lithium Ringer's is substituted for sodium Ringer's, the resting respiration begins a slow decline. Tetanization produces almost no increase in rate; there may result a slowing or interruption in the decline of the resting respiration, but over all the effect of activity during exposure to lithium Ringer's is certainly not more than 5% of the effect measured in sodium Ringer's. The observation suggests strongly that lithium ions are extruded from frog nerve very slowly or not at all. Swan and Keynes[13] have reported that lithium ions are extruded from frog muscle much less rapidly than are sodium ions. Another conclusion from this experiment is that energy demand originating in the excitability cycle is less than 5% of the total. Therefore, it appears unlikely that the fast recovery components of the three lower curves of Fig. 3 result from the cessation of such a demand.

ION TRANSPORT AND ENERGY REQUIREMENTS

In Table I are shown data from the work of Hurlbut and Asano on water distribution, ion distribution and fluxes, and the net ion changes which result from activity or exposure to an altered environment. The water distribution refers to intact nerve. The assignment of water and ions is based upon the assumption that the nerve consists of two compartments, intracellular and extracellular, the latter occupied by bathing solution. The internal concentrations of ions are based upon measurements in which the extracellular space had been washed free of sodium and potassium ions and include a small correction for sodium lost during this washing.[14] The fluxes of sodium and of potassium were measured as outfluxes from resting nerve which had previously been equilibrated with radioactive ions.

At the bottom of Table I the sodium gained and the potassium lost during activity and during exposure to oxygen-free and K-free environments are compared. It is apparent that in each case the sodium gain is nearly equal to the potassium loss. Asano and Hurlbut[15] have shown that ionic recovery does take place in the three or four hours following activity (50 volleys/sec, 1 hr, in 2 mM K^+ Ringer's). Figure 5 indicates that recovery from rather large ionic shifts takes place in such a way that the potassium movement in one direction balances

TABLE I. Frog nerve.

Extracellular water content	45% wet weight[a]
Intracellular water content	29% wet weight[a]
Dry weight	26% wet weight[a]

	Concentration: Intracellular ($\frac{\text{mmole}}{\text{kg intracell water}}$)	Concentration: Extracellular (mM)	Flux ($\frac{\text{mmole}}{\text{kg intracell water, hr}}$)
Sodium	47[a]	116	23[b]
Potassium	159[a]	2	10–28[b]

	Net changes resulting from: Activity 50 volleys/sec 1 hr	Asphyxia 5 hr	K-free Ringer's 5 hr
Sodium gain ($\frac{\text{mmole}}{\text{kg intracell water}}$)	18[c]	35[a]	31[b]
Potassium loss ($\frac{\text{mmole}}{\text{kg intracell water}}$)	21[c]	41[a]	34[b]

[a] Reference 14.
[b] W. P. Hurlbut and T. Asano (unpublished observations).
[c] Reference 15.

the sodium movement in the other. The rates of ionic recovery during the first hour or two (Fig. 5) produce concentration changes of about 12 mM (intracellular) per hour. This net ionic exchange that is measured during recovery is presumably superimposed upon the normal fluxes, measured in resting nerve, of about 23 mM (intracellular) per hour for sodium and probably about the same for potassium.

For a two-compartment system, the thermodynamic expression for the energy required to move one mole of sodium from inside (i) to outside (o) and one mole of potassium in the opposite direction is

$$RT\left(\ln\frac{Na_o}{Na_i}+\ln\frac{K_i}{K_o}\right),$$

where Na_o, . . . etc., are ion concentrations (strictly activities). This expression has a value of about 3000 cal/mole for the concentrations of sodium and potassium given in Table I. If the energy available from the hydrolysis of one mole of ATP to ADP, under intracellular conditions, is 7000 to 12 000 cal, then energetically it should be possible for some 2 to 4 sodium ions to be extruded for each molecule of ADP produced. Assuming, conservatively, that one ADP is produced for each sodium ion transported, it may then be asked whether the observed oxygen consumption appears to be capable of providing sufficient energy, via phosphorylated intermediates, to transport ions at the rates observed. For frog nerve at rest, the oxygen uptake is about 1.5 μmole/g (wet)/hr and the sodium flux about 6.6 μmole/g (wet)/hr. Thus the ratio Na:O is $6.6/(2\times1.5)=2.2$. If, in the functioning cell, mitochondria maintain an average P:O ratio (phosphate acceptors phosphorylated to oxygen atoms reduced) of about 3, as isolated mitochondria do,[16] then it would seem that oxidation provides adequate energy for transporting one sodium ion per ATP broken down. If the Na:P ratio is actually unity, the comparison implies that only about 30% of the resting respiration is available for processes other than ion transport.

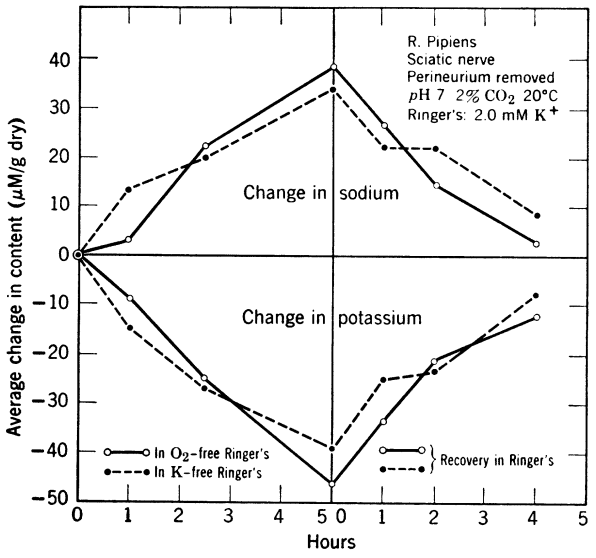

FIG. 5. Time courses of the changes in ionic contents of stripped nerves during and after exposure to oxygen free or potassium-free Ringer's solution. (Partially unpublished observations of W. P. Hurlbut and T. Asano.) Ordinate scale symbol μM means micromole.

An estimate of the increase in rate of sodium extrusion during a period of activity can be made from other observations of Asano and Hurlbut.[15] They found that after one hour of activity at 50 volleys/sec the net gain of sodium averaged 32 μmole/g (dry) when the nerve was bathed with K-free solution and only 16 when the Ringer's contained 5 mM K^+. This is an equivalent difference in rate of 4.2 μmole/g (wet)/hr. If, in the two solutions, about the same amount of sodium entered the nerve during each impulse, the observed difference may be attributed to effective, restorative transport taking place during activity. The ratio of this difference in rate to the increase in respiration during activity in 5 mM K^+ is approximately $Na:O=4.2/(2\times.8)=2.6$. This figure suggests that, in the case of active nerve also, there may be little energy utilized for purposes other than ion transport.

It should be emphasized that only one plausible line of thought has been followed in making these estimates and comparisons and that assumptions without direct experimental support have been invoked. Alternative lines of reasoning may ultimately prove to be more acceptable than the one outlined here.

POST-TETANIC HYPERPOLARIZATION

Experiments which indicate that there are measurable electrical changes associated with the ionic transport events of recovery[17] are now described.

A stripped frog nerve is mounted in a plastic chamber so as to pass through five compartments (separated by grease-seals) containing oxygenated solutions. The first and fifth compartments are connected via calomel half-cells to a stable dc amplifier (chopper amplifier, time constant about 1 sec). The first compartment usually, and the second, fourth, and fifth compartments always contain a 5 mM K^+ Ringer's, in the experiments to be described. The nerve is stimulated before it enters the first compartment and the impulses are blocked in the third which contains a choline chloride (sodium-free) Ringer's. By this arrangement, it is possible to follow the changes in potential developed by an active or recovering region of nerve, the steady potential of an inactive region serving as a reference.

Figure 6 shows the potential changes observed during and following a 25-min period of activity at 50 volleys/sec. The downward deflection at the beginning of the tetanus is the time average of the negative action potentials. Once each minute the tetanus was interrupted for 5 sec and the recorded potential showed a positive deflection which reached its peak within this

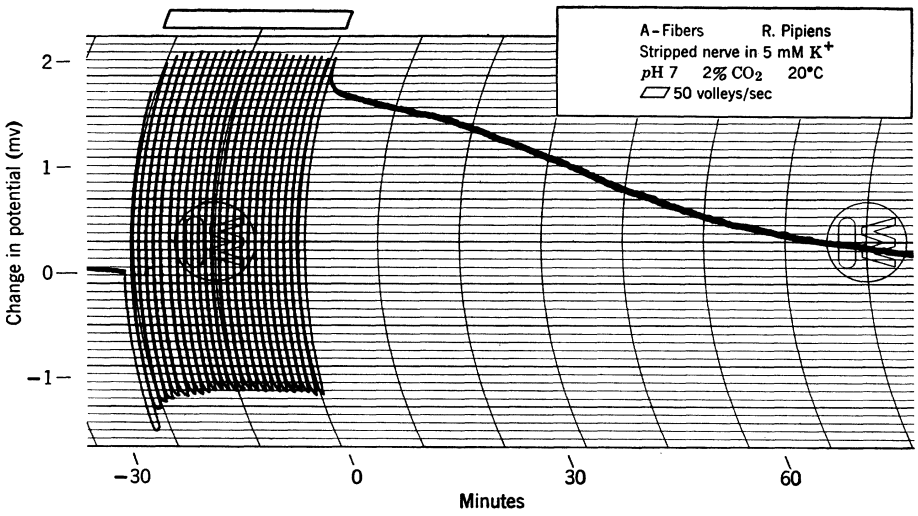

FIG. 6. Changes in average membrane potential recorded during and after 25-min tetanus. Tetanus interrupted for 5 sec each minute [from C. M. Connelly, "Post-tetanic hyperpolarization in frog nerve," in *Proceedings of the National Biophysics Conference, 1957* (Yale University Press, New Haven, to be published)].

interval. The amplitude of this hyperpolarization (net increase in membrane potential) reached a maximum after about 4 min and thereafter changed little. After the end of the tetanus, the hyperpolarization lasted for more than an hour; its recovery time course cannot be described as exponential.

Figure 7 shows how the prolonged positive after-potential is affected by the level of K^+ in the bathing solution (in the first compartment). A stimulation of 25 min in a solution containing 5 mM K^+ was first carried out as a control, and after recovery the solution was changed either to 8.5 mM K^+ (upper) or to K free (lower). The potential changes recorded during tetanus under the modified conditions have been superimposed on the control observations. The amplitude of the hyperpolarization varies appreciably with the level of potassium; recovery is rapid in high potassium and appears to be very slow and prolonged in K-free solution. During recovery from activity, the kinetics of oxidative recovery and the kinetics of the positive after-potential are both affected in much the same way.

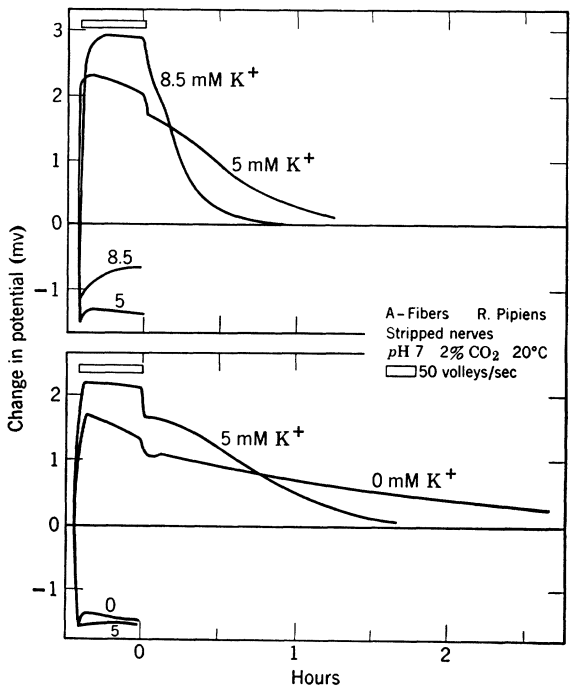

FIG. 7. Superimposed tracings of changes in average membrane potential recorded during and after 25-min tetani in Ringer's solutions containing different levels of K^+. Traces shown during period of tetanus are envelopes of changes similar to those shown in Fig. 6. In each of the two experiments the first tetanus was in Ringer's with 5 mM K^+, and the second tetanus carried out after 30 to 40 min exposure to the second solution. Zeroes of potential adjusted to superimpose at beginning of tetanus.

Other characteristics of this after-potential have been examined. It has been found that the observed amplitude of hyperpolarization approaches a maximum, or "saturation level" as the frequency of tetanus is increased above about 25/sec.[17] This maximum is about 2 mv. Depolarization produced by introducing isotonic potassium chloride into the first compartment averages 10 mv. Thus, maximum hyperpolarization corresponds to an increase in membrane potential of about 20%.

Post-tetanic recovery may be rapid or long-lasting depending on duration and frequency of tetanus. At frequencies above about 25/sec the effect of duration is striking, as illustrated in Fig. 8 which shows, superimposed, the recoveries from four tetani of different durations, at 50 volleys/sec. The longer the tetanus, the longer recovery takes, as if continued activity resulted in an accumulation of something, the final dissipation of which has associated with it an emf. Areas under the total hyperpolarization-time curves (i.e., area above the zero-potential line, including the periods of tetanus and recovery) have been measured as a function of duration of tetanus. Within experimental limits, area varies linearly with duration suggesting that the magnitude of hyperpolarization is approximately a linear measure of the rate of a recovery process.

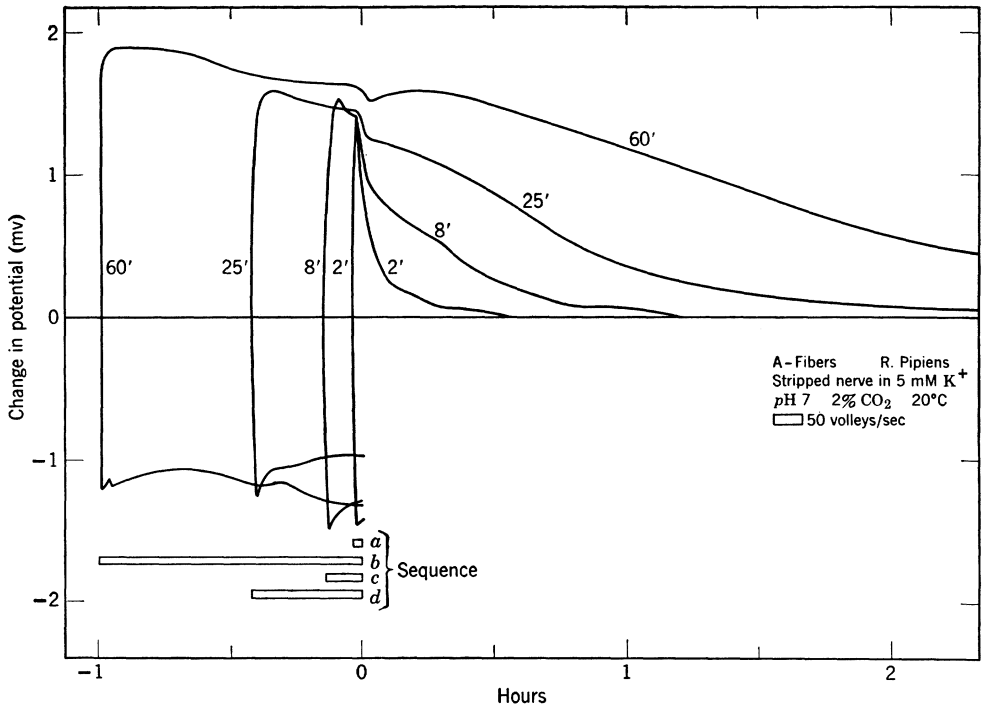

FIG. 8. Superimposed tracings of changes in average membrane potential recorded during and after a series of tetani of different durations. Traces shown during period of tetanus are envelopes of changes similar to those shown in Fig. 6 [from C. M. Connelly," Post-tetanic hyperpolarization in frog nerve," in *Proceedings of the National Biophysics Conference, 1957* (Yale University Press, New Haven, to be published)].

A variety of observations appears to be consistent with the idea that the recovery process involved is the outward transport or extrusion of sodium ions. One experiment which has a direct bearing on the question is illustrated in Fig. 9. The positive afterpotential associated with a tetanus is effectively eliminated upon complete substitution of lithium for sodium in the bathing Ringer's. The parallelism between this result and that described earlier on the effect of lithium Ringer's on activity respiration furnishes strong support for the idea that the activity respiration and the positive after-potential both have their origin in the process involving the outward transport of sodium coupled to the inward movement of potassium.

One further point should be discussed. Ritchie and Straub,[18] in studying the positive afterpotential of

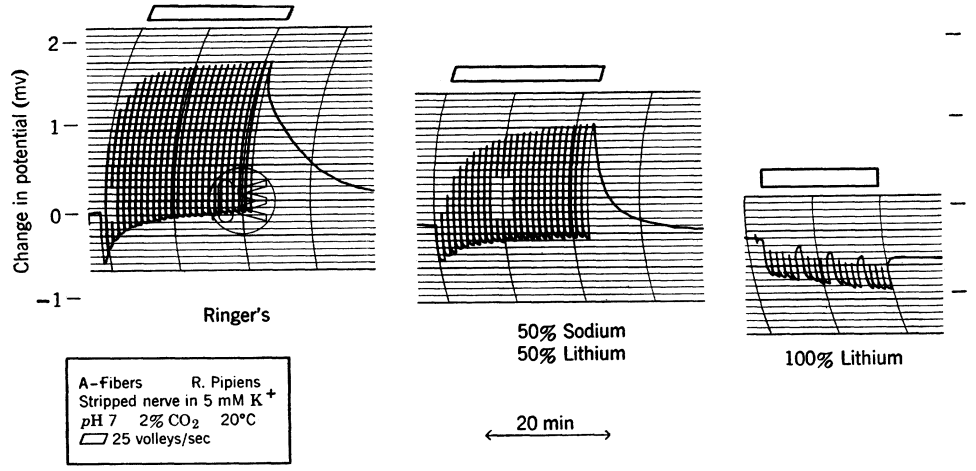

FIG. 9. Changes in average membrane potential recorded during and following tetani in solutions having different proportions of sodium and lithium ions. Potential zero common to the three records. During intervals (about 30 min) between records, nerve was bathed in the next solution. First and second records, 25-min tetani interrupted for 5 sec each minute. Third record, 20-min tetanus with 13 interruptions of 5 sec and 3 interruptions of 1 min each [from C. M. Connelly, "Post-tetanic hyperpolarization in frog nerve," in *Proceedings of the National Biophysics Conference, 1957* (Yale University Press, New Haven, to be published)].

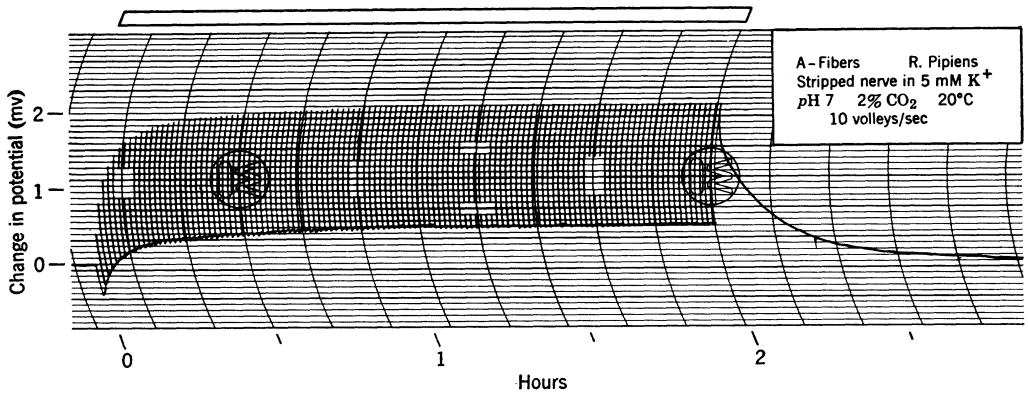

FIG. 10. Changes in average membrane potential recorded during and after 2-hr tetanus at 10 volleys/sec. Interrupted 5 sec each minute.

mammalian C-fibers, came to the conclusion that their observations could be explained solely by an electroneutral pump operating to restore the normal sodium-potassium balance following activity. The after-positivity was described as a variation of membrane potential in response to the variation in the concentration of potassium in the fluid immediately outside the nerve membrane. This happens as follows: during recovery, the pump produces a net flow of potassium inward across the membrane. By this action, the concentration of potassium at the outside surface of the fiber is lowered below its value in the body of the bathing solution. Since it is known that a decrease in external concentration of potassium does produce an increase in membrane potential, the conclusion appears to be unassailable, qualitatively. One must agree that any ion pumping system, whether electroneutral or inherently electrogenic, must have this property. It is to be taken

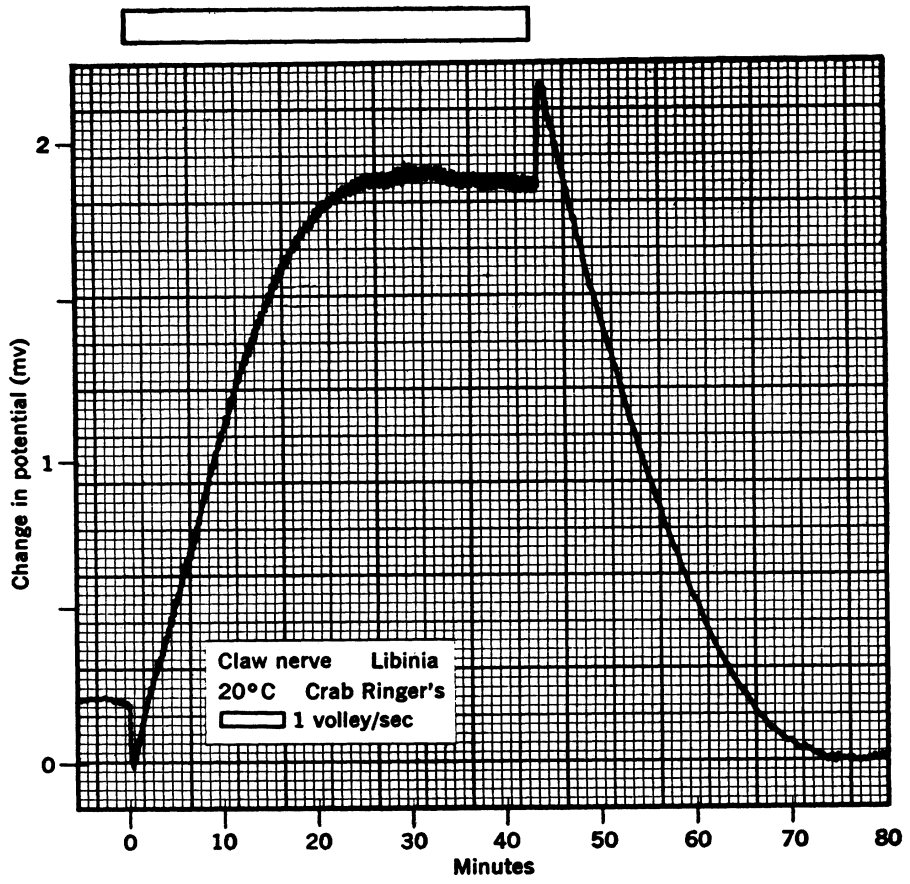

FIG. 11. Changes in average membrane potential of spider-crab nerve recorded during and following 43-min tetanus at 1 volley/sec. Not interrupted during tetanus. Measured depolarization of spider-crab nerve in isotonic potassium chloride about 30 mv.

for granted that the transport system in frog nerve produces some potential by this mechanism. On the other hand, Fig. 10 illustrates an experiment in which the Ritchie and Straub mechanism is not sufficient to explain the observed variations of potential. During a tetanus, the average membrane potential (exclusive of the action potentials) should be either negative or zero, according to the Ritchie and Straub scheme. The average should be negative during periods in which there is a net loss of potassium from the fibers and would approach zero if the system approached a steady state (i.e., K^+ pumped back inside during pulse interval $\equiv K^+$ lost/impulse). The lower envelope of the record in Fig. 10 shows that the membrane became hyperpolarized four minutes after the beginning of the tetanus and remained so for the remainder of it. With correction for the apparent depolarization introduced by averaging the action potentials, the statement could be made that hyperpolarization began at the beginning of the tetanus and reached a final average level (in the 100-msec interval between pulses) of almost one millivolt. Thus, the ionic transport system of frog nerve appears to be inherently electrogenic (positive outward).

A similar statement, based on similar evidence applies to unmyelinated limb nerve of the spider crab, shown in Fig. 11. Here the membrane also develops a net hyperpolarization during low-frequency tetani.

If one takes as a working hypothesis the proposition that the magnitude of hyperpolarization is proportional to the rate of sodium extrusion, how can the shapes of the recovery curves of Fig. 8 be explained? The kinetic behavior of a simple reaction mechanism showing saturation kinetics is portrayed in Fig. 12. It is the classical enzyme-substrate reaction of Michaelis, the rate of which is given by the second expression in the figure, where K_M is the concentration of substrate that produces half-maximal rate. The curve describes the rate of reaction as a function of time as an initially large concentration of substrate decreases. If nerve were to extrude the sodium remaining within it with the kinetics of this mechanism, the hyperpolarization should follow this curve exactly, starting at a point appropriate to the amount of sodium accumulated during the tetanus. But, the recovery curves of Fig. 8 cannot be superimposed by displacement along the time axis, and, therefore, do not conform to this model.

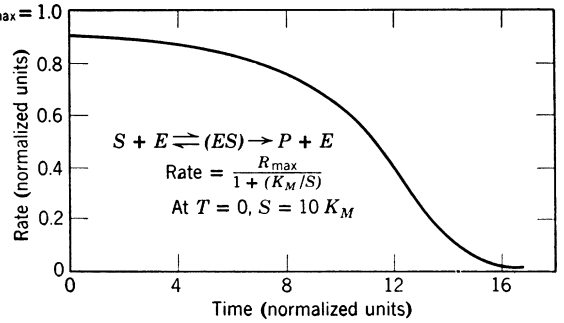

FIG. 12. Calculated time course of rate of disappearance of substrate with Michaelis type of enzyme-substrate reaction mechanism. K_M is concentration of substrate producing half-maximal rate [from C. M. Connelly, "Post-tetanic hyperpolarization in frog nerve," in *Proceedings of the National Biophysics Conference 1957* (Yale University Press, New Haven, to be published)].

If sodium ions enter the active fiber at the nodes and are also extruded principally from the nodes, then ions not extruded immediately after entry must tend to diffuse into the myelin-insulated internodal space. To determine the possible effect of diffusion in the internode on the kinetics of ionic recovery, the analog model shown in Fig. 13 was constructed. The model corresponds to one-half a node and to the adjacent one-half internodal space. A pentode, whose plate current-plate voltage curve is approximately a hyperbolic saturation curve of the Michaelis type, simulates the ion-extruding mechanism at the node; a filter network of 10 *RC* units is the analog of the diffusion field in the internode. Positive charges correspond to sodium ions; potentials at various points in the plate and filter circuits to sodium concentrations; plate current to rate of sodium extrusion; and injection of a constant current to the plate circuit simulates a tetanus. Seconds in the model correspond to minutes in the nerve. Figure 14 shows superimposed records of the output of the analog circuit, with parameters chosen to duplicate as closely as possible the

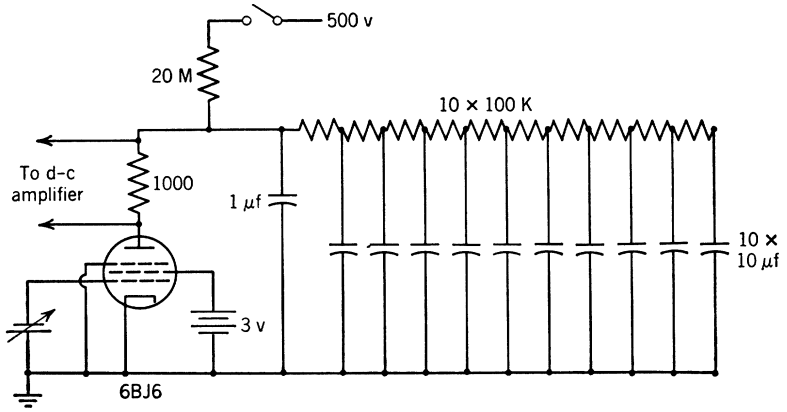

FIG. 13. Analog circuit [from C. M. Connelly, "Post-tetanic hyperpolarization in frog nerve," in *Proceedings of the National Biophysics Conference, 1957* (Yale University Press, New Haven, to be published)].

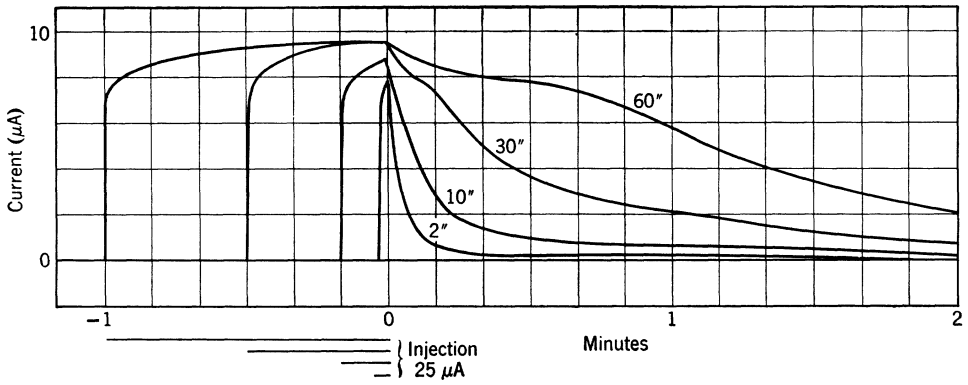

FIG. 14. Superimposed records of changes in plate current of analog circuit of Fig. 13, during and after 25-μA injections of different durations. Grid bias adjusted to give maximum plate current of 10 μA [from C. M. Connelly, "Post-tetanic hyperpolarization in frog nerve," in *Proceedings of the National Biophysics Conference, 1957* (Yale University Press, New Haven, to be published)].

observations of Fig. 8. The time constant of the network in this case is about twice as large as the corresponding time constant for the diffusion of sodium chloride in the internodal space (estimated assuming free aqueous diffusion and node-to-node spacing of 2 mm). It is encouraging that the shape of the recovery curve changes more or less in the proper manner as the duration of tetanus is increased. This oversimplified analysis tends to support the hypothesis that the positive after-potential has its origin in a saturable, ion extruding mechanism operating at the nodes.

CONCLUDING REMARKS

The events of recovery may be described tentatively as follows: Sodium ions enter a fiber during activity. The increase in internal concentration accelerates a saturable ion-transport process in which sodium ions and high-energy phosphate compounds are obligatory participants. Sodium ions are liberated to the exterior, and potassium in the exterior medium participates in the over-all cycle, being transferred to the interior. The operation of the mechanism generates an emf, directed positively outward. Oxidative metabolism maintains the supply of high-energy phosphate compounds.

The observations outlined in this discussion have emphasized the coupling between oxidative metabolism as an energy source and ionic transport processes as an energy sink. The molecular mechanisms of active ionic transport across membranes are almost a complete mystery. Proposed mechanisms range from shuttling carriers to micro-pinocytosis to enzymatic modification of pore configuration or charge distribution. This is an outstanding problem in cellular and molecular biology today.

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