1948

Rafael Lorente de No, 1946

The Rockefeller University

CORRELATION OF NERVE ACTIVITY WITH POLARIZATION PHENOMENA[1]

RAFAEL LORENTE DE NÓ
Member, The Rockefeller Institute for Medical Research

a. *Basic Facts and Terminology.*—Nerve physiology has been analyzed chiefly on the basis of (1) the results of measurements of differences of electric potential between points on the surface of the nerve and (2) the effects of electric currents applied to the nerve through electrodes on its surface. In all cases the interpretation of the experimental results requires the use of the theory of electrotonus.

The basic phenomenon of the theory is illustrated by diagram *I* of figure 1. An electric current supplied by a polarizing circuit is applied to the nerve through electrodes on its surface (p_1, p_2). Although the shape of the electrodes is rather unimportant, the situation is visualized best when one thinks of the electrodes as thin cylindrical bands surrounding the nerve. In the case of an homogeneous cylindrical conductor of the same diameter as the nerves ordinarily used in experimental work (about 1 mm.), the applied current would flow practically only in the segment of conductor extending between the polarizing electrodes; therefore, a recording instrument (*osc.*) connected in the manner indicated in figure 1, *I*, would not measure any potential difference during the flow of the applied current. In the case of nerve, however, even when the distance between electrodes p_1 and r_1 is made as great as 30–40 mm., the measuring instrument detects a potential difference which is usually called the *electrotonic potential.* On the other hand, if electrode r_2 is located at a great distance from p_1 and electrode r_1 is successively placed at a number of points of the p_1r_2 segment, it is found that the measured potential difference decreases in an exponential fashion when the p_1r_1 distance is increased.

These facts indicate that the nerve fibers are core conductors,

[1] Lecture delivered October 24, 1946.

i.e., cable-like structures (fig. 1, *II*), composed of an internal longitudinal conductor or core, a membrane and an external longitudinal conductor (interstitial tissue fluid). From these three conductors the membrane has the lowest conductivity; it plays the rôle of the insulating sheath of a cable and forces the

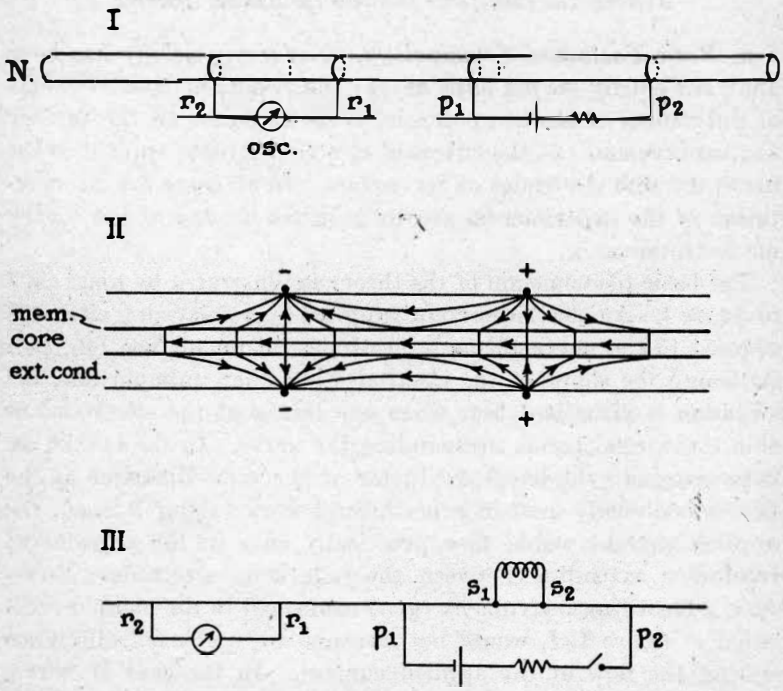


FIG. 1. Diagrams indicating the arrangement of electrodes and circuits used in experiments on nerve.

applied current to spread along the nerve in the manner indicated in figure 1, *II*.

Since the diameters of the nerve itself and of the individual nerve fibers are small, the longitudinal conductors may be assumed to be linear (linear in the sense of a geometrical line). If it is further assumed that the external and the internal longitudinal conductors have the properties of ohmic resistances

and that their resistances per unit length are r_e and r_i ohms respectively, an elementary analysis leads to the following important conclusion. Regardless of the nature of the membrane, the potential difference V_e measured on the surface of the nerve between electrodes r_1 and r_2 is related to the values V_1 and V_2 of the membrane potential at these two points by this simple formula:

$$V_e = \frac{r_e}{r_e + r_i} (V_2 - V_1)$$

Thus, if the membrane potential V_2 at r_2 is constant, the measured external electrotonic potential V_e is proportional to the change in the membrane potential V_1 at r_1.

In strict sense this simple interpretation of the external potential V_e is not correct since distributed electromotive forces appear in the core of the nerve fibers but it is adequate for the qualitative discussion to be made in this presentation. Indeed, within this narrow frame also the simplification is permissible to regard the nerve as containing only one nerve fiber (fig. 1, *II*).

In addition to the electrotonic potentials produced by applied currents, we will have to consider *demarcation potentials* arising from differences in the properties of adjacent segments of nerve and *action potentials* resulting from conduction of nerve impulses.

The simplest procedure to create a demarcation potential is to injure the nerve at one point, for example, by heating. In figure 2, *I*, the horizontal line *N* represents a nerve that had been injured at point *h* and the curve V_e, the demarcation potential measured at various distances from the injury. These differences, of course, were the result of the flow of demarcation current indicated in figure 2, *II*. Detailed analysis of the demarcation current and potential would necessitate consideration of an extensive experimental material. The main conclusion of the analysis, however, can be stated briefly. In resting uninjured nerve a difference of electrostatic potential exists between the internal longitudinal conductor and the interstitial tissue fluid; this potential difference is usually called the *membrane potential.* In the experiment illustrated by figure 2 a demarcation current flowed because the injury had destroyed the membrane and therefore the potential

difference between the core and the interstitial tissue fluid had been reduced to the value of a small junction potential. Demarcation currents also flow, of course, when the value of the membrane potential of a segment of nerve is altered by means of suitable chemical agents (figs. 5 to 7).

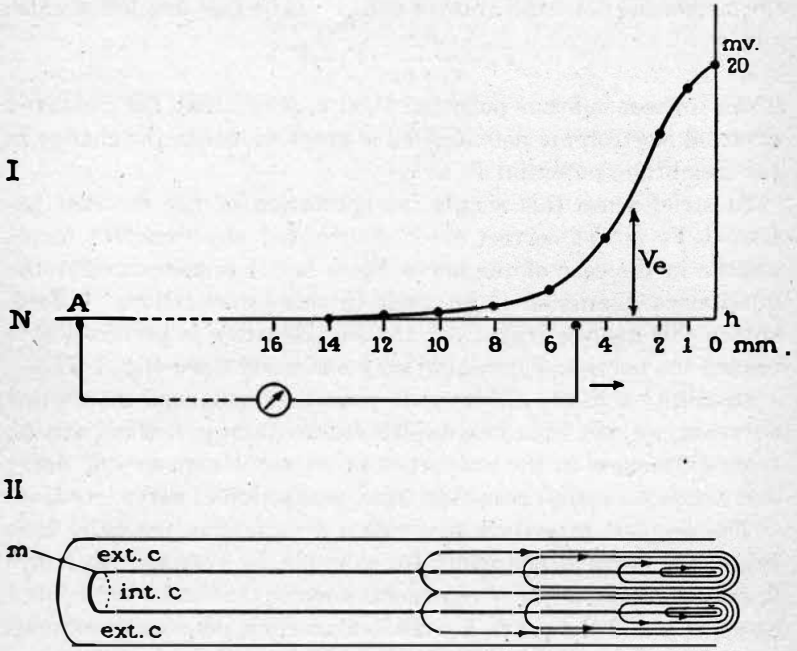


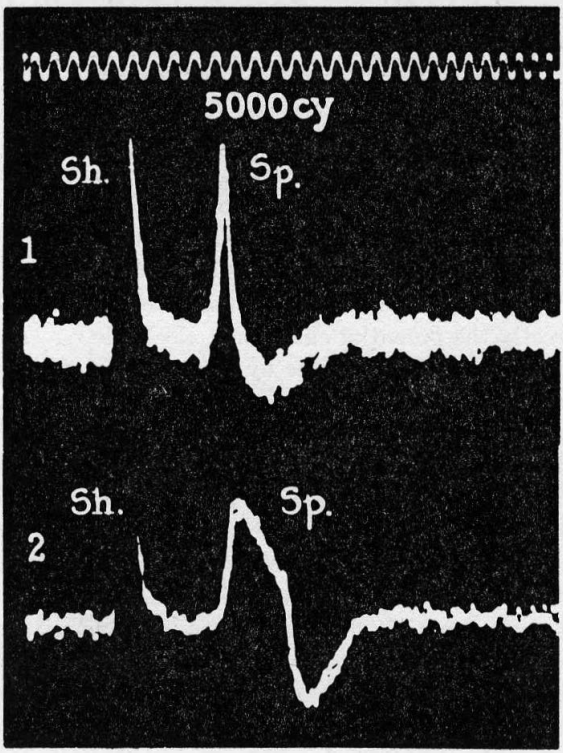
FIG. 2. *I.* Plot of the demarcation potential V_e against distance from the injured point *h*; *N*, nerve. *II.* Diagram indicating the distribution of demarcation current along the nerve; *m*, membrane; *ext. c.*, external longitudinal conductor, *int. c.*, internal longitudinal conductor (core).

The experimental arrangement ordinarily used to study action potentials is indicated in figure 1, *III.* A stimulating circuit (s_1, s_2) delivers to the nerve an electric shock of suitable strength which initiates a nerve impulse at the cathode (s_1). The impulse propagates itself along the nerve. It is clear that successive passage of the disturbance through points r_1 and r_2 must result in

the recording of a diphasic action potential (fig. 3, *2*) from which the potential changes at each electrode can be ascertained (fig. 3, *3*). If the distance between electrodes r_1 and r_2 is of the order of 2 or 3 mm., the diphasic record (fig. 3, *1*) can be regarded as the first derivative of the action potential; therefore, graphical integration can be used to determine the shape of the action potential. Ordinarily, however, the r_1r_2 distance is made relatively large and the nerve is injured at the level of electrode r_2 so that except for a relatively small artifact (fig. 20, *5, d.a.*) caused by the approach of the impulse to electrode r_2, the record gives directly the potential changes at electrode r_1. The diphasic artifact can be made negligible by treatment of the end segment of the nerve with KCl.

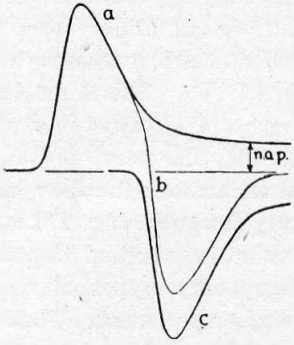
Analysis of the spike leads to the conclusion that during the conduction of the nerve impulse an alteration of the nerve fiber takes place which results in a sudden negative variation of the membrane potential. The recovery from the alteration and consequently the restoration of the membrane potential also takes place with great rapidity; however, each impulse leaves a small deficit of membrane potential which is restored at a relatively low rate. This residual depolarization is usually called the negative after-potential. The negative after-potential accumulates during conduction of trains of impulses (fig. 4). Under certain conditions the negative after-potential, after passing through a maximum, remains at a practically constant level even during 10 second tetani (fig. 4, *11, 12*); but under other conditions the negative after-potential continuously increases with increasing duration of the tetanus (fig. 4, *5, 6*). This second situation is theoretically very important since a progressive increase of the negative after-potential indicates that the nerve is passing into a state of depression or stated in a more familiar language, that the nerve is becoming severely fatigued (fig. 17).

The recovery from the residual depolarization ordinarily occurs through a temporary hyperpolarization of the membrane called the positive after-potential. Under certain conditions the positive after-potential includes a large R_3 defiection, under other



0.2 msec.

3



conditions, however, it may include only a brief R_2 deflection. In this presentation the term positive after-potential will be used only to denote the R_3 deflection.

b. *Membrane Potential.*—In the literature on nerve physiology it is customary to explain the resting membrane potential as a diffusion potential that results from the difference in the concentrations of potassium inside and outside the nerve fibers. This hypothesis was first enunciated by Bernstein (*cf.* Bernstein, '12) and although at present the hypothesis has several versions it is sufficient to discuss the original one which also is the most frequently used.

Bernstein's hypothesis is based on one fact and two assumptions. The fact is that the concentration of potassium inside the nerve fibers is much greater than the external concentration. According to modern estimates, the ratio of the two concentrations is 65:1 (*cf.* data by Fenn, Cobb, Hegnauer and Marsh in Gasser, '37). The first assumption is that the nerve membrane is semipermeable; it is permeable to potassium ions and impermeable to anions and to sodium ions. If the additional assumption is made that the membrane potential is maintained by outward diffusion of potassium ions, it is found that the value of the membrane potential would be proportional to the logarithm of the ratio of the internal and external concentrations of potassium, the factor of proportionality being 0.058 v. at 18° C.

Probably because the value calculated for the membrane potential is in agreement with experimental measurements, the diffusion hypothesis has received wide acceptance without having been submitted to more than perfunctory experimental tests. The hypothesis, however, cannot withstand a systematic analysis.

FIG. 3. Spikes of single nerve fibers of the VIIth spinal nerve of a bullfrog recorded with electrodes 3 mm. apart (*1*) and 20 mm. apart (*2*). Both records are photographs of a number of successive oscillograph sweeps. Since the impulses were being propagated with constant speed, record *1* may be regarded as the first differential of the spike. *3*, reconstructions of the spikes at the first recording electrode (*a*) and at the second recording electrode (*c*) made on the basis of an enlarged tracing (*b*) of record *2*; *n.a.p.*, negative after-potential. Nerve in 95% O_2 and 5% CO_2.

Figure 5 illustrates the effect of increasing the external concentration of K^+ ions upon the membrane potential of bullfrog sciatic nerve. The curves measure the demarcation potentials established between a segment of nerve in contact with Ringer's solution and another segment in contact with a mixture of Ringer's solution and an isotonic (0.11 M) solution of KCl. The detailed analysis of the demarcation potential curves is a difficult problem, but within the frame of this discussion it is sufficiently accurate to assume that the ordinates of the curves are propor-

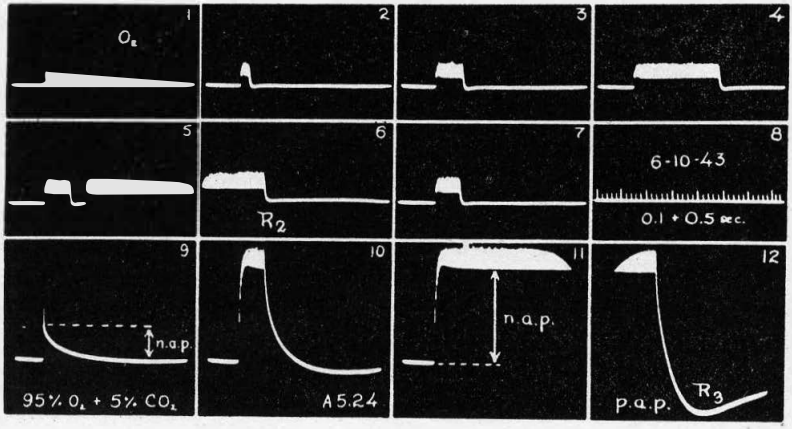


FIG. 4. After-potentials produced by single volleys of impulses (*1*, *g*) and by rhythmic trains of impulses (*2* to *7*; *10* to *12*); the trains used for records *5*, *6*, and *11*, *12* were 10 seconds long. *n.a.p.*, negative after-potential; *p.a.p.*, positive after-potential. Records *1* to *7* were obtained with the nerve in oxygen and records *9* to *12* with the nerve in 95% O_2 and 5% CO_2.

tional to the decrease of the membrane potential of the segment of nerve in contact with the KCl solution.

The diffusion potential hypothesis predicts that if the external concentration of potassium is increased, the membrane potential of the nerve fibers will decrease. This prediction is in agreement with the experimental results presented in figure 5. In two essential points, however, the experimental results are in disagreement with the predictions of the hypothesis.

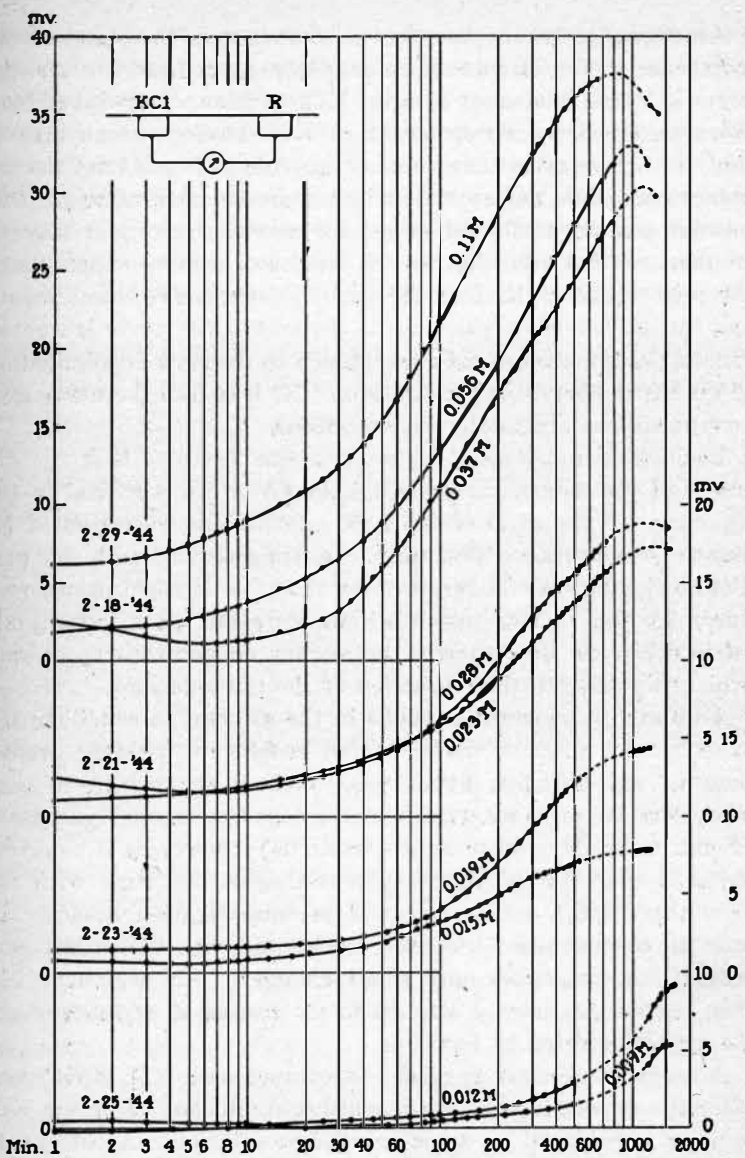


FIG. 5. Demarcation potentials resulting from the action of solutions of KCl upon a segment of the nerve. Ascent of the curves indicates depolarization of the treated segment.

According to the hypothesis, the decrease in the value of the membrane potential should take place immediately after the nerve is placed in contact with the KCl solution. In point of fact, however, the depolarizing action of KCl develops at an exceedingly low rate; even in the case of isotonic KCl (0.11 M) the depolarization does not approach its maximum until after the test solution has been allowed to act for several hours. It may be emphasized that according to the results of control experiments, the penetration of K^+ ions through the connective tissue sheath and the inter-fibrilar spaces up to the axis of the nerve is a rapid process; as a matter of fact, in relation to the slow depolarization of the nerve fibers, the penetration of K^+ ions into the nerve may be regarded as practically instantaneous.

The diffusion potential hypothesis also predicts that the decrease of the membrane potential should be proportional to the logarithm of the ratio of the new external concentration of K^+ ions to the normal concentration. In disagreement with this prediction the curves of figure 5 show that the depolarizations produced by the various test solutions were not on a logarithmic relationship to the external potassium concentrations at any arbitrarily selected time of action of the test solutions.

An assumption could be made in the attempt to conciliate the experimental observations presented in figure 5 with the predictions of the diffusion hypothesis. Nerves are known to gain potassium when the external potassium concentration is increased (Fenn, Cobb, Hegnauer and Marsh, '36); therefore it could be assumed that the membrane potential does not vary with the logarithm of the external potassium concentration because the internal concentration increases so that the ratio of the two concentrations undergoes only small changes. An argument like this, however, cannot be applied to the results of experiments of the type illustrated by figure 6.

A segment of nerve is placed in contact with 0.11 M KCl and after the depolarization has reached a significant value the KCl solution is replaced by a potassium-free solution. According to Bernstein's hypothesis, since the internal concentration of po-

tassium has been increased, the membrane potential should acquire a value in excess of the normal one immediately after the nerve has been placed in contact with the potassium-free solution; thereafter the membrane potential should decrease towards the normal value paralleling the outward diffusion of the excess of K^+ ions. These predictions are in sharp disagreement with the experimental results. The curves of figure 6 show that during the first

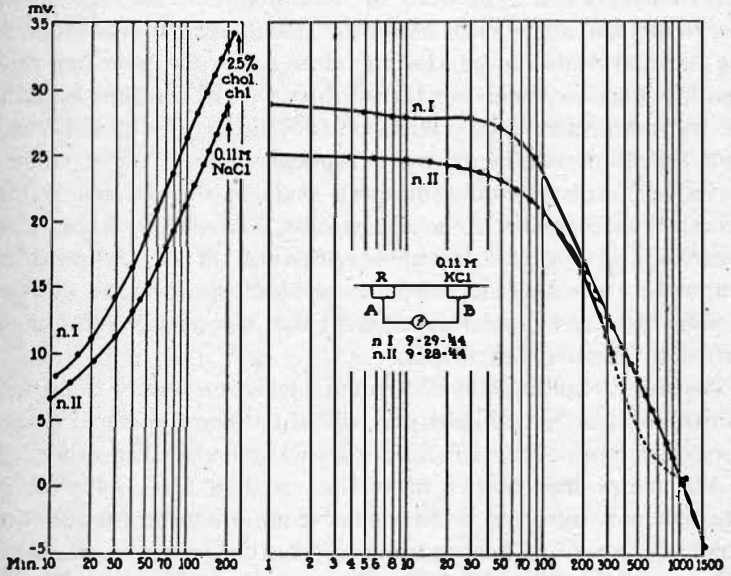


FIG. 6. Depolarization of the nerve by isotonic KCl and repolarization in a potassium-free solution.

hour after the nerves had been placed in contact with the potassium-free solutions, the membrane potential remained practically constant at the low value that it had reached during the action of the excess of K^+ ions; thereafter a slow repolarization process began which required many hours for completion. The unavoidable conclusions to be drawn from the results of this experiment are: (1) that the value of the membrane potential is not directly dependent upon the ratio of the internal and external

concentrations of potassium and (2) that the membrane potential is not maintained by outward diffusion of potassium ions.

A number of years ago Koch ('27) and Gerard ('30) demonstrated that nerve deprived of oxygen undergoes a depolarization which is reversible; i.e., after oxygen is again made available to the nerve a repolarization takes place. In the past, this remarkable phenomenon has been placed in agreement with the diffusion potential hypothesis by assuming that the rôle of oxidative metabolism is only indirect. Metabolism would maintain the semipermeability of the membrane thereby creating those conditions under which outward diffusion of K^+ ions can establish the membrane potential. However, detailed study of the anoxic depolarization and the oxidative repolarization of nerve under a variety of experimental conditions leads to the conclusion that oxidative metabolism is the mechanism that directly creates the membrane potential. Complete presentation of the evidence cannot be made here, it will be convenient, however, to mention the results of one type of experiment that also yields a test of the diffusion potential hypothesis.

The three experiments illustrated by figure 7 were done with pairs of nerves. In all cases one half of the nerve was maintained in oxygen in contact with Ringer's solution while the other half of the nerve was placed in contact with a KCl solution, its atmosphere being oxygen for one nerve and nitrogen for the other nerve of the pair. The demarcation potential curves show that in all cases the combined effects of anoxia and excess of K^+ ions caused a greater depolarization than the excess of K^+ ions alone. However, after oxygen was admitted into the nerve chamber, the KCl-treated segment was able to perform an oxidative repolarization, its membrane potential rapidly becoming higher than that of the nerve kept in oxygen. Indeed, in two instances the oxidative repolarization temporarily brought the membrane potential above that of the segment of nerve in Ringer's solution. The detailed curves of the oxidative repolarization presented in the right half of figure 7 show the exceedingly high rate of the repolarization; in all cases the membrane potential reached its

maximum after 10–15 minutes of respiration. The brevity of this interval of time renders the assumption impossible that the repolarization was accompanied by significant changes in the

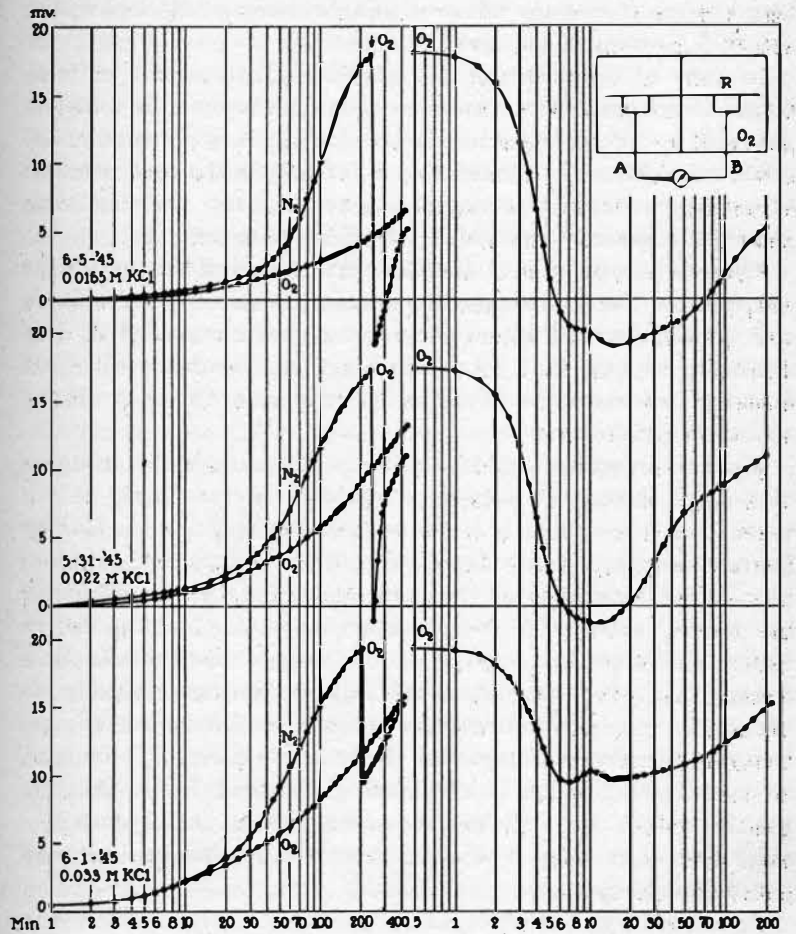


FIG. 7. Effect of an increased concentration of KCl upon the membrane potential of bullfrog sciatic nerve in the presence and in the absence of oxygen. The curves of the oxidative repolarization of the anoxic nerves have been reproduced in the right half of the figure on an extended time scale.

internal concentration of potassium; but regardless of any assumption that could be made in reference to the internal concentration of potassium there is no doubt that oxidative metabolism can counteract the effect of a large excess of K^+ ions in the external medium of the nerve fibers.

In view of these results and of others that cannot be mentioned in a brief report, there can be no doubt that the diffusion potential hypothesis must be discarded. The membrane potential is not referable to the existence of differences in the concentration of potassium inside and outside the nerve fibers; the membrane potential is directly established by oxidative metabolism.

The rôle played by potassium in nerve function is a matter for conjecture. The abundance of potassium inside the nerve fibers and the depolarizing action of an extracellular excess of K^+ ions decidedly suggest that potassium plays an important rôle; but however important this rôle may be, it is subordinate to that of oxidative metabolism.

Many assumptions could be made to account for the existence of a large amount of potassium inside the nerve fibers; among them there is one which is exceedingly interesting. In his Harvey Lecture Hastings ('40) described a drastic experiment to emphasize the importance of the intracellular ionic environment for the normal activity of intracellular enzymes. Making use or rather abuse of this concept, let us assume that potassium is present inside the nerve fibers at a high concentration simply because this concentration creates optimal conditions for the activity of the enzymatic systems of the nerve fibers. To be sure, an assumption like this is so exceedingly radical that it has little positive value. Nevertheless, the assumption is useful since consideration of it helps to discard from one's mind the diffusion potential hypothesis.

An often forgotten experimental fact can also be mentioned in order to emphasize that no greater importance for nerve function should be attached to potassium than to any other constituent of the nerve fiber. A frog nerve maintained in potassium-free Ringer's solution remains excitable for the same length of time

as a companion nerve maintained in Ringer's solution with the normal amount of potassium (usually 36 hours at 20° C.); a nerve, however, which is maintained in a sodium-free medium becomes inexcitable after 10–12 hours and regains its excitability when and only when Na^+ ions are made available to it (Overton, '02; Lorente de Nó, '44). If this experiment should be considered in isolation it would have to be concluded that for nerve function the most important ions are Na^+ ions.

When it comes to explain how oxidative metabolism establishes the membrane potential one finds that at the present state of knowledge, a detailed hypothesis cannot and should not be formulated. From the study of electrotonic potentials and action potentials, however, a general view is obtained that can serve (1) as a tool to integrate into a body of knowledge experimental observations of great diversity and (2) as a working hypothesis to predict the occurrence of phenomena which can be submitted to experimental analysis. Although the logical procedure would be to present first the evidence and then the working hypothesis, it will be simpler to describe first the hypothesis and then use it to discuss experimental results.

An important fact is that the membrane potential consists of several fractions; for the purpose of this report, however, it will be sufficient to consider only the two main fractions Q (quick) and L (labile). This division is justified by a number of experimental reasons among which the following is important. During the passage of the nerve impulse the membrane potential collapses. A part of the potential is restored during the descending limb of the spike while another smaller part is restored during the period of the negative after-potential. That part of the membrane potential which is restored during the descending limb of the spike is the Q fraction, and the other part which is restored during the negative after-potential is the L fraction. The division thus made is not entirely accurate since a small deficit of Q fraction remains after the end of the spike and a small part of L fraction is restored during the descending limb of the spike; fine details, however, need not be taken into account at this time.

Since the membrane potential is not a diffusion potential, it must be maintained at static double layers; a conclusion which is in full agreement with the results of polarization experiments

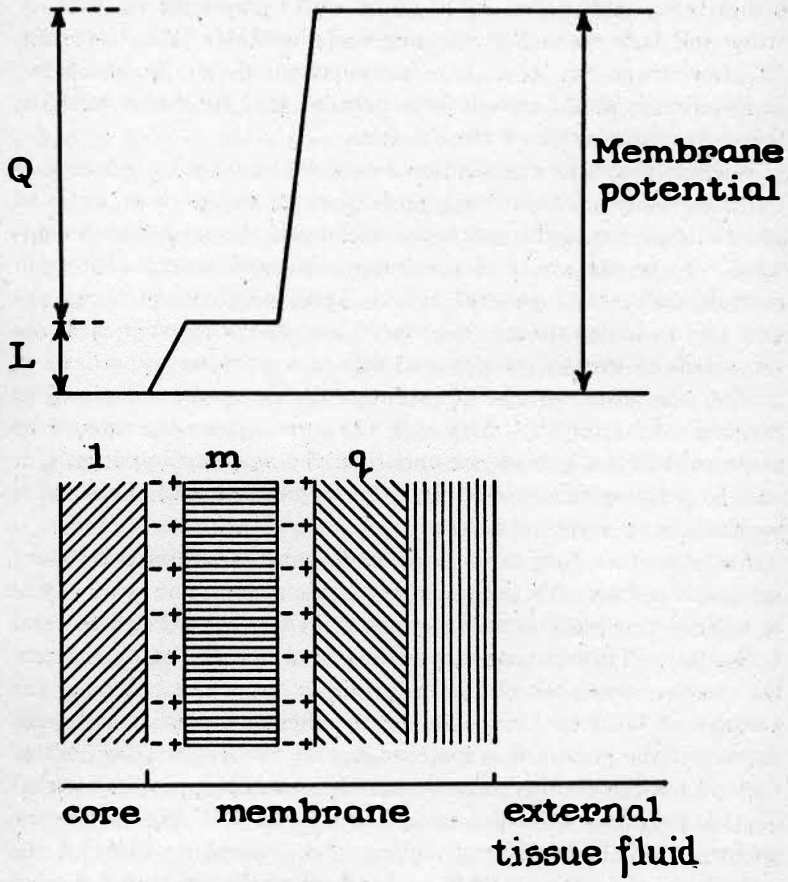


FIG. 8. Diagrams illustrating the working hypothesis on the nature of the membrane potential.

(see later). In view of the fact that the membrane potential consists of two fractions, it may be postulated that the membrane includes two boundaries (fig. 8). The Q fraction is maintained

at a double layer at the boundary between the q and m phases and the L fraction at the boundary between the m and l phases. There are experimental facts to justify the assumption that the l phase is the internal one, it is perhaps the core itself.

In an important respect the differences of electrostatic potential Q and L which with resting nerve exist across the double layers represent equilibrium potentials. Since the charged particles of a double layer are under the influence of electrostatic (Coulomb) forces of attraction, the double layer could not exist unless non-Coulomb (chemical) forces should also exist at the boundary and should tend to separate charged particles of the opposite sign. Therefore, the value of the potential difference existing across a double layer is determined by the equilibrium of the Coulomb and non-Coulomb forces that act upon the electrically charged particles (*cf.* Helmholtz, 1847, 1853).

This situation can also be described in another manner. The transfer of a positively charged particle x and of a negatively charged particle y, for example, across the l-m boundary is a chemical reaction $lx, my = ly, mx$. Since the L double layer consists of x particles in the m phase and y particles in the l phase, it is clear that when this reaction proceeds in the direction $lx, my \rightarrow ly, mx$, the free energy of the system decreases. For this reason we may say that non-Coulomb or chemical forces act upon the particles x and y. On the other hand, the transfer of the charged particles across the boundary requires that a certain amount of work be done against the electric forces. If the decrease of free energy is greater than the electric work, the transfer of particles will take place in the indicated direction; if the decrease of free energy is smaller than the electric work, the transfer of particles will take place in the opposite direction; the equilibrium condition will be reached when the electric work and the decrease of free energy are equal.

It is thus clear that the resting or equilibrium value of the L fraction of the membrane potential is determined by the chemical reaction $lx, my \leftrightharpoons ly, mx$. On the other hand, a change in the value of the L fraction will drive the chemical reaction in one

direction or the other. In particular, if a short circuit is established between the external tissue fluid and the core so that a demarcation current can flow, the L potential will decrease since charged particles will be carried away from the double layer by the current; the double layer would rapidly disappear were it not that the decrease of the L potential allows the boundary reaction to proceed in the direction $lx, my \rightarrow ly, mx$ so as to replace those charged particles which are carried away by the current. In view of this situation we may say that the non-Coulomb forces constitute the E.M.F. of the membrane. Similar considerations apply, of course, to the Q fraction of the membrane potential.

The rôle played by oxidative metabolism can now be easily described. The chain of reactions of the oxidative metabolism determines the chemical composition of the l, m and q phases and therefore creates the E.M.F. of the membrane. If the nerve is deprived of oxygen, the E.M.F. of the membrane decreases with the result that the double layers collapse. Postanoxic respiration reestablishes the E.M.F. of the membrane and therefore charged particles of the opposite sign are again separated into double layers. Since during the postanoxic repolarization the charged particles need be displaced only through distances of the order of magnitude of ionic radii, it is quite understandable that the repolarization can take place in a few minutes; it also is understandable that postanoxic repolarization can occur in the presence of a wide range of concentrations of potassium ions.

In the following discussion of electrotonic potentials the properties of the L fraction of the membrane potential will be analyzed first. Since the fluctuations of the value of the Q fraction take place at a much greater speed than the fluctuations of the L fraction, the fluctuations of the Q fraction cannot be photographed at the low sweep speed which is convenient for the study of the L fraction; they are included in the discontinuities of the records at the make and at the break of the applied current.

c. *Electrotonus and After-potentials.*—Ether anesthesia causes important changes in the electrotonus (Biedermann, '96, p. 694). In figure 9 the pairs of records *1* and *2* reproduce electrotonic

potentials in normal nerve, while the other pairs of records (*3* to *12*) reproduce the electrotonic potentials observed at the indicated intervals of time after the introduction of ether vapor into

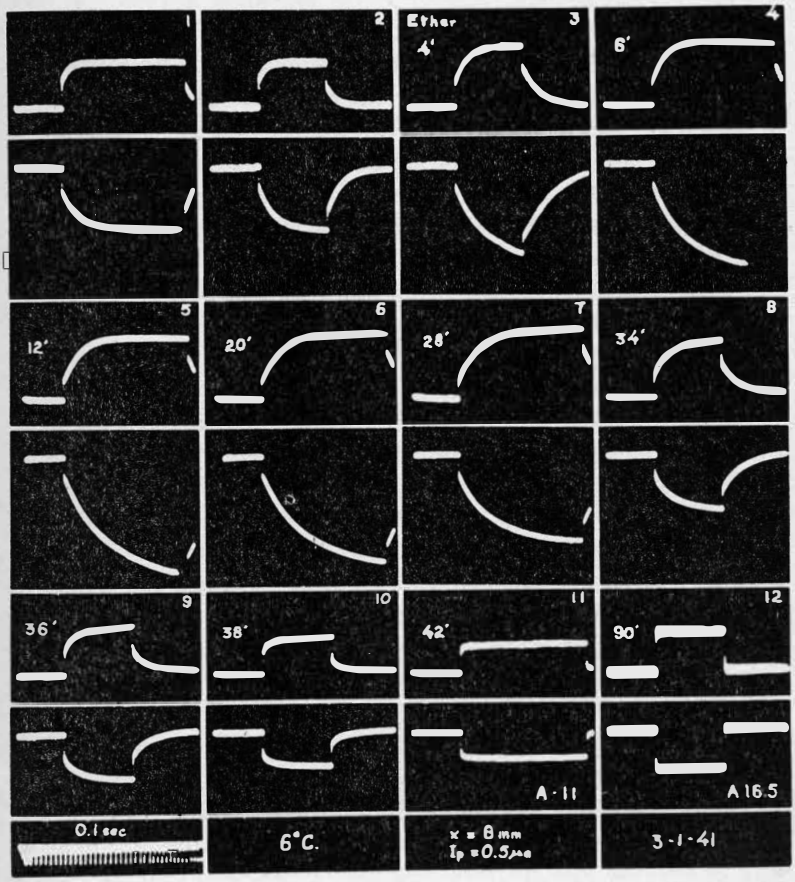


FIG. 9. Effect of ether anesthesia upon the electrotonic potential of bullfrog sciatic nerve. *A 11, A 16.5,* amplifications measured in millimeters of the original reproduction per millivolt input.

the nerve chamber. The partial pressure of ether was low during the first part of the experiment; it was increased in the interval between records *6* and *7*.

It will be noted in records *1* and *2* that the electrotonic potential displayed two components of widely different temporal course: (1) a fast component that appeared as discontinuities of the records at the make and at the break of the applied current and (2) a slow component that had a remarkably low rate of establishment and decay. The fast component of the electrotonus was modified relatively little by ether anesthesia; the slow component, however, underwent spectacular changes.

During the initial phase of the anesthesia (records *3* to *5*) the height of the slow component increased and the asymmetry of catelectrotonus and anelectrotonus became very pronounced (records *5*). During the second phase (records *6, 7*) the anelectrotonus decreased markedly while the catelectrotonus remained almost unchanged; consequently, the two potentials became symmetrical (records *7*). During the third phase (records *8* to *12*) the slow electrotonus decreased continuously; ultimately the electrotonic potentials displayed practically only fast components (records *11, 12,* note the higher amplification used for records *12*). At the slow sweep speed that was being used, the shape of records *12* was practically identical to the shape of records obtained by measuring the potential difference established by the applied current across an ohmic resistance which was included in the circuit in series with the nerve.

The effect of ether upon the electrotonic potentials is not specific; it is referable to the changes that ether causes in the value of the membrane potential. Initially ether increases the membrane potential, while later it causes a progressive depolarization. Any agent that increases the membrane potential causes an increase in the height of the electrotonic potential similar to that observed during the initial phase of ether anesthesia; likewise, any agent that causes depolarization of the membrane decreases the height of the slow electrotonus. In particular, it should be mentioned that electrotonic potentials of the type illustrated by records *11* and *12* of figure 9 also are observed with nerves that have undergone a far-reaching anoxic depolarization.

The difference in the behavior of the fast and the slow com-

ponents of the electrotonic potential clearly shows that the applied current establishes the two components by different mechanisms. Let it be assumed that the fast component which is recorded with depolarized nerve, measures the resistivity of the membrane.

The dependence of the slow electrotonus upon the value of the membrane potential suggests that the slow electrotonus is a polarization potential comparable to those which are measured by electrochemists at the electrodes of galvanic cells. Two main types of electrode polarization are considered by electrochemists, concentration polarization and chemical polarization or overpotential. The first mechanism can play only an insignificant rôle in the case of the slow component of the electrotonus since this component does not appear in nerves in the state of ether anesthesia nor in nerves that are conducting trains of impulses at frequencies above 40–50 per second (fig. 11, *6*). Therefore the slow electrotonus must belong to the group of overpotentials. As a matter of fact, there is evidence to show that the slow electrotonus consists in fluctuations of the L fraction of the membrane potential.

In the experiment illustrated by figure 10, a comparison was made at two different temperatures of (1) the electrotonic potentials produced by 30-second pulses of current, cathodal (electrode p_1, cathode) in the case of records *1, 2* and *9, 10* and anodal (electrode p_1, anode) in the case of records *3, 4* and *11, 12,* and (2) the after-potentials produced by conduction of 30-second trains of impulses at the frequency of approximately 100 per second (records *6, 7, 14, 15*).

If records *1, 2* are compared with records *6, 7* it will be found that the tracing of the catelectrotonus during the flow of the applied current and the negative after-potential during the tetanus, i.e., the height of the white band in records *6, 7,* had the same temporal course; a lowering of the temperature to 27° C. modified both the catelectrotonus (records *9, 10*) and the negative after-potential (records *14, 15*); the change, however, was the same in the two instances.

After the end of the tetani the negative after-potential was replaced by the positive after-potential (records *7, 15*), i.e., by a hyperpolarization of the membrane; similarly, after the end of the applied currents the slow electrotonus reversed its sign (records *2, 10*). Thus, in the two instances the depolarization of

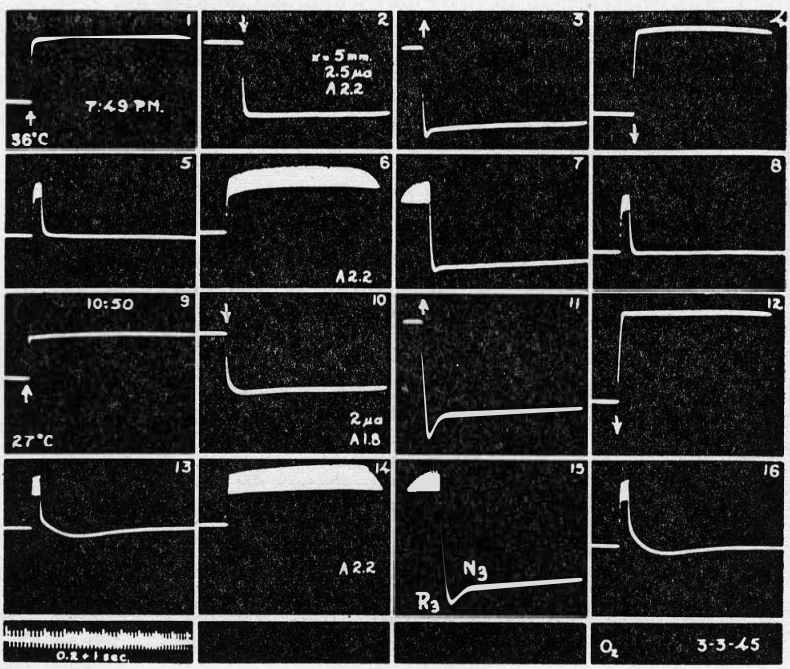


FIG. 10. Comparison of electrotonic potentials and after-potentials at 36° and at 27° C.

the membrane initiated a process that ultimately resulted in a hyperpolarization.

Although at the two temperatures the post-cathodal overshooting and the positive after-potential had similar temporal courses, the deflections in records *2, 7* and *10, 15* were not exactly identical. By properly choosing the magnitude and duration of the applied current and the duration of the tetanus it would have

been possible to produce identical post-cathodal overshootings and positive after-potentials. In this experiment, however, the conditions were chosen so that the temporal course of the positive after-potential would be reproduced by the slow electrotonus created by the anodal current. The experiment was successful, since the anelectrotonus (records *3, 11*) duplicated the positive

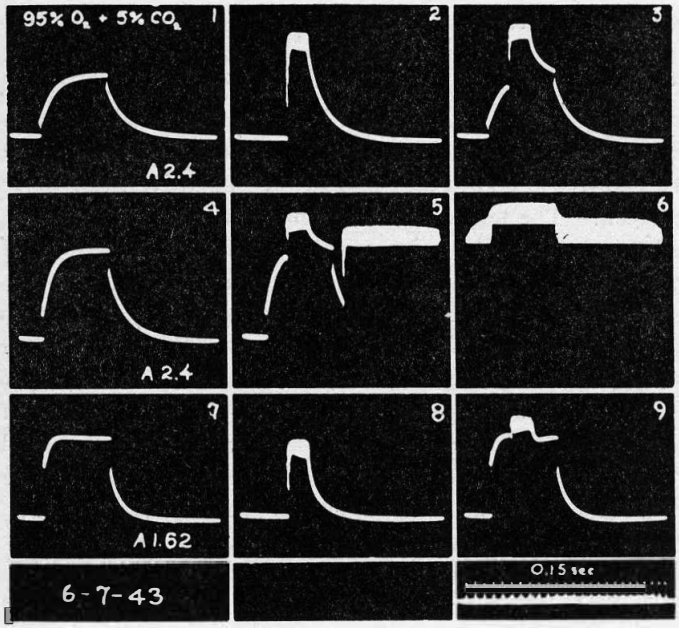


FIG. 11. Effects of the superposition of pulses of cathodal current and of rhythmic trains of impulses.

after-potential (records *7, 15*) at the two temperatures. Thus, we can reach an exceedingly important conclusion which also is in agreement with the results of a number of other experiments. The flow through the membrane of an applied anodal current produces changes in the membrane potential identical with those which are produced by the metabolic mechanisms of the nerve fibers during the recovery of the loss of membrane potential created by conduction of a train of impulses.

A convenient procedure to analyze the relationship of the slow catelectrotonus to the negative after-potential is to superpose the effects of pulses of applied current and rhythmic trains of impulses. In figure 11 record *1* presents the catelectrotonus produced by a rectangular pulse of current and record *2*, the negative after-potential produced by a brief tetanus. Record *3* presents the result of superposing the tetanus upon the applied current; as can readily be noted, the sum of slow catelectrotonus and negative after-potential in record *3* is equal to the negative after-potential in record *2*. Record *5* presents the result of superposing the tetanus of record *2* upon the catelectrotonus of record *4;* again the sum of slow catelectrotonus and negative after-potential in record *5* is equal to the negative after-potential in record *2*. The tetanus was recommenced during the sweep of record *5* and was continued through the sweep of record *6*. During the sweep of record *6* the rectangular pulse of current was superposed upon the tetanus; it produced only a rectangular deflection, the height of which was that of the fast component in record *4*. Thus, it can be concluded that the slow electrotonus and the negative after-potential represent the same change in the value of the membrane potential. The limiting value of both slow catelectrotonus and negative after-potential is that of the *L* fraction. This fact is shown again in records *7* to *9*. The current used to obtain record *9* was slightly greater than the rheobase of the nerve; since a current of this magnitude reduces the *L* fraction of the membrane potential to a very small value, the tetanus of record *8* produced only a very small negative after-potential when it was superposed upon the applied current (record *9*).

d. *The Nerve Reaction.*—In view of the fact that the slow electrotonus consists in changes in the value of the *L* fraction of the membrane potential, the mechanism of its production becomes readily understandable. It is clear that if the ions that carry the current were transported across the *L* double layer at the rate at which they reach the *l–m* boundary, the strength of the double layer and consequently the value of the *L* fraction would remain

constant during the flow of the applied current. Therefore, the slow electrotonus is a polarization potential of the same nature as the overpotential of electrodes (for modern theory of the overpotential *cf.* Gurney, '31, Butler, '40, Glasstone, Laidler, Eyring, '41). It is produced because the transport of ions across the *L* double layer is a relatively slow process and consequently the flow of the current is accompanied by changes in the strength of the double layer. An important consequence of this proposition is that the slow electrotonus is established without measurable changes in the concentration of ionized solutes; its establishment requires only a change in the number of charged particles in the two layers of the double layer and this change is far too small to be detected by the methods of analytical chemistry.

At the present state of knowledge no detailed assumption may be made regarding the process of transport of the current across the *L* double layer. Certain general features of the process, however, are understandable. With resting nerve the value of the *L* potential determines the equilibrium point of a chemical reaction at the *l–m* boundary insofar as the decrease of free energy that would result from the combination of charged particles of the *l* and *m* phases with constituents of the *m* and *l* phases respectively, is equal to the work that would be done in transporting those particles across the electrostatic potential difference existing at the boundary. When the slow electrotonus is established, i.e., when the value of the *L* fraction is changed, equilibrium ceases to exist at the *l–m* boundary so that the chemical reaction will progress in one or the other direction according to whether the slow electrotonus represents a decrease or an increase of the *L* potential. This situation is essentially identical to that which arises when the difference of potential between an electrode and a solution of its ions is changed. If the potential difference is changed in one direction the metallic cores go into solution as ions; if the electrode potential is changed in the opposite direction, the ions of the solution are deposited on the electrode.

Certain important details of the relationship of the electrotonus

to the magnitude of the applied current are illustrated by the series of records of figure 12. The magnitude of the current was varied from 0.03 μa (records *1* and *2*) to 3 μa (records *11* and

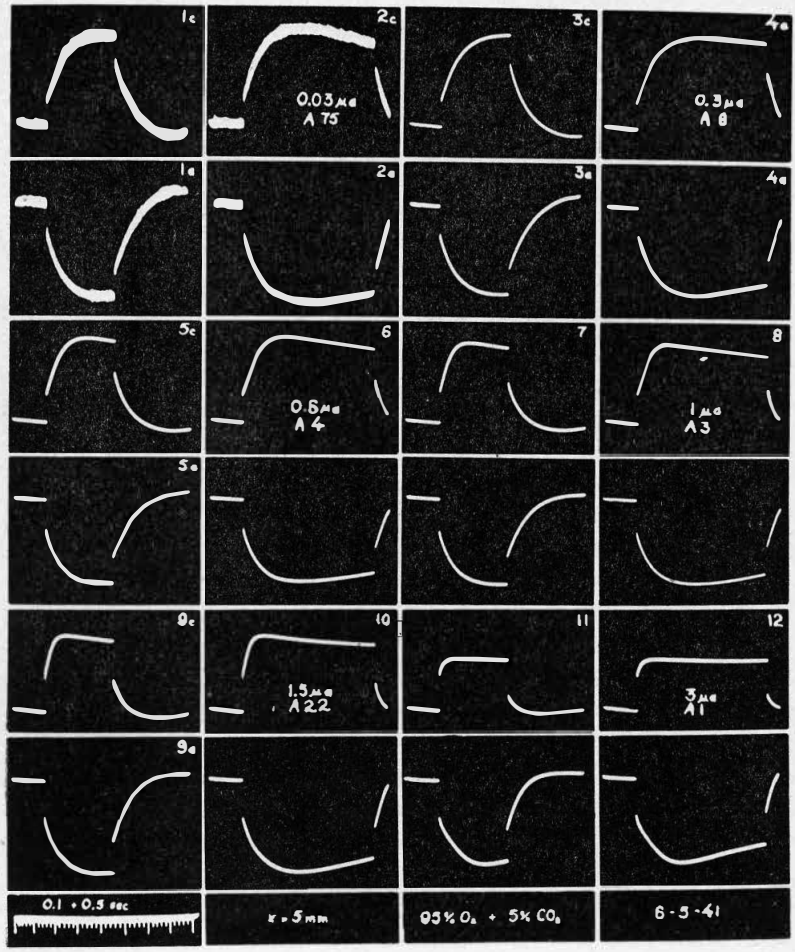


FIG. 12. Electrotonic potentials produced at 5 mm. from electrode p_1 by rectangular pulses of current of the indicated magnitude. Amplifications given in multiples of the amplification used for records 12.

12). The latter current still was subliminal for a large majority of the fibers of the nerve; on the other hand, when the applied current was decreased to 0.01 μa and the amplification was increased, the electrotonic potentials still displayed essentially the temporal course that appears in records *1*. The electrotonic potentials produced by smaller currents were of the order of magnitude of the tube noise of the amplifier; therefore, the series of records of figure 12 covers the whole practical range of subliminal currents. Pulses of current of two different durations were used: short pulses in order to observe the overshooting after the end of the applied current (records of the first and third columns) and long pulses in order to observe the maximum of the potential during the flow of the applied current (records of the second and fourth columns).

If in examining figure 12 the amplification factors are taken into account it will be found that the fast component of the electrotonus was approximately proportional to the applied current while the relationship of the slow electrotonus to the applied current was markedly non-linear, with the noteworthy peculiarity that with small currents the catelectrotonus was higher than the anelectrotonus while with large currents the anelectrotonus was higher than the catelectrotonus. There still are in figure 12 a number of details that deserve consideration; within the frame of this report, however, emphasis can be placed only upon these two, (1) in all cases the slow electrotonus passed through a maximum during the flow of the applied current and (2) in all cases the slow electrotonus reversed its sign after the end of the applied current.

Since the nerve fibers are core conductors, the theoretical analysis of the potential changes recorded at one point of the nerve offers considerable difficulties; the problem, however, becomes simple after the instantaneous distribution of the electrotonic potential along the nerve has been ascertained. The technical procedure is not difficult, one determines experimentally the electrotonic potential at a number of points of the nerve and the experimental records are used to prepare families of curves such as those reproduced in figure 13.

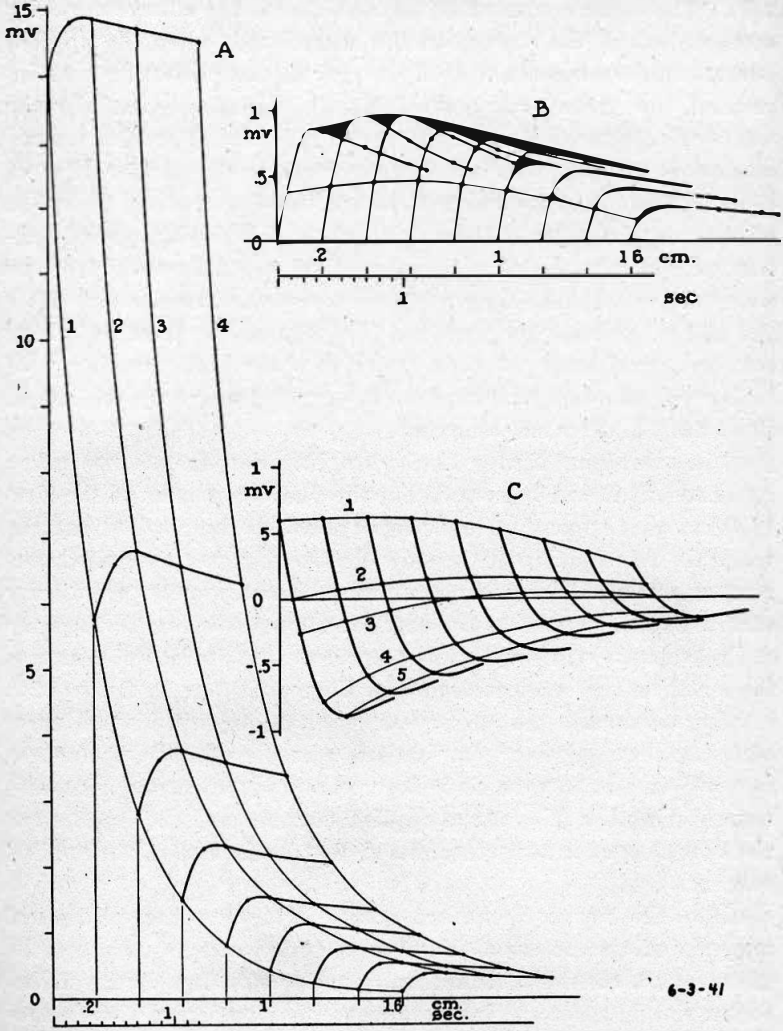


FIG. 13. Plots of the temporal course and of the longitudinal distribution of the electrotonic potential in the extrapolar segment of the nerve. *A*, *B*, electrotonic potential during the flow of the applied current; *C*, electrotonic potential after the end of the polarization. Curves *A* measure the total potential; curves *B* and *C* only the slow components.

Family of curves *A* figure 13 gives both the temporal course and the longitudinal distribution of the electrotonic potential during the flow of the current and family *C* the temporal course and longitudinal distribution of the slow electrotonus after the end of the applied current. Family *A* shows the remarkable fact that the electronic potential passed through its maximum almost at the same time at all points of a 16-millimeter segment of nerve. In addition, since the slope of the longitudinal distributions *1* to *4* is proportional to the current in the longitudinal conductors, it is clear that the decrease of the electrotonic potential below its maximum was accompanied only by negligible changes in the flow of longitudinal current.

Similar phenomena are illustrated by family of curves *C*. Since the slope of the longitudinal plots (*1* to *5*) is negligible, there can be no doubt that (1) the decrease of the slow catelectrotonus and the reversal of its sign took place almost simultaneously at all points of a long segment of the nerve and (2) the overshooting of the electrotonic potential occurred in the absence of a significant flow of longitudinal current. Under conditions such as these the only possible explanation of the overshooting is the following. At the end of the applied current the E.M.F. of the membrane had a greater value than in resting nerve, so that in the absence of the applied current the membrane potential increased beyond the value of the resting membrane potential. Later both the membrane E.M.F. and the membrane potential returned gradually to the normal value.

The fact that the E.M.F. of the membrane increased during the flow of the applied current also explains the production of the maximum of the electronic potential. On the one hand, the increase of the E.M.F. of the membrane resulted in the decrease of the electrotonic potential below its maximum and on the other, the increase of the E.M.F. of the membrane resulted in the establishment of a new condition at the polarizable boundary so that the electrotonic potential remained at a steady level during the further flow of the applied current. In the case of the anelectrotonus, the maximum and the overshooting of the electrotonic potential are referable to a decrease of the E.M.F. of the membrane.

Thus, analysis of the electrotonic potential reveals an exceedingly important fact. The nerve fiber has the ability of regulating the E.M.F. of its membrane in response to and to oppose impressed changes of its membrane potential. This process may be called the "nerve reaction." The action of a cathodal current is to decrease the membrane potential and the corresponding reaction of the nerve fiber consists in an increase of the E.M.F. of the membrane; similarly, the action of an anodal current is to increase the membrane potential and the corresponding reaction of the nerve fiber consists in a decrease of the E.M.F. of the membrane. In both cases the reaction tends to oppose the effect of the applied current so as to prevent a change in the value of the membrane potential. In view of the records presented in figure 12, it may be stated that the nerve reaction is a process that has no threshold of initiation; it is elicited by the flow of any current, however small.

Since the value of the electrotonic potential is determined by an action-reaction interplay, it should be expected that, at least under given conditions, the slow electrotonus would display oscillatory behavior. In point of fact, decremental oscillations of the slow electrotonus are frequently observed. A condition that favors their appearance is a subnormal value of the resting membrane potential.

Records *1* to *4*, *6* and *9* of figure 14 present examples of oscillatory behavior of the postcathodal overshooting. In addition, they illustrate the important fact that the duration of the applied current plays the rôle of an independent variable in the determination of the magnitude and temporal course of the overshooting. Records *11* and *12* present the behavior of the slow electrotonus during the flow of the anodal current. The similarity between records *6* and *11* is in agreement with the general principle according to which the processes established by the metabolic mechanisms in order to restore losses of membrane potential can be duplicated by applying to the nerve an anodal current. Finally, if record *7* is examined with some attention it will be found that during the flow of the cathodal current, the catelectro-

tonus passed through a series of oscillations of smaller magnitude but of the same frequency as those of the anelectrotonus (record 11).

As should be expected, when the postcathodal overshooting displays oscillatory behavior the positive after-potential also has an oscillatory course.

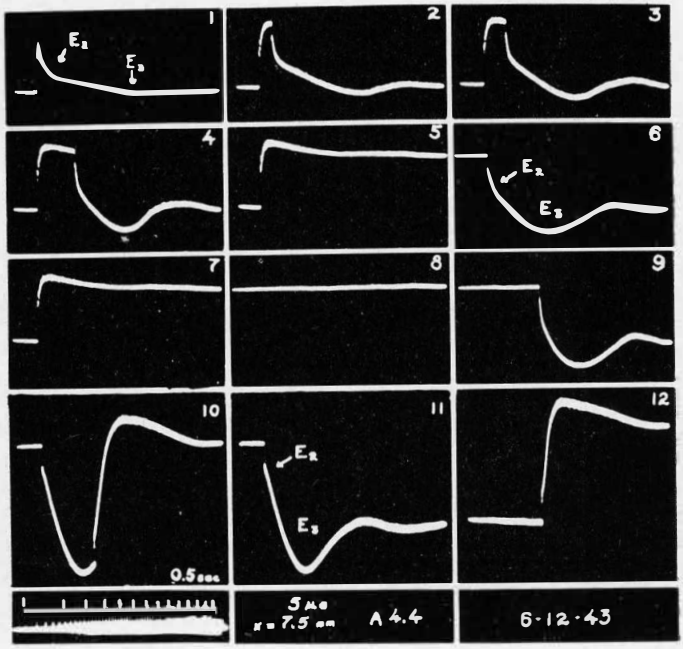


FIG. 14. Illustration of oscillatory behavior of the slow electrotonus.

e. *Slow Electrotonus and Excitability.*—An important feature of the slow electrotonus is that its fluctuations are accompanied by changes in the excitability of the nerve fibers. The relationship between the value of the *L* fraction of the membrane potential and the excitability of the nerve is so intimate that it can be defined in the following manner. Any change in the value of the *L* fraction causes a change in the excitability of the nerve

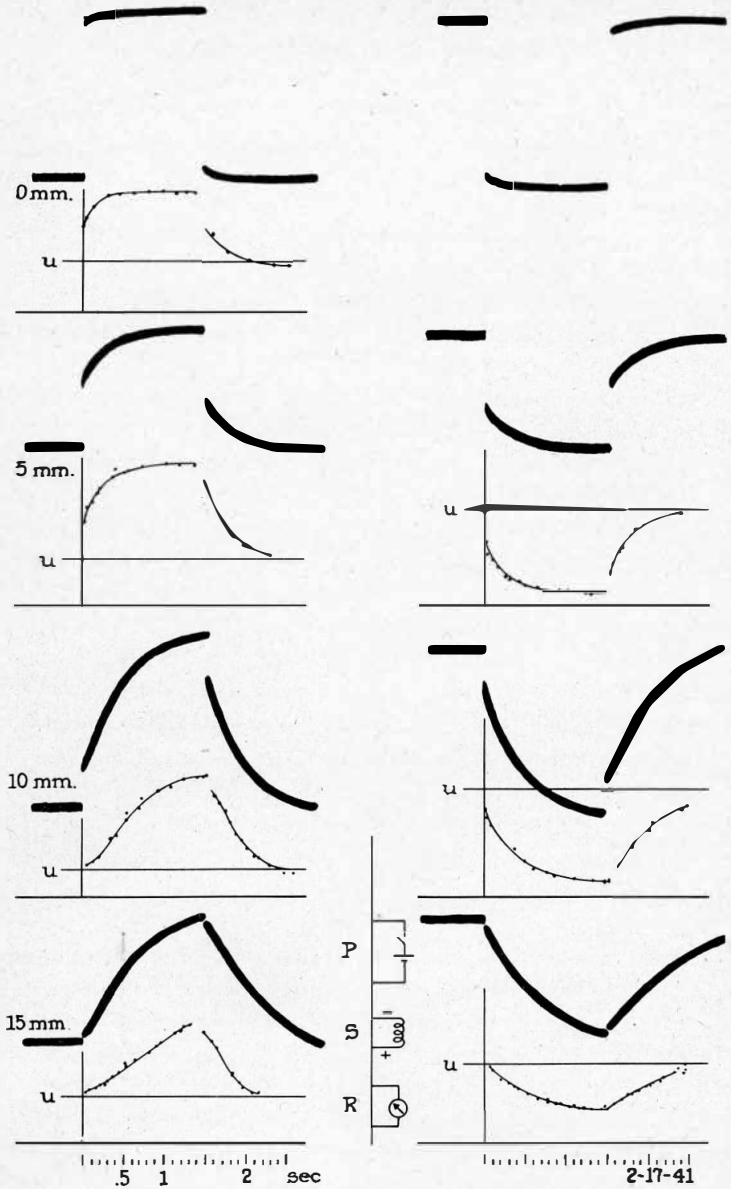


FIG. 15. Illustration of the relationship of the excitability of the nerve to the slow electrotonus.

fibers and conversely a change in the excitability of the nerve fibers, which takes place at the rate of the fluctuations of the slow electrotonus, may be taken as a sign that the value of the L fraction of the membrane potential is undergoing a change.

The curves and the tracings of records of electrotonic potentials reproduced in figure 15 illustrate the relationship between the excitability, i.e., the reciprocal of the stimulation threshold and the changes in the value of the L fraction of the membrane potential. The observations were made with the arrangement of electrodes indicated in the diagram, the distances between the polarizing electrode (p_1) and the cathode of the stimulating circuit (s_1) being those which are indicated on the left side of figure 15. In each case the electrotonic potentials were recorded at the stimulating cathode. As can readily be noted, the excitability curves faithfully paralleled the tracings of the slow electrotonus.

The relationship illustrated by figure 15 is generally true for nerves under ordinary experimental conditions, i.e., for nerves that have a membrane potential in the neighborhood of the normal value and are polarized at room temperature with applied currents of magnitude not exceeding more than a few times the rheobase of the nerve and of duration not exceeding a few seconds. Under these conditions a decrease in the value of the L fraction of the membrane potential causes a decrease in the threshold of stimulation regardless of whether the L fraction has been decreased by an applied cathodal current or by the operation of the E_3 reaction after the end of an applied anodal current (post-anodal overshooting). Conversely, an increase in the L fraction of the membrane potential causes an increase in the threshold of stimulation regardless of whether the L fraction has been increased by an applied anodal current or by the operation of the E_3 reaction after the end of an applied cathodal current (post-cathodal overshooting).

The relationship between the slow electrotonus and the excitability of the nerve fibers makes it possible to express Pflüger's well known rule in terms of directly measurable quantities.

Pflüger's rule is this, "The establishment of the catelectrotonus and the disappearance of the anelectrotonus increase the excitability while the establishment of the anelectrotonus and the disappearance of the catelectrotonus decrease the excitability of the nerve fibers." In Pflüger's formulation the terms catelectrotonus and anelectrotonus denoted states of the nerve which heretofore could not be directly related to the value of the membrane potential. At present, insofar as the slow electrotonus is concerned, the situation can be accurately described in this manner: catelectrotonus is a state in which the *L* fraction of the membrane potential has a lower value than in resting nerve and anelectrotonus, a state in which the *L* fraction has a higher value than in resting nerve. In reference to the excitability of resting nerve, the excitability is increased in the catelectrotonic state and decreased in the anelectrotonic. This formulation would not have to be modified if catelectrotonus and anelectrotonus should be defined in reference to the resting blood-perfused nerve.

A rule has recently been given by Gasser ('37) to relate the excitability of the nerve fibers during the recovery after conduction of the impulses to the sign of the after-potential. "The excitability of the nerve fibers is increased during the negative after-potential and decreased during the positive after-potential." An illustration of this relationship is presented in figure 16. Record *1* gives the height of the unconditioned testing spike. The nerve was submitted to continuous tetanic stimulation that was interrupted 3 seconds before the start of the sweep of record *16*. In addition, in order to test the excitability of the nerve fibers, the stimulation was interrupted shortly before or during each one of the sweeps of records *3* to *15*. Since all the records of figure 16 have the zero potential level in common, the differences between the heights of the base line of record *1* and of the base lines of the other records measure increments and decrements in the membrane potential. As can readily be noted, the excitability of the nerve was increased and consequently the testing spike was higher than in record *1* whenever the testing stimulus was applied during the negative after-potential, while the excitability was decreased during the positive after-potential.

A priori, one is inclined to think that Pflüger's and Gasser's rules apply to entirely different situations since Pflüger's rule describes changes in excitability produced by applied currents which may be subliminal while Gasser's rule describes changes in

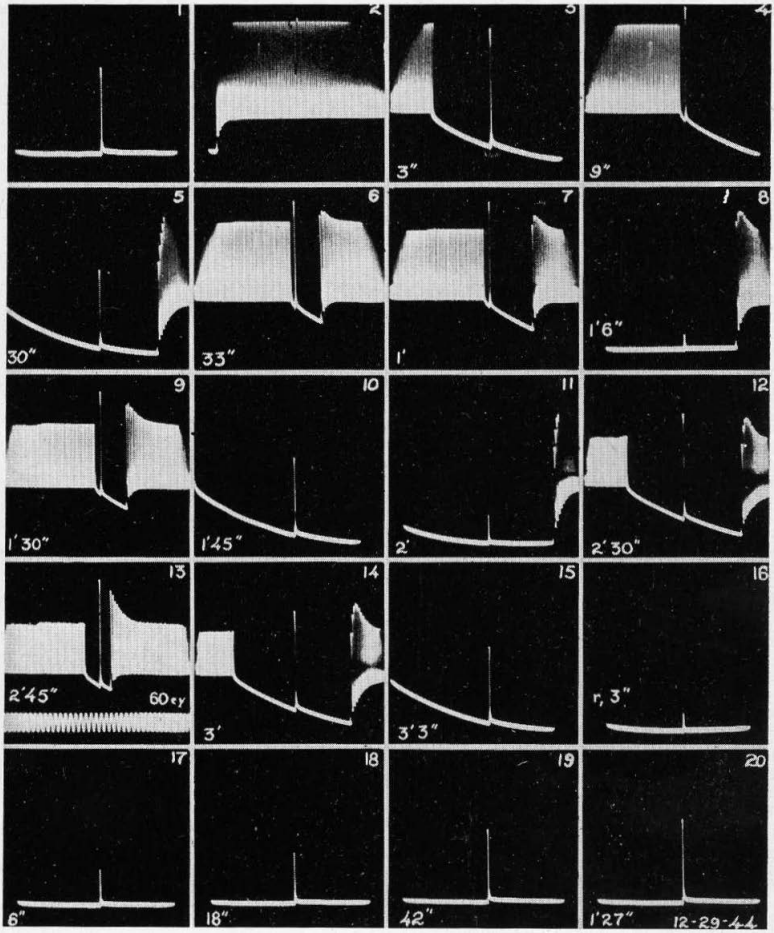


FIG. 16. Illustration of the relationship of the excitability of the nerve to the after-potentials in a nerve in which the *L* fraction of the membrane potential had been increased by the introduction of 5% CO_2 in the atmosphere.

excitability occurring during the recovery after conduction of impulses. However, if it is taken into account that under certain conditions the major part of the after-potentials consist of a change in the value of the *L* fraction of the membrane potential,

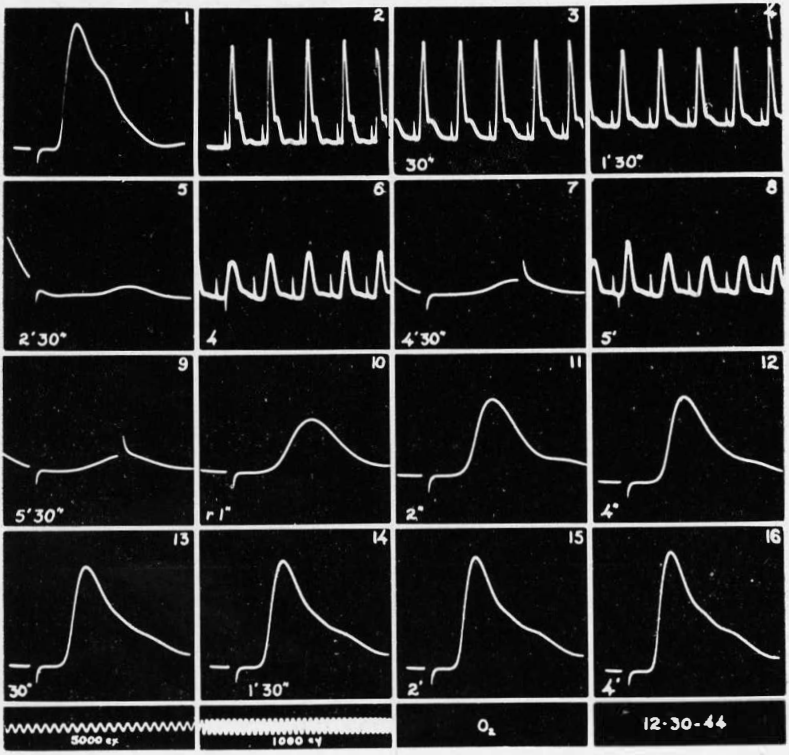


FIG. 17. Development of the state of cathodal depression by long lasting rhythmic conduction of impulses.

it becomes clear that under those conditions the excitability changes during the period of after-potentials must resemble the changes which accompany the slow electrotonus produced by subliminal currents. Therefore the contents of the rules of Pflüger and Gasser are essentially identical.

It should be emphasized that Pflüger's rule holds true when it is applied to changes in the membrane potential of nerves that are at or near to the normal state. There are, however, two important conditions under which the rule ceases to apply. If the nerve has been depolarized to such an extent that its excitability is depressed, the anelectrotonus will produce an increase of the excitability. Examples of this situation will be mentioned later. On the other hand, if with a nerve in the normal state, the applied cathodal current produces a catelectrotonus which includes a deficit of the *Q* fraction exceeding a certain limit, the catelectrotonus will cause a decrease of the excitability. This phenomenon was called in the classical literature "Werigo's cathodal depression."

The passage of an impulse leaves a deficit of *Q* fraction which is sufficient to produce cathodal depression or as it is usually called, relative refractoriness to stimulation. Under the experimental conditions that are used most frequently, the recovery of the deficit of *Q* fraction takes place quite rapidly so that the relatively refractory period is short but if the nerves are submitted to long-lasting tetanic stimulation, the cumulative loss of *Q* fraction may bring the nerve into a state of severe depression which may properly be called "fatigue." An example of this situation is presented in figure 17. Record *1* presents the spike elicited in the resting nerve by a supramaximal A shock and records *10* to *16*, the spikes elicited by the same shock at the indicated intervals of time after the end of a rhythmic stimulation that lasted for 5.5 minutes. Records *2* to *4*, *6* and *8* present the spikes of the conditioning tetanus which were initiated by shocks delivered through a second pair of stimulating electrodes. During the tetanus the fibers underwent a progressive loss of *Q* fraction, the extent of which is measured by the difference in the heights of the base lines of records *10* and *1;* the slow rate of decay of the negative after-potential (residual depolarization) can be observed by noticing the downward displacement of the base line in records *10* to *16*. Since in this case the negative after-potential consisted in a deficit of *Q* fraction, the excitability was

depressed as long as the negative after-potential was detectable. In addition, the spike height and the speed of conduction of impulses were subnormal.

The results of the experiment illustrated by figure 17 forcibly demonstrate that the interpretation of the excitability changes observed during the recovery after conduction of impulses must be made on the basis of this fact. The residual depolarization or negative after-potential left by the passage of nerve impulses includes two deficits, one of *Q* fraction and one of *L* fraction. A deficit of *L* fraction is always accompanied by a decrease of the excitation threshold while a deficit of *Q* fraction causes a decrease of the excitation threshold if it is small and an increase of threshold if it is large. The deficit of *Q* fraction left by the passage of an impulse always is sufficient to produce depression of excitability (relatively refractory period) but with nerves in which the *L* fraction is large, the effect of the deficit in *L* fraction may obscure the effect of the deficit in *Q* fraction. Indeed, the deficit in *L* fraction may be sufficient to lower the threshold below that of resting nerve.

Figure 18 illustrates the results of a reproducible experiment. All attempts to interpret these results met with failure until the work on the electrotonic potential had progressed so far that it became possible to postulate the existence of *Q* and *L* fractions in the membrane potential. Recovery curves *I* and *II* were obtained with the nerve in air immediately after excision, i.e., with the nerve in a state in which the *L* fraction of the membrane potential is very small and consequently the stimulation threshold is low. At this stage of the experiment the threshold of stimulation during the recovery was determined almost exclusively by the restoration of the deficit of *Q* fraction; therefore, no similarity existed between the recovery curve and the tracings of the after-potentials. After the nerve had been submitted to some experimentation the *L* fraction underwent a spontaneous increase so that when curves *III* and *IV* were obtained the effect of the deficit in the *L* fraction became demonstrable in the form of a shortening of the relatively refractory period. The atmosphere of the nerve was

then changed to 95% O_2 and 5% CO_2, a procedure that causes a large increment of the *L* fraction and therefore a large increase of the stimulation threshold. Under conditions such as these the threshold of stimulation during the recovery is determined mainly by the changes in the value of the *L* fraction, i.e., by the deficit

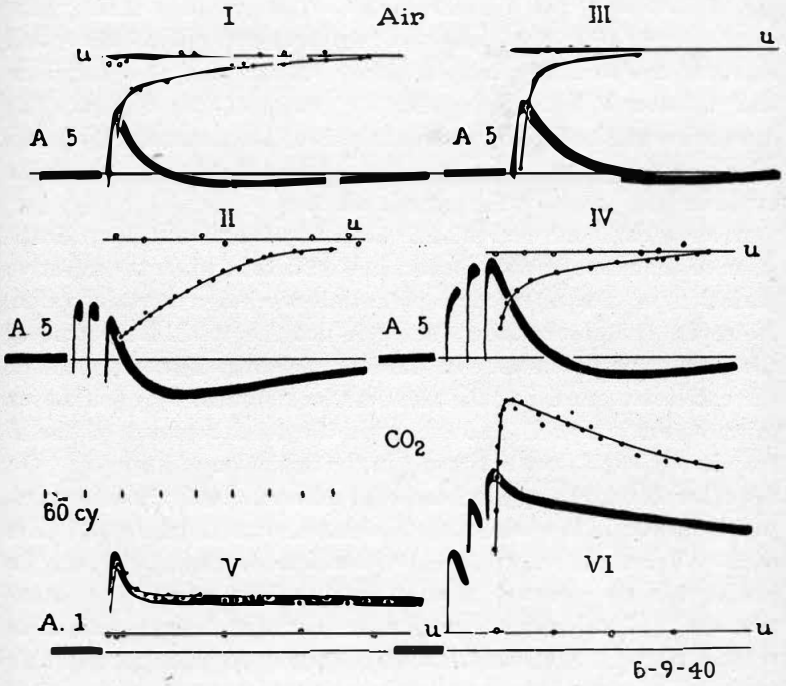


FIG. 18. Tracings of the after-potentials and curves of the recovery of excitability observed with the nerve in air (*I* to *IV*) and with the nerve in 95% O_2 and 5% CO_2 (*V*, *VI*).

of the *L* fraction during the negative after-potential and the excess of *L* fraction during the positive after-potential; consequently recovery curves *V* and *VI* paralleled the course of the negative after-potential. At the peak of curve *VI* the threshold of stimulation was approximately equal to the resting threshold at the time when curve *I* was obtained.

Between the extreme situations illustrated by curves *I, II* and *V, VI* of figure 18 there are a number of intermediate situations that can be created by judicious choice of the experimental conditions. A detailed discussion of the problem would overstep the frame of this report.

f. *Initiation of the Nerve Impulse.*—The problems that appear in the study of the initiation of the nerve impulse by applied currents are so many and of nature so varied that a complete analysis cannot be made within the frame of this report. The discussion will be limited to an analysis of the elementary process which initiates the nerve impulse. According to the available evidence this process is the same in all cases.

After a cathodal current has been applied to the nerve initiation of the nerve impulse occurs at the instant when the negative variation of the membrane potential reaches a certain value. However, the determining factor is not the total decrement of the membrane potential. In the first place the E_3 component of the catelectrotonus, i.e., the slow catelectrotonus, by itself never initiates an impulse. Indeed, even the total removal of the L fraction by itself does not result in the initiation of impulses. On the other hand, the applied cathodal current usually initiates impulses before it has been able to remove more than a small part of the L fraction and, under given conditions, the impulse may be initiated in the presence of an L fraction increased to the extent that the total value of the membrane potential is greater than in resting nerve. The initiation of the nerve impulse is referable to a component of the catelectrotonus which has a more rapid temporal course than the E_3 component; it will be called the E_1 component.

Figure 19 presents at two different sweep speeds the electrotonic potentials produced by rectangular pulses of cathodal current (cathode, electrode p_1) of progressively increasing duration. The pulse used to obtain records *13* and *14* was 2 seconds long. In all those records of figure 19 which were obtained with pulses of sufficient duration the electrotonic potential displayed a maximum during the flow of the applied current and an overshooting after

the end of the current. The maximum and the overshooting of record *6* of figure 19 are quite similar to the maximum and the overshooting in record *5c* of figure 12; in view of the fact, however, that the time scales used in the two instances were nearly in the ratio 500:1, it is obvious that the fluctuations of the

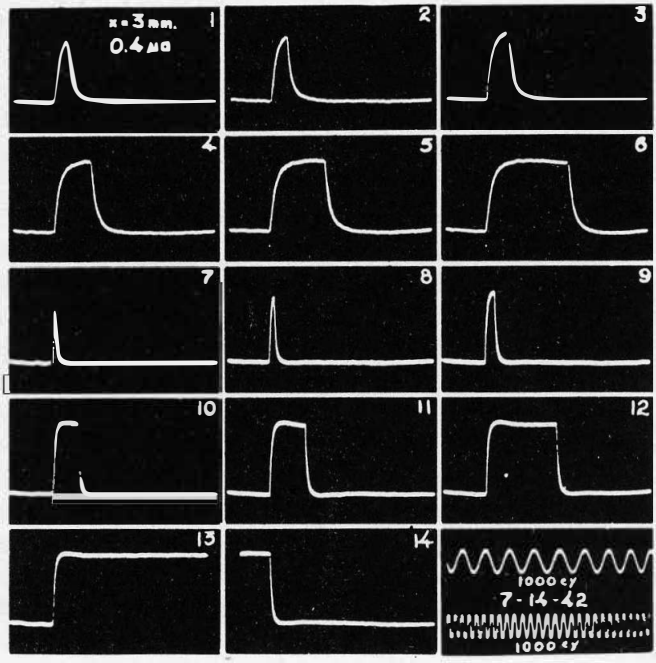


FIG. 19. Catelectrotonic potentials produced at 3 mm. from electrode p_1 by a subliminal current.

electrotonic potential must have been caused by different processes. The fluctuations that appear in the records of figure 19 were included in the discontinuities of the records of figure 12, i.e., in the "fast electrotonus."

In view of the existence of maxima and overshootings, it is clear that the fast electrotonus cannot be referable solely to the flow of current through layers of the membrane which have a low

conductivity. Since anesthesia, anoxia, lack of Na^+ ions, and a number of chemical agents cause the disappearance of the fluctuations of the fast electrotonus, it is reasonable to conclude that the fast electrotonus includes an F component that, loosely speaking, may be said to measure the resistivity of the membrane and an E_1 component which is a polarization potential comparable to the E_3 component or slow electrotonus. Unfortunately, no experimental procedure has been found that could lead to a satisfactory estimate of the relative heights of the F and E_1 components of the fast electrotonus. The evidence, however, leaves no doubt that the value of the E_1 component also is the result of an action-reaction interplay.

The applied current produces a polarization potential which is a change in the value of the Q fraction of the resting membrane potential. This change initiates a response, the E_1 reaction which results in a change of the E.M.F. that maintains the Q potential at the q–m double layer. The change in the E.M.F. is such as to oppose the change in the Q potential, that is to say, when an applied cathodal current decreases the Q potential, the E_1 reaction increases the E.M.F. of the membrane, while, when an applied anodal current increases the Q potential, the E_1 reaction decreases the E.M.F. of the membrane. In either case the nerve reaction removes part of the polarization potential created by the current and changes the conditions at the q–m boundary so that further flow of the current does not increase the polarization potential. In other words, the E_1 reaction is a process by means of which a dynamic equilibrium is established between the applied current and the nerve fiber. When the equilibrium is broken because the applied current is interrupted, the changed value of the E.M.F. of the membrane results in an overshooting of the electrotonic potential.

The significance of the E_1 reaction becomes apparent when it is observed that passage of nerve impulses initiates the same fluctuations of the Q fraction of the membrane potential as the flow of an applied current, therefore, the E_1 reaction which causes the overshooting after the end of a cathodal pulse is precisely

that process by means of which the activity of the metabolic mechanisms tends to restore the loss of Q potential left by the passage of the nerve impulse.[2] On the other hand, it can be shown that the flow of an applied current duplicates the effect of the activity of the metabolic mechanisms.

Figure 20 presents records of the spike of an alpha volley of impulses at three different sweep speeds and at two amplifications. Since the second recording electrode (r_2) was on a point of the nerve that had only been injured by sharp crushing, the record was the result of the superposition of the spike at electrode r_1

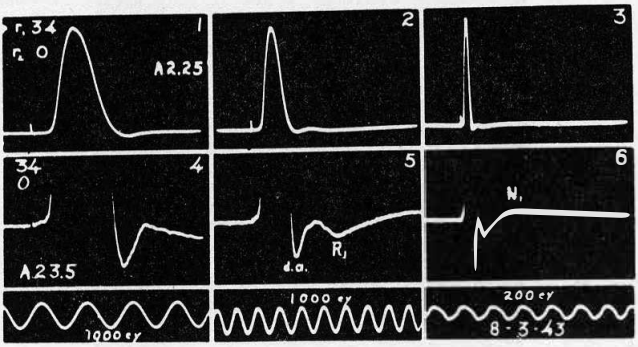


FIG. 20. Illustration of the diphasic artifact, *d.a.* and the E_1 deflection of the spike.

(point *34*) and a small change of potential at electrode r_2 (point *0*). This change produced that positive phase of the spike which is usually called the diphasic artifact (fig. 20, *5*, *d.a.*). The artifact, however, was followed by another positive deflection,

[2] The fact that the fluctuations in the value of the Q fraction take place with great rapidity seems to be in contradiction to the fact that a deficit of Q fraction may persist for a considerable period of time during which the nerve is in a state of depression (fig. 17). The explanation of the discrepancy is that the operation of the E_1 reaction is sufficient only to restore small losses of Q fraction, while the restoration of greater losses involves as a preliminary step the creation of an increment of the L fraction, a part of this increment being converted at a relatively slow rate into Q fraction. Presentation of the evidence that is available to support this conclusion cannot be made within the frame of a brief report.

R_1', that denoted a temporary increase of the Q fraction at point *34*. Ordinarily the R_1 deflection is submerged in the diphasic artifact. In this experiment the artifact and the R_1 deflection

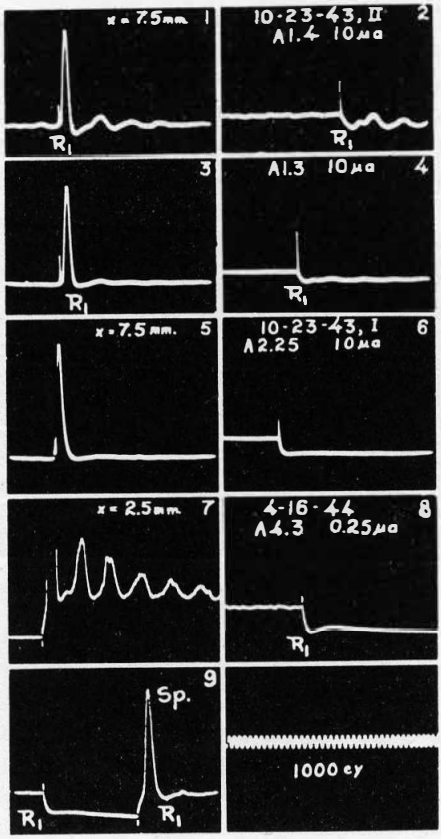


FIG. 21. Illustration of the similarity of the fluctuations of the membrane potential produced by the passage of a volley of impulses and by applied currents.

were separated by choosing the r_1r_2 distance so that the R_1 deflection would follow after the artifact. Another procedure that can be used to demonstrate the existence of the R_1 deflection is to

treat the end segment of the nerve with KCl so as to prevent changes in the potential of electrode r_2. Since thereby the appearance of the diphasic artifact is prevented, the R_1 deflection is recorded without distortion. This procedure was used to obtain the records of figure 21.

In the experiments illustrated by figure 21, *1* to *6*, a comparison was made of the R_1 deflection produced by the passage of the nerve impulse and the overshooting of the electrotonic potential after the end of applied cathodal currents. The state of the oxalate-treated nerves was changed by removing the oxygen from the atmosphere. The similarity between the behavior of the membrane potential after the spikes and after the end of the cathodal currents was striking. In the case of records *1* and *2* the R_1 deflection was followed by rhythmic fluctuations of the membrane potential accompanied by firing of impulses; lack of oxygen modified the R_1 deflection and the following fluctuation of potential in the same manner (records *3, 4*); finally, in a more advanced stage of anoxia, both the spike and the cathodal pulse of current were followed only by faint R_1 deflections (records *5, 6*).

Records *7* and *8* were obtained also with an oxalate-treated nerve with the use of a 2-second pulse of current. Record *7* illustrates an important phenomenon. The flow of the applied current initiated a rhythmic discharge of impulses, each spike starting immediately after the R_1 deflection of the preceding spike. The explanation of this phenomenon is the following. The R_1 deflection actually is the first half-wave of a decremental oscillation; often it is followed by a sharp negative crest, N_1 (fig. 20, *6*), and not rarely the R_1–N_1 sequence is followed by decremental oscillations, the intervals between successive negative crests being about 5 to 8 msec. at 20° C. If the membrane potential is low, impulses are initiated at or shortly before the N_1 crest, the stimulus for the initiation of the impulse being the variation of the Q fraction that occurs during the transition from the R_1 deflection to the N_1 crest. The rhythmic discharge is therefore self-maintained in so far as each impulse supplies

the stimulus for the initiation of the following one. The fundamental frequency of the discharge is that of the R_1–N_1 oscillation; slower rhythms, however, can also appear since modulation of the R_1–N_1 oscillations by slower potential fluctuations may result in the initiation of impulses at the second or third N_1 crest instead of at the first.

The rhythmic discharge ceased during the interval between records *7* and *8* so that record *8* presents only the R_1–N_1 sequence after the end of the cathodal pulse. R_1 deflections also are present in record *9* which was obtained by the use of a short pulse of anodal current. There is in record *9* an R_1 deflection immediately after the make of the anodal current and another R_1 deflection following after the spike that was initiated by the break of the current. Thus, record *9* illustrates two important facts: (1) the flow of an anodal current produces an E_1 fluctuation the sign of which is opposite to that of the E_1 fluctuation at the make of the cathodal current and equal to that of the E_1 fluctuation after the break of the cathodal current, and (2) the flow of an applied anodal current initiates that sequence of potential changes which occurs during the recovery of the deficit of Q potential left by the passage of the nerve impulse; i.e., the flow of the anodal current duplicates the effect of the activity of the metabolic mechanisms also in the case of the E_1 component of the electrotonus.

The E_1 fluctuations of the electrotonic potential are accompanied by large changes in the excitability of the nerve fibers. In the experiment illustrated by figure 22 a comparison was made of the electrotonic potentials and the excitability of the nerve fibers. Curves *Ic* and *IIc* present the excitability changes at the make (*Ic*) and after the break (*IIc*) of the cathodal current and curves *Ia* and *IIa*, the excitability changes at the make (*Ia*) and after the break (*IIa*) of the anodal current. Curves *IIIc* and *IIIa* present with a slower time line the excitability changes during the flow of the cathodal (*IIIc*) and of the anodal (*IIIa*) currents. The tracings labelled *P* present the fluctuations of the electrotonic potential. As can readily be noted, the

excitability curves faithfully paralleled the E_1 fluctuations of the electrotonic potential.

The excitability changes illustrated in figure 22 also are in agreement with Pflüger's rule. If in reference to the E_1 com-

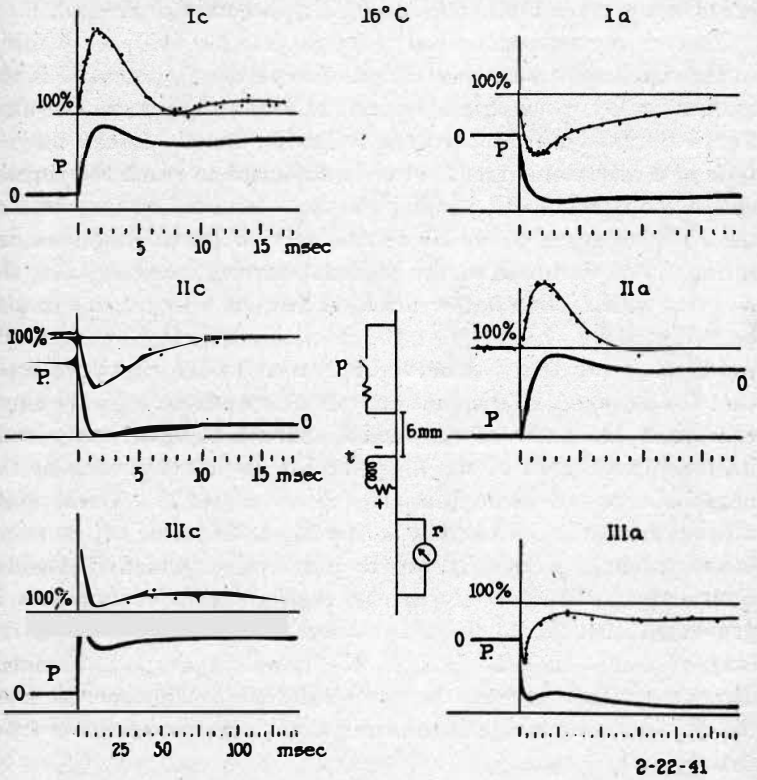


FIG. 22. Relationship of the excitability of the nerve to the E_1 fluctuations of the electrotonic potential.

ponent of the electrotonus, catelectrotonus and anelectrotonus are defined as states in which the Q fraction of the membrane potential is decreased and increased, respectively, Pflüger's rule can be stated in this manner. The excitability is increased in the catelectrotonic and decreased in the anelectrotonic state regard-

less of whether the change in the Q fraction has been produced directly by the applied current or by the operation of the E_1 reaction after the end of the applied current. It should never be forgotten, however, that if the decrease of the Q fraction exceeds a certain limit, the excitability becomes depressed.

There is a considerable body of experimental evidence leading to the conclusion that the creation of negative E_1 potential is the mechanism by which applied currents initiate the nerve impulse. Particularly significant are the following facts. If the magnitude of a cathodal current is barely sufficient to reach the threshold of the nerve fibers, the impulses are initiated at the crest of the E_1 fluctuation, never later; similarly, when the impulses are initiated by the break of the cathodal current, they arise at the N_1 crest which follows after the R_1 deflection. Impulses can also be initiated by the make of the anodal current; the necessary condition being that the nerve be so near to the rhythmic state that the E_1 potential displays an oscillatory course. Under these conditions the make of an anodal current of small magnitude initiates oscillations of the E_1 potential, impulses arising at the negative crests of the oscillations (fig. 23, *2* to *12*). If the magnitude of the applied current is increased, the value of the membrane potential is rapidly raised to a level at which E_1 oscillations cannot take place, whereby the initiation of impulses is prevented (fig. 23, *13* to *15*). It is therefore clear that in the case of small anodal currents the impulses are not initiated directly by the current, they are initiated by the operation of the E_1 reaction which creates a negative variation of the Q fraction.

The nature of the accommodation of the nerve to the cathodal current is now readily understandable. Accommodation is the result of the operation of the E_1 reaction. The applied current creates the E_1 potential while the E_1 reaction first opposes the growth of the E_1 potential and then removes a significant part of the E_1 potential that has already been created. If, at the time when it passes through its maximum, the E_1 potential does not reach the threshold of the nerve fiber, the impulse will not

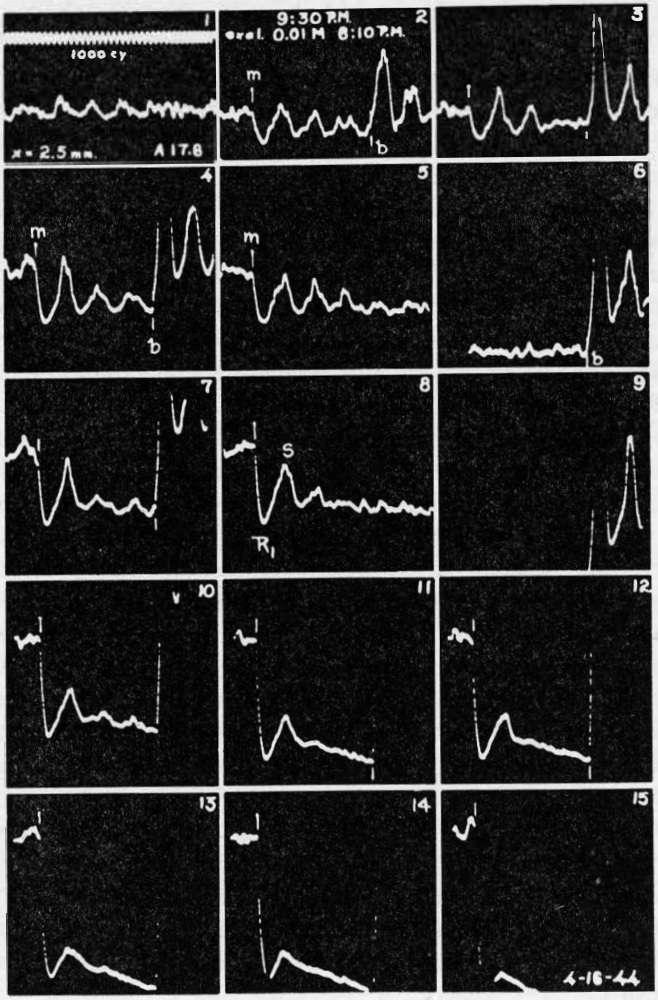


FIG. 23. Effect of anodal currents of progressively increasing magnitude upon oxalate-treated nerve shortly after the onset of spontaneous rhythmic activity. *1*, the base line in the absence of stimulation. The make and the break of the current are indicated by short vertical lines labelled *m* and *b* in several records. The R_1 deflection has been labelled in record *8*; *s* in this record denotes the spikes initiated by the operation of the nerve reaction.

be initiated since the E_1 reaction changes the conditions at the q–m boundary in such a manner that further flow of the current will not produce an increase of the E_1 potential. Thus, according to this view, accommodation is the result of an active process, the E_1 reaction, which removes an important fraction of the stimulating agent, the E_1 potential, created by the current.

It may be of interest to mention that the hypothesis of the "local cathodal response" (Katz, '37), recently put forward to explain the initiation of the nerve impulse, is untenable. The hypothesis states that the nerve fiber acts as a passive structure for anodal currents of any magnitude and for cathodal currents of magnitude below 50% of the rheobase, while cathodal currents of greater magnitude initiate an active "local response" consisting of a depolarization which adds itself to the depolarization produced by the current. All parts of this hypothesis are erroneous. (1) The nerve fibers never act as passive structures; they produce a response to any current, cathodal or anodal, however small, and (2) the response that they produce is precisely the opposite of that which is assumed in the hypothesis of the "local cathodal response." The response of the nerve fibers, the nerve reaction, opposes the effect of the applied current. If the nerves of invertebrates were comparable at all to vertebrate (frog, mammalians) nerves, Hodgkin's ('38) hypothesis of the "local cathodal response" also would have to be discarded.

Let this point be made perfectly clear. Cathodal currents produce two essentially different responses in vertebrate nerve. The response to subliminal currents is the nerve reaction that opposes the effect of the applied current. If the magnitude of the current is increased so that the nerve reaction becomes insufficient to oppose its effect, a nerve impulse is initiated. Thus, there is a sharp discontinuity between the effects of subliminal and of liminal cathodal currents. Even more, there is a sharp discontinuity in the response of the nerve fiber to a liminal current; before the impulse is initiated, the nerve fiber opposes the depolarizing action of the current while, when the nerve impulse arises, the nerve fiber undergoes a sudden depolarization.

g. *Critical Excitability Level of the Membrane Potential.*— Emphasis has already been placed upon the fact that if the decrease of the *Q* fraction produced by a cathodal current exceeds a certain limit, the excitability of the nerve becomes depressed; ultimately, the flow of the cathodal current renders the nerve inexcitable. The effect of the cathodal current is not specific, since it can be duplicated by any depolarizing agent. In general, it can be said that a segment of nerve submitted to the action of a depolarizing agent becomes inexcitable when the demarcation potential measured between the treated segment and untreated nerve reaches the value of about 8–10 mv. The value of the membrane potential at which the excitable mechanism becomes inoperative may be called the "critical excitability level."

The fact that the excitability of a depressed nerve can be increased by an applied anodal current has been known for a long time since it was discovered by Bilharz and Nase in 1862; in addition, there is considerable literature on the restoring action of the anodal current. In particular, it should be mentioned here that in 1922 Thörner demonstrated that anodal polarization may delay the onset of the inexcitability of a nerve deprived of oxygen. It was not known, however, that the anodal current may repolarize and render excitable nerves that have been depolarized far beyond the critical excitability level by the effect of anoxia alone or combined with that of metabolic inhibitors. In other words, it was not known that to a large extent an applied anodal current may substitute for the activity of the metabolic mechanisms of the nerve fibers.

The observations illustrated by figure 24 were begun after the nerve had been deprived of oxygen for more than three hours. Since the anoxic depolarization had decreased the membrane potential below the critical excitability level the nerve was inexcitable. For this reason record *1* displays only the shock deflection, i.e., the deflection produced by electrotonic spread of the stimulating shock along the nerve. At the instant indicated by the arrow on record *2*, a 12 μa anodal current was applied to the nerve which was not interrupted until after record *11* had been

obtained. Since the nerve was in an advanced state of depolarization, the polarizability of the membrane was low; consequently the anodal current produced an increment of the membrane

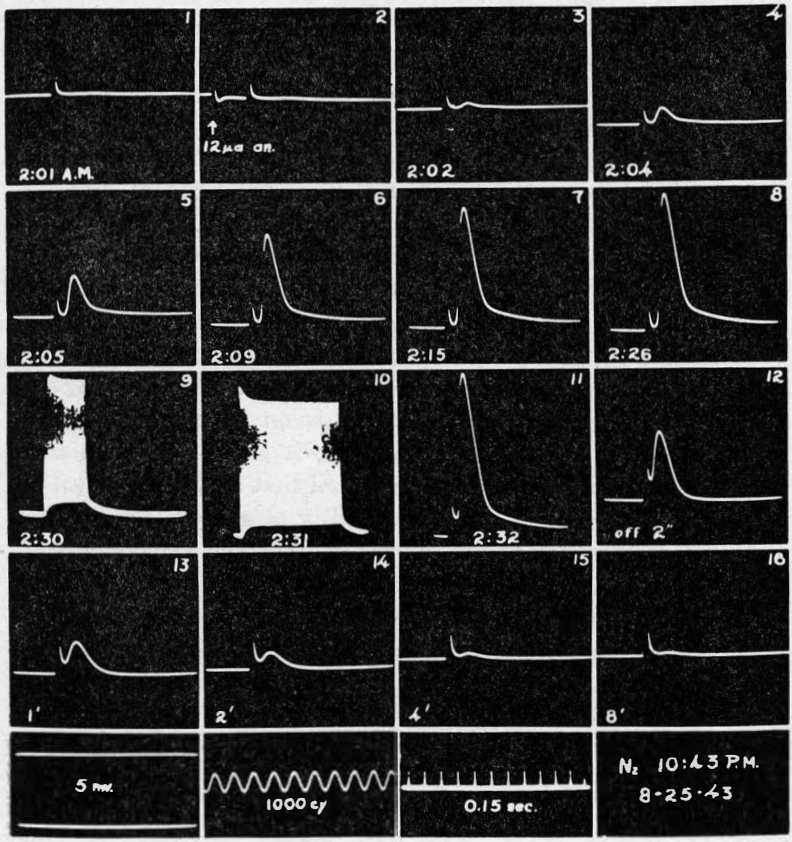


FIG. 24. Restoration of the excitability of anoxic nerve by an applied anodal current.

potential at a much slower rate than in the case of normal nerve. The increment of the membrane potential revealed itself in the form of a progressive displacement of the base line of records *2* to *11*. Soon a few fibers of the nerve became excitable (record

3); the number of excitable fibers increased progressively (records *4* to *7*) until finally, after the anodal current had been allowed to flow for 25 minutes, a large majority of the nerve fibers were able to produce impulses (record *8*). The stimulating shock was then strengthened and many fibers were found to be able to produce impulses in response to a train of shocks at the frequency of 100 per second. The applied current was inter-

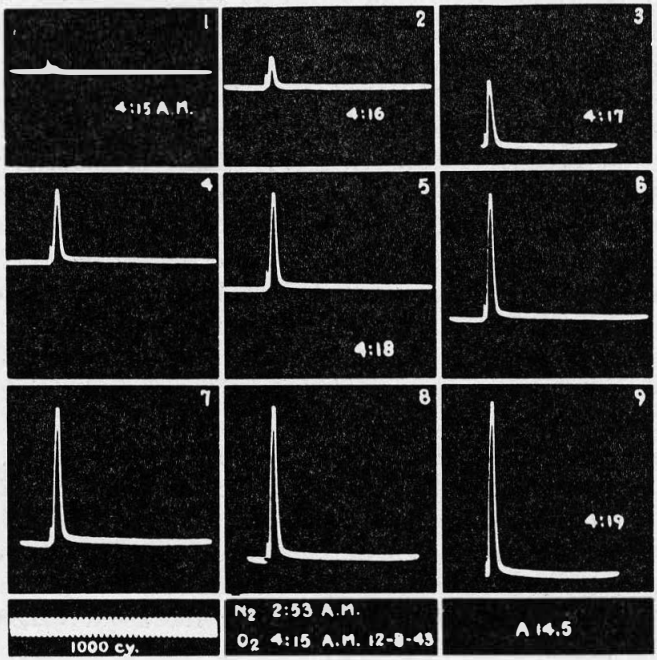


FIG. 25. Restoration of the excitability of anoxic nerve by respiration.

rupted immediately after the sweep of record *11*. In the absence of the applied current the nerve again became depolarized. The decrease of the membrane potential revealed itself in the progressive ascent of the base line of records *12* to *16*. As can readily be noted, the progressive depolarization was accompanied by a decrease of the number of excitable fibers, until finally the nerve again became inexcitable.

There is a remarkable similarity between the effects of anodal polarization and oxidative metabolism. In the experiment illustrated by figure 25 the nerve was deprived of oxygen until only a few fibers were excitable (record *1*). Oxygen was then introduced into the nerve chamber. Respiration resulted in a rapid increase of the membrane potential which was accompanied by restoration of the excitability of the nerve fibers.

In view of these results the following assumption seems to be justified. Nerve deprived of oxygen undergoes depolarization because in the absence of oxygen certain oxidized substances become reduced. Anoxic nerve is repolarized and rendered excitable by the anodal current because the flow of the anodal current results in oxidation of the reduced substances or, otherwise stated, because the flow of the anodal current produces changes in the physico-chemical structure of the nerve fiber similar to those which are produced by the uptake of atmospheric oxygen.

The results of experiments of the type illustrated by figure 26 suggest that indeed the anodal current produces its effect because the potential difference that it establishes across the membrane drives chemical reactions so that the changes underlying the polarization are reversed.

In the experiment illustrated by figure 26 the nerve was treated with iodoacetamide (0.001 M) and, after the symptoms of the poisoning had become patent, the nerve was deprived of oxygen. The observations recorded in figure 26 were made after the nerve had been deprived of oxygen for 180 minutes. Since the nerve was inexcitable, record *1* presents only the shock deflection. Anodal polarization was then applied throughout the intervals between records *2* to *17*. The magnitude of the current was increased and decreased several times in order to follow the progress of the restoration of excitability. During these trials it was observed that the number of excitable fibers was not directly dependent upon the value of the increment of the membrane potential; to be sure, there was at all stages of the restoration a value of the membrane potential at which the response was greatest, but restoration of the excitability of all the A fibers (record *15*)

was not obtained until the current had been allowed to flow for 11 minutes. This fact that can also be observed with unpoisoned nerve (fig. 24) clearly indicates that the restoration requires two

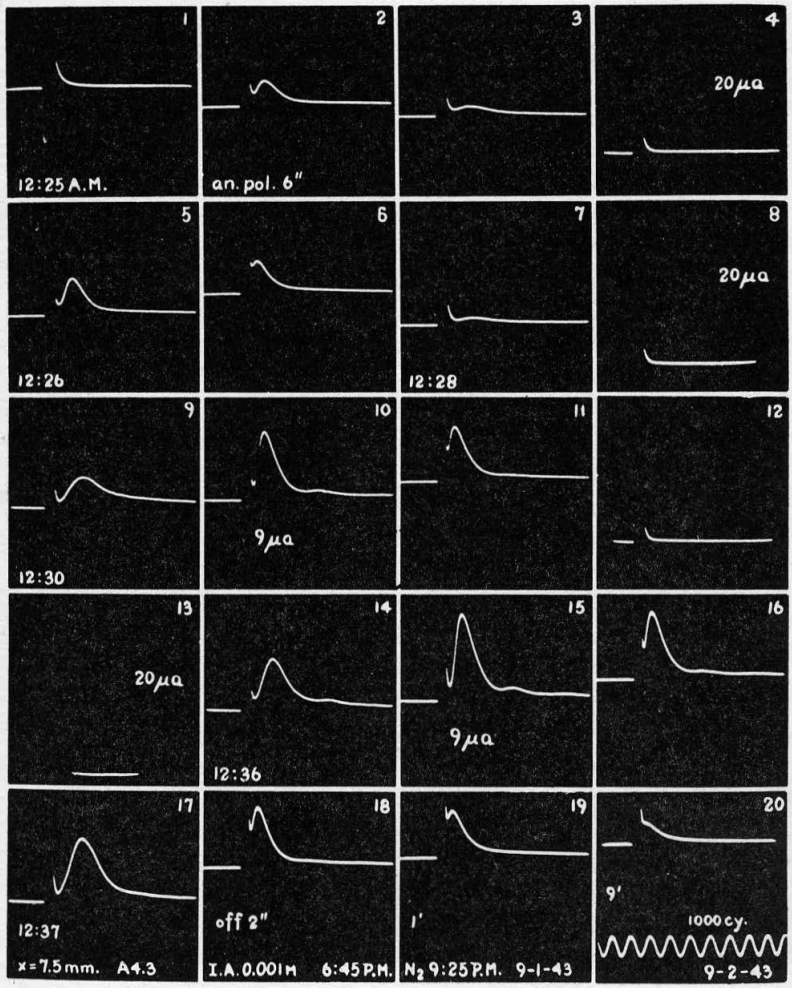


FIG. 26. Restoration of the excitability of nerve poisoned with iodoacetamide and deprived of oxygen, by an applied anodal current.

conditions: (1) that the membrane potential be raised above the critical excitability level, and (2) that the flow of current be maintained until chemical changes have taken place in the artificially repolarized membrane.

The extent by which the applied anodal current may substitute for the activity of the metabolic systems is illustrated by figure 27. It can be seen in figure 26, *18* to *20*, that after the interruption of the anodal current, the decrease of the membrane potential again rendered the nerve inexcitable. One hour later, the nerve having been maintained in nitrogen all the time, restoration of the excitability was again effected by means of the applied anodal current of optimal magnitude (9 μa). Record *1* of figure 27 presents the spike obtained in response to a maximal alpha plus beta shock. Without interrupting the flow of the applied current, the nerve was submitted to continuous tetanic stimulation that was maintained for two hours (records *2* to *24*). During the tetanus a progressive decrease of the value of the membrane potential took place (cf. base lines of records *2* to *5, 8, 9, 12, 13; 16, 17*) which was referable to the inability of the current to restore fully during the intervals between impulses the loss of membrane potential produced by each impulse. The loss of membrane potential resulted in the inexcitability of a number of fibers, but after the level of the membrane potential had been increased by means of a brief period of polarization with a greater current (records *6, 7; 10, 11; 14, 15; 18, 19*) the spikes regained practically their initial height (records *8, 12, 16, 20*). The applied current was interrupted immediately after the sweep of record *20;* the resulting depolarization of the membrane resulted in a rapid decrease of the excitability (records *21, 22*), but a 2-second period of polarization with a large current (record *23*) markedly increased the height of the response. Since the rhythmic stimulation of the nerve had been maintained for 127 minutes, the number of impulses which had been produced in the responding fibers may be estimated at no less than 150,000. Thus, there can be no doubt that in the absence of oxygen and in the presence of an inhibitor of glycolysis, the nerve fibers can produce an ex-

ceedingly large number of impulses provided only that their membrane potential be maintained at the appropriate level by means of an externally applied anodal current.

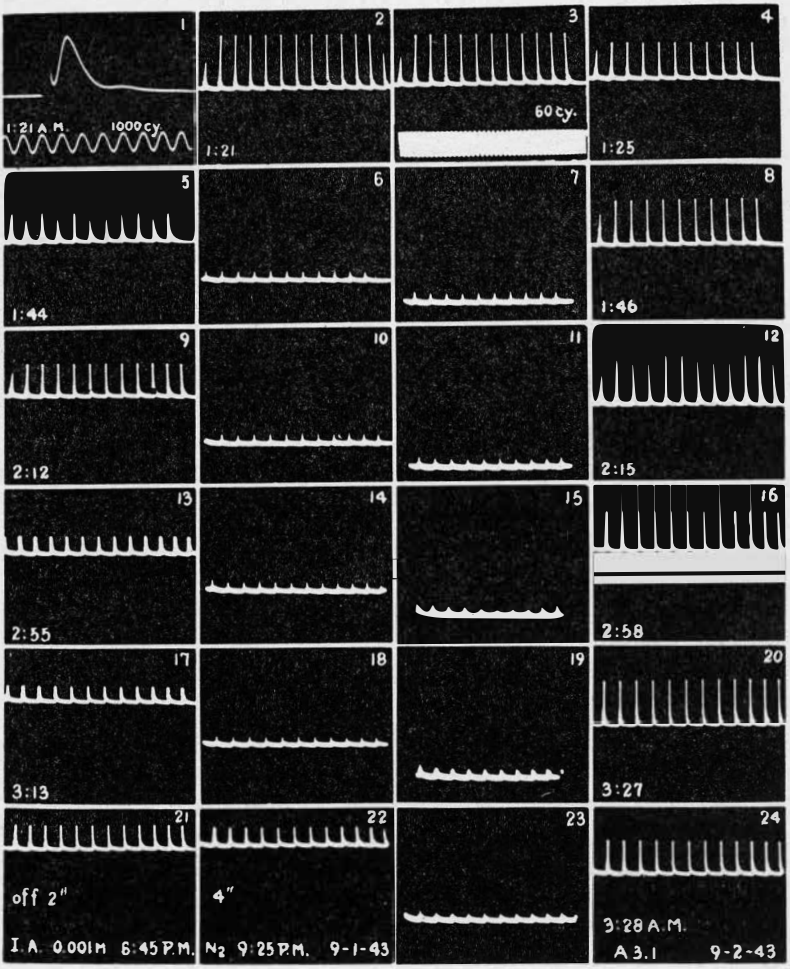


FIG. 27. Rhythmic activity of poisoned and anoxic nerve in the presence of applied anodal currents.

The significance of the experiment illustrated by figures 26 and 27 is increased by this fact. When oxygen was admitted into the chamber, the nerve failed to perform a successful oxidative repolarization; thus, there could be no doubt that the presence of iodoacetamide had blocked key reactions of the oxidative metabolism; nevertheless, anodal polarization still was able to effect restoration of excitability. Obviously, the anodal current may produce its effect even after important links of the chain of respiratory systems have been blocked.

The experiments illustrated by figures 26 to 27 were done with frog nerves; similar results, however, can be obtained with mammalian nerves. The observations presented in figure 28 were done with a human radial nerve that was dissected 13 hours after death. When the nerve was brought to the laboratory by Dr. Tarlov it was inexcitable; a small demarcation potential, however, was measured between the center of the nerve and a freshly injured point near one of the ends. The existence of this demarcation potential indicated that although the nerve had undergone a far reaching depolarization, a disintegration of its structure had not taken place yet; therefore, it was expected that if the nerve were artificially repolarized by an applied anodal current, it would regain its ability to produce impulses. The expectation proved to be correct.

Record *1* of figure 28 shows the response obtained after a several minutes long period of polarization with a 440 μa anodal current had begun to restore the excitability of the nerve fibers. The number of fibers which were able to produce impulses was exceedingly small; it increased, however, during the renewed flow of the applied current (records *2* to *4*). The break of the anodal pulse used to obtain records *5* to *6* initiated impulses in a number of fibers, some of which also were able to respond to the induction shock used to obtain record *7*. During a following period of anodal polarization (records *8* to *11*) the spike was seen to increase and after the end of the applied pulse the break response (record *11*) was greater than it had previously been (record *6*). A comparison of records *7* and *12* shows the increase of the num-

ber of fibers which had become able to respond in the absence of applied polarization.

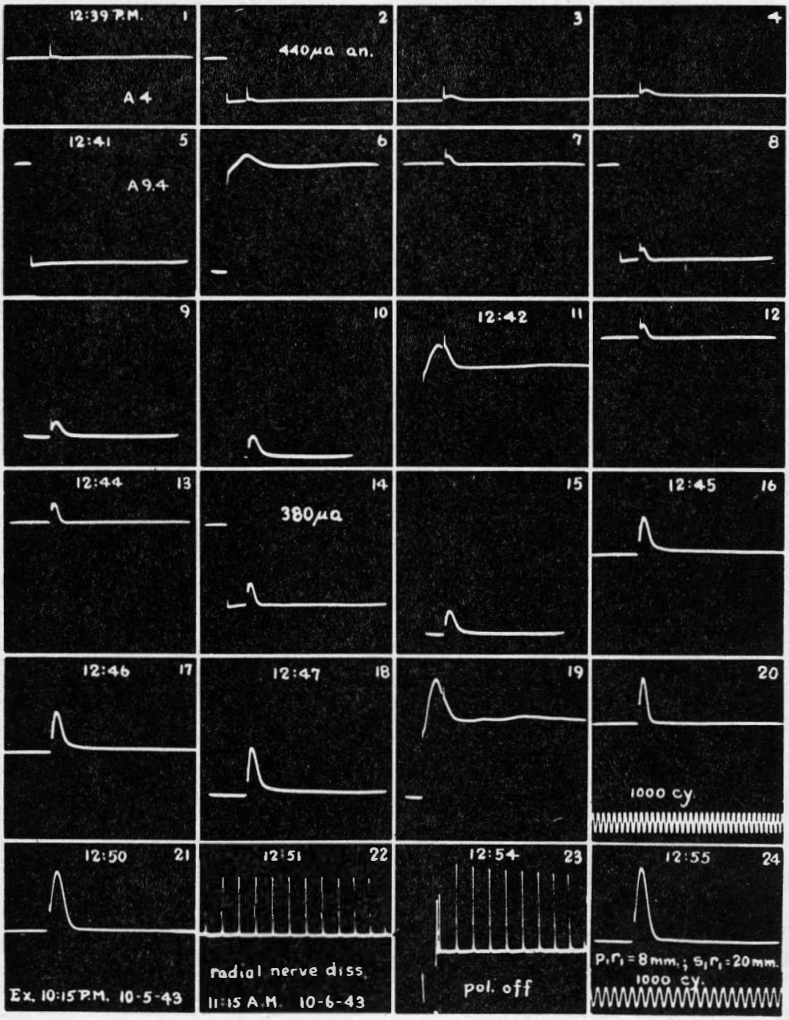


FIG. 28. Restoration of the excitability of a human nerve by the applied anodal current.

This number was further increased by a new period of polarization in the interval between the sweeps of records *12* and *13*. Since the state of the nerve was being rapidly improved, the magnitude of the applied current was decreased in order to avoid damage to the nerve. Restoration to an important degree was effected by the anodal pulse used to obtain records *14* to *19* and further improvement of the state of the nerve was produced by another pulse applied in the interval between the sweeps of records *20* and *21*. Records *22* and *23* present the responses to a rhythmic train of shocks at the frequency of 17 per second; anodal polarization was applied immediately after the sweep of record *22* and was interrupted during the sweep of record *23*.

One minute after the end of the polarization the spike produced in response to single shocks still had the great height shown by record *24*. In the absence of polarization the nerve fibers gradually became inexcitable; no difficulty was found, however, in restoring their excitability by anodal polarization. The shape of the spikes recorded in figure 28 as well as the results of appropriate tests done by displacing electrode r_1 away from the polarizing electrode (p_1), proved that the impulses were produced only in that segment of nerve which had been repolarized by the applied current.

h. *Concluding Remarks.*—Two general questions may now be considered. The first question is that of the relationship of the nerve impulse to the enzymatic systems of nerve. A complete answer to this question cannot be given at the present state of knowledge; a partial answer, however, is possible. Observations have been made on nerves that had been poisoned with a variety of metabolic inhibitors. Di-isopropyl fluorophosphate at the concentration 0.001 M or eserine up to the concentration 0.002 M do not prevent the conduction of impulses even after they have been allowed to act upon the nerve for 24 hours. Eserine at the exceedingly high concentration 0.01 M, fluoride at the concentration 0.02 M, cupric chloride at the concentrations 0.005 and 0.01 M, cyanide at the concentration 0.001 to 0.05 M and veratrine at the concentration 1:50000 cause a depolarization of the membrane

and consequently render the nerve fibers inexcitable. In all cases, however, anodal polarization is able to restore the excitability of the nerve fibers; therefore, the enzymatic reactions which are inhibited by those substances are not directly involved in the production of the nerve impulse. In point of fact, as the evidence stands at present, it is exceedingly unlikely that any of the enzymatic reactions which are now known could play a direct rôle in the production of the nerve impulse.

There can be hardly any doubt that the nerve impulse is the result of a reversible electro-chemical reaction, but the nature of this reaction still is a matter for conjecture. A successful approach to the problem could perhaps be made by studying the action of cocaine and similar anesthetics, since cocaine blocks the production of the nerve impulse selectively; that is to say, cocaine renders the nerve fibers inexcitable with little interference with other aspects of nerve function.

The second question is that of the relationship of the membrane potential to metabolism. There is no doubt now that the membrane potential is directly established by oxidative metabolism. It also can be taken for granted that applied currents modify the state and the properties of the membrane because by changing the value of the membrane potential they alter the course of reactions of the oxidative chain.

The effect of the cathodal current is opposed by the nerve reaction which tends to maintain the membrane potential at the normal level. If the nerve reaction is sufficient, that is to say, if the metabolic mechanisms are able to supply the energy required to maintain the strength of the double layers of the membrane at a constant level, the cathodal current fails to cause a progressively increasing depolarization of the nerve fibers. If the nerve reaction, however, is insufficient, that is to say, if the metabolic mechanisms are unable to replace the charged particles that are removed from the double layers by the current, the nerve fibers undergo a progressively increasing depolarization, the observed changes in the properties of the nerve fibers being exactly those which appear after the nerve is deprived of oxygen. On the other

hand, a nerve that has undergone anoxic depolarization is repolarized by the anodal current much in the manner that it is repolarized by oxidative metabolism.

Since the value of the membrane potential determines the equilibrium point of a system of chemical reactions, the membrane potential must be regarded as a component of the system, the other components being, of course, the chemical species present in the membrane and core of the nerve fiber. The chemical constitution of the nerve fiber and the value of the membrane potential or rather of its fractions cannot be regarded as separate entities since a change in the chemical constitution must necessarily be accompanied by a change in the membrane potential and conversely, a change in the membrane potential must necessarily cause a change in the chemical constitution. Likewise, the membrane potential and the structure of the membrane must be regarded as inseparable entities since the membrane potential determines the physico-chemical structure of the membrane and conversely.

Under conditions such as these it is clear that (1) a description of the properties of the membrane must always include a statement of the value of the membrane potential, and (2) a distinction between "chemical" and "electrical" processes in nerve should not be made. To be sure, ordinary chemical reactions may take place in nerve but those reactions which are directly related to the maintenance of the resting membrane potential and to the production of the nerve impulse are electro-chemical reactions that take place at organized boundaries and can be driven in one direction or the other by a difference of electrostatic potential.

Only in one sense and solely for the purposes of theoretical analysis can a distinction be made between electrical and chemical processes. The resting membrane potential, i.e., the electrostatic potential difference that exists across the membrane is a measure of work done against Coulomb forces in separating charged particles of the opposite signs; consequently the membrane potential is the measure of an amount of free energy that has been released by degradation of certain chemical species. The existence

of a membrane potential indicates that free energy of metabolic substrates has been converted into electrical energy; according to the evidence which is now available this step is essential in the utilization of metabolic energy by the nerve fibers; it might also be important in the case of other cells.

REFERENCES

Bernstein, J., 1912, Elektrobiologie, Braunschweig, Friedr. Vieweg und Sohn.

Biedermann, W., 1895, Elektrophysiologie, Jena, Gustav Fischer.

Bilharz, A. and Nasse, O., 1862, *Arch. Anat., Physiol. u. wissensch, Med.*, 66.

Butler, J. A. V., 1940, Electrocapillarity, New York, Chemical Publishing Company.

Fenn, W. O., Cobb, D. M., Hegnauer, A. H. and Marsh, B. S., 1934, *Amer. J. Physiol., 110*, 74.

Gasser, H. S., 1937, Sequence of Potential Changes, Chapter IV, in Erlanger, J. and Gasser, H. S., Electrical Signs of Nervous Activity, Philadelphia, University of Pennsylvania Press, 130.

Gerard, R. W., 1930, *Amer. J. Physiol., 92*, 498.

Glasstone, S., Laidler, K. J. and Eyring, H., 1941, The Theory of Rate Processes, New York, McGraw-Hill Book Company.

Gurney, R. W., 1931, *Proc. Roy. Soc. London, Series A, 134*, 137.

Hastings, A. B., 1940–1941, *Harvey Lectures, Series 34.*

Helmholtz, H., 1847, Über die Erhaltung der Kraft, Sitzung der Phys. Ges. zu Berlin, Berlin, G. Reimer; reprinted in Helmholtz, H., 1882, Wissenschaftliche Abhandlungen, Leipzig, Johann Ambrosius Barth, *1*, 12.

Helmholtz, H., 1853, *Ann. Phys. u. Chem., 89*, 211 and 353; reprinted in Helmholtz, H., 1882, Wissenschaftliche Abhandlungen, Leipzig, Johann Ambrosius Barth, *1*, 475.

Hodgkin, A. L., 1938, *Proc. Roy. Soc. London, Series B, 126*, 87.

Katz, B., 1937, *Proc. Roy. Soc. London, Series B, 124*, 244.

Koch, E., 1927, *Arch. ges. Physiol., 216*, 100.

Lorente de Nó, R., 1944, *J. Cell and Comp. Physiol., 24*, 85.

Lorente de Nó, R., 1947, A Study of Nerve Physiology, The Studies from the Rockefeller Institute for Medical Research, *131, 132.*

Overton, E., 1902, *Arch. ges. Physiol., 92*, 346.

Thörner, W., 1922, *Arch. ges. Physiol., 197*, 159.