

VOLTAGE CLAMP BEHAVIOR OF IRON-NITRIC ACID SYSTEM AS COMPARED WITH THAT OF NERVE MEMBRANE

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ABSTRACT

The current-voltage relation for the surface layer of an iron wire immersed in nitric acid was investigated by the voltage clamp technique. Comparing the phase of nitric acid to the axoplasm and the metallic phase to the external fluid medium for the nerve fiber, a striking analogy was found between the voltage clamp behavior of the iron-nitric acid system and that of the nerve membrane. The current voltage curve was found to consist of three parts: (a) a straight line representing the behavior of the resting (passive) membrane, (b) a straight line representing the fully excited (active) state, and (c) an intermediate zone connecting (a) and (b). It was shown that in the intermediate zone, the surface of iron consisted of a fully active patch (or patches) surrounded by a remaining resting area. The phenomenon corresponding to "repetitive firing of responses under voltage clamp" in the nerve membrane was demonstrated in the intermediate zone. The behavior of the cobalt electrode system was also investigated by the same technique. An attempt was made to interpret the phenomenon of initiation and abolition of an active potential on the basis of the thermodynamics of irreversible processes.

INTRODUCTION

The similarity between the process of nervous conduction and that of propagation of the activation wave along an iron wire immersed in nitric acid has been worked out by many previous investigators, notably by Heathcote (9), Lillie (12), Bonhoeffer (3), Yamagiwa (22), and Franck (7). As Heathcote pointed out more than half a century ago, this similarity arises obviously from the situation that, both in the nerve fiber and the iron wire model, the local current flowing between the active and the resting areas plays a decisive role in the process of propagation. Recently, Bonhoeffer (4) and Franck (7) stressed the similarity in the kinetics of initiating activity between the two systems. Tasaki (17) also pointed out the similarity between the two systems with regard to the phenomenon of initiation and abolition of activity. Some time ago, Bartlett (2) compared transient current flows in the iron-sulfuric acid system with those in plant and nerve cells.

The present paper deals with determination by the method of voltage clamp of the current-voltage relationship of the iron-nitric acid system. Since the currently prevailing concepts of nerve excitation are based mainly on the results of observations made by the use of this method (5, 10, 20), it seemed desirable to investigate the behavior of the iron-nitric acid system under "potentiostatic" (or voltage-clamp) conditions.

The attempt to analyze the iron electrode system by the method of voltage clamp is not entirely new. Several years ago, Professor U. F. Franck made some preliminary observations on this electrode system (quoted in reference 4). The present investigation, which is an extension of the work done by these German electrochemists, brought out several new phases of striking similarity between the behavior of the nerve membrane and that of the surface layer of a piece of iron immersed in nitric acid. The behavior of cobalt immersed in the mixture of hydrochloric acid and chromium trioxide was also investigated in this connection.

Methods

The experimental arrangement used is illustrated in Fig. 1. A piece of iron wire of 1 mm. in diameter (kindly supplied to us by Professor U. F. Franck) was covered tightly with a glass tubing except at the tip. The narrow space between the glass tubing and the wire was filled with melted paraffin. The exposed tip of the wire was polished with a fine grinding stone. This smooth, slightly convex metal surface was immersed in a nitric acid solution of approximately 1.33 in specific gravity. The other end of the iron wire was grounded by means of a thick copper wire.

Two platinum electrodes were also immersed in the nitric acid. The small electrode (P_t in the figure) consisted of a 100 μ (or 200 μ) platinum wire covered with a glass tubing except for a length of about 0.3 mm. at the tip; this exposed tip of the small platinum (potential) electrode was fixed at a distance of about 0.1 mm. from the surface of the iron near the lower edge of the glass tubing covering the iron wire. The other, larger electrode consisted of a platinum wire of about 1 mm. in diameter and about 20 cm. in length was placed at some distance away from the tip of the iron wire.

The electrical circuits connected to these three electrodes are illustrated in Fig. 1, top. The arrangement was made in such a way that the potential difference between the grounded iron wire and the potential electrode could be altered along rectangular time courses of desired amplitudes by automatically controlling the current fed into the large platinum (current) electrode. The potential electrode was connected to one of the inputs of a differential amplifier, A_1 in the figure, through a potentiometer (5000 ohms) and a 1.5 volts battery. This potentiometer provides a means of compensation of the steady potential difference between the iron and the platinum. The potential electrode was connected also to the cathode ray oscilloscope through a resistor of about 10 kilohms. The other input of the differential amplifier A_1 was led to a source of rectangular voltage pulses (Grass stimulator, type S4A). The current flowing into the current electrode was measured by recording the IR -drop across

a small resistance (r in the figure) which was as a rule 1 ohm (sometimes 2, 10, or 100 ohms).

Amplifier A_1 was of a Tektronix 112 type operated at a gain of 250 to 400. The output of this commercially available d.c. amplifier was led to a low output-impedance amplifier, A_2 in the figure, designed specially for the present experiment. This amplifier consisted of 6L6 and 6SN7 tubes at the input stage followed by fourteen cathode followers made with 6CL6 tubes connected all in parallel. These cathode followers were operated at +350 volt plate and -250 volt cathode return voltage;

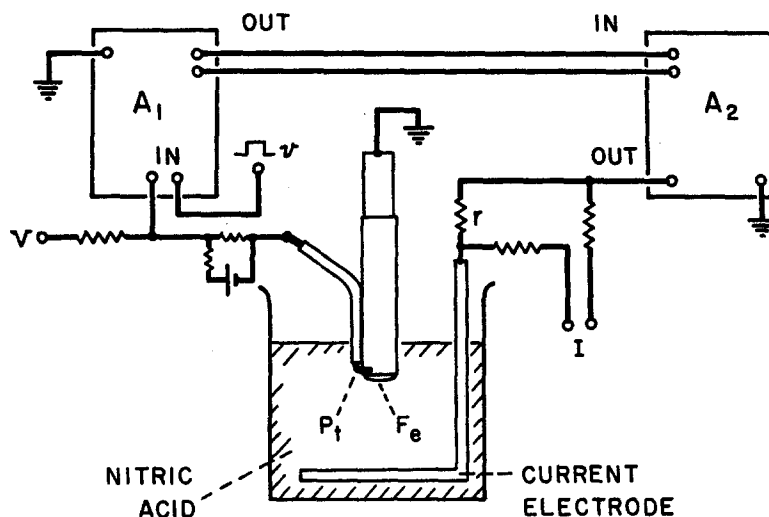


FIG. 1. Experimental arrangement used for clamping the potential of the iron-nitric acid system. P_t represents the platinum potential electrode, F_e the exposed surface of the iron wire. The current electrode was made with large platinum wire. When a visual observation of the surface of F_e was required, the iron electrode was held nearly horizontally to the surface of nitric acid. Further description in text.

the common cathode resistance of 1000 ohms carried a steady current of 250 ma. The transconductance of the 6CL6 tube was 7000 micromhos under the working conditions.

Voltage clamp by this arrangement depends on the property of the differential amplifier, A_1 , to amplify the potential difference between the two inputs. This potential difference, after being amplified by a factor of approximately 300, gives rise to a current flowing in the direction which tends to reduce the potential difference between the two inputs of A_1 . The potential difference between the two inputs can be maintained at an extremely small value if the gain of A_1 is high enough and, at the same time, if the output impedance of A_2 is sufficiently low. In other words, the potential difference V between the iron and small platinum electrodes should closely follow the time course of the potential v generated by the source of rectangular pulses under these experimental conditions. A simple analysis of the circuit employed indi-

cates that the imperfection of voltage clamping, defined by $(v - V)/V$, is given approximately by the following formula:—

$$\frac{(v - V)}{V} = \frac{1}{n m g_m X}$$

in which n is the over-all gain of the amplifier A_1 , m the number of cathode followers in A_2 , g_m the transconductance of each tube in A_2 and X the resistance of the iron electrode system. The resistance X was found to be close to 5 ohms at the peak of activation. Introducing $n = 300$, $m = 14$, $g_m = 0.007$, and $X = 5$, it is found that the imperfection should be of the order of 0.7 per cent at the peak of activation.

The procedure of adjusting the arrangement prior to the start of voltage clamping is as follows: With the current electrode disconnected from the output of A_2 , the d.c. level in A_1 was adjusted to give nearly zero potential at the output of A_2 . Then the output of A_2 was connected to the current electrode. After confirming that there was not appreciable current through the current electrode at this moment, a rectangular voltage pulse was applied to the second input of A_1 . Since the iron electrode is traversed by a current of the order of 50 ma., it is necessary to keep the resistance between different ground wires in the circuit below 0.1 ohm.

The time courses of the potential, V , and of the current, I , were recorded simultaneously with a Du Mont oscillograph (type 322) and with a polaroid camera. An interval of 5 minutes or more was allowed for the recovery between successive trials.

The method used for the cobalt electrode system is essentially the same as the one described above. All the observations were made at room temperature (23°C.).

RESULTS

1. Terminology Employed.—Before starting the description of the results obtained, we define the terms employed in the following description. Our terminology is based purely on the comparison of this iron electrode system to the analogous situation in voltage clamp experiments on the nerve membrane. If one compares the phase of the iron to the external fluid medium of the squid giant axon and the phase of nitric acid to the axoplasm carrying two internal (potential and current) electrodes, it is possible to adopt physiological terms to describe the electrochemical phenomena in the iron electrode system.

Both in the squid giant axon and in the single node preparation of the toad, the external fluid medium has high electrical conductivity and is nearly equipotential; this is quite true in the present inanimate system. In the case of the squid axon, there is a slight IR -drop in the axoplasm when the membrane is traversed by a strong current; an analogous situation exists in the present iron electrode system. The nerve membrane is activated by an outward directed current through the membrane; this is also true in the case of the iron electrode system if the substance of iron is regarded as being "outside" the "excitable surface membrane" of the iron.

In the records presented in this paper, as well as in our previous papers (17–21), the upward deflection of the potential trace means positivity of the

potential electrode referred to ground (*i.e.* that iron is less anodic). According to this convention, the all-or-none "response" in the iron electrode system can be represented by an upward (positive) swing of the potential of the fluidal phase (axoplasm). In the current trace, the upward deflection represents current flowing "outward" through the "excitable membrane" (*i.e.* from the fluidal phase in the beaker into iron); this convention was adopted because the behavior of an ohmic resistor can then be expressed by the parallelism between the deflections of the current and potential traces.

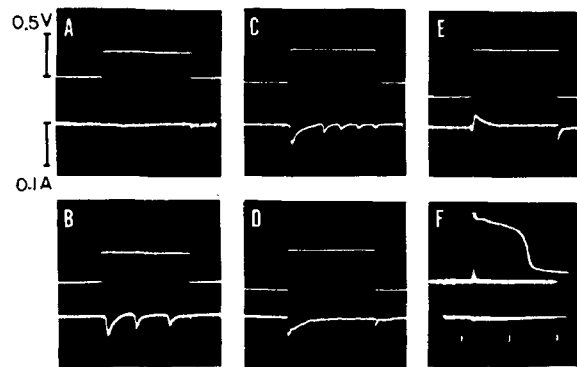


FIG. 2. Simultaneous recording of the current and the voltage of the iron-nitric acid system. In records *A* to *D*, the potential of the system was varied along rectangular course by the use of the arrangement of Fig. 1; the duration of the voltage pulse is 1 second. Record *F*, the "action potential" of the system; the time marker below shows 1 second interval.

2. Current-Voltage Relation in the Iron-Nitric Acid System.—Prior to the start of voltage clamp experiments on the iron-nitric acid system, "electric responses" of the system were recorded. This was done by disconnecting the current electrode in Fig. 1 from the output of amplifier A_2 and by applying voltage pulses of 30 to 50 volts (approximately 5 msec. in duration) through a 10 kilohm resistor. It was found that the "threshold membrane potential" of the system was approximately 0.5 volt above the resting potential level. The duration of the response was between 1 and 3 seconds and its peak amplitude was close to 1 volt. These findings agree with those described by previous investigators (3, 7).

As can be seen in the example of Fig. 2, record *F*, the response showed an initial peak followed by a long plateau. The response came to an end by a more or less abrupt fall in potential from the level of the plateau. This configuration of the response in this electrode system resembles that of the squid axon under tetraethylammonium chloride (TEA) or that of the nodal membrane treated with a trace of nickel or cobalt ion (19, 15).

The duration of the clamping pulse employed was as a rule 1 second in duration. When the clamping potential level was above the threshold membrane potential but below the peak amplitude of the all-or-none response, a surge or surges of inward currents (flowing from iron to acid) were observed following the start of the rectangular clamping pulse (see Fig. 2, records C and D). This situation is analogous to what has been observed in the nerve membrane. We found that this analogy between the nerve membrane and the iron-nitric acid system was far more profound when we plotted the peak amplitude of the

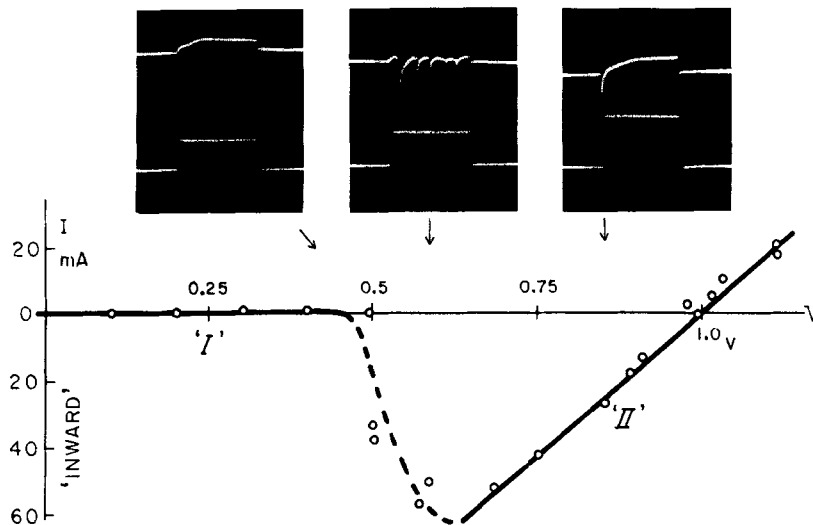


FIG. 3. Relation between the amplitude of the clamping voltage pulse, V , and the peak amplitude of the inward current, I , determined by the method of voltage clamp on the iron-nitric acid system. Three samples of records on which the measurements were made are given. The surface area of iron in nitric acid was approximately 1 mm^2 .

inward current against the clamping potential above the resting level (see Fig. 3).

The current-voltage relation in the iron-nitric acid system was found to consist of three distinct parts, straight line 'I' in Fig. 3, the intermediate zone shown by the broken line in the figure, and straight line 'II'. It is evident that straight line 'I' represents the behavior of the passive surface of the iron; since the resistance of this resting surface of the system is high (2 to 10 kilohms), the slope of this line is not clear in the figure. The intermediate portion of the current-voltage relation was labile and it was difficult to obtain consistent readings by repeating the measurement. This lability in the intermediate zone will be discussed in the next section.

Undoubtedly straight line 'II' represents the behavior of the activated state

of the electrode system. When the amplitude of the rectangular clamping pulse was larger than about 1 volt, the sudden jump in the current trace (following the capacitive surge) was plotted against the voltage. This plot yielded an approximately linear extension of straight line 'II' in the region of high voltages. In some cases, however, there was a considerable divergence of the straight line in this region. (This divergence may be attributed to the violent chemical reaction on the surface of the electrode which can be observed in this high voltage range.) The resistance of the system determined from the slope of straight line 'II' was 4 to 5 ohms for the surface of approximately 1 mm.² An appreciable fraction of this resistance is considered to represent the resistance of the nitric acid between the potential electrode and the surface of the iron.

Bonhoeffer (3) and Franck (7) regard the level of the resting potential in this electrode system, E_I , as representing the oxidation-reduction potential determined by the concentration ratio of the nitric acid to the nitrous acid. The peak potential of the response in this system E_{II} , is determined by the concentration of the ferrous ion in the immediate vicinity of the surface of the iron. The two straight lines, 'I' and 'II' in the figure, can then be expressed formally by the equations

$$I = g_I(V - E_I) \quad (1)$$

and

$$I = g_{II}(V - E_{II}) \quad (2)$$

in which g_I and g_{II} represent the conductance of the electrode system in the passive and active states respectively.

The mechanism of the fall of potential in the descending phase of the electric response is not altogether clear. Bonhoeffer (3, p. 79) mentions that it is probably connected with the generation of nitrous acid during activity affecting the surface of the iron electrode. Accumulation of nitrous acid in the medium is expected to alter the conductance of the system gradually, because the polarizability of the system is reduced by the presence of nitrous acid in nitric acid (3). (With the present experimental arrangement, the chemical product from the surface of the iron may also affect the surface of the potential electrode.) From these arguments, it follows that both g_{II} and E_{II} should vary gradually when the potential V is maintained at a high constant level. The gradual change in the current during the early period of maintained V can therefore be ascribed to such gradual changes in g_{II} and E_{II} . In the later period I can change with time because of gradual repassivation of the surface.

3. Discrete and Repetitive Phenomena in the Iron-Nitric Acid System.—When the amplitude of the clamping voltage pulse was far below the threshold level (*i.e.* when it is less than about 0.1 volt), the iron electrode system was

found to behave roughly like an ohmic resistor (with some parallel capacity). With slightly stronger clamping pulses, a phenomenon which corresponds to the delayed rectification in the nerve membrane was observed: the current intensity was found to increase gradually under voltage clamp (see the record in Fig. 3, left), while a clamping pulse of the same amplitude but of the opposite polarity might give rise to a weak, roughly constant current.

When the clamping level was very close to the threshold potential, records were often obtained showing variable, subthreshold inward current under voltage clamp which resemble those in the squid axon (21). (Note that in record A in Fig. 2 the current trace goes below the base line near the beginning of the rectangular clamping pulse.)

Clear inward currents appeared always in a discrete fashion. When the amplitude of the clamping pulse was around 0.5 volt, inward surges of currents of the order of 20 to 50 ma. could be observed through the surface area of about 1 mm.² But, when pulses of the same amplitude were repeated at an interval of 5 minutes, records showing no or very small inward current (less than about 5 ma.) could also be obtained. There was a distinct threshold under the voltage clamp conditions, although the threshold might fluctuate to some extent when the clamping pulses were repeated. At threshold the latency (*i.e.* the time from the start of the rectangular pulse to the beginning of the discrete inward current) showed a large variation. The longest latency observed was around 0.5 second at room temperature.

In the range of pulse amplitude between the threshold for a discrete inward current and about 0.1 volt above it, multiple inward surges were frequently observed during one period of a clamping pulse. Within this range of voltage, the frequency of repetition of inward surges tended to be higher for higher pulse amplitudes (see records B and C in Fig. 2). When the frequency of repetition was higher, the amplitude of the individual surges decreased more rapidly with time. The interval between individual surges varied between 0.15 and 0.3 second in most of our experiments. Between these individual surges there was a period during which there was no appreciable current. Sometimes the rhythm of the repetitive surge was irregular; on a few occasions a regular alternation of large and small surges was observed. In all cases, the rhythm of repetitive surges under voltage clamp was far higher than that of repetitive firing under constant current conditions.

All the characteristics of the discrete and repetitive phenomena in the iron-nitric acid system under voltage clamp closely resemble those of the corresponding phenomena in the nerve membrane, particularly in the squid axon treated with TEA (20, 21).

By direct visual observations of the surface of the iron in the nitric acid under a dissecting microscope (40 ×), it was found that small inward surges of currents in the intermediate zone derive from activation of restricted areas

on the metal surface. The resting surface of the metal was silvery white. When the surface was activated, there was a transient darkening of the surface, sometimes leaving a brownish color for a considerable period of time. When the amplitude of the inward surge was distinctly smaller than the maximum (in clamping with a pulse of approximately 0.5 volt), there was a clearly discernible demarcation line between the activated and non-activated area. The activated area was usually circular. There was a close proportionality between the darkened area and the amplitude of the observed inward current. When the inward current gradually increased with time, there was a gradual expansion of the dark area.

The results of these visual observations prove beyond any reasonable doubt that the intermediate zone of the current-voltage relation represents a gradual increase in the surface area activated by the clamping pulses. An entirely analogous situation has been demonstrated in the squid axon membrane (20).

At the boundary of an active patch surrounded by the resting area, the potential drop across the surface layer of the iron electrode has to be continuous. The potential drop at the margin of an expanding active patch should be around 0.5 volt in the present system (see Fig. 3). On the resting side of the margin, the surface layer should be traversed by an "outward" current compatible with Equation 1 in the preceding section. On the active side of the margin, there should be an "inward" current strong enough to lower the voltage (V in Equation 2) to the same level as on the resting side. During a transient period, the surface density of an inward current in a limited area can be somewhat greater than the maximum value shown in Fig. 3, since the maximum in this figure represents the average for the whole surface layer of the iron electrode.

4. Observations on Cobalt-Hydrochloric Acid System.—Franck (7) reported that a cobalt wire immersed in a mixture of a chromium trioxide solution and hydrochloric acid can be used as a model of the excitable tissue (*cf.* also reference 17). The arrangement used for the following observations on the cobalt electrode system was the same as that used for the iron-nitric acid system (Fig. 1). A piece of cobalt wire was inclosed in a glass tubing exposing a portion of about 0.5 mm. in a bath of 2 N hydrochloric acid. When a small amount of 1 M CrO_3 solution was added to the hydrochloric acid, the potential of the fluid in the beaker started to oscillate. (At this moment, the current electrode was not connected to the feedback amplifier.) On further addition of CrO_3 into the beaker, there was an increase in the amplitude of the oscillatory potential with occasional firing of full sized responses (see Fig. 4, left top). Similar records have been obtained by Heathcote (see Fig. 22 in reference 9) in the iron-nitric acid system more than 50 years ago. The analogous phenomenon in the nerve membrane has been observed by many investigators, notably by Arvanitaki (1).

A further addition of CrO_3 into the beaker increased the frequency of firing. Addition of more CrO_3 resulted in an increase in the "active period" of each response. Finally, a stage was reached in which the original "active" phase was so long compared with the "resting" period that the polarity of the "response" appeared to be reversed (see record in Fig. 4, top right). Spontaneously induced responses at this stage were also preceded by small oscillatory potential variations; this subthreshold oscillation was smaller in amplitude than that observed when the concentration of CrO_3 was low, but its general character was similar.

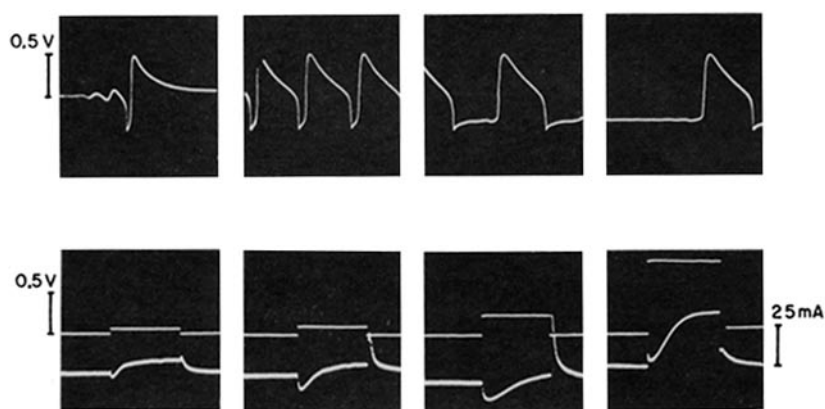


FIG. 4. Top: Gradual change in the pattern of repetitive firing of responses of the cobalt electrode system (under unclamped conditions) caused by successive addition of CrO_3 . Bottom: Voltage clamp behavior of the cobalt electrode system.

As is well known, CrO_3 is a strong oxidizing agent. The progressive change in the pattern of potential variations shown in Fig. 4, top, can be interpreted as due to increasing stabilization of the cobalt electrode system in the oxidized state with successive additions of CrO_3 in the presence of chloride ions. These records illustrate that, depending upon the relative length of time during which the system is either in the reduced or oxidized state, the response can either be "depolarizing" or "hyperpolarizing."

The records in the lower row in Fig. 4 were taken after the last records in the upper row had been made. The current electrode now was connected to the output of the feedback amplifier (A_2 in Fig. 1) to clamp the potential at the lower (resting) level. Then, the currents necessary to alter the potential difference across the surface layer of the cobalt along rectangular courses were determined by the procedure described under Methods.

Under the present experimental conditions, the voltage clamp behavior of the cobalt electrode system was very different from that of the iron electrode

system. The current observed was found to be a continuous function of the amplitude of the clamping voltage pulse. As can be seen in Fig. 4, bottom, the peak amplitude of the inward current first increased continuously with increasing pulse amplitude. It reached a flat maximum in the region 30 to 50 mv. above the resting level. On further increase in the amplitude of the clamping pulse, the inward current decreased with the pulse amplitude.

When the polarity of the clamping pulse was reversed, namely, when the fluid in the beaker was made more negative by the rectangular pulse, a new phenomenon was encountered. At pulse amplitudes less than about 20 mv., a transient outward current followed by a long inward current could be observed following the start of the pulse. Such a transient outward current resembled in its time course the outward current seen at the end of the rectangular pulses in the middle two records of Fig. 4, bottom. With larger negative clamping pulses, there were inward currents which quickly reached their large final intensity.

The voltage clamp behavior of the cobalt electrode system can be explained in the following manner: When a piece of cobalt is immersed in a mixture of CrO_3 and hydrochloric acid, there are continuous chemical reactions on the surface of the metal. (Note that the corrosion of the cobalt electrode is much faster than that of the iron electrode.) As a consequence, the metal surface is covered by patches in the reduced state mixed with the remaining oxidized area. When the potential of the electrode system is clamped at the resting (lower) level, the metal surface is still in such a mixed state. When the clamping level of the potential is altered by a rectangular voltage pulse, the proportion of the active surface area to the resting changes rapidly.

Let α denote the active portion in the unit surface area. Let g_I and E_I be the conductance and the effective E.M.F. of the system in the resting state and let g_{II} and E_{II} be the corresponding figures in the active state. Then, the relation between the current, I , and the voltage, V , of the system is given formally by

$$I = (1 - \alpha)g_I(V - E_I) + \alpha g_{II}(V - E_{II}). \quad (3)$$

When the voltage V was clamped at the resting level, the total current I is zero. (Note that $0 < \alpha < 1$ and $E_I < V < E_{II}$ under these conditions.) When the voltage is suddenly shifted from V to $V + \Delta V$, the current should be altered by the amount given by

$$\Delta I = [g_I(1 - \alpha) + \alpha g_{II}]\Delta V - [g_I(V - E_I) + g_{II}(E_{II} - V)] \frac{d\alpha}{dV} \Delta V. \quad (4)$$

Since α is a monotonically increasing function of V , the second term in this expression for ΔI can give rise to transient currents flowing in the direction opposite to what is expected from Ohm's law. The transient deflections of the current trace following the start and the end of the clamping pulses in the

two middle records of Fig. 4, bottom, can therefore be explained as indicating that the second (negative) term in Equation 4, right, is larger than the first (positive) term in these cases.

One of the major differences in the behavior between the iron electrode system and the cobalt system appears to be that g_{II} is far greater than g_I in the iron system while in the cobalt system g_{II} is not much different from g_I . The previous A.C. impedance measurement on the cobalt system showed that the difference in the two conductances is only 30 to 40 per cent. This may partly account for the differences observed. In addition, however, it should be kept in mind that the chemistry of the cobalt-chromic acid system is quite different from that of iron. Depending upon the potential level more than just one type of chromium compound can cover the surface of the cobalt.

In the cobalt electrode system, oscillatory membrane currents under voltage clamp were observed when the duration of the clamping pulse was relatively short. Using clamping pulses of about 0.03 volt in amplitude and 0.2 to 0.5 second in duration, we found that the membrane current often oscillates (at a frequency of 5 to 10 cycles per second) in the period following the end of the clamping pulse during which the potential is clamped at the initial (resting) level. We interpret this phenomenon as a sign of synchronization of the incessant transitions between active and resting states at individual spots on the surface of the cobalt.

DISCUSSION

It is evident from what has been mentioned under Results that there is striking analogy between the electrical behavior of the nerve membrane and the behavior of the iron or cobalt electrode system. Although the actual electrochemical reactions involved are obviously different in these cases, the kinetics of the process proposed for one system can be applied to the other system to explain the corresponding phenomena. In fact, the explanations of the observations presented in this paper are based on the concepts which have been found useful for the explanation of the behavior of the nerve membrane.

It should be emphasized in this connection that the analogy between the nerve membrane and the metal electrode systems is based on the existence of the *eddy currents* between the active and resting surface areas. Obviously, the difference in the (effective) E.M.F. of the system between the active and resting states is the cause of the eddies. The current intensity of these eddies depends also on the surface conductances in the active and the resting states. When the conductance in the active state is larger than that in the resting state, the currents through small active spots in the membrane should tend to activate the neighboring area and to form larger active patches. The appearance of an active patch would tend to throw the whole area of the membrane into the active state if there were no powerful interference arising from the experi-

mental arrangement. The voltage clamp arrangement is naturally a very effective device for suppressing the process of activation of the resting surface by the eddy currents.

Active patches have been demonstrated both in the squid axon (20) and in the iron-nitric acid system (see Results) under the so called voltage clamp conditions. The demonstration of smaller active spots in the nerve membrane is somewhat indirect; it is based on the observation that a discrete inward surge under voltage clamp (appearance of a patch) is preceded by a graded inward current which can be strongly suppressed by low sodium or by narcosis (21). In the inanimate systems, the existence of microscopic active spots has been proven beyond any reasonable doubt. Stephenson and Bartlett (16) demonstrated spotwise passivation and activation of the smooth surface of chemically pure copper immersed in hydrochloric acid. In a personal communication, Matsumoto and his coworkers in Japan demonstrated microscopically observable active spots on the surface of iron in nitric acid by an ingenious chemical technique. Since this observation by Matsumoto and his coworkers has important bearing upon the problem to be discussed later, we shall discuss their results in some detail.

A small piece of iron which had been coated with an oxide layer was immersed in a mixture of nitric acid and potassium ferricyanide. The silvery surface of the iron in the mixture was examined under a microscope (50 to 100 \times). When a weak stimulating current pulse was applied to the iron, small blue spots, randomly scattered over the entire surface, were seen under the microscope. Undoubtedly, appearance of these blue spots is the sign of a chemical reaction between the ferricyanide in the solution and the ferrous ions released from the active spots. The number of these spots was found to increase with increasing stimulus intensity. When the density of these spots reached a certain level, a violent reaction started over the entire surface, turning the silvery color of the surface into deep blue; at this moment a full sized action potential was developed.

Morphologically speaking, the nerve membrane is not uniform nor smooth. Furthermore, at the molecular level, changes in the membrane (caused by a stimulating current) have to be discrete and all-or-none in character. It would not seem unreasonable to assume that similar spotwise activation can occur in the excitable nerve membrane. A somewhat similar view has been expressed by Grundfest (8) in his discussion of the general properties of the electrically excitable membrane.

Historically speaking, the concept of a subthreshold response originates with Rushton (14) who argued, based on the idea of restimulation by eddy currents, that an active process limited in a small area of the membrane should fail to excite the neighboring resting region. Rushton's original concept was once abandoned, when subthreshold phenomena were demonstrated in a macroscopically uniform (spatially clamped) piece of membrane (11). The argument developed above can be regarded as a modification of Rushton's concept with an additional assumption of spotwise activation of the excitable membrane.

From the thermodynamical point of view, a living nerve fiber is an open system; there is a continuous exchange of matter between the axoplasm and the surrounding medium. The process of action potential production is associated with various irreversible phenomena, such as heat production, diffusion, chemical reactions, etc. We made an attempt to interpret the phenomenon of excitation in the nerve membrane, as well as that in the iron-nitric acid system, in terms of the modern thermodynamical theory of irreversible processes (13, 6).

Let us consider, in the first place, the case in which a piece of excitable membrane is traversed by a short pulse of stimulating current. At the moment when the current pulse comes to an end, there will be a number of active spots in the membrane. The membrane potential at this moment will be given by the value of V in Equation 3 into which the condition $I = 0$ is introduced. Substituting this value of V in the last term in Equation 3, it is found that the total eddy current, i , is given by

$$i = \frac{(1 - \alpha)g_I \alpha g_{II}(E_{II} - E_I)}{(1 - \alpha)g_I + \alpha g_{II}}, \quad (5)$$

in which α is the active fraction and the other terms represent the conductances and the (effective) E.M.F. in the two states, resting and active. Equation 5 shows how the eddy current varies with α . When the entire membrane is at rest, *i.e.* when $\alpha = 0$, its intensity is zero. When α is small, the eddy current increases with an increase in α . The intensity reaches a maximum when $\alpha = 1/(1 + \sqrt{g_{II}/g_I})$, and then it decreases with increasing α .

The next problem is to estimate the rate of increase in the entropy of the whole system associated with the irreversible processes caused by the eddy current. It is simple to calculate Joule's heat caused by the current; the entropy production due to this heat generation shows a single maximum at a definite value of α . The entropy production arising from the electrochemical reaction in the resting area (which tends to increase α) is proportional to the product of the electrochemical affinity of the reaction, A_I , and the current intensity i (see p. 133 in reference 6). If A_I is independent of i , the entropy production by this reaction should reach a maximum at the value of α giving the maximum of i . (It is possible that A_I increases with decreasing i , since the affinity is defined as a measure of the remoteness from equilibrium.) In an analogous manner, the entropy production at the active spots should show a single maximum approximately at the value of α for the maximal i . It is therefore evident that the total entropy production is a function of α with a single maximum at a certain finite value of α ; we denote this value of α which gives rise to a largest entropy production by α_m .

According to the thermodynamics of irreversible processes (see p. 200 in reference 6), a system tends to undergo an evolution towards a state of lowest

entropy production compatible with the conditions imposed upon the system (uniform temperature, zero membrane current, etc., in the present case). If the value of α at the moment of cessation of the applied current is smaller than the critical value α_m , it is expected that the system undergoes an evolution toward the resting state. If the stimulating current is strong enough to raise the active fraction at the end of the pulse above the critical value α_m , on the contrary, the subsequent evolution of the system should be toward the uniformly active state. The threshold, therefore, corresponds to the state in which the entropy production associated with the eddy current is maximal. It is thus found that the formal, quasi-quantitative argument developed above gives us a reasonable explanation of the mechanism of nerve excitation.

In an entirely analogous manner, the phenomenon of abolition of an action potential can be interpreted as a transition from the upper stable state (designated by the suffix II) to the lower (I). Like the process of initiation of an action potential, abolition is an all-or-none phenomenon. It is known that, in the early phase of an action potential, the threshold membrane potential for abolition roughly coincides with the threshold potential for initiation (*cf.* p. 871 in reference 19). This fact is consistent with the present hypothesis, because there is no change in the constants in Equation 5 characterizing the two states (g 's and E 's) at this moment. The subsequent gradual rise in the threshold membrane potential for abolition is due to the slow fall in g_{II} during activity (and probably to other factors).

It seems possible to expand this formal argument to explain other phenomena known to occur in the nerve membrane. In the argument above, it was not necessary to make any assumption other than the assumptions inherent in the postulates of the existence of two stable states in the membrane.

Finally, we shall comment on the main difference between the "two stable state hypothesis" discussed above and the generally accepted theory of excitation proposed by Hodgkin and Huxley (10). As has been pointed out previously (19), the equation dealing with the relation between the membrane current and the voltage, Equation 3 in the present paper, is analogous to that employed in the sodium theory. Thus, E_I in the present paper represents the resting potential across the nerve membrane which is known to be close in the diffusion potential for potassium ions. Similarly, E_{II} represents the e.m.f. of the active membrane which can be strongly affected by the sodium concentration outside the membrane. However, g_{II} does not necessarily represent a conductance for any special ion species, because the transition to the active state is assumed to be caused by a "reaction" in the membrane (analogous to reduction of the oxide layer on the surface of iron by an electric current). Another important feature in the present hypothesis is that not only g_{II} but also E_{II} can undergo a gradual change during activity, because it does not represent an equilibrium potential for any specific ion species. In the present

hypothesis, we are dealing essentially with a discontinuous system; the voltage clamp experiments, therefore, do not play as critical a role as they did in the formulation of the sodium theory.

The arguments developed above, based on the analogy between the electric behavior of the iron electrode system and that of the nerve membrane, do not of course prove the validity of the present hypothesis. We simply stress that none of the known experimental facts on the nerve membrane conflicts with the hypothesis mentioned above.

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