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DEMONSTRATION OF TWO STABLE STATES OF THE NERVE MEMBRANE IN POTASSIUM-RICH MEDIA

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This paper deals primarily with the current–voltage relationship of the nerve membrane immersed in potassium-rich media. The existence of a discontinuous or threshold phenomenon in the nerve membrane which had been rendered 'inexcitable' by potassium has been reported recently by three independent workers, namely by Stämpfli (1958) and Mueller (1958*a*, *b*) in the frog nerve fibre, and by Segal (1958) in the squid giant axon. The essential feature of the phenomenon may be illustrated by Segal's observation on squid axons immersed in a solution containing 100 or 400 mM potassium acetate and 0·6 mM potassium bicarbonate, made isotonic by adding sucrose. Upon application of anodal (hyperpolarizing) current pulses above a certain 'threshold' intensity, the axons were found to develop large 'hyperpolarizing responses'. These responses could be 'abolished' by cathodal current pulses and were followed by a kind of refractoriness.

Stämpfli regarded this phenomenon as having been expected from the sodium theory (Hodgkin & Huxley, 1952): the discontinuity was explained as possibly due to the shift of the membrane potential from the 'equilibrium level' determined by potassium ions to the level determined by chloride ions. Obviously, this explanation is not quite consistent with Segal's results on the squid axon, in which the discontinuity was shown in the absence of chloride in the medium. Furthermore, the hyperpolarizing response reported by Segal was associated with a large change in the membrane conductance, while the discontinuity observed by Stämpfli was attributed to a change in the e.m.f. of the membrane.

Mueller was interested mainly in demonstration of long 'depolarizing responses' in the absence of sodium in the medium. His explanation was based on the assumption that the action potential is generated by chemical processes, the equilibrium of which is affected by the membrane potential (Mueller, 1958*b*).

The main purpose of the present investigation was to analyse the mechanism underlying this discontinuous phenomenon in the squid giant axon and in the node of Ranvier of the toad myelinated nerve fibre. As the analysis of the data progressed, it became evident that this and other allied phenomena can best be interpreted on the basis of the two-stable-state hypothesis (Tasaki & Hagiwara, 1957; Tasaki & Spyropoulos, 1958). In this hypothesis it is assumed that the nerve membrane is capable of undergoing a reversible physico-chemical change which gives two stable configurations. The process of initiation and abolition of the action potential is interpreted as transitions between the two stable states of the membrane (Tasaki, 1956). The present analysis indicates that the nerve membrane immersed in potassium-rich media is in the 'upper stable state' of the membrane, which normally represents the excited state, and that anodal current pulses are capable of inducing transitions to the 'lower stable state' of the membrane.

In the Appendix of this paper it will be shown that 'hyperpolarizing responses' similar to those in the nerve membrane can be demonstrated in the electro-chemical model of the two stable states, namely in the iron–nitric acid or iron–sulphuric acid systems.

METHODS

Observations on squid giant axons. The material used was North Atlantic squid, *Loligo pealii*, available at the Marine Biological Laboratory at Woods Hole. A giant axon 0·4–0·6 mm in diameter and approximately 40 mm in length was partially cleaned after removal from the body of the animal. The axon was then mounted horizontally on a glass plate about 35 mm wide. A set of electrodes consisting of two silver wires each 50 μ in diameter was introduced into the axon by the technique described previously (Tasaki & Hagiwara, 1957). One of the wires had an exposed surface extending over 17 mm of its length, and the other had a bare region of 1 mm in the middle of the bare surface of the other wire. The wire with the long exposed surface was used to apply pulses of constant current to the axon and the other wire with the short exposed surface was used to record the potential difference across the membrane.

Circuit *A* in Fig. 1 was used to measure the membrane impedance at the same time as the membrane potential. This circuit is an a.c. Wheatstone bridge, the surface membrane of the axon comprising one arm. The terminals shown at the top of the figure were connected to the sources of rectangular voltage pulses (Tektronix pulse generator Type 161) and of sinusoidal waves (General Radio beat-frequency oscillator Model 130 XA). Resistance R_1 in the ratio arm was 0·5 MΩ; this high resistance served to keep the membrane current constant when the membrane resistance varied during the period of current flow. The other resistance, R_2 in the figure, was 50 kΩ, the ratio of R_1 to R_2 thus being 10. The variable resistance, R_p, was an ordinary decade resistance box (General Radio Type 1432-N) and the variable capacitor varied between 0·01 and 10 μF.

The output of the bridge (*Z* in the figure) was amplified with a differential amplifier (Tektronix type 122, operated at a gain of 100) used in conjunction with paired cathode-follower stages. The frequency of the bridge a.c. was between 0·5 and 6 kc/s. The amplitude of the bridge a.c. across the axon membrane was not allowed to go above 1–2 mV. The non-sinusoidal components in the impedance bridge output were eliminated by using an electronic filter (Spencer Kennedy Laboratory, Model 302) tuned to the bridge frequency. The simultaneous record of the membrane potential (*V*) was obtained by connecting the output of one of the cathode followers directly to the input of an oscilloscope.

When a simultaneous recording of the membrane potential and the current was required, a small resistor (approximately 5 Ω) was inserted between ground and the large electrode in the surrounding sea water; the *IR* drop across this resistor was taken as the measure of the membrane current. In such cases, a second Ag–AgCl (agar) electrode was introduced into the surrounding sea water for differential recording of the membrane potential.

Photographic records of the membrane current, the impedance and the potential were made with a DuMont dual-beam oscilloscope and a Grass camera at room temperature (21–22° C).

Observations on toad single node preparations. The experimental arrangement used for recording potential variations across the nodal membrane of a single node preparation of the toad myelinated nerve fibre was similar to that used in a previous experiment (Tasaki & Bak, 1958). The node to be studied, N_1 in Fig. 1 *B*, was immersed in a long narrow pool of Ringer's solution which in most of the experiments was approximately 0·8 mm wide. One of the adjacent nodes, N_0, was in a large

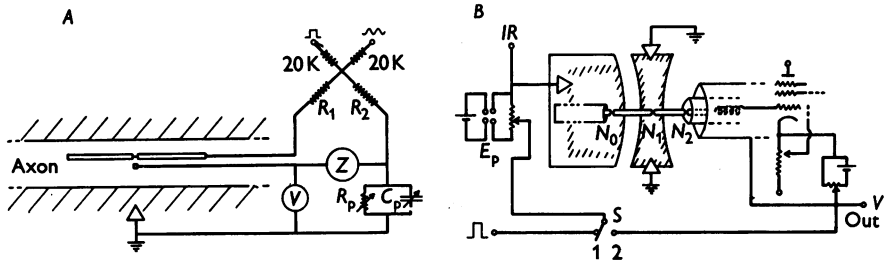

Fig. 1. *A.* Arrangement used for impedance measurements on the squid giant axon treated with potassium-rich solutions. The drawings of the axon and of the internal electrodes are not to scale. The exposed surfaces of the internal electrodes are shown as thicker portions inside the axon. For further description see text.

B. Arrangement used to record potential variations at node of Ranvier, N_1, of a toad myelinated nerve fibre. The portions including N_0 or N_2 are in a dilute cocaine-Ringer solution. *V* indicates the output of a unity-gain cathode-follower. The potential level of the terminal labelled '*IR*' was taken as a measure of the current applied to the node N_1. E_p consists of a 1·5 V battery and a Helipot potentiometer. Position (1) of switch *S* was used to apply rectangular current pulses to the node, and position (2) to record the variation of the resting potential of the node.

pool filled with 0·2 % cocaine-Ringer solution. Node N_2, on the other side of N_1, was in the small pool of Ringer's solution (usually containing 0·05 % cocaine) at the tip of the grid electrode (which was made of glass tube of 3 mm inside diameter). The small middle pool was grounded with a large electrode at each end of the glass plate. The electrode in the large pool was normally connected to a source of square voltage pulses (Tektronix pulse generator). A 'polarizing circuit', E_p in the figure, was used to apply a constant current to the node under study. The length of the nerve fibre between N_0 and N_1 exposed to the air was approximately 0·2 mm and the portion in the air between N_1 and N_2 was about 0·5 mm.

A unity-gain pre-amplifier (Bak, 1958), slightly modified for the present purposes, was used throughout. Switch *S* in the figure was used to connect the output of the unity-gain pre-amplifier to the electrode in the large pool. When the potential of the large pool follows that of the grid electrode, the flow of current between N_0 and N_1 caused by a change in e.m.f. at N_1 should be almost completely suppressed. Further details of this method of measuring the resting potential will be described under Results.

Most of the experiments were carried out at room temperature (20–22° C). Since the time constant of the recording system was approximately 50 μsec with a 50 MΩ input resistance, the action potential of the node could be recorded without any appreciable loss. A Tektronix dual-beam oscilloscope (Type 551) and a Grass camera were used for recording.

The Ringer's solution used in most experiments contained (mM): NaCl 110, $KHCO_3$ 2 and $CaCl_2$ 1·5; pH adjusted to approximately 7·4, usually by addition of NaH_2PO_4. Chloride-free Ringer's solution was prepared by using sodium acetate or sodium glutamate instead of NaCl, and calcium acetate in place of $CaCl_2$ Tetraethylammonium (TEA) chloride solutions were prepared either from the commercial product 'Etamon' or by neutralizing a solution of TEA hydroxide (obtained from Eastman Organic Chemicals) with hydrochloric acid.

RESULTS

PART I. Observations on squid giant axons

Demonstration of hyperpolarizing responses

In most experiments in the present investigations, the amount of potassium ions in the medium was altered by mixing isomotic (550 mM) KCl solution with normal sea water in volume ratios of 1:6 to 6:1. Frequently axons were used into which a tetraethylammonium chloride (TEA) solution had been injected by the technique described previously (Tasaki & Hagiwara, 1957). On a few occasions, the axon was treated with a mixture of isotonic KCl solution and sodium-free sea water (in which sodium ions in artificial sea water had been replaced with choline). In these potassium-rich media 'hyperpolarizing responses' were observed whenever strong inward current pulses were applied through the axon membrane. There were, however, some quantitative differences between the responses observed under different conditions.

An example of hyperpolarizing responses obtained with the arrangement of Fig. 1*A* is presented in Fig. 2. The axon was immersed in sea water, the potassium content of which was raised to 110 mM by adding isotonic KCl to normal sea water. The Wheatstone bridge was balanced for the impedance of this partially depolarized membrane. When pulses of outwardly directed current were applied to the axon membrane, the axon showed no sign of a physiological response. In other words, the axon was found to be 'inexcitable' in the ordinary sense of the word (top records in the figure). When pulses of inwardly directed current were applied to the membrane, however, potential variations were observed which resembled ordinary (depolarizing) responses in their configuration (lower records).

The hyperpolarizing response that was obtained in 110 mM-K sea water had a more or less sharp initial peak followed by a long plateau; in this respect it resembled the (depolarizing) response of a TEA-treated axon. In all cases the initial peak was preceded by a slow gradual potential change which resembled a 'subthreshold response'. When the applied current was withdrawn, the membrane potential returned nearly to its initial level 4–8 msec after the end of the current pulse. This return of the membrane potential was preceded by a depolarizing potential variation which had all the properties (except for its smaller size) of a 'break response' in a normal axon. Some details of the potential changes at the end of the applied current pulse will be discussed in the following pages.

Simultaneous impedance measurements indicated, in agreement with Segal's observation (1958), that there was a change in the membrane impedance associated with a hyperpolarizing response. The Wheatstone bridge, balanced for the membrane impedance at rest, went off balance when there was a sub-threshold or a full-sized hyperpolarizing response. The initial peak in the response was associated with a reduction in the width of the impedance trace, indicating that the membrane impedance tends to return towards its normal value at this moment.

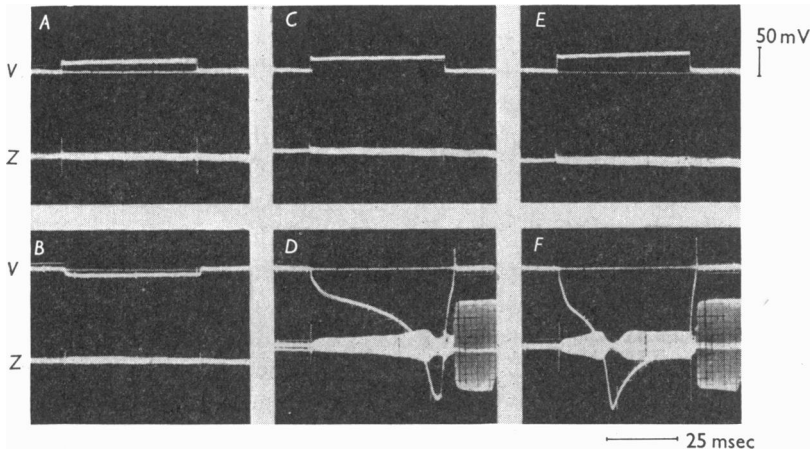

Fig. 2. Simultaneous recording of the change in the membrane impedance (trace Z) and the membrane potential (V) obtained from a squid giant axon immersed in a mixture of one part of isosmotic KCl solution and approximately 4 parts of normal sea water. The impedance bridge of Fig. 1 A was used. The bridge a.c. frequency was 6 kc/s, and the bridge was balanced for the impedance of the membrane at rest ($C_p = 2{\cdot}6\ \mu F$, $R_p = 20\ \Omega$, $R_1 = 500\ k\Omega$ and $R_2 = 50\ k\Omega$). The intensities of the applied current pulses (in μA) were: in record A +30 (cathodal), in B −15 (anodal), in C +40, in D −35, in E +50, and in F −45, respectively.

When the membrane potential returned to the original level after the end of the applied pulse, there was a marked bridge unbalance which lasted for 1–3 min. It was easy to show that this marked bridge unbalance represented a decrease in the membrane impedance. During this prolonged period of decreased membrane impedance, the membrane was 'relatively refractory' to a second pulse of inward current (Segal, 1958); in other words, no hyperpolarizing response could be obtained unless much stronger pulses were used for stimulation.

It was found, by means of trials repeated at intervals of 2–3 min, that the membrane impedance was increased during the hyperpolarizing response. Except at (or near) the peak of the hyperpolarizing response, the increase in the parallel resistance (namely, the value of R_p giving a perfect bridge balance)

was 1·2–3 times. (This impedance measurement was complicated by the finding that the parallel capacity, C_p in the figure, increased slightly during the hyperpolarizing response; however, the increase in R_p was large enough to raise the total impedance above the resting level.) When a hyperpolarizing response was preceded by a long 'subthreshold response' (as in record *D* in Fig. 2), the peak of the response was smooth and the membrane impedance showed little (or sometimes no) tendency to return to the original value at this moment.

Further analysis of the behaviour of TEA-treated axons

The hyperpolarizing response described in the preceding section differs from the ordinary (depolarizing) response in the following respects. It not only has an opposite polarity, but also has the property that the response can be maintained as long as the stimulating (anodal) current lasts. In a previous investigation (Tasaki & Hagiwara, 1957), it was recognized that the response of a TEA-treated axon could be maintained practically indefinitely by passing a weak *outward* current through the membrane. An example of this type of observation is reproduced in Fig. 3.

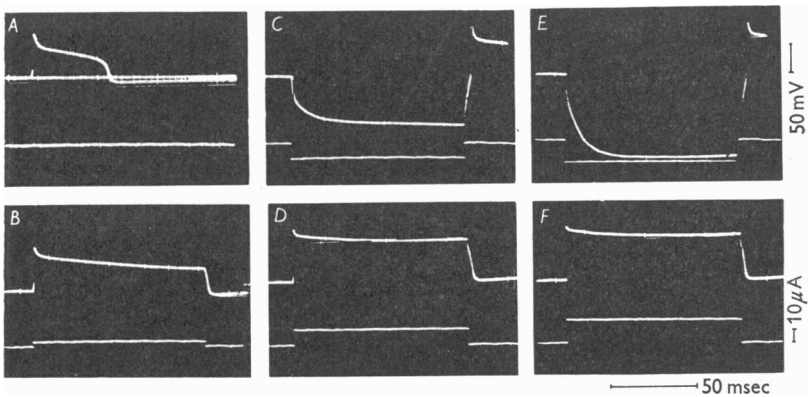

Fig. 3. Simultaneous recording of the membrane potential (upper trace) and the membrane current (lower trace) in a squid giant axon treated with an intracellular injection of tetraethylammonium chloride (TEA). The axon was immersed in normal sea water. The internal current electrode was 17 mm long and the axon diameter was 550 μ.

In this experiment, approximately 3 mm^3 of isosmotic TEA chloride solution (stained with chlorphenol red) was injected into the entire length of a squid axon 35 mm long. This resulted in a prolongation of the response of the axon (evoked by a short cathodal pulse) to approximately 50 msec. (Note that the duration of the response of a normal squid axon, excluding the 'undershoot', is 0·6–0·7 msec at room temperature.) When a constant current of about 10 μA/cm^2 was sent outward through the membrane of such an axon, the

plateau of the response was prolonged further and in many cases the plateau level was maintained until the membrane current was turned off (see the lower row in Fig. 3).

When the amount of TEA injected into the axon was insufficient, the prolongation of the response was less marked than in that shown in Fig. 3, and a weak outward current could not maintain the plateau indefinitely. In such axons a stronger outward current evoked a train of responses of which the first member was much longer in duration than the rest. Obviously, the behaviour of such axons is intermediate between the normal and heavily-TEA-treated axons.

In TEA-treated axons immersed in normal sea water, application of *inward* currents through the membrane did not bring about any discontinuous potential variations; the membrane potential changed continuously with increasing intensity of the applied current (Fig. 3, records *C* and *E*). When a hyperpolarizing current pulse was suddenly withdrawn, there was always a 'break response'.

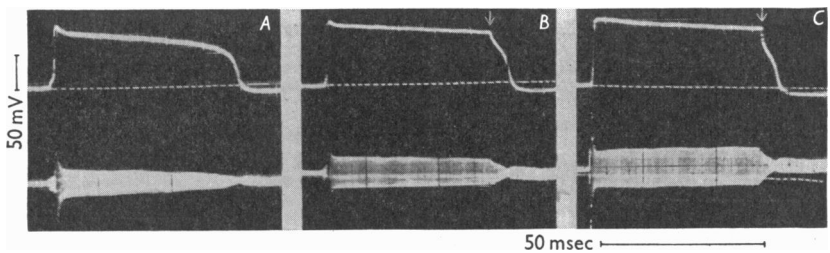

Fig. 4. *A*. Change in the membrane impedance associated with a prolonged response in a squid giant axon treated with TEA (evoked by a short stimulating pulse). *B* and *C*. The effect of a constant cathodal current pulse of 50 msec duration upon the axon; the current intensity used was approximately (in *B*) 18 and (in *C*) 75 μA/cm^2. The bridge a.c. frequency was 5 kc/s and the balance was obtained with R_p in Fig. 1 set at 22·5 Ω and C_p at 2·56 μF.

With the arrangement of Fig. 1*A*, the impedance of the membrane of TEA-treated axons was measured under various experimental conditions. Figure 4 shows the behaviour of the membrane impedance of an axon whose response was modified by a long outward membrane current. Record *A* shows that a response evoked by a short cathodal pulse is associated with a large impedance loss during the initial peak, followed by a rather small impedance loss during the plateau. (The large bridge unbalance during the initial peak cannot be seen in the figure because the excursion of the beam is too large.) When the potential level during the plateau was raised by an outward current of 25–100 μA/cm^2, there was an increase in the bridge unbalance during the plateau, indicating that the impedance loss was augmented by the applied current.

It is seen in record *C* of Fig. 4 that upon withdrawal of the applied current

(marked by the arrow) the membrane potential starts to fall immediately. Following this rapid fall, the membrane potential stays at about the level of the terminal portion of the plateau for a period of a few milliseconds before it falls another step to reach the level of the 'undershoot'. It is obvious that the first step in the potential fall is due to the disappearance of the IR-drop on withdrawal of the applied current. In other words, the potential level which is attained after the first fall represents the 'effective e.m.f.' of the membrane which is still in the active state.

The preferential use of the term 'effective e.m.f.' in place of the more commonly employed term 'equilibrium potential' is prompted by the reticence to consider the nerve as being in the state of thermodynamical (or rather thermostatic) equilibrium. The expression 'effective e.m.f.' when employed in the present treatment indicates the potential difference across the membrane less the IR-drop; it may be regarded, in the case mentioned above, as corresponding approximately to the weighted average of the sodium and potassium equilibrium potentials,

$$(g_K E_K + g_{Na} E_{Na})/(g_K + g_{Na}),$$

in the sodium theory (Hodgkin & Huxley, 1952).

It has been pointed out in previous papers (Tasaki & Hagiwara, 1957; Tasaki & Spyropoulos, 1958) that the phenomena of initiation and abolition of an action potential can be regarded as transitions between two stable states of the membrane. (A state is called 'stable' when the membrane returns directly to its original state, after a weak perturbing pulse causing a small passive change in the membrane is withdrawn.) When the stability of the membrane state was examined by passing current pulses of various intensities and polarities, it was found that there are in the membrane only two 'stable states'. The effective e.m.f.'s and the conductances in these two states are labelled E_1, g_1, E_2, g_2, respectively. Although these values characterizing the states of the membrane vary in general with time, a transition between the state indicated by (E_1, g_1) and that represented by (E_2, g_2) in every spot in the membrane is considered as a discontinuous phenomenon.

With the terminology mentioned above, the results shown in Fig. 4 can be restated in the following manner. At the end of the plateau in record C, the membrane potential is given by the sum of E_2 and i/g_2, where i is the current sent through the membrane. When the current is turned off, the membrane potential approaches the value E_2 with the time constant of the membrane. Later, there is a transition from potential E_2 to E_1. The gradual change in the membrane potential in this transition is interpreted as due to the presence of mixed states, in which some part of the membrane is in one state and the remaining part in the other state.

Similarly, the break excitation shown in records C and E in Fig. 3 can be expressed in the following manner. During the period of inward current flow, the axon membrane is still in the lower stable state characterized by E_1, g_1 As is shown by the records of Fig. 5, the membrane impedance does not show

any large change on sudden withdrawal of the inward current, indicating that the potential variation at this moment is due to a rapid loss of IR-drop. A transition from the lower to the upper state takes place when the membrane potential starts to rise rapidly again; there is a large change in the membrane e.m.f. and in the conductance at this moment. The formation of a peak and the subsequent potential variation are regarded as transient (i.e. non-stationary) phenomena. This is discussed below.

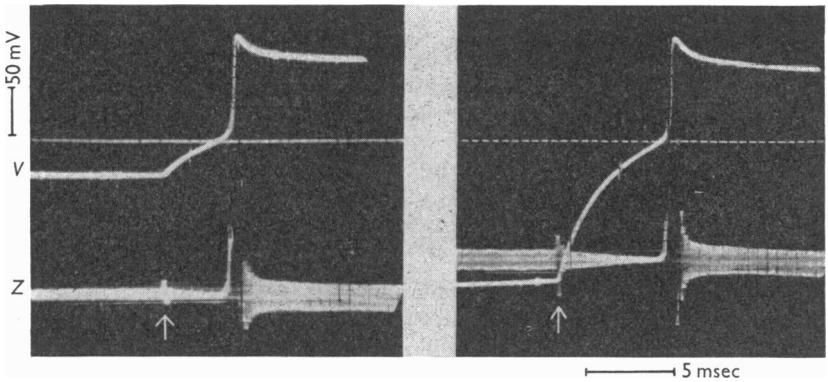

Fig. 5. Break responses at the end of anodal current pulses of 40 msec duration. The bridge frequency was 6 kc/s, and the balance of the membrane at rest was obtained with C_p of 2·5 μF and R_p of 22·5 Ω. The end of the anodal pulse is marked with arrows. The axon was treated with TEA (intracellular injection) and was kept in normal sea water.

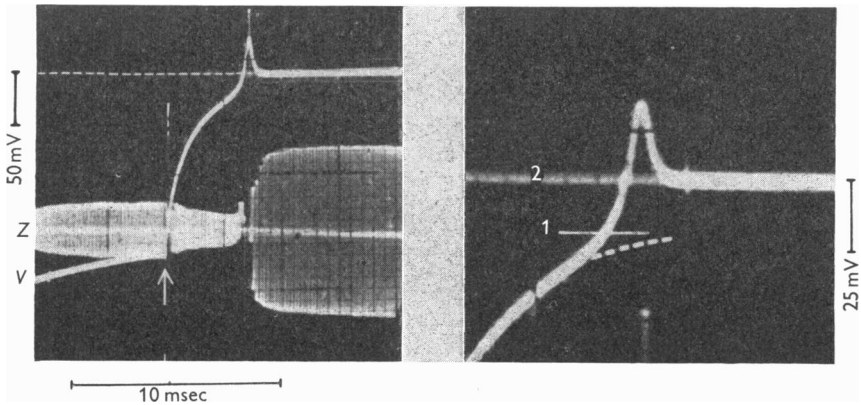

Fig. 6. Demonstration of the two steps in the membrane potential (V) and the change in the membrane impedance (Z) following withdrawal of an anodal current pulse which evoked a hyperpolarizing response. The record on the right is an enlarged photograph taken from the same axon but at a different time. The horizontal line marked '1' represents the asymptote of the first step in the potential variation. The line marked '2' shows the initial level of the membrane potential. The axon was in sea water containing 110 mM-KCl with no previous treatment with TEA. The bridge a.c. frequency was 4 kc/s.

Comparison of hyperpolarizing responses with responses of TEA-treated axons

It has been pointed out in the description of the experiment of Fig. 2 that the rise in the membrane potential following withdrawal of the anodal current is not immediate nor simple. Figure 6 shows two examples, recorded at high speed, of the membrane potential and impedance near the end of the applied anodal current. These records were taken from an axon in a potassium-rich medium. The axon was not treated with TEA; however, the records taken from TEA-treated axons in potassium-rich media were very similar to those in Fig. 6. It is seen in the figure that, at the moment of withdrawal of the current, there was no discontinuous change in the membrane impedance. At the moment when the 'break response' was produced, there was a sudden decrease in the membrane impedance which slowly returned to the original level within approximately 3 min. The break responses were found to be far smaller in axons immersed in potassium-rich solutions than in axons in normal sea water (Figs. 2, 3, 5 and 6).

In axons immersed in a mixture of isosmotic KCl solution and sodium-free sea water, hyperpolarizing responses similar to those shown in Fig. 2 were observed. In other words, sodium ions are not required in the medium for production of hyperpolarizing responses. On withdrawal of the applied current after production of a hyperpolarizing response, the membrane potential again rose in two steps. However, the break response or the 'overshoot' (which raised the membrane potential above the initial stationary level) was completely suppressed by the absence of sodium ions in the medium. The effect of sodium was therefore to give rise to a transient potential elevation when the membrane had reached the initial state after the end of the hyperpolarizing pulse.

It is interesting to compare the time course of the membrane impedance shown in Fig. 6 with that in Fig. 5. In both cases the membrane impedance is seen to fall suddenly at the moment when the break response started. It is also clear that the configuration of the potential trace is very similar in the two figures. At the moment of withdrawal of the applied anodal current, the membrane potential rises rapidly. Obviously this is due to the disappearance of the IR-drop in the membrane. Following this, the membrane potential approaches an intermediate level between the initial and the final levels. Then the rate of potential rise increases again, and a break response is produced. As has been mentioned above, the amplitude of the break response is far smaller in potassium-rich media than in the normal sea water. Other differences in the time course of the break response in these two cases are due partly to the effect of TEA injected into the axon in the experiment of Fig. 5.

Based on the similarity mentioned above, the experimental results shown in Fig. 6 can be described in the following manner: As in the experiment of

Fig. 5, the potential level before the withdrawal of the applied anodal current is determined by the membrane e.m.f. in the lower stable state and the *IR*-drop across the series resistance in the membrane. When the applied current is withdrawn, the membrane potential approached the e.m.f., E_1, of the lower stable state. Following this a rapid transition from the lower stable state to the upper takes place, resulting in a rapid rise in the membrane potential. The membrane conductance is higher in the upper stable state than in the lower.

From this statement it follows immediately that the production of a hyperpolarizing response can be regarded as a transition of the membrane from the upper state to the lower. It should be stressed in this connexion that, unless there was a hyperpolarizing response during the period of current flow, a two-step potential change could not be demonstrated on withdrawal of the anodal current pulse. This fact supports the view that the axon immersed in a potassium-rich medium is in the upper stable state and that a strong anodal current pulse causes a transition to the other state.

In Figs. 5 and 6 the faint horizontal lines represent the level of the membrane potential in the initial stationary state. In Fig. 5 this level is close to the terminal portion of the first step of the two-step potential variation; this level corresponds (by definition) to the e.m.f. of the lower stable state. In Fig. 6 the potential level in the initial state (marked '2' in the figure) is higher than the terminal portion of the first step (marked '1'). The difference between the two levels, 1 and 2, is 20–25 mV in this figure; there is some ambiguity in determination of this potential difference.

The measurement of the membrane impedance associated with a hyperpolarizing response (Fig. 2) indicates that the membrane impedance is lower at the initial peak than on the plateau of the response. The *IR*-drop across the resistance of the membrane should therefore be smaller at the peak of the response than at the end. It follows from this that the potential variation between the peak and the plateau is due to a change in the membrane e.m.f. rather than that in the membrane resistance.

In conclusion, the interpretation of the hyperpolarizing response suggested by the experiments described above is: (1) that the membrane of the axon immersed in a potassium-rich medium is in the upper of the two stable potential states of the membrane, (2) that an anodal current causes a transition from this state to the lower one, and (3) that the membrane which has been brought to the lower stable state undergoes a transition to the upper stable state on withdrawal of the applied current. The implication of this interpretation is that it is the e.m.f. in the upper stable state that is affected by potassium and sodium ions in these experiments (see Discussion).

PART II. Observation on toad single node preparations

Properties of the hyperpolarizing response in the nodal membrane

In the following experiments, the arrangement illustrated in Fig. 1*B* was used. When the potassium concentration of the surrounding medium was raised above about 30 m-equiv/l., the node under study (N_1) showed, as a rule, no sign of an electric response to outward-directed (cathodal) currents. In response to stimulating currents of the reversed polarity, however, the membrane potential exhibits a kind of discontinuous variation at a certain current intensity. In this respect, the present observation is in agreement with that of Stämpfli (1958).

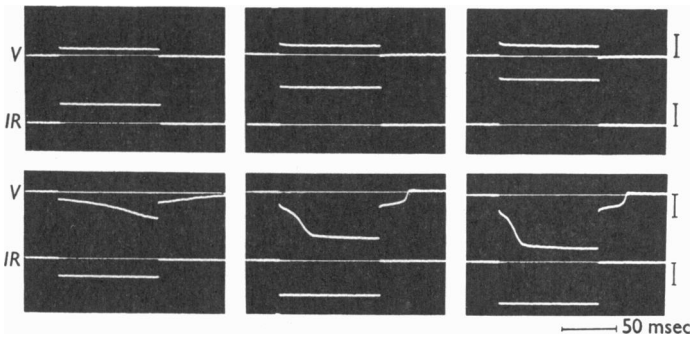

Fig. 7. Variations in the membrane potential of a single node preparation in a potassium-rich medium caused by long rectangular current pulses. The arrangement of Fig. 1*B* was used. The medium contained (mM) KCl 75, NaCl 35, $CaCl_2$ 1·5 and $NaHCO_3$ 2. Note large potential variations in response to anodal current pulses (lower records). The calibrating bar for the potential trace (*V*) indicates 50 mV and that of the current trace (*IR*) represents 100 mV divided by the resistance (*R*) of the fibre.

An example of the records obtained with a solution containing (mM) KCl 75, NaCl 35 and $CaCl_2$ 1·5 (pH adjusted with bicarbonate) is given in Fig. 7. The lower oscillograph trace (*IR*) indicates the time courses of the stimulating voltage pulses applied between the node under study (N_1) and the adjacent one (N_0); the size of the upward deflexion of this trace represents the intensity of the outward current through the node multiplied by the resistance between the two nodes. The upper trace indicates the variation of the potential of the nodal axoplasm (at N_1) referred to the grounded fluid medium. The appearance of disproportionately large potential variations in response to applied inward (anodal) current pulses (see the lower row in the figure) is obvious.

It should be noted that in this figure, when the applied anodal current was withdrawn following production of a 'hyperpolarizing response', the membrane potential did not return to its original level immediately, but it rose in two

steps. This was a constant finding in all the observations on the node. It is a strange fact that this has never attracted our attention previously.

As in the corresponding observation on the squid axon membrane (Fig. 6), the first step of the potential rise following withdrawal of the anodal current pulse (Fig. 7, bottom) is evidently caused by the disappearance of the *IR*-drop across the nodal membrane. The potential level reached after the first step (E_1 in the present terminology) was found to last much longer in single node preparations than in the case of squid giant axons. The interval between the

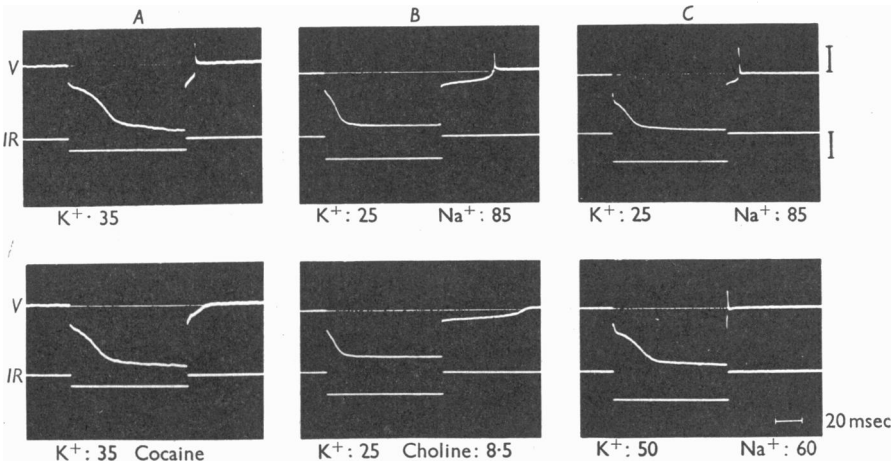

Fig. 8. Records showing the effects of narcosis (*A*), of sodium deprivation (*B*) and of a change in the potassium concentration (*C*) upon the time course of the potential variation following withdrawal of an anodal current pulse. In *A*, top, the concentration of KCl in the medium was 35 mM and that of NaCl 85 mM; in *A*, bottom, cocaine HCl 0·1 % was added to the medium. In *B*, top, the concentration of KCl was 25 mM and that of NaCl 85 mM; in *B*, bottom, NaCl was replaced with choline chloride. In *C* the ratio of the concentration of KCl to the concentration of NaCl in the medium was altered from 25:85 to 50:60. The calibrating bar for *V* is 50 mV and that for *IR* 100 mV.

end of the applied anodal pulse and the end of level E_1 was in some cases 50 msec or more. In other cases, the interval was much shorter (a few milliseconds) or the end of level E_1 was not clear because of a gradual transition from E_1 to the initial level. The difference between the quasi-stationary level E_1 and the initial level was between 20 and 30 mV.

When the node under investigation was immersed in a potassium-rich medium with nearly normal sodium content, the transition from the level of E_1 to the final stationary level was preceded by a transient potential variation which had the properties of a 'break response'. These transients are seen as vertical straight lines in Fig. 8 rising above the initial potential level. The duration of the transient was of the order of 1 msec at room temperature, and

its amplitude varied from preparation to preparation. Unlike the corresponding observations on the squid giant axon, observations of this type could be repeated at a frequency of one or two per second.

The possibility was mentioned that the behaviour of the nodal membrane described above might be due to a transition from the thermodynamic 'equilibrium potential' determined by potassium ions to that determined by chloride ions (Stämpfli, 1958). If this were the case, the phenomenon under study should be strongly modified by changes in the concentration of the chloride ion in the fluid medium. When a test was carried out, however, by replacing the chloride in the medium either with glutamate or with a mixture of glutamate and acetate, it was found that the phenomenon under investigation was not at all affected by changes in the chloride in the medium (see also the next section).

In Fig. 8 are shown the effects of several agents which were found to affect the process of transition from the level of E_1 to the final stationary level. When a narcotic in sufficient concentration to suppress the ordinary response of the node (e.g. 0·1 % cocaine) was added to the medium, the transient 'overshoot' above the initial level was completely and reversibly eliminated (Fig. 8*A*). The effect of replacing the sodium ions in the medium with choline was similar to that of cocaine; the hyperpolarizing response to an anodal current pulse remained essentially unaltered, while the transient 'overshoot' was reversibly suppressed by sodium deprivation. The similarity between the effect of narcosis and that of sodium deficiency has been pointed out previously by Tasaki & Bak (1958).

Column *C* in Fig. 8 shows an example of the effect of variation in the potassium concentration upon the duration of the level E_1. It has been pointed out above that this duration varies enormously from preparation to preparation. For a given preparation, however, the duration was found to decrease with increasing potassium concentration. This observation on the potassium effect was carried out both with and without compensation of the d.c. level with the polarizing circuit (E_p in Fig. 1*B*).

The effect of increased potassium just mentioned is easy to understand if it is recalled that the node in normal Ringer's solution is in the lower stable state (with an e.m.f. of E_1) and that the increase in the potassium content in the medium brings the membrane to the upper stable state (E_2). The fact mentioned above simply indicates that the transition from E_1 to E_2 is accelerated by increasing the concentration of potassium in the medium.

When the potassium concentration of the medium was between 15 and 25 m-equiv/l., it was frequently found that a weak cathodal current pulse gave rise to a small 'depolarizing response'. In such cases, an anodal pulse evoked a somewhat dubious 'hyperpolarizing response' leaving a clear negative shift of the base line on withdrawal of the current. An example of such behaviour is furnished in Fig. 9.

The behaviour of the membrane just mentioned is interpreted as the result of its being in a mixed state. The portion of the membrane in the lower stable state can respond to a cathodal current pulse while the portion in the upper state can give rise to a hyperpolarizing response.

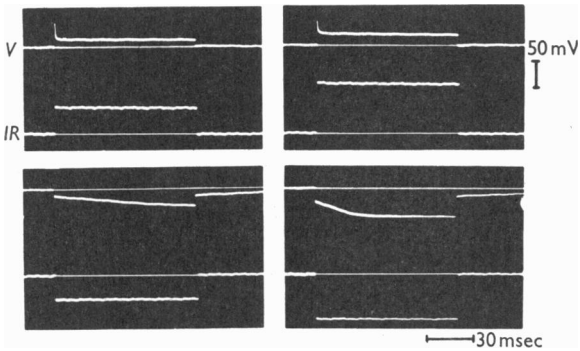

Fig. 9. Similar to the records in Fig. 7, but taken at a lower concentration of KCl. The medium contained (mM) KCl 25, NaCl 85, $NaHCO_3$ 2 and $CaCl_2$ 1·5. Note the initial peak in the potential (upper) trace at the onset of cathodal current pulses and also the lowered potential level after the end of anodal current pulses. The vertical bar subtending 50 mV applies to both traces.

Effect of maintained polarization

The effect of maintained polarization upon the physiological properties of the node in potassium-rich media has been reported recently by Mueller (1958*a*) and by Stämpfli (1958). They observed discontinuous variations in the membrane potential when the strength of the anodal polarizing current through the node was gradually increased. In the present investigation the effect of polarization was examined under various environmental conditions.

When a node immersed in a medium containing 25–30 m-equiv/l. of potassium was slowly hyperpolarized by gradually increasing the external voltage source (E_p in Fig. 1*B*), an abrupt fall was encountered in the observed membrane potential as the applied voltage reached a value between 40 and 65 mV. When the strength of the voltage source was slowly and smoothly decreased, there was a sudden rise in the observed membrane potential as E_p reached a level between 20 and 30 mV. The extent of the discontinuous change in the membrane potential was 30–40 mV. These observations on the discontinuity and the hysteresis are in good agreement with those of Stämpfli.

When the applied voltage was maintained at the level just below the critical value for a discontinuous (upward or downward) change, the membrane potential was found to show irregular variations. Occasionally a sudden jump in the membrane potential took place from one of the peaks of the irregular potential variations. An abrupt upward potential variation was associated,

when there were enough sodium ions in the medium, with a transient 'overshoot' of the membrane potential above the final stationary level.

Records *A* and *C* in Fig. 10 show examples of spontaneous transitions observed at the critical level of hyperpolarization. Record *B* shows that such transitions could be induced by weak current pulses superposed upon the constant hyperpolarizing current. It was clear that when the membrane was in the lower stable state only a cathodal (depolarizing) pulse could induce an abrupt potential variation, and the variation induced was always upward

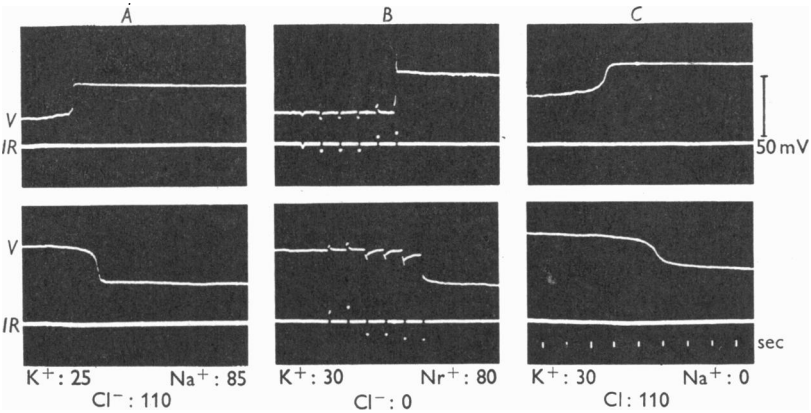

Fig. 10. Discontinuous changes in the membrane potential of nodes immersed in potassium-rich media. The nodes were hyperpolarized by an external source of current at the critical intensity. The concentrations of sodium, potassium and chloride ions in the medium are given in m-equiv/l. In the top record of *A*, the polarizing voltage (E_p in Fig. 1 *B*) was approximately 24 mV, in bottom of *A* about 40 mV, in top of *B* about 40 mV, in bottom of *B* about 60 mV, in top of *C* about 25 mV and in bottom of *C* about 38 mV. The calibrating bar applies to both channels.

(depolarizing). When the membrane was in the upper stable state as the consequence of such an upward transition, the only possible discontinuous variation in the membrane potential was an abrupt fall. In this sense the existence of two stable states in the membrane was obvious. On a few occasions, repetitive spontaneous transitions between the two stable states were observed at a constant level of hyperpolarization.

As in the observations described in the preceding section, a variation in the chloride concentration of the medium brought about, essentially, no effect upon the phenomenon observed (see an example in Fig. 10 *B*). Replacement of the sodium ions in the medium with choline resulted in a loss of the transient 'overshoot' of the membrane potential associated with a transition from the lower state to the upper (Fig. 10 *C*). Except for this short transitional period, the discontinuous phenomena at the node remained unaffected by sodium deprivation.

By means of subthreshold current pulses 100 msec in duration repeated at 1 sec intervals, the difference in the membrane resistance in the two different states was determined. The potential variations caused by such pulses were found to be reduced by a factor of 2–3 when the membrane in the lower stable state underwent an upward transition. Similar results were obtained previously by Mueller (1958*a*).

It should be noted that, because of the difference in the membrane resistance in the two states, the observed jumps in the membrane potential do not represent the difference in the e.m.f. of the membrane in the two states. There is a continuous current through the nodal membrane under these experimental conditions. The observed jump in the membrane potential consists of a change in the *IR*-drop superposed on the difference in the e.m.f. in the two states.

Factors affecting the resting potential in the nodal membrane

When the node of Ranvier under study (N_1 in Fig. 1*B*) is depolarized by application of a potassium-rich saline solution, a continuous inward current begins to flow through the node as a consequence of the potential difference between the adjacent narcotized node (N_0) and the region treated with potassium. The *IR*-drop caused by this inward current through the node tends to reduce the observed amount of depolarization by potassium. The current can, however, be eliminated by adjusting the polarizing voltage (E_p in Fig. 1*B*) so that the voltage becomes equal (and opposite in sign) to the potential difference caused by potassium. This procedure of d.c. compensation was frequently used in the experiments of Figs. 7, 8 and 9 described above.

In the following series of experiments, this procedure of d.c. compensation was done automatically by taking advantage of the unity-gain high input impedance pre-amplifier employed in the present investigation. While the node under study (N_1) was still in normal Ringer's solution (with the neighbouring nodes N_0 and N_2 immersed in cocaine-Ringer solution), the output d.c. level of the unity-gain pre-amplifier was adjusted to zero (ground); then, using the switch *S* (Fig. 1) the output of the pre-amplifier was connected to the electrode in the large pool which had hitherto been effectively grounded. Under these circumstances, changes in the e.m.f. of the membrane could be recorded faithfully by the pre-amplifier because there should be no current through the node under investigation.

With this new method of measuring the resting potential of a single node preparation, attempts were made to examine the effects of various chemical agents upon the node in potassium-rich media. The chemicals which were found in the present series of investigations to be capable of modifying the resting potential are monovalent TEA ions and divalent nickel ions (also cobalt and beryllium).

The remarkable effect of nickel ions upon the action potential of the myeli-

nated nerve fibre was discovered by Spyropoulos & Brady (Brady, Spyropoulos & Tasaki, 1958) and is illustrated in the upper part of Fig. 11. The main features of the alteration of the action potential by nickel ions are (*a*) enormous prolongation of the action potential forming a 'plateau', and (*b*) formation of a pronounced 'shoulder' from which the membrane potential falls rapidly toward the resting level. At low temperatures the spike duration of a nickel-treated node can frequently reach 1 sec or more. External application of TEA to the node does prolong the action potential but only slightly (cf. Lorente de Nó, 1949). Its main effect is to reduce or eliminate the 'shoulder' which exists in the normal action potential of the node: that is to say, under TEA, the membrane potential falls more smoothly from its peak to the base line.

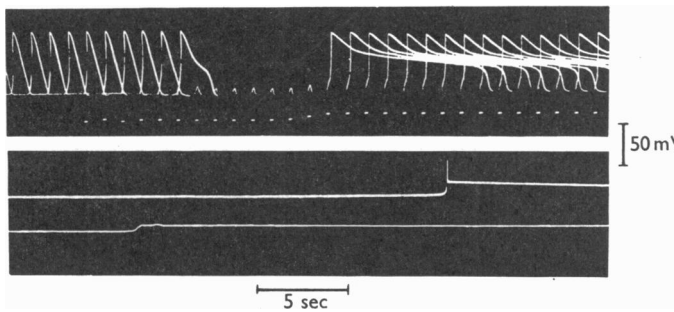

Fig. 11. Prolongation of the action potential of a single node by application of 0·4 mM-$NiCl_2$ (top) and the effect of a gradual increase in the potassium concentration in the medium upon the resting potential of the node treated with nickel (bottom). Application of $NiCl_2$ was started in the upper record when the time marks (1 sec apart) were turned on; the stimulus strength had to be increased because of a rise in threshold. The spike duration changed from approximately 1 to 10 msec. In the lower record the concentration of KCl in the medium was increased from 15 to 30 mM at the rise of the lower signal. Calibration applies to all the traces.

The node whose action potential had been prolonged by application of a dilute (approximately 0·4 mM) $NiCl_2$-Ringer solution was found to be highly resistant to potassium depolarization. When the prolongation was greater than about twentyfold, the change in the resting potential caused by application of a 40 mM-KCl solution (containing also (mM) NaCl 70, $CaCl_2$ 1·5 and $NiCl_2$ 0·3) was generally between 5 and 10 mV. Even an isotonic KCl solution (containing 0·4 mM-$NiCl_2$) was occasionally found to depolarize the node by only 15 mV or less. The extent of prolongation of the action potential, as well as the resistance to potassium depolarization, varied enormously from preparation to preparation. In some preparations, a gradual increase in the potassium concentration brought about an abrupt depolarization accompanied by a transient 'overshoot' above the final stationary level of the membrane potential.

The lower record in Fig. 11 shows an example of abrupt depolarization of the nodal membrane in a medium containing high potassium and a small amount of $NiCl_2$. It is evident that the sudden rise in the membrane potential in the figure cannot be explained in terms of a rapid increase in the potassium concentration in the medium. In this case the replacement of the external fluid (removal of 15 mM-KCl and application of 30 mM-KCl) was completed approximately 17 sec before the observed jump in the membrane potential. The presence of the transient 'overshoot' above the final stationary level suggests that this jump is due to a transition of the membrane from the lower stable state to the upper.

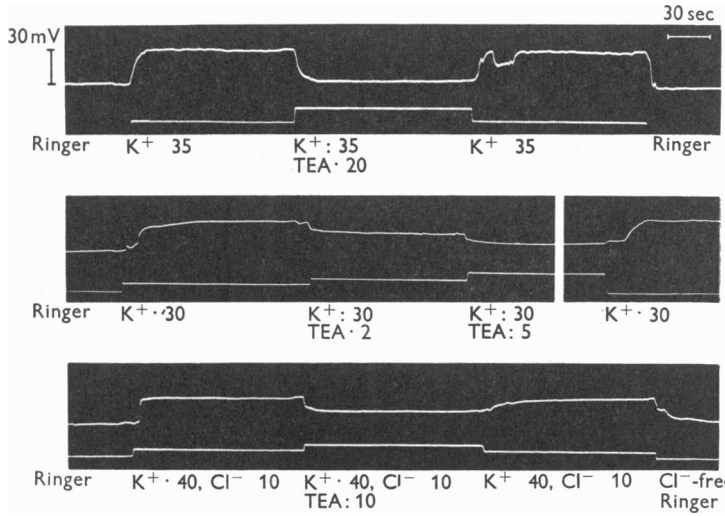

Fig. 12. Restoration of the resting potential of single nodes in potassium-rich media by tetraethylammonium (TEA). The arrangement of Fig. 1 *B* was used, with switch *S* in position 2. The concentrations (m-equiv/l.) of potassium and TEA are indicated. In the bottom record the effect of TEA was demonstrated in a medium which contained 100 mM-glutamate and 10 mM-chloride. These records were taken from three different preparations. The nodes were kept in each test solution for a period of approximately 2 min.

In contrast to the effect of nickel, the effect of TEA upon the node depolarized by potassium was highly reproducible. In all the single node preparations employed (approximately 20), the effect could be demonstrated without any exceptions. Addition of a small amount of TEA in the medium containing 30–60 mM-KCl was found to restore the resting potential of the node reversibly. The extent of restoration increased as the TEA concentration in the medium was increased. The observation could be repeated as many times as was desired on each preparation.

In Fig. 12 are furnished three examples of the records showing the effect of TEA upon the nodes in potassium-rich media. As can be seen in the figure, a

variation in the concentration of chloride in the medium had essentially no effect upon the process of restoration of the resting potential by TEA. (There was a fall of a few millivolts in the membrane potential when the major portion of the chloride ions in the medium was replaced with glutamate ions.) Replacement of sodium ions with choline had no effect on this property of the node either.

Tetramethylammonium and tetrabutylammonium ions were tested in a similar manner. No clear repolarization of the potassium-treated nodal membrane was observed with these quaternary ammonium ions.

DISCUSSION

It is obvious that there is a great degree of similarity in the *biophysical* properties between the two different nerve fibres examined in the present investigation. However, it should be stressed that the *chemical* properties of the two systems show marked differences. For example, in the squid axon membrane, it was not possible to demonstrate any clear change in the action potential by application (either intra- or extracellular) of nickel, whereas in the nodal membrane application of nickel ions is the most powerful means of prolonging the action potential (Fig. 11). External application of TEA did not bring about any clear effect in the squid axon, but in the nodal membrane there is a definite prolongation.

The time course of the response of a TEA-treated squid axon (Figs. 3*A* and 4*A*) resembles that of the nickel-treated node (Fig. 11); but the membrane conductance of a TEA-treated squid axon is nearly normal except during the initial peak, whereas in a nickel-treated node it gradually returns to normal. The squid axon whose response has been prolonged by intracellular injection of TEA shows no clear resistance to potassium depolarization, but a nickel-treated node is highly resistant to potassium.

At present it does not seem possible to analyse how these differences in chemical properties arise. In the following discussion we limit ourselves to the formal, biophysical aspects of the problem that are more or less common to both the squid giant axon and the toad myelinated nerve fibre.

The effect of potassium upon the resting potential has been thoroughly investigated by direct methods (see Hodgkin, 1951). The relationship between the logarithm of the external potassium concentration and the resting potential is shown diagrammatically by the continuous line in Fig. 13. When the nerve fibre is immersed in a normal medium (either normal Ringer's solution or normal sea water), the resting potential is about -60 mV or slightly more; under these conditions, the membrane is considered to be in the lower stable state labelled (1) in the figure. When the potassium concentration in the medium is raised well above the normal level, the resting potential changes

linearly with the logarithm of the potassium concentration. According to the analysis stated under Results, the membrane is in the upper stable state (2) in this range of potassium concentration.

By recording the quasi-stationary potential levels observed after withdrawal of anodal current pulses, it has been shown that the lower stable state of the membrane in potassium-rich media is approximately 25 mV below the level of the resting potential; the difference between the two levels is almost independent of the potassium concentration used. The level of the lower stable state revealed by this method is shown by the portion labelled '*a*' in the figure.

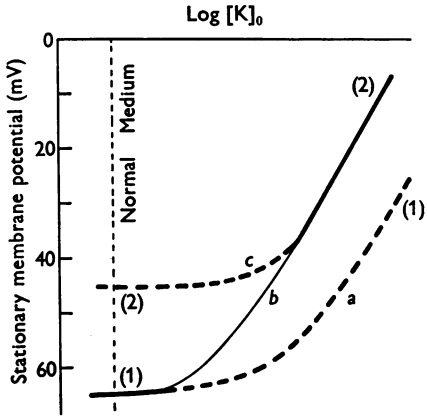

Fig. 13. Schematic diagram showing the relationship between the logarithm of the external pottasium concentration and the stationary or quasi-stationary potential levels across the nerve membrane. The continuous line represents the 'resting potential'. The broken lines indicate the quasi-stationary potential levels, portion '*a*' being determined by sudden withdrawal of anodal current pulses and portion '*c*' determined from the level of the 'shoulder' of a prolonged response. The thick line labelled (1) represents the potential level in the upper stable state and (2) that in the lower stable state. Portion '*b*' represents the mixed state.

The 'plateau' of a prolonged response of the nerve fibre in the normal medium can be regarded as the quasi-stationary potential level of the membrane in the upper stable state. The level of the 'shoulder' of a prolonged response is 20–30 mV above the level of the resting potential; this level is shown by the portion labelled '*c*' in the figure.

The portion labelled '*b*' indicates that the nerve membrane is in the mixed state. The amount of displacement of the base line caused by anodal current pulses (Fig. 9) can be taken as a measure of the fraction of the membrane in the upper stable state. In this range this displacement increases with increasing potassium concentration. This portion may therefore be regarded as bridging the two thick lines representing the pure states (1) and (2).

It should be noted that the curves in Fig. 13 are by no means hypothetical ones; they are merely a composite graphical presentation of the stationary

and quasi-stationary potential levels directly observed in the absence of a membrane current. These stationary and quasi-stationary potential levels of the membrane are not affected by the concentration of sodium outside the membrane. Sodium deprivation, as well as narcosis, affects the transient potential variation associated with a transition from the lower stable state (1) to the upper (2). An action potential observed in a normal medium consists of this transient potential variation followed by a downward transition from (2) to (1). In potassium-rich media the upward transition from (1) to (2) after withdrawal of an anodal current pulse is accompanied by a transient potential variation but is not followed by a downward transition.

The records furnished in Fig. 2 show that in the squid axon a downward transition is accompanied by a large transient potential variation which constitutes the peak of the hyperpolarizing response. Similarly, a downward transition at the end of a response of a squid axon in normal sea water is associated with a transient potential variation which forms the 'undershoot' of the action potential. In the nodal membrane a downward transition is not accompanied by a conspicuous transient; there is no peak in the hyperpolarizing response and no 'undershoot' at the end of a spike potential.

When there is a strong electric current through the membrane, the stable potential levels are displaced by the *IR* drop across the membrane. It has been shown that the major portion of the potential variation associated with a hyperpolarizing response is nothing more than a change in the *IR* drop.

When an upward transition is caused by a strong cathodal current pulse applied to the membrane in a normal medium (in the current or voltage clamp situation), the membrane is expected to stay in the upper stable state as long as the depolarizing pulse is maintained. This should then result in a maintained high membrane conductance. The fact that a maintained depolarization increases the membrane conductance was shown first by Cole & Curtis (1941).

Finally, the effect of nickel and TEA ions upon the nodal membrane will be briefly discussed. The main feature of the action of nickel ions upon the node is formation of a long 'plateau' and a prominent 'shoulder' in the action potential (Fig. 11). Under the influence of nickel a gradual increase in the potassium concentration in the medium caused either no distinct depolarization or an abrupt depolarization. These effects of nickel ions can be stated as the result of an action tending to shorten the time spent by the system in the mixed state. When the effect of nickel is very pronounced, the membrane appears to stay in the lower state (with reduced potassium dependence) even in a medium containing a high concentration of potassium.

The effect of TEA on the node can be regarded as the result of an action tending to favour the existence of a mixed state of the membrane. Depending on the amount of TEA added to the medium, the system can take any stationary level between portion (*a*) of line (1) in Fig. 13 and the portion of (2)

above (*a*). In metal-oxide models of the nerve membrane the conditions tending to favour the appearance of a mixed state can also be demonstrated (Franck, 1956; Tasaki & Bak, 1959).

At present nothing is known as to the physico-chemical nature of the two stable states of the membrane. In no way does ignorance of its nature affect the interpretation of the experimental results presented in this paper. The transition from one state to the other may be caused by chemical dissociation of a radical on the inner surface of the membrane: this situation is analogous to the process taking place in the cobalt and iron models of the nerve membrane. Alternatively, the two stable states may simply represent two stable configurations of the lipo-protein layer in the membrane. Professor T. Teorell (personal communication) claims that two stable states can be demonstrated in his physical model of the excitable membrane. It is interesting to note that in both states the stationary and quasi-stationary potential levels are affected primarily by the potassium ions in the medium. Direct physico-chemical studies of the two states of the membrane are highly desirable.

SUMMARY

1. Both in the squid giant axon and in the single node preparation of the toad, the nerve membrane immersed in potassium-rich media is capable of developing 'hyperpolarizing responses' to anodal current pulses.

2. The major portion of the potential variation associated with a 'hyperpolarizing response' is a change in the *IR* drop in the membrane; the change in the membrane e.m.f. is 20–30 mV and is approximately independent of the concentration of potassium in the medium.

3. Analyses of the stability of the system indicate that the nerve membrane immersed in potassium-rich media is in the 'upper stable state' and that a 'hyperpolarizing response' represents a transition to the 'lower stable state'.

4. In the toad single node preparation immersed in potassium-rich media, it was shown that the resting potential can be reversibly altered by addition of tetraethylammonium (TEA) ions to the medium. This effect was interpreted as the result of bringing the system into a mixed state.

5. The nodal membrane whose action potential has been prolonged by nickel ions is insensitive to potassium depolarization; sometimes the membrane responds to a gradual increase in the potassium concentration with a sudden depolarization.

6. Chloride ions in the medium have little effect upon the potential levels in the two states. Changes in the sodium concentration in the medium, as well as narcosis, alter the transient potential variation associated with a transition from the lower stable state to the upper.

APPENDIX

Demonstration of hyperpolarizing responses in the iron-electrode system

In the well known electrochemical model of the nerve membrane formed at the interphase between iron and strong nitric acid, a normal 'action potential' represents a transient rise of the potential of the nitric acid relative to that of the iron (see Franck, 1956). In this model the phase of nitric acid is comparable to the axoplasm of a living nerve fibre and the substance of iron to the surrounding fluid medium. The state in which the surface of the iron is covered with an oxide layer corresponds to the lower state and the state in which the oxide layer is removed corresponds to the upper state.

When the nitric acid is diluted to the extent that the oxide layer can no longer stay on the surface of iron, a current flowing 'inward' (from metal to acid) across the iron surface is expected to oxidize the surface, causing a transition from the upper stable state to the lower. Since the oxide layer shows a high electric resistance to penetrating currents and since there is a difference in the effective e.m.f. in the two states, this transition is expected to give rise to potential variations similar to the hyperpolarizing response observed in the nerve membrane.

The diagram in Fig. 14, left, illustrates the experimental arrangement used for demonstrating 'hyperpolarizing responses' in the iron-wire model of the nerve membrane. A wire of soft iron approximately 1 mm in diameter (kindly supplied by Professor U. F. Franck) was enclosed in a glass tube and the space between the glass tube and the wire was filled with melted paraffin. The commercially available nitric acid was diluted to obtain a solution of the specific gravity of approximately 1·335. Two large platinum wire electrodes were immersed in the nitric acid. One of the platinum electrodes was used to pass a known amount of current through the surface of the iron in nitric acid and the other for recording the potential difference between the fluid in the beaker and the iron wire.

First, by passing a short pulse of 'outward' current (flowing from acid to iron), normal 'action potentials', which looked similar to those of a TEA-treated axon, were observed. Next, the nitric acid in the beaker was diluted ten times with distilled water. In this dilute nitric acid it was no longer possible to elicit an 'action potential' from the model by application of 'outward' current pulses. When a long pulse of 'inward' current was applied to the system, a large fall in the potential of the acid was observed when the pulses were stronger than a certain 'threshold intensity'. The potential variation observed under these experimental conditions resembled those in Figs. 2 or 7 except in its time course after the break of the applied current. Apparently this difference derived from the situation that the oxide layer formed by the passage of current did not disappear soon after the end of the applied pulse;

the system stayed in the lower state much longer than in the corresponding observation on the nerve membrane.

The transition from the lower stable state to the original upper state was found to take place much sooner when sulphuric acid was employed instead of nitric acid. The behaviour of the iron immersed in sulphuric acid has been worked out by Franck (1956). When the concentration of the sulphuric acid was about 0·5 N or slightly less, records which look very similar to those

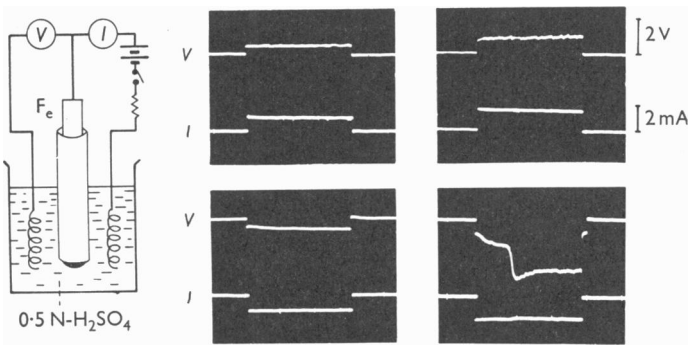

Fig. 14. Demonstration of 'hyperpolarizing responses' in the iron electrode immersed in 0·5 N sulphuric acid. In the records an upward deflexion of the potential trace (V) represents a rise in the potential of the platinum electrode in the acid relative to that of the grounded iron wire. An upward deflexion of the current trace (I) represents a flow of current from acid to iron. Note the appearance of the intermediate potential level, E_1, following withdrawal of 'anodal' current pulse. The duration of the current pulses was 6 sec; the temperature of the fluid in the beaker was approximately 30° C.

obtained from the nerve membrane were obtained (see Fig. 14). It is obvious from what has been stated above that the formal interpretation of the phenomenon in terms of e.m.f. and resistance of the surface membrane is similar in the iron wire model and in the nerve membrane.

The author wishes to express his gratitude to Dr C. S. Spyropoulos for his valuable help in the present investigation. Part of the work presented in this article was carried out at the Marine Biological Laboratory, Woods Hole, Massachusetts.

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