The Ionic Basis of Nervous Conduction

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Trinity College, Cambridge, which I entered in 1932, has a long-standing connection with neurophysiology. As an undergraduate I found myself interested in nerve and was soon reading books or papers by Keith Lucas (*1*), Adrian (*2*), Hill (*3*), and Rushton (*4*), all of whom are, or were, fellows of Trinity. I had a particular reason for looking at Lucas's papers because my father and Lucas had been close friends and both lost their lives during the first world war. My reading introduced me to Bernstein's membrane theory (*5*), in the form developed by Lillie (*6*), and I thought it would be interesting to test their assumptions by a simple experiment. A central point in the theory is that propagation of the impulse from one point to the next is brought about by the electric currents which flow between resting and active regions. On this view, the action potential is not just an electrical sign of the impulse, but is the causal agent in propagation. Nowadays the point is accepted by everyone, but at that time it lacked experimental proof. By a roundabout route I came across a fairly simple way of testing the idea. The method depended on firing an impulse at a localized block, and observing the effect of the impulse on the excitability of the nerve just beyond the block. It turned out that the impulse produced a transient increase in excitability over a distance of several millimeters, and that the increase was almost certainly caused by electric currents spreading in a local circuit through the blocked region (*7*). More striking evidence for the electrical theory was obtained later, for instance when it was shown that the velocity of the nerve impulse could be changed over a wide range by altering the electrical resistance of the external fluid (*8*). But this is not the place to describe these experiments, and I would like to take up the story again in 1938, when I had the good fortune to spend a year in Gasser's laboratory at the Rockefeller Institute in New York. Before leaving Cambridge I had found, by a lucky accident, that it was quite easy to isolate single nerve fibers from the shore crab, *Carcinus maenas*. This opened up several interesting lines of investigation, and I became increasingly impressed with the advantages of working on single nerve fibers. *Carcinus* fibers are very robust, but they are at most 1/30 millimeter in diameter and for many purposes this is inconveniently small. There was a good deal to be said for switching to the very much larger nerve fibers which J. Z. Young (*9*) had discovered in the squid and which were then being studied by Cole and Curtis (*10*) in Woods Hole. Squids of the genus *Loligo* are active creatures, 1 or 2 feet long, which can swim backward at high speed by taking water into a large cavity and squirting out a jet through a funnel in the front of the animal. The giant nerve fibers, which may be as much as a millimeter in diameter, run in the body wall and supply the muscles that expel water from the mantle cavity. Although these fibers are unmyelinated, their large size makes them conduct rapidly, and this may be the teleological reason for their existence. It should be said that large nerve fibers conduct faster than small ones (*11*) because the conductance per unit length of the core increases as the square of the diameter, whereas the electrical capacity of the surface increases only as the first power.

You may wonder how it is that we get along without giant nerve fibers. The answer is that vertebrates have developed myelinated axons in which the fiber is covered with a relatively thick insulating layer over most of its length, and the excitable membrane is exposed only at the nodes of Ranvier. In these fibers, conduction is saltatory and the impulse skips from one node to the next. I regret that shortage of time does not allow me to discuss this important development, with which the names of Kato, Tasaki, and Takeuchi (*12*) are particularly associated.

Early in 1938, K. S. Cole asked me to spend a few weeks in his laboratory at Woods Hole where squid are plentiful during the summer. I arrived in June 1938 and was greeted by a sensational experiment, the results of which were plainly visible on the screen of the cathode-ray tube. Cole and Curtis (*13*) had developed a technique which allowed them to measure changes in the electrical conductivity of the membrane during the impulse; when analyzed, their experiment proved that the membrane undergoes a large increase in conductance which has roughly the same time course as the electrical change (Fig. 1). This was strong evidence for an increase in ionic permeability, but the experiment naturally did not show what ions were involved, and this aspect was not cleared up until several years after the war. At first sight, Cole and Curtis's results seemed to fit in with the idea that the membrane broke down during activity, as Bernstein and Lillie had suggested. However, there was one further point which required checking. According to Bernstein, activity consisted of a momentary breakdown of the membrane, and on this view the action potential should not exceed the resting potential. Huxley and I started to test this point early in 1939. We measured external electrical changes from *Carcinus* fibers immersed in oil with a cathode-ray tube, direct-current amplifier, and cathode followers as the recording instrument. The resting potential was taken from the steady potential between an intact region and one depolarized by injury or by isotonic potassium chloride. To our surprise we found that the action potential was often much larger than the resting potential—for example, 73 millivolts for the action potential as against 37 millivolts for the resting potential. [Although I was not aware of it until much later, Schaefer (*14*)

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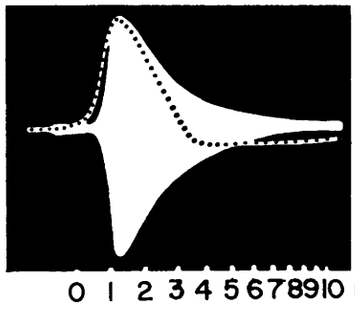

Fig. 1. Action potential (dotted curve) and increase in conductance (white band) in squid axon at about 6°C. [From Cole and Curtis (*13*)]

had previously reported a similar discrepancy in the sartorius and gastrocnemius muscles of the frog.] Our results did not give the absolute value of the membrane potentials because of the short-circuiting effect of the film of sea water which clings to a fiber in oil. However, there is no reason why short-circuiting should affect one potential more than another, and the discrepancy seemed much too large to be explained by some small difference in the way the two potentials were recorded. Nevertheless, we were extremely suspicious of these results with external electrodes, and before they could be published both of us were caught up in the war.

Before going further with the discrepancy, it seemed important to establish the absolute value of the membrane potentials by recording potential differences between an electrode inside the nerve fiber and the external solution. Osterhout and his colleagues (*15*) had recorded internal potentials by introducing electrodes into the vacuoles of large plant cells, but for obvious reasons the comparable experiment had not been attempted with nerve. The best preparation on which to try such an experiment was the giant axon of the squid, and the first measurements of this kind were made during the summer of 1939 by Curtis and Cole (*16*) at Woods Hole, and by Huxley and myself (*17*) at Plymouth. There were minor differences in technique, but the general principle was the same. A microelectrode consisting of a long glass capillary, filled with saline or metal, was inserted at one end of the fibre and pushed in for a distance of 10 to 30 millimeters. The fiber was damaged at the point where the capillary entered it, but an insertion of 10 to 30 millimeters was sufficient to take the electrode into intact nerve. During the insertion the electrode had to be kept away from the membrane; if it scraped against the surface the axon was damaged. However, if kept clear of the membrane, the electrode did no harm, and it has since been shown that axons will conduct impulses for many hours after being impaled in this way. Figure 2*A* shows an electrode inside an uncleaned axon; Fig. 2*B* is similar, but the small nerve fibers round the giant axon have been removed and dark ground illumination has been used.

In 1939 both the Woods Hole and Plymouth groups found that large action potentials could be recorded between an internal electrode and the external solution, thus providing strong evidence for the idea that the action potential arises at the surface membrane. With this technique Huxley and I again obtained the disturbing result that the action potential was much greater than the resting potential (*17*). Figure 3, which illustrates one of these experiments, shows an action potential of 86 millivolts and a resting potential of 45 millivolts. In their 1939 experiments Curtis and Cole (*16*) recorded the action potential with a condenser-coupled amplifier; later measurements with a d-c amplifier gave an average action potential of 108 millivolts and an average resting potential of 51 millivolts (*18*). Curtis and Cole also showed that the resting potential could be abolished, reversibly, by increasing the external potassium concentration until it was about the same as that in the axoplasm; at high concentrations the membrane behaved like a potassium electrode, as predicted by Bernstein's theory.

The small size of most nerve or muscle fibers made it difficult to extend the technique employed for the giant axon to other preparations. However, another very convenient and powerful method was developed by Graham, Gerard, and Ling, who showed that extremely small glass capillaries could be inserted transversely into muscle fibers without causing appreciable damage (*19*). In order to obtain consistent results it is desirable that the electrodes should have an external diameter of less than 0.5 micron. This small diameter means that the electrodes have a high resistance, and special precautions must be taken with the recording system. Initially the electrodes were used to measure the resting potential, but increasing the concentration of the potassium chloride in the electrode to 3 molar enabled the action potential to be recorded as well (*20*). Many types of excitable cell have now been examined, and in nearly every case it has been found that the action potential exceeds the resting potential, often by 40 to 50 millivolts.

Yet another method is required for myelinated nerve fibers, which do not take kindly to impalement. A useful way of eliminating external short circuiting was introduced by Huxley and Stämpfli in 1950 (*21*), and their method has been refined in a very elegant way by Frankenhaeuser (*22*). The val-

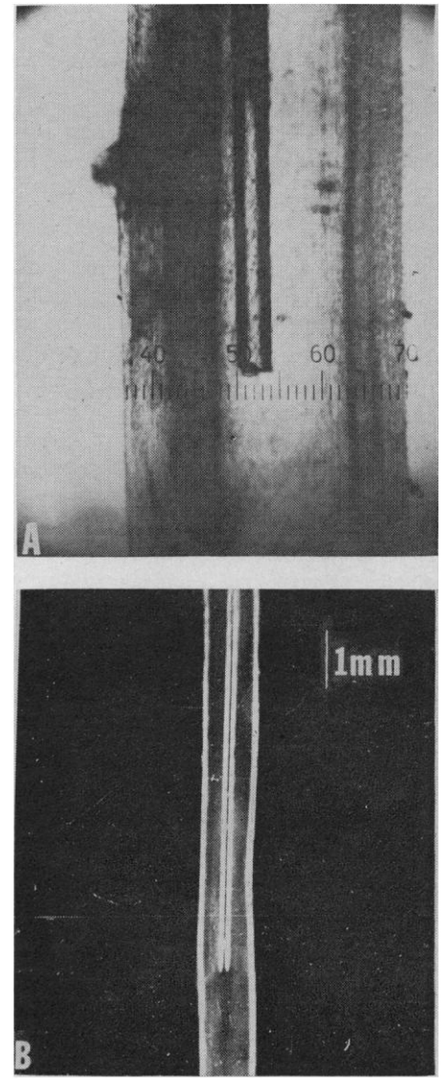

Fig. 2. (*A*) Photomicrograph of a recording electrode inside a giant axon of *Loligo forbesi*. The giant axon, which shows as a clear space, was left with small nerve fibers on either side; one division = 33 microns. [From Hodgkin and Huxley (*17*)] (*B*) Cleaned giant axon of *Loligo forbesi* with glass tube 0.1 millimeter in diameter inside it; dark ground illumination. [From Hodgkin and Keynes (*55*)]

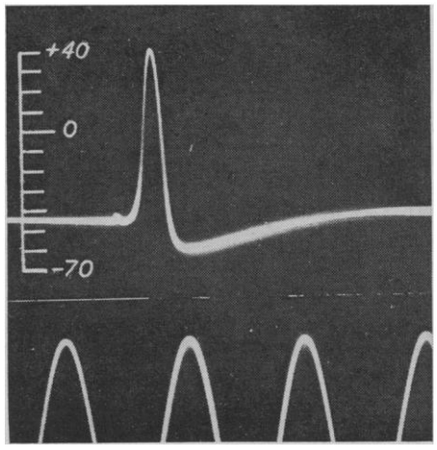

Fig. 3. Action potential and resting potential recorded between inside and outside of axon with capillary filled with sea water. Time marker, 500 cy/sec. The vertical scale indicates the potential of the internal electrode in millivolts, the sea water outside being taken as at zero potential. [From Hodgkin and Huxley (*17*); see also *23*]

ues found by applying these methods to amphibian nerve fibers are: action potential, 120 millivolts; resting potential, 70 millivolts. Absolute values for mammalian nerve fibers are not known, but they are probably not very different from those reported for frog.

At the end of the war, the position was that several of Bernstein's assumptions had been vindicated in a striking way, but that in one major respect the classical theory had been shown to be wrong. By 1945 most neurophysiologists agreed that the action potential was propagated by electric currents, and that it arose at the surface membrane; it was also clear that the resting potential was at least partly due to the electromotive force of the potassium concentration cell. On the other hand, there was impressive evidence that in both crab and squid fibers the action potential exceeded the resting potential by 40 to 50 millivolts (*17, 18, 23*). This was obviously incompatible with the idea that electrical activity depended on a breakdown of the membrane; some process giving a reversal of electromotive force was required.

The Sodium Hypothesis

There were several early attempts to provide a theoretical basis for the reversal, but most of these were speculative and not easily subject to experimental test. A simpler explanation, now known as the sodium hypothesis, was worked out with Katz and Huxley and tested during the summer of 1947 (*24*). The hypothesis, which undoubtedly owed a good deal to the classical experiments of Overton (*25*), was based on a comparison of the ionic composition of the axoplasm of squid nerve with that of blood or sea water. As in Bernstein's theory, it was assumed that the resting membrane is selectively permeable to potassium ions and that the potential across it arises from the tendency of these ions to move outward from the more concentrated solution inside a nerve or muscle fiber. In the limiting case, where a membrane which is permeable only to potassium separates axoplasm containing 400 mM K from plasma containing 20 mM K, the internal potential should be 75 millivolts negative to the external solution. This value is obtained from the Nernst relation

$$V_K = \frac{RT}{F} \ln \frac{[K]_o}{[K]_i} \qquad (1)$$

where V_K is the equilibrium potential of the potassium ion defined in the sense internal potential minus external potential and $[K]_o$ and $[K]_i$ are potassium concentrations (strictly, activities) inside and outside the fiber. Resting potentials of 70 millivolts have been observed in undissected squid axons (*26*); the smaller values found in isolated axons may be explained by a leakage of sodium into the fiber. If the permeability to sodium were 1/12 that to potassium, a potential of about 50 millivolts is predicted for an isolated axon in sea water (350 mM K, the mM Na in axoplasm 10 mM K, 450 mM Na in sea water).

From Bernstein's theory it might be assumed that when the membrane broke down, the ratio of the permeabilities to Na and K would approach that of the aqueous mobilities of these ions, about 0.7 to 1. In that case, the action potential could not exceed the resting potential and would in fact be less by at least 8 millivolts. However, it is simple to rescue the hypothesis by assuming that the active membrane undergoes a large and selective increase in permeability to sodium. In the extreme case, where the membrane is much more permeable to sodium than to any other ion, the potential should approach that given by the Nernst formula; that is,

$$V_{Na} = \frac{RT}{F} \ln \frac{[Na]_o}{[Na]_i} \qquad (2)$$

This gives a limiting value of + 58 millivolts for the tenfold concentration ratio observed by Steinbach and Spiegelman (*27*) and accounts satisfactorily for the reversal of 50 millivolts commonly seen in intact axons.

A simple consequence of the sodium hypothesis is that the magnitude of the action potential should be greatly influenced by the concentration of sodium ions in the external fluid. For the active membrane should no longer be capable of giving a reversed electromotive force if the concentration of sodium is equalized on the two sides of the membrane. The first quantitative tests were made with Katz, in the summer of 1947. They showed that the action potential, but not the resting potential, was reduced by replacing external sodium chloride with choline chloride or with glucose. If all the external sodium was removed the axon became reversibly inexcitable, in agreement with Overton's experiment on frog muscle. Figure 4 illustrates one of the experiments. In the physiological region, the overshoot varied with external sodium concentrations in the same manner as a sodium electrode (*24*).

It was also shown that a solution con-

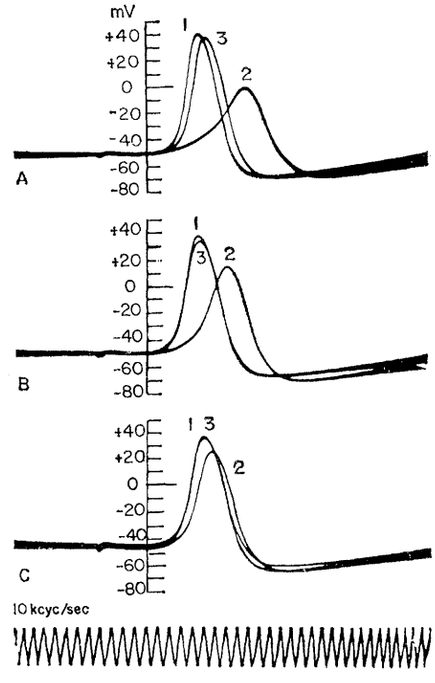

Fig. 4. Effect of sodium-deficient external solutions on the action potential of a giant axon. Records labeled 1 and 3 were with the axon in sea water; A2, with 0.33 sea water, 0.67 isotonic dextrose; B2, with 0.5 sea water; 0.5 isotonic dextrose; C2, with 0.7 sea water, 0.3 isotonic dextrose. [From Hodgkin and Katz (*24*)]

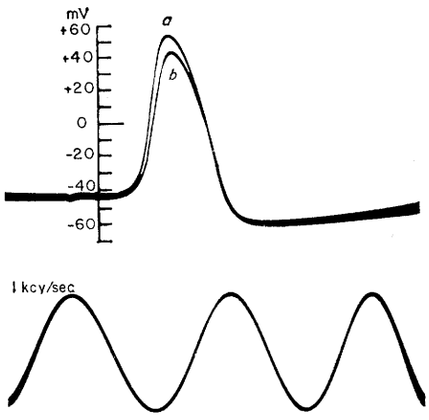

Fig. 5. Effect of sodium-rich external solution on the action potential of a giant axon. Record *a*, in sea water; record *b*, 50 seconds after applying sea water containing additional NaCl (Na concentration, 1.56 times that in sea water). [From Hodgkin and Katz (*24*)]

taining extra sodium increased the overshoot by about the amount predicted by Eq. 2. This is a particularly satisfactory result, because it seems most unlikely that an increase beyond the normal could be brought about by an abnormal solution. Figure 5 illustrates one of these experiments. Later Stämpfli (*28*) showed that at the node of Ranvier an increase of about 35 millivolts in the overshoot is brought about by a fourfold increase of external sodium.

The effect of varying external sodium concentration has now been studied on a number of excitable tissues: for example, frog muscle (*20*), myelinated nerve (*29*), Purkinje fibers of the heart (*30*), and crustacean nerve (*31*). In all these cases the results were very similar to those in the squid axon.

There are at least two cases where the mechanism is thought to be basically different. These are crab muscle, in which an entry of calcium, or other divalent cations, provides the inward current (*32*), and the plant cell, *Chara*, where an exit of chloride ions from the vacuolar sap may be the primary process (*33*).

Ionic Movement during Activity

During the period 1947–51 several investigators started to measure the effect of stimulation on the movements of labeled sodium across the surface membrane of giant axons. As often happens, work proceeded independently and more or less simultaneously on the two sides of the Atlantic, the principal investigators being Keynes (*34*) in England and Rothenberg (*35*) and Grundfest and Nachmansohn (*36*) in America. In 1949 Keynes reported that stimulation of *Sepia* axons at 100 per second caused a 15-fold increase in the rate of uptake of ^{24}Na. There was also a substantial increase in the outflow of labeled sodium, and at first it was difficult to decide whether activity was associated with a net uptake of sodium. Keynes and Lewis (*37*) resolved the difficulty by measuring the sodium concentration in axoplasm by activation analysis, and there is now general agreement that at 20°C the net entry of sodium in one impulse amounts to 3 to 4×10^{-12} mole per square centimeter. Other experiments showed that a similar quantity of potassium ions leave the fiber during an impulse (*38*). It is perhaps easier to get an idea of what these quantities mean by saying that one impulse is associated with an inward movement of 20,000 sodium ions through 1 square micron of surface.

An entry of 4×10^{-12} mole of sodium per square centimeter is more than enough to account for the action potential. From the work of Cole and his colleagues it is known that the electrical capacity of the membrane is about 1 microfarad per square centimeter (*10*). The quantity of charge required to change the voltage across a 1-microfarad condenser by 120 millivolts is 1.2×10^{-7} coulomb; this is equivalent to 1.2×10^{-12} mole of monovalent cation, which is only one-third of the observed entry of sodium. A discrepancy in this direction is to be expected. In addition to charging the membrane capacity during the rising phase of the action potential, a good deal of sodium exchanges with potassium, particularly during the early part of the falling phase. From the quantitative theory which Huxley and I developed, the size of the ionic movements can be predicted from electrical measurements. As Huxley describes (*39*), the theoretical quantities turn out to be in reasonable agreement with experimental values.

The quantity of sodium which enters a myelinated axon during an impulse is much less than in an unmyelinated fiber of comparable size (*40*). This is presumably because the ionic exchange is confined to the node of Ranvier and the capacity per unit length of the axon is reduced by the thick myelin sheath.

Analysis of Membrane Currents: Voltage Clamp Experiments

In pursuing the evidence for the ionic theory I have departed from the strict order of events. During the summer of 1947 Cole and Marmont (*41, 42*) developed a technique for impaling squid axons with long metallic electrodes; with this technique they were able to apply current uniformly to the membrane and to avoid the complications introduced by spread of current in a cable-like structure. Cole (*41*) also carried out an important type of experiment in which the potential difference across the membrane is made to undergo a steplike change and the experimental variable is the current which flows through the membrane. In Cole's experiments a single internal electrode was used for recording potential and

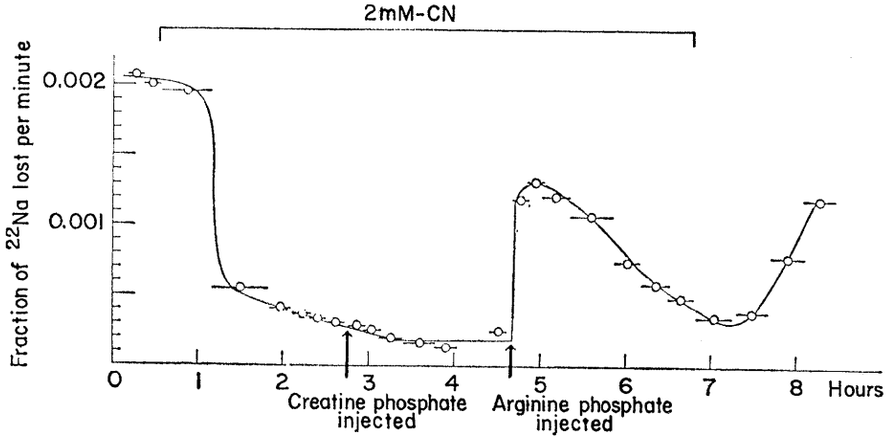

Fig. 6. Effect on outflow of sodium of (i) poisoning with cyanide; (ii) injecting creatine phosphate; (iii) arginine phosphate; (iv) removal of cyanide. The mean concentrations in the axon after injection were 15.3 m*M* creatine phosphate and 15.8 m*M* arginine phosphate. [From Caldwell *et al.* (*48*)]

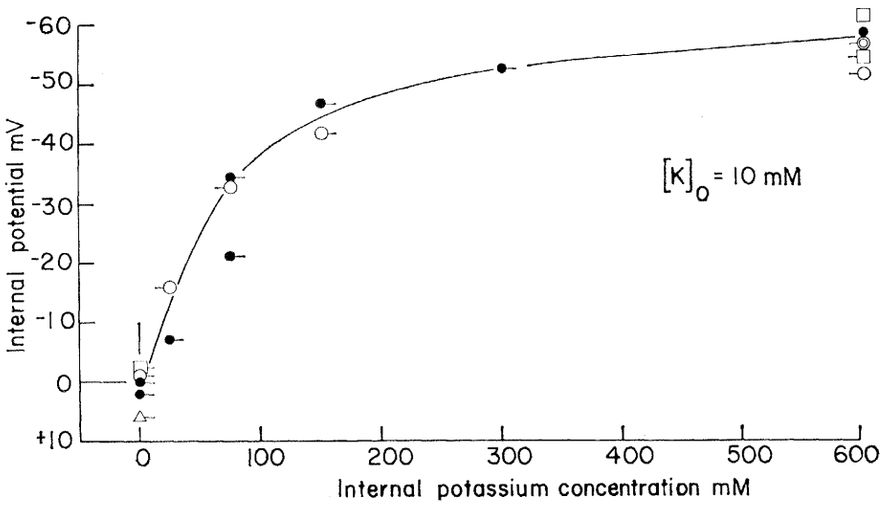

Fig. 7. Effect of varying internal potassium concentration on the resting potential. External solution, sea water containing 10 m*M* K; internal solution, NaCl-KCl solutions isotonic with sea water. Note that the resting potential reaches a limiting value of about −55 millivolts at potassium concentration greater than 150 m*M*. [From Baker *et al.* (*54*)]

passing current; since the current may be large, electrode polarization introduces an error and makes it difficult to use steps longer than a millisecond. However, the essential features of the experiment, notably the existence of a phase of inward current over a range of depolarizations, are plainly shown in the records which Cole obtained in 1947 (*41*). It was obvious that the method could be improved by inserting two internal electrodes, one for current the other for voltage, and by employing electronic feedback to supply the current needed to maintain a constant voltage. Cole, Marmont, and I discussed this possibility in the spring of 1948, and it was used at Plymouth the following summer by Huxley, Katz, and myself (*43*). Further improvements were made during the winter, and in 1949 we obtained a large number of records which were analyzed in Cambridge during the next 2 years (*44*). Huxley describes these results in more detail (*39*); here all that need be said is that by varying the external ionic concentrations it was possible to separate the ionic current flowing through the membrane into components carried by sodium and potassium, and hence to determine how the ionic permeability varied with time and with membrane potential.

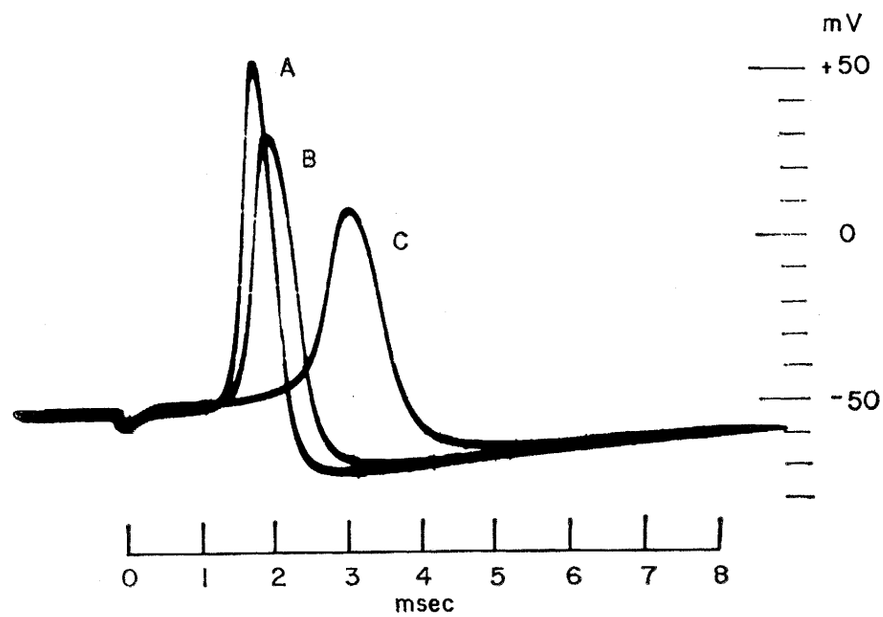

Fig. 8. Effect on action potential of replacing internal potassium with sodium ions. (*A*) Isotonic potassium sulfate; (*B*) 0.25 K replaced by Na; (*C*) 0.5 K replaced by Na. The records were obtained in the order *B, A, C*. [From Baker *et al.* (*54*)]

To begin with, we hoped that the analysis might lead to a definite molecular model of the membrane. However, it gradually became clear that different mechanisms could lead to similar equations and that no real progress at the molecular level could be made until much more was known about the chemistry and fine structure of the membrane. On the other hand, the equations that we developed proved surprisingly powerful, and it was possible to predict much of the electrical behavior of the giant axon with fair accuracy. Examples of some of the properties of the axon which are fitted by the equations are: the form, duration, and amplitude of the action potential; the conduction velocity, impedance changes, ionic movements; and subthreshold phenomena, including the oscillatory behavior.

Experimental Work on Giant Axons Since 1952

In the last part of this lecture I should like to mention some of the more recent developments in the ionic theory of nervous conduction. One major problem, which has interested a number of physiologists and biochemists, is to find out how cells use metabolic energy to move sodium and potassium ions against concentration gradients. In excitable tissues this process is of particular interest because it builds up the ionic concentration differences on which conduction of impulses depends. When a nerve fiber carries an impulse it undergoes a rapid cycle of permeability changes which allow first sodium and then potassium ions to move down concentration gradients. In giant axons, the changes associated with an impulse are exceedingly small, as can be seen from the fact that a 500-micron axon loses only one-millionth of its internal potassium in a single impulse. Large fibers can therefore conduct many impulses without recharging their batteries by metabolism. Nevertheless, if they are to be of any use to the animal, nerve fibers must be equipped with a mechanism for reversing the ionic exchanges that occur during electrical activity. The necessity for such a system was foreseen by Overton in 1902 when he

pointed out that human heart muscle carried out some 2.4×10^9 contractions in 70 years, yet, as far as he knew, contained as much potassium and as little sodium in old age as in early youth (*25*). Forty years later Dean introduced the idea of a sodium pump and showed that the distribution of potassium and chloride in muscle might be a passive consequence of an active extrusion of sodium, but that active transport of potassium or chloride ions would by themselves be inadequate (*45*). The concept was developed further by Krogh (*46*) and Ussing (*47*) and is now supported by experiments on a wide range of animal tissues.

Giant nerve fibers provide excellent material for studying ion pumping. One approach is to inject radioactive sodium ions and to collect the labeled ions which emerge from the fiber. Such experiments show that if the fiber is poisoned with cyanide or dinitrophenol it stops pumping, and sodium ions gradually accumulate inside. The fiber remains excitable for many hours because sodium and potassium can still move downhill during the impulse. But any sodium which gets into the fiber remains there and is not extruded as it would be in an unpoisoned axon. The ability to extrude sodium depends on the presence of adenosine triphosphate (ATP), and with an axon in which all the ATP has been broken down, sodium extrusion can be restored by injecting energy-rich phosphate in the right form (*48*). Figure 6 illustrates one of these experiments. It shows that the outflow of sodium is reduced to a low value by cyanide and can be restored by the molluscan phosphagen, arginine phosphate, but not by the vertebrate phosphagen, creatine phosphate. This is a satisfactory result since it is known that creatine phosphate is not handled by the enzyme which catalyzes the transfer of phosphate from arginine phosphate to adenosine diphosphate (*49*).

The molecular nature of the pumping mechanism is unknown, but there is much evidence to show that in most cells it is driven by compounds containing energy-rich phosphate, such as ATP or phosphagen. Recent interest in this field has been focused by Skou on an ATP-splitting enzyme which is present in the membrane and has the interesting properties of being activated by sodium and potassium and inhibited by substances which interfere with sodium transport (*50*).

Perfusion of Giant Axons

In conclusion I should like to mention an interesting new method which has been developed during the last few years. Since the action potential of a nerve fiber arises at the surface membrane it should possible to replace the protoplasm inside the fiber with an aqueous solution of appropriate composition. Methods for perfusing axons were worked out by Tasaki and his colleagues at Woods Hole (*51*) and by Baker and Shaw at Plymouth (*52*). The technique used at Plymouth is based on the observation (*53*) that most of the axoplasm in giant nerve fibers can be squeezed out of the cut end. This has been known since 1937, but until fairly recently no one paid much attention to the electrical properties of the thin sheath which remained after the contents of the nerve fiber had been removed. Since extrusion involves flattening the axon with a glass rod or roller it was natural to suppose that the membrane would be badly damaged by such a drastic method. However, in the autumn of 1960 Baker and Shaw (*52*) recorded action potentials from extruded sheaths which had been refilled with isotonic solution of a potassium salt. On further investigation (*54*) it turned out that such preparations gave action potentials of the usual magnitude for several hours, and that these were abolished, reversibly, by replacing potassium with sodium in the internal solution. As can be seen from Figs. 7 and 8, the resting potential and action potential vary with the internal concentrations of potassium and sodium in a manner which is consistent with the external effect of these ions.

A point of some general interest is that, although about 95 percent of the axoplasm had been removed, axon membranes perfused with isotonic potassium solutions were able to carry some 300,000 impulses. This reinforces the idea that chemical reactions in the bulk of the axoplasm are not essential for conduction of impulses and that ionic concentration gradients provide the immediate source of energy for the action potential. Huxley (*39*) tells more about the way in which this is done.

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56. A more complete bibliography can be found in A. L. Hodgkin, *Conduction of the Nervous Impulse* (Liverpool Univ. Press, Liverpool, 1963); also in *Biol. Rev. Cambridge Phil. Soc.* **26**, 339 (1951) and *Proc. Roy. Soc. London* **B148**, 1 (1958). From the bibliography it is evident that the development of the ionic theory has been very much a cooperative effort, and I wish to thank all those who have contributed to it. For more direct help, my particular thanks are due to W. A. H. Rushton, K. S. Cole, H. J. Curtis, A. F. Huxley, B. Katz, and R. D. Keynes. I am very grateful to the professors of physiology at Cambridge, Sir Joseph Barcroft, Lord Adrian, and Sir Bryan Matthews, and to the director and staff of the laboratory at Plymouth. My thanks are also due to the Rockefeller Foundation, the Nuffield Foundation, Trinity College (Cambridge), and the Royal Society for financial support. I should record my gratitude to R. H. Cook for the design and construction of apparatus and for his unfailing help.

Excitation and Conduction in Nerve: Quantitative Analysis

A. F. Huxley

Professor Hodgkin has told you how he was influenced as an undergraduate by the writings of four fellows of Trinity College, Cambridge. I too was an undergraduate at Trinity, but by the time I was taking physiology seriously, in my final year in 1938–39, there was yet another fellow of the College who influenced me even more directly than the ones mentioned by Hodgkin, and that was Hodgkin himself. He was one of my teachers during that year, and my first introduction to research was the short period that we spent together at the Marine Biological Laboratory at Plymouth in the summer of 1939, when we succeeded in recording the resting and action potentials of the giant nerve fiber of the squid with an internal microelectrode. This work was brought to a stop by the war, but we joined up again at Cambridge early in 1946, and almost the whole of my share in the work for which the prize was given was done jointly with him during the succeeding 5 or 6 years.

The "Voltage-Clamp" Method

Hodgkin has spoken about the ionic theory of the nerve impulse from a broad point of view, and I propose to go into greater detail on the quantitative aspects of the theory that we developed (*1*). The measurements on which this was based were made by a feedback method which has become known as the "voltage clamp." In this, a pair of wires is introduced along the axis of the giant nerve fiber, as shown diagrammatically in Fig. 1. The potential difference across the membrane is measured between one of these wires and an electrode in the sea water just outside the fiber, while the other wire is used for passing current through the membrane to another external electrode. The voltage wire is connected to the input of an amplifier whose output goes to the current wire, the direction of the connections being such that any accidental change of membrane potential is almost completely annulled by the current that the amplifier sends through the membrane. Rectangular pulses can also be fed into the amplifier through a second input. When this is done, the amplifier automatically sends through the current wire whatever current may be needed to make the membrane potential undergo stepwise changes proportional to those which are applied through the second input. This current is then displayed on a cathode-ray oscilloscope and photographed.

The net result is the same as would be obtained if a single ideal electrode was placed inside the fiber and connected to a low-impedance source of voltage steps, and the current was recorded. A method of this kind was indeed tried by Cole and Marmont in 1947 (*2*), and gave useful results, but it is not suitable for quantitative work because the current density that the electrode has to pass is really quite large and no one has yet made an electrode that is sufficiently free from polarization troubles.

Analysis of the Currents through the Nerve Membrane

In order to create a nearly instantaneous change in the potential difference across the membrane, the membrane capacity has to be charged or discharged by the passage of a substantial quantity of electricity in a very short time. This pulse of capacity current can be recorded by the voltage-clamp method, but in the figures reproduced here most of it cannot be seen because of its rapid rise and fall, and its very short total duration, which is only a few microseconds. Analysis of these pulses has confirmed the existence of the capacity in the membrane of about 1 microfarad per square centimeter which had been demonstrated many years earlier with alternating-current methods by Curtis and Cole (*3*). Our present concern is, however, with the currents which flow in the first few milliseconds after the completion of this capacity current, while the membrane potential is held constant by the feedback system.

The general features of these components of the current are illustrated in Fig. 2. The right-hand side of the figure shows that when the normal potential difference across the membrane is increased by 40 millivolts (the inside of the fiber thus being made more negative), the currents are very small. They are barely visible at the amplification used in these records, but with

The author is head of the department of physiology, University College of London. This is the lecture which he delivered in Stockholm, Sweden, 11 December 1963, when he received the Nobel prize in physiology and medicine, a prize which he shared with A. L. Hodgkin and Sir John Eccles.