THE EFFECT OF SODIUM AND POTASSIUM IONS ON THE IMPEDANCE CHANGE ACCOMPANYING THE SPIKE IN THE SQUID GIANT AXON*

By HARRY GRUNDFEST, ABRAHAM M. SHANES, AND WALTER FREYGANG‡

(From the Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare, Bethesda, and the Marine Biological Laboratory, Woods Hole)

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INTRODUCTION

The transient changes which take place in the giant axon during the development and decline of the spike now appear to involve a series of physicochemical phenomena of hitherto unsuspected complexity. According to the formulation of Hodgkin and Huxley (10), the first event is a process involving an influx of sodium ions followed by an "inactivation" which causes this influx to subside; a delayed but prolonged outflux of potassium completes the cycle and restores the electrical condition of the fiber to its original state. The measured fluxes of the ions (12, 13) are roughly in accord with this hypothesis.

The impedance change during nerve activity is due solely to an alteration in membrane conductance and is practically proportional to the latter (1). Its direct measurement under a variety of experimental conditions provides additional data for a more precise delimitation of mechanism, or may lead to the recognition of additional factors. The work presented here seems in large part to be explicable on the basis of the theory advanced by Hodgkin and Huxley (10), but from the data both of the present report and of the following (14) it appears that additional processes are involved in the manifestation of the action potential.

A preliminary report of some experiments, in which membrane impedance changes during activity were examined in low sodium solutions, recently was given (5). The present study is an amplification of these earlier experiments

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Present address: National Institute of Mental Health of the National Institutes of Health, Public Health Service, Federal Security Agency, Bethesda.

and deals with the quantitative effects of different external concentrations of potassium as well as of sodium. Simultaneously, the threshold and spike amplitude were followed.

The data to be presented show that both ions are important factors controlling the amplitude of the impedance (or conductance) change during activity. The effect of *lack* of Na^+ or *excess* of K^+ on the impedance change and spike amplitude is similar but not identical. In addition, potassium delays the return to resting conductance.

Methods

All data are based on transverse impedance measurements at 25 kilocycles on clean segments of the giant axon from the hindmost stellar nerve of *Loligo pealii*. A plexiglass chamber similar to that described by Cole and Curtis (1) was used. The electrodes were of platinum. The two impedance electrodes, 300 micra high and 420 micra long, were set into opposite sides of a trough 450 micra high, 480 micra wide, and 1.5 cm. long. This trough, into which was placed the cleaned segment of the axon, was made deeper and wider at the ends to accommodate the uncleaned portions of the stellar nerve. Stimulating and pick-up electrodes projected from below into the floor of the channel; the former were 1 cm. from the impedance electrodes and the latter consisted of one electrode directly between the impedance electrodes and a second 1.6 cm. beyond. The nerve was fixed in position by inserting the thread tied to its ends into small cork blocks at the extreme ends of the channel. After the top of the channel had been closed off with Scotch electrical tape, a continuous flow of solution through the chamber was begun. Within 30 minutes or less the impedance drift became sufficiently low, so that an experimental run could be started. The equilibration solution was alternated with media modified with respect to either sodium or potassium, and thereby served as a repeated point of reference. In the potassium series the reference solution contained 520 mM NaCl, 17 mM KCl, and 30 mM $CaCl_2$ per liter, and, as in all other solutions, a small quantity of bicarbonate to bring the pH to about 7.8. The potassium concentration was changed without altering the sodium level. In sodium runs the reference solution was the same except for the KCl, which was 10 mM/liter. Sodium was replaced with choline by dilution of the reference solution with a choline chloride solution containing the same quantities of KCl and $CaCl_2$. The resistivity of this choline chloride solution was found to be 20 per cent greater than that of NaCl artificial sea water.

The block diagram in Fig. 1 illustrates the general arrangement of electrical components. The 25 kilocycle signal of a model 202B Hewlett-Packard oscillator, metered with a microvolter, supplies an A.C. Wheatstone bridge, one arm of which is composed of the nerve in series with the impedance electrodes. The bridge output is then fed to an amplifier followed by a 25 kilocycle "balanced T," R-C rejection filter, and finally to one channel of a DuMont type 322 dual beam oscilloscope. The output of the filter was down 30 per cent at 8 and 40 kilocycles, thereby substantially reducing noise, harmonics, and distortion. This made high amplifications feasible, a factor of particular importance in the study of low level impedance changes described in the subsequent paper (14).

The response time of the bridge circuit and associated equipment was judged to be

of the order of 100 to 200 microseconds, as determined by suddenly introducing or removing a small resistor in series with the axon on the input of the bridge. This was of the order of the rise time found for the spike impedance change at room temperature, which ranged from 25 to 28°C.

The stimulating shock was a 100 microsecond rectangular pulse, and the threshold was read from the linearly calibrated output control.

Photographs were taken (*a*) of the bridge unbalance during the spike simultaneously with a timing signal, (*b*) of a calibration in the form of the oscilloscope deflec-

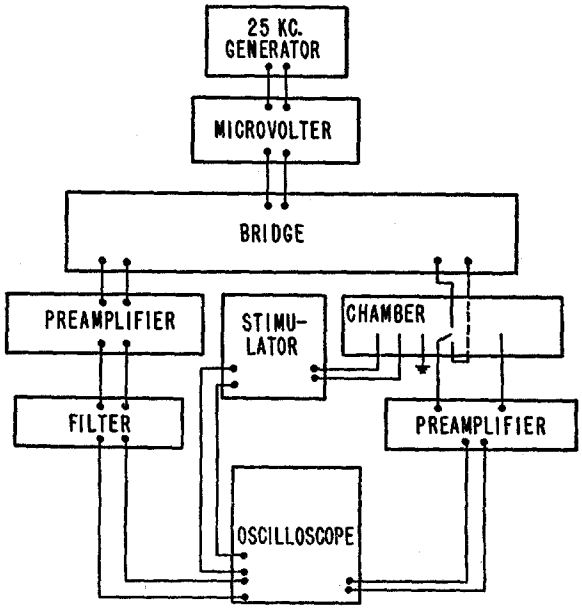

FIG. 1. Diagrammatic arrangement of components for the measurement of impedance changes and spikes of nerve fibers.

tion for a given change in the resistance of the bridge, and (*c*) of the spike and the bridge output in the absence of the 25 kilocycle bridge input. The deflection observed in the impedance measuring circuit in the absence of the 25 kilocycle signal provided a check on the possible distortion of the impedance record by pick-up of the impulse passing between the impedance electrodes. Such distortion was small (Figs. 2 and 6) and did not affect the measurements, which were made from peak to peak of the records.

Repeated series were run on a fiber as long as the experimental effects were reversible. Changes of solution required about a minute, at which time equilibration appeared complete and photographs were taken. Measurements were made on the projected 35 mm. records. The amplitudes of the spike, the impedance change, and the half-time under experimental conditions were always computed relative to the means of the control values obtained immediately before and after each change.

Cole and Curtis (1) have pointed out that under the conditions of our experiments

the bridge unbalance, as indicated by the oscilloscope deflection, is proportional to the conductance change of the fiber membrane. Hence, the relative amplitudes of the spike-induced unbalance, described in the following figures, represent the relative conductance change. Absolute determinations were not attempted, but such values are obtainable from our data by assigning 40 mmho/cm.^{2} to the conductance change in sea water (Cole and Marmont, quoted in reference 2) or in a solution containing 520 mM/liter NaCl and 10 mM/liter KCl.

RESULTS

Potassium.—Typical records are given in Fig. 2. Comparison of the upper traces of A (zero KCl) and C (17 mM/liter KCl) illustrates the increase in spike amplitude with a lowering of the potassium level of the medium; B and D show the corresponding increase in the amplitude of the spike impedance change as well as the decrease in recovery time.[1] The converse effects with an increase in potassium concentration are apparent in C to F, records E and F representing the changes produced by increasing the KCl to 35 mM/liter. The spikes (records A, C, E), though diphasic, are asymmetrical. This asymmetry is probably due to the recording conditions, for the proximal electrode was on a cleaned fiber lying in a narrow channel, while the distal electrode was on the stellar nerve in a larger body of fluid.

The temporal characteristics of the spike were not studied in detail. However, it can be seen in Fig. 2 A, C, and E that the time from the stimulus artifact to the first peak is lengthened by increasing the extracellular potassium concentration. However, the interval between the maximum and minimum of the diphasic action potential remained practically unchanged, indicating little alteration in the conduction velocity. The variation in time to the first peak may therefore have been due to variation in latency or in the rate of rise of the spike. The complexity of the recording conditions did not permit further analysis.

The electrical artifact, picked up by the bridge in the absence of the 25 kilocycle signal as the spike passed between the impedance electrodes, is given by the small deflection on the lower traces appearing with the spikes in Fig. 2 A, C, and E. It is small compared with the bridge unbalance and, as can be seen, would leave the much larger deflection in the impedance records (B, D, F) practically undisturbed.

The effect of graded changes of potassium concentration on the amplitude of both the spike and of its maximum impedance change, relative to their values in 17 mM/liter, is summarized for eight nerves in Fig. 3. Because 45 mM/liter KCl blocked in some cases, the mean at this concentration is based on the

[1] In the absence of potassium, a prolonged unbalance of the bridge is evident after the major impedance change (Fig. 2 B). Data recently obtained demonstrate that this is an enhanced "initial after-impedance" (see reference 14).

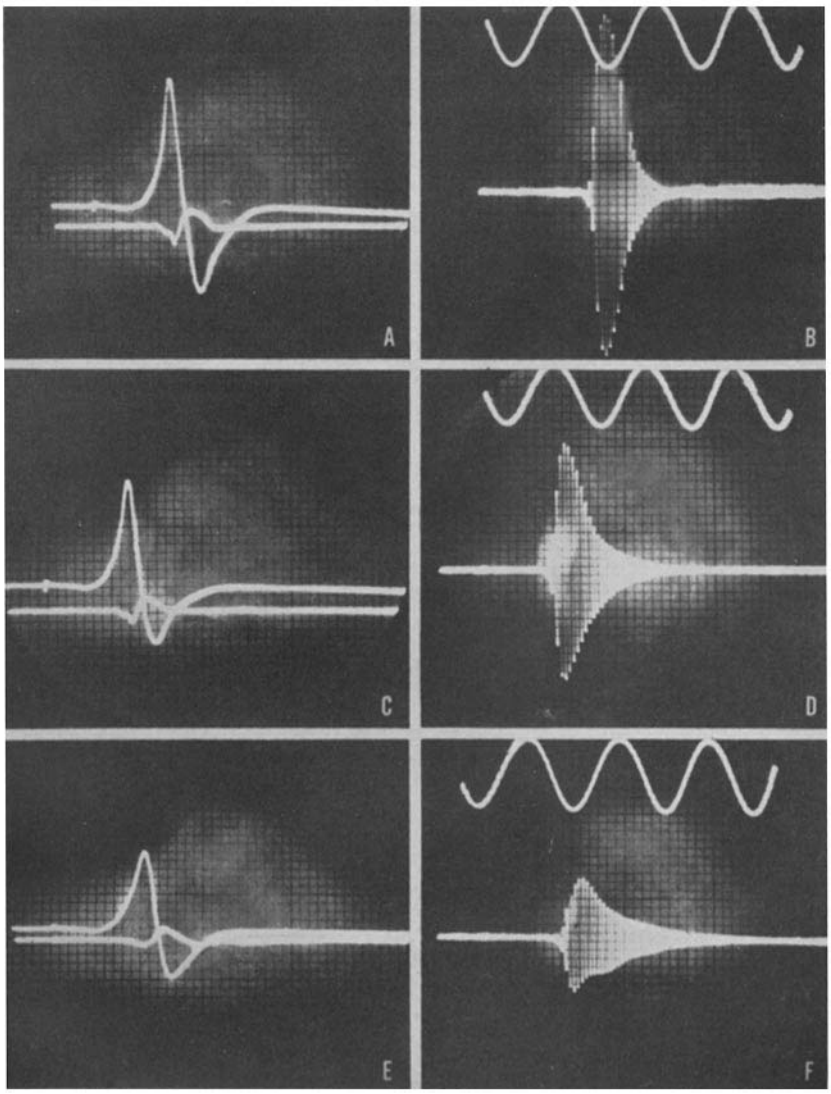

FIG. 2. A, C, E, diphasic spikes (upper traces) and bridge artifacts (lower traces) at zero, 17, and 35 mM/liter potassium concentration, respectively; B, D, F, corresponding bridge unbalances accompanying the spike. Interval between time markers, 1 msec.

fewest determinations. In this and subsequent figures, the parentheses contain the number of determinations which provided the mean at each concentration, and the vertical lines give ± the standard error.

As the potassium concentration is increased above 17 mM/liter, the spike height and the spike impedance change decline, the latter somewhat more rapidly. When the level is decreased below 17 mM/liter, the two curves behave quite differently. The spike impedance change continues to increase along the nearly linear curve obtained for high potassium levels, whereas the spike

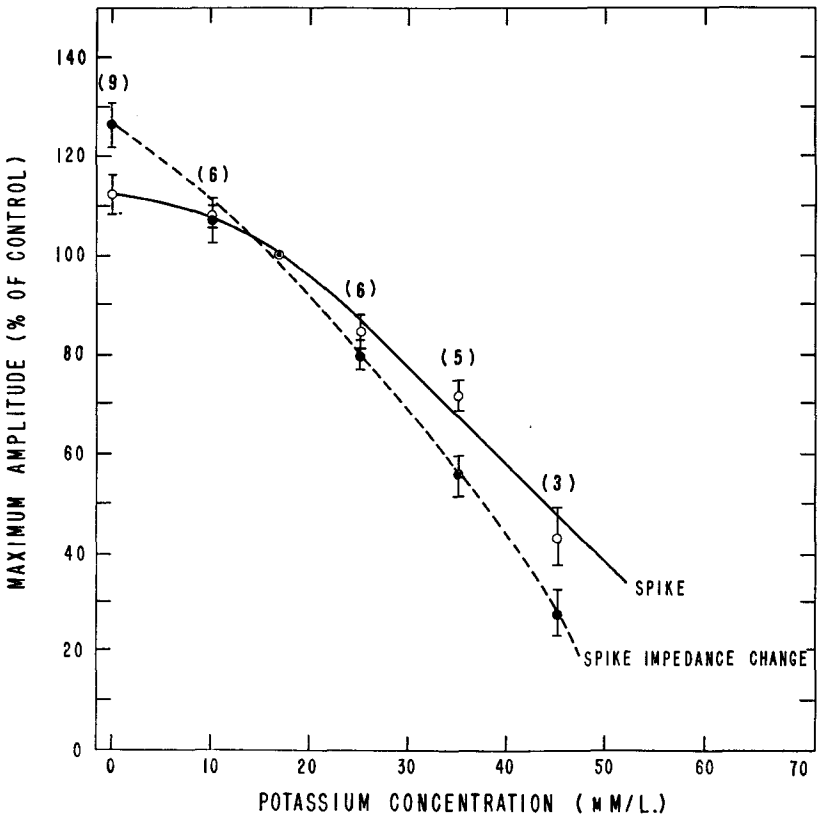

FIG. 3. Variation of the amplitude of the spike and its impedance change, relative to the values at 17 mM/liter, with extracellular potassium concentration.

becomes relatively insensitive to potassium, as is also the case for the resting potential (4, 15).

Corresponding data, showing that the half-time of the spike impedance change (*i.e.*, the time from the beginning of the increased conductance to the time when the bridge unbalance has dropped to half its maximum value) increases with an elevation of potassium in the medium, are plotted in Fig. 4. These values also are given relative to the control value in 17 mM/liter potassium. The half-time of the 41 controls averaged 407 ± 7 microseconds.

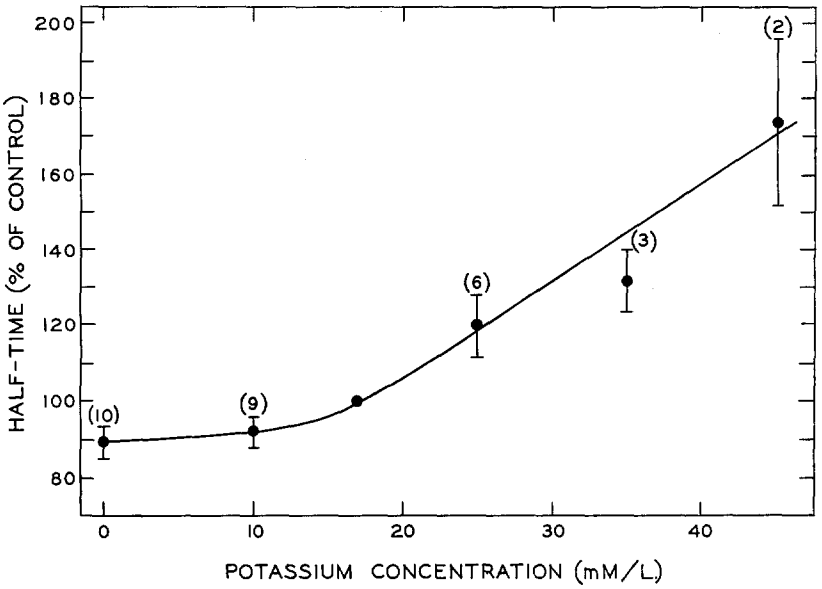

FIG. 4. The dependence on extracellular potassium concentration of the half-time of decline of the impedance change, relative to that at 17 mM/liter, measured from the initiation of the bridge unbalance.

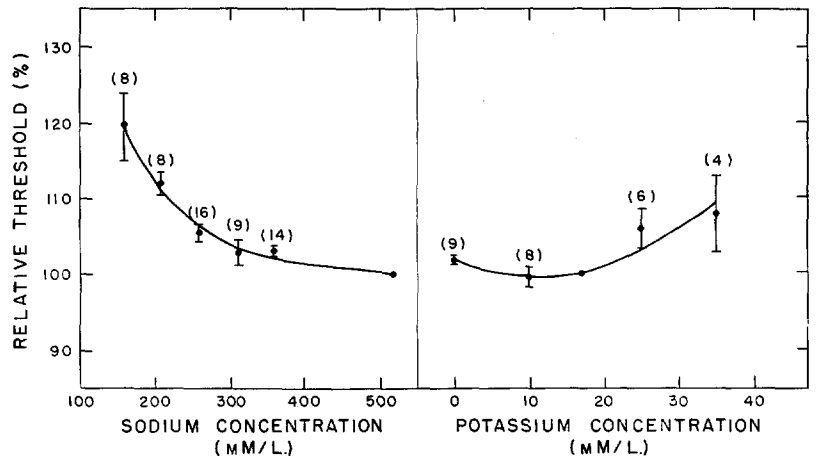

FIG. 5. Threshold, as a function of potassium and sodium concentration, relative to that at 17 and 520 mM/liter, respectively.

It should be noted that the half-time includes a rise time of about 150 microseconds. The frequency response of the bridge and associated circuits was of this order, as was the transit time of the spike along the effective length

of the impedance electrodes. Satisfactory information therefore is unavailable as to the actual rise time of the conductance increase. Correction for this would steepen the curve in Fig. 4, particularly at low potassium levels.

Fig. 5 summarizes the effect of potassium on the threshold. The threshold is relatively insensitive to the potassium ion until blocking concentrations are approached.

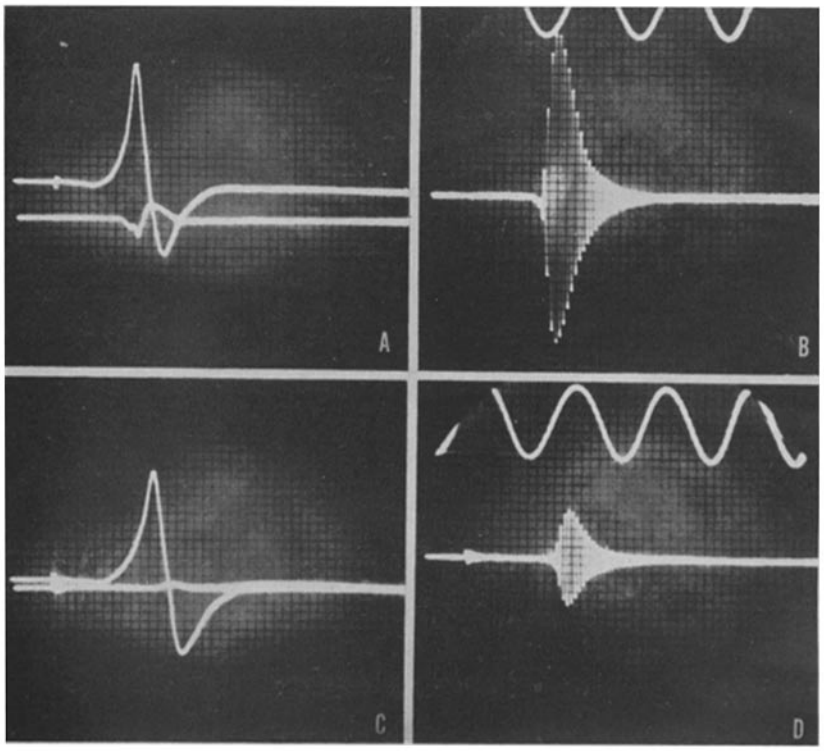

FIG. 6. A and C, diphasic spikes (upper traces) and bridge artifacts (lower traces) at 520 and 260 mM/liter sodium concentration; B and D, corresponding bridge unbalance. Interval between time markers, 1 msec.

Sodium.—Representative records, obtained with the same axon used for the potassium records in Fig. 2, are shown in Fig. 6. In accord with the observations of Grundfest *et al.* (5), they demonstrate the decrease in the spike impedance change as well as that of the spike (11) with decreasing sodium. No effect is apparent on the declining phase of the bridge unbalance.

The averaged data from seven nerves are the basis of the curves in Fig. 7. Only 4 values contributed to the lowest figure because of the tendency of some fibers to block at such low sodium levels. The next three points are each based on 8 to 10 determinations, some having been repeated on the same preparations.

The decrease in spike height as the sodium content of the medium is lowered conforms closely to the curve obtained by Hodgkin and Katz (11). The relationship of the spike impedance change to sodium concentration is almost identical with that for spike amplitude. The impedance data are consistently lower than those for spike height; however, the individual points are not

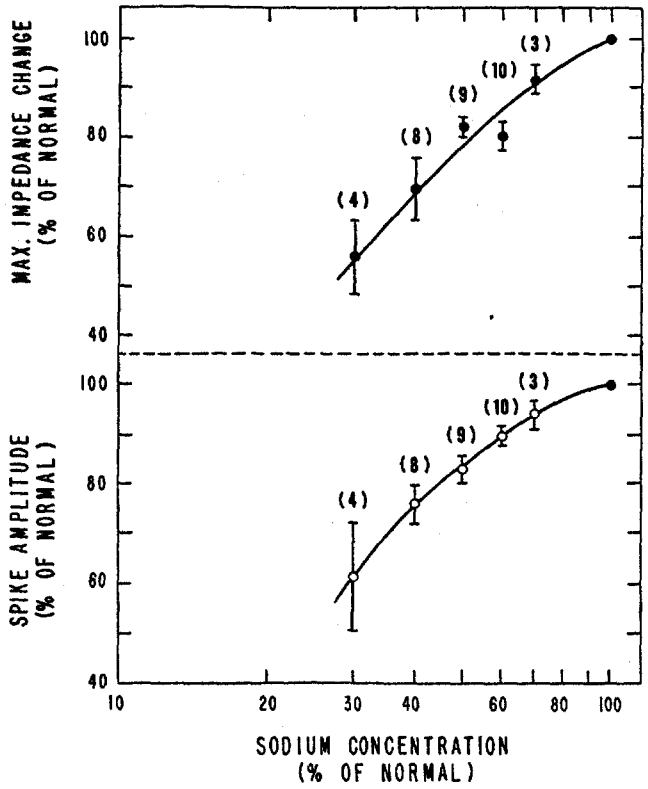

FIG. 7. Variation of the amplitude of the spike and its impedance change, relative to the value at 520 mM/liter, with extracellular sodium concentration. The relative sodium concentration is shown on a logarithmic scale, while in Fig. 3 the potassium concentration is plotted linearly.

significantly different. Nevertheless a real difference may exist between the two curves since a correction, which should be applied for the reduced shunting by choline solutions, is greater for the spike impedance change than for the spike. Thus, calculations, based on the relation between the spike impedance change and conductance of the medium (1) and the relation between spike amplitude and intra- and extrafibrillar resistance, indicate that because of the reduced shunting the spike impedance change measured 10 per cent and the spike 5 per cent greater in the weakest sodium solution employed; *viz.*,

156 mM/liter. The corrected curve for the spike impedance change therefore would be steeper than that for the spike.

As may be seen in Fig. 5, the threshold rises gradually as sodium is removed from the medium, and more steeply when the blocking level is approached.

DISCUSSION

The theoretical basis for interpreting these results is summarized in a recent review (2), which also lists earlier literature. The frequency characteristics of the impedance of a simple geometrical system like that of a resting axon surrounded by sea water indicate that the axoplasm is of relatively high conductivity and is surrounded by a membrane of high capacitance shunted by high resistance. Cole and Curtis (1) have shown that during the nerve impulse the capacitance remains constant whereas the parallel resistance drops. The most recent measurements (2) give a decrease during activity to $\frac{1}{60}$ of the resting value. The effect of low sodium or high potassium, therefore, is to reduce the extent of the resistance change.

Physical Significance of the Decreased Resistance Change.—The decrease in the amplitude of the spike impedance changes under the conditions of our experiments may be the result (*a*) of a decrease in the resting resistance, the low resistance during activity being unaffected, (*b*) of an increased resistance during activity, the resting resistance being unaffected, or (*c*) of both effects. Available data and theoretical considerations favor the increase in minimum resistance during activity as the major factor. Thus, an extrapolation of Hodgkin's data (6) suggests that lowering the sodium concentration decreases the resting conductance, which in case *a* could cause only an increase in the amplitude of the spike impedance change. In the experiments with increased potassium, in which the resting membrane resistance is appreciably reduced (6, 16), some influence of this factor can be expected, but is easily shown to be small. For example, in 10 mM/liter potassium the resting conductance is 0.7 mmho/cm^2. (2); an increase of potassium to 45 mM/liter, the maximum used in the present work, would increase this conductance to about 4 mmho/cm^2. (16).[2] During the impulse, in 10 mM/liter potassium, the conductance rises to 40 mmho/cm^2. (2); on the assumption that the same maximum value is attained in the high potassium solution, the conductance change would be 39 mmho/cm^2. in 10 mM/liter potassium, and 36 mmho/cm^2. in 45 mM/liter, or more than 90 per cent of the original change. As may be seen in Fig. 3, the effect of raising the potassium concentration is much greater, so

[2] Dr. K. S. Cole (personal communication) points out that this figure may be an overestimate for our conditions of measurement. The high frequency locus may not only show a smaller effective decrease of the rest impedance in elevated potassium, as compared to the D.C. data employed above, but an actual increase. In that case, the effect of potassium on the resting level of impedance would be negligible.

that at 45 mM/liter the maximum conductance during activity is 25 per cent of that in 10 mM/liter, or about 10 mmho/cm². The conclusion appears justified, therefore, that the decrease in the spike impedance change represents a larger minimum resistance during the spike.

Nature of the Decrease in Amplitude of the Impedance Change.—The interpretation of these results hinges on the sequence of ionic events believed to occur during the spike. The first possibility to be considered is that there is an interference with the increase in ion movement which is known to occur with activity. From calculations of the sodium conductance (7), low sodium appears to have only a slight effect on the mobility change. The major critical variable therefore is the number of sodium ions available in the membrane to carry current. The slopes of the I_{Na}-V curves in sea water and 30 per cent sea water at the sodium potential (Fig. 13 in reference 7) may be taken as a rough estimate of the maximum sodium conductance under these two conditions (Hodgkin, personal communication); on this basis, the maximum conductance is decreased from 23 to 10 mmho/cm². in 30 per cent sodium. Thus, the predicted value in low sodium is about 45 per cent of that in sea water as compared with the 50 per cent actually found after correction for the reduced shunting in low sodium (*cf.* our Fig. 7). This is within the variability of the data, but a detailed analysis similar to that carried out by Hodgkin and Huxley (10) for other conditions is required to ascertain the extent of the agreement.

The action of potassium on the conductance change of activity is probably related to the earlier finding that the effectiveness of this ion on spike height is greater than can be accounted for by the alteration in resting potential alone (11). A possible explanation is that the external potassium level, by virtue of its effect on the resting potential, modifies the "inactivation process" in the same way as do small, electrically induced changes in membrane potential (9). The membrane potentials associated with the different potassium levels employed in the present experiments can be determined approximately from the E-log K curve of Curtis and Cole (4).[3] The relative changes in the corresponding sodium current (from Fig. 5 in reference 9) can then be obtained as an estimate on the effect on peak conductance. On this basis, a change of the external fluid to one with low potassium would increase the peak conductance by 35 per cent, while a change to an external medium of 45 mM/liter potassium would decrease the peak conductance to 35 per cent. These figures are close to the corresponding experimental data in our Fig. 3.

Temporal Course of the Impedance Change.—The slowing of the rate of return of the conductance to normal in high potassium is of interest in the light of the absence of a corresponding effect on the spikes (11). This appears explicable as the consequence of a slowing of the rate of subsidence of potassium and

[3] After correction of their relative potassium scale to a potassium level in sea water of 10 mM/liter rather than the 13 mM/liter originally assumed.

sodium conductances. The elevated potassium can achieve this in two ways: (*a*) by depolarizing the membrane and (*b*) by decreasing the positive phase following the spike. From Figs. 9 and 14 of Hodgkin and Huxley (8), the time constant of decline of both conductances may be expected to be lengthened by these effects. The half-time of decline of the impedance change (0.4 msec.) corresponds closely to the duration of the spike (0.3 to 0.5 msec.) as measured with an internal electrode at the same temperatures (Grundfest *et al.*, unpublished).[4] In view of this, the large changes in the decay constant of sodium conductance (Fig. 9, reference 8) may contribute substantially in prolonging the early part of the falling phase of the impedance change.

Low sodium, in contrast to elevated potassium, alters the configuration of the spike (11) without an appreciable effect on the temporal course of the conductance change. Additional data appear necessary for an evaluation of these findings.

Threshold.—The effect of elevated potassium on the conductance change has been pointed out to be explicable in terms of enhanced inactivation. In that case, however, the development of the local response, and hence the threshold, would be expected to be modified in a similar fashion. Our Fig. 5, however demonstrates a negligible effect by potassium except at extreme concentrations.

The increase in threshold at low sodium concentrations is of interest in the light of the finding by Crescitelli (3) that the effectiveness of anesthetics is enhanced by low sodium concentrations. This synergistic action would be accounted for by an additive effect in raising threshold.

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SUMMARY

Decrease of the sodium concentration of the medium depresses both the spike and the associated impedance change in almost identical fashion. Elevation of the potassium level also depresses both phenomena, but affects the impedance change more than the spike; it slows the return to the initial impedance level. The effects on the threshold to brief square waves are also described. These results appear largely accounted for by the observations of Hodgkin and Huxley with the voltage clamp technique and by their recent hypothesis as to nature of the spike processes.

[4] The half-time of the decline of membrane conductance calculated by Hodgkin and Huxley (Fig. 17, reference 10) occurs earlier than found experimentally, which suggests either that the sodium conductance change lasts somewhat longer or that the potassium conductance arises later than assumed by these investigators.

REFERENCES

1. Cole, K. S., and Curtis, H. J., Electric impedance of the squid giant axon during activity, *J. Gen. Physiol.*, 1939, **22,** 649.
2. Cole, K. S., and Curtis, H. J., Bioelectricity: Electric physiology, *in* Medical Physics, (O. Glasser, editor), Chicago, The Year Book Publishers, Inc., 1950, **2,** 82.
3. Crescitelli, F., Modification in responses to sodium of nerve fibers treated with drugs, *Am. J. Physiol.*, 1952, **169,** 638.
4. Curtis, H. J., and Cole, K. S., Membrane resting and action potentials from the squid giant axon, *J. Cell. and Comp. Physiol.*, 1942, **19,** 135.
5. Grundfest, H., Guttman, R., Hendley, C. D., and Wilson, I. B., Membrane resistance changes in the course of axonal spikes modified by low Na^+ concentration, *Science,* 1952, **115,** 522.
6. Hodgkin, A. L., The effect of potassium on the surface membrane of an isolated axon, *J. Physiol.*, 1947, **106,** 319.
7. Hodgkin, A. L., and Huxley, A. F., Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo, J. Physiol.*, 1952, **116,** 449.
8. Hodgkin, A. L., and Huxley, A. F., The components of membrane conductance in the giant axon of *Loligo, J. Physiol.*, 1952, **116,** 473.
9. Hodgkin, A. L., and Huxley, A. F., The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo, J. Physiol.*, 1952, **116,** 497.
10. Hodgkin, A. L., and Huxley, A. F., A quantitative description of membrane current and its application to conduction and excitation in nerve, *J. Physiol.*, 1952, **117,** 500.
11. Hodgkin, A. L., and Katz, B., The effect of sodium ions on the electrical activity of the giant axon of the squid, *J. Physiol.*, 1949, **108,** 37.
12. Keynes, R. D., The role of electrolytes in excitable tissues, Inst. Biophysics, Univ. Brazil, 1951, **9,** 1.
13. Rothenberg, M. A., Studies on permeability in relation to nerve function. II. Ionic movements across axonal membranes, *Biochem. et Biophysic. Acta,* 1950, **4,** 96.
14. Shanes, A. M., Grundfest, H., and Freygang, W., Low level impedance changes following the spike in the squid giant axon before and after treatment with "veratrine" alkaloids, *J. Gen. Physiol.*, 1953, **37,** 39.
15. Shanes, A. M., and Hopkins, H. S., Effect of potassium on "resting" potential and respiration of crab nerve, *J. Neurophysiol.*, 1948, **11,** 331.
16. Weidmann, S., Electrical characteristics of *Sepia* axons, *J. Physiol.*, 1951, **114,** 372.