LOW LEVEL IMPEDANCE CHANGES FOLLOWING THE SPIKE IN THE SQUID GIANT AXON BEFORE AND AFTER TREATMENT WITH "VERATRINE" ALKALOIDS*

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

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

(Received for publication, February 16, 1953)

INTRODUCTION

While the impedance changes associated with the spike have received considerable attention, few data are available for the events which follow the spike. The only published results appear to be those of Tasaki and Mizuguchi (16) on single vertebrate fibers. The present study is concerned with a correlation of the impedance changes with the later electrical phenomena in giant axons from *Loligo pealii*.

The accentuation of the negative after-potential by the alkaloid mixture, veratrine, has long been known. More recently, the two pure alkaloids derived from it—cevadine and veratridine—have been demonstrated to produce characteristically different negative after-potentials in nerve (14). Thus, the after-potential, which disappears exponentially in both alkaloids, does so at a considerably slower rate in veratridine than in cevadine, the time constant being eightfold greater. The amplitude is twice as high in an equivalent concentration of cevadine compared to veratridine, but this is still only 3 per cent of the spike height obtained under similar conditions of measurement (11).

The results to be described were obtained by the application of extremely sensitive methods to the transverse impedance technique employed by Cole and Curtis (2). This has led to the following observations: (*a*) In untreated axons, a decrease in conductance below that at rest follows the well known increase accompanying the spike; (*b*) a conductance increase, with a time course identical with that of the after-potential, accompanies the alkaloid-induced negative after-potential; and (*c*) this secondary conductance increase, in

* This work was carried out at the Marine Biological Laboratory partly under a grant to one of us from the United States Atomic Energy Commission under contract AT (30-1)1076.

‡ Aided by a Fellowship from the National Foundation for Infantile Paralysis.

cevadine, attains a maximum about 3 per cent of the amplitude of the spike conductance change, which is the percentage of the after-potential peak relative to the spike height.

Methods

Except for the manner in which the axons were treated with experimental solutions and mounted in the chamber, the procedure and equipment for impedance measurements were identical with those described in the preceding paper (5). The limited amplitude of the changes encountered required maximum sensitivity. Therefore, solutions were not run through the troughs after the axon was placed in the chamber. Instead, the axon was laid in position with such fluid as succeeded in clinging to it, and then covered. By this means the amplitude of the impedance change was made 3 to 4 times greater than with the flow technique. Under these conditions, bridge unbalance corresponding to less than 0.1 per cent change in the resistance of one arm could be measured. All experiments were performed at room temperature, which ranged from 24–27°C.

Sea water was the basic medium employed in this study, and cevadine[1] and veratridine[1] were added to it from concentrated, neutral stock solutions as described earlier (14). After control observations had been made on an axon, it was removed from the chamber and exposed for several minutes to cevadine or veratridine at a concentration of one part in a million (0.1 mg. per cent), then washed for an equal period in alkaloid-free sea water, and finally remounted. This procedure was demonstrated previously to give stable after-potentials with reproducible characteristics (14).

Impedance changes in the axon have been found to be due to alterations in the resistive component of the membrane (1, 2, 6). The measurements to be described are considered to be of the same nature. The small impedance changes found in this study could be balanced merely by altering the resistance arm of the bridge.

RESULTS

Normal Fibers.—In general, a tenfold increase in amplification over that employed for the spike impedance change sufficed to bring the phenomena of interest within reach. Fig. 1 shows typical records. Fig. 1 A demonstrates a bridge unbalance, at low gain and with a high sweep speed, which corresponds to the major transitory decrease studied by Cole and Curtis (2). This is shown with ten times greater gain and with a slower time base in Fig. 1 B. A secondary unbalance, distinct from the initial major one, is now evident. It does not appear to represent the same process causing the large impedance change since its maximum occurs after the spike conductance change has almost completely subsided. Moreover, this small unbalance was found to be corrected by an increase in the compensating resistance rather than by the decrease required by the preceding phenomenon (Fig. 2). Hence, the increase in

[1] Kindly supplied by Professor O. Krayer.

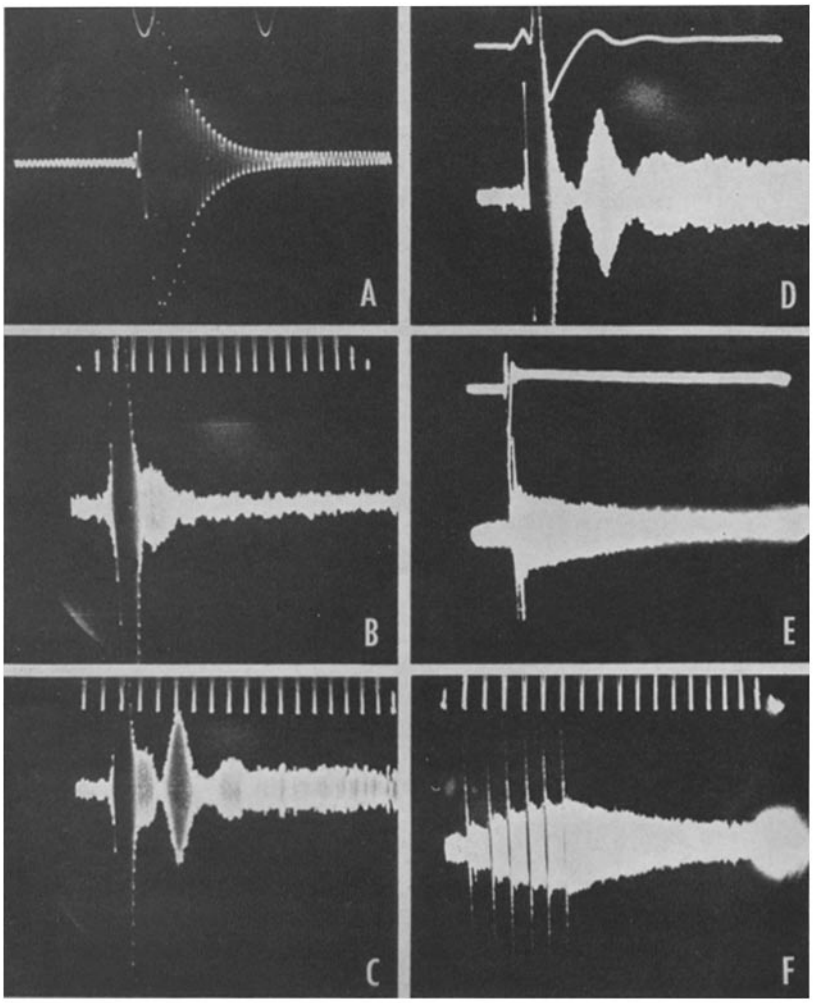

FIG. 1. A, typical bridge unbalance observed at low gain during passage of a single impulse. Interval between time markers, 1 msec. Bridge frequency, 25 kc./sec. B, as in A, with slower sweep and ten times more gain. C, as in B, but after exposure to 0.1 mg. per cent cevadine. D, as in C, but more typical of cevadine action; the monophasic action potential, simultaneously observed at high gain at a point 7.5 mm. distant, exhibits the "positive overshoot" (downswing following the spike) and bioelectrical oscillations. E, as in D, but tenfold slower sweep. F, addition of negativity after-impedances during a brief volley at 100 shocks/sec. Interval between time markers, 10 msec.

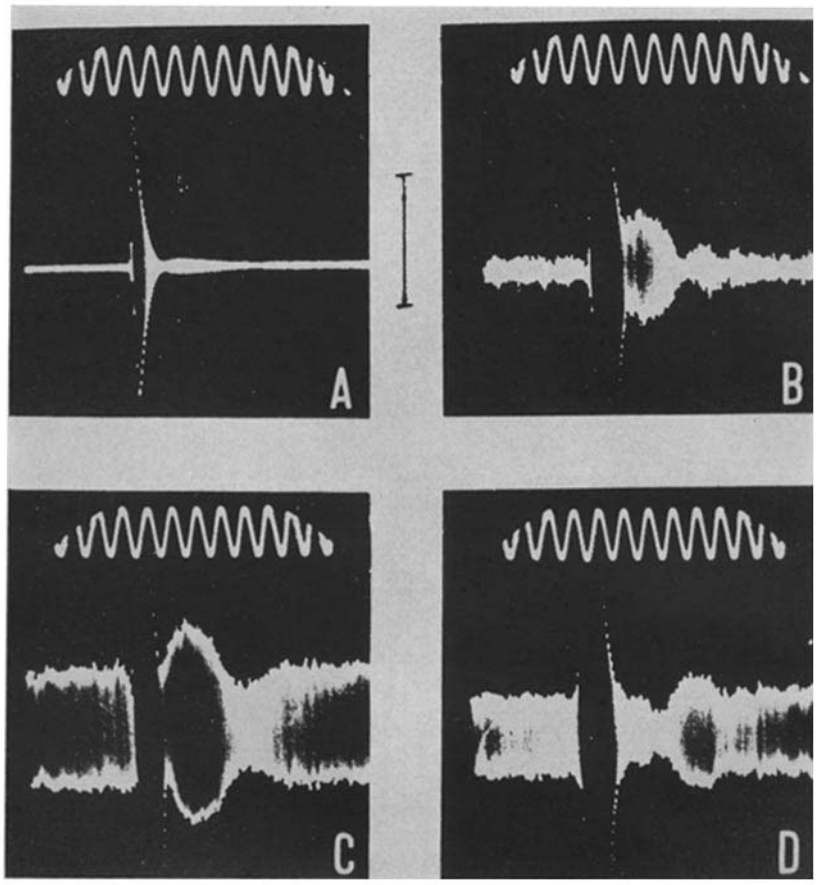

FIG. 2. A, typical bridge unbalance, during passage of a single impulse, observed at low gain and with a slow sweep. The vertical line gives the extent of the bridge unbalance resulting from a resistance change of 3.7 per cent. Timing signal in this and succeeding records, 1 kc./sec. B, as in A, with ten times more gain. C, as in B, but bridge initially unbalanced by a *decrease* in resistance in the compensating arm. D, as in B, but bridge initially unbalanced by an *increase* in the compensating resistance, thereby balancing out the initial after-impedance. The absence in B of an intermediate balance point corresponding to the transition from the high conductance of the spike-impedance to the decreased conductance of the initial after-impedance may have been due to the limited response time (100 to 200 microseconds) of the circuits (5).

conductance characteristic of the spike process is followed by a decrease to below the resting level, a sequence seen in all preparations kept in sea water.

At this point a simplified terminology will be adopted. The word "change"

will be dropped but implied in the expression "spike-impedance" ("spike-conductance," "spike-resistance"), used to refer to the large change during the spike. "After-impedance" ("after-conductance," "after-resistance"), will refer to subsequent alterations. Thus, the small unbalance described above is an after-impedance. To distinguish it from another to be described shortly, it will be called the initial after-impedance.

TABLE I

A Comparison of the Amplitude and Temporal Characteristics of the Impedance Changes Accompanying the Action Potential of the Squid Giant Axon in Sea Water before and after Treatment with Alkaloids

Variability is expressed as the standard error, and the number of determinations on which it is based is given. The amplitude of the spike-impedance is given by the percentage change in bridge resistance required to give the same deflection, while that of the after-impedances is given relative to the spike-impedance amplitude; t is the half-time, T the total duration, and τ the time constant.

Alkaloid	Spike-impedance			After-impedance			
				Initial		Negativity	
	Amplitude	t	T	Amplitude	T	Amplitude	τ
	per cent	*msec.*	*msec.*	*per cent*	*msec.*	*per cent*	*msec.*
None.....	8.0 ± 0.6	0.40	1.4 ± 0.1	2.8 ± 0.4	3.0 ± 0.3	0	—
Number..	14	8	16	11	14		
Cevadine...	5.6 ± 0.9	0.39	1.6 ± 0.2	0*	—	2.6 ± 0.3	47 ± 2‡
Number..	11	6	10	8		16	13
Veratridine..						1.3 ± 0.3	414 ± 25§
Number..						3	6

* Two of the eight preparations failed to lose this component of the after-impedance.

‡ Two records obtained following brief repetitive bursts (8 and 30 impulses at 50 and 100/sec.) gave approximately the same time constant and therefore were included.

§ Two values are based on recovery following a brief repetitive burst as for cevadine.

In Table I are summarized the duration of the initial after-impedance and its amplitude relative to the spike-impedance. For comparison are given the half-time and duration of the spike-impedance, both measured from the beginning of the unbalance. The half-time is the time required for the spike-impedance to decline to half its maximum; the total duration was considered to terminate at the beginning of the initial after-impedance, although it probably extended another 15 per cent into the after-impedance, as shown by removal of the latter with cevadine (see below and Table I).

The spike-impedance, which lasts 1.4 msec., is about triple the duration of the spike itself at our experimental temperatures (Grundfest *et al.*, unpublished). The initial after-impedance is twice as long as the former, so that the combined duration of the impedance changes is practically identical with that

of spike and positive overshoot (11). No other impedance changes, large or small, were seen with sea water as the medium; this was also the case with repetitive stimulation for several seconds at as high a rate as 100 shocks per second.

Cevadine-Treated Fibers.—The pure alkaloid exerts a negligible effect on the temporal characteristics of the spike impedance (Table I); the amplitude, however, is significantly lower, but this is probably due to a secondary effect; *viz.*, the leakage and accumulation of potassium around the fibers. This conclusion is based on the following observations. (*a*) In many fibers mounted immediately following exposure to cevadine, the spike and spike-impedance declined with time, occasionally to the point of block. Both could be restored by dipping the axon once or twice in sea water. (*b*) In two fibers, under conditions of continuous flow of sea water, introduction of the alkaloid did not affect the spike-impedance. (*c*) Increase of the extracellular potassium concentration is known to depress the spike-impedance (5). (*d*) The alkaloids can increase the resting leakage of potassium from nerve fibers (15).

The effect of cevadine on after-impedance was much more marked. As may be seen in Fig. 1 D and Table I, the initial after-impedance usually was absent after treatment of the axons with the alkaloid. Fig. 1 C shows one of the two instances in which the initial after-impedance persisted. Two new conductance changes made their appearance—a recurrent one, which in Fig. 1 D obviously is related to oscillations in bioelectrical potential, and a prolonged one associated with the negative after-potential (Fig. 1 E). The last will be designated the negativity after-impedance.

The negativity after-impedance, like the after-potential, disappears exponentially, as shown by the linearity of the semilogarithmic plot in Fig. 3. In Table I is given the mean time constant of decay (47 milliseconds), which agrees with the figure obtained for the after-potential (14). The maximum amplitude of the after-impedance is about 3 per cent of the spike-impedance, which is approximately the ratio of the after-potential to the spike height (*cf.* spike data in reference 11 with after-potential data in reference 14).

The negativity after-impedance resembles the negative after-potential in being additive, as may be seen in Fig. 1 F. Brief repetitive activity, such as shown, was followed by the usual exponential recovery and with a time constant about that of the single action potentials.

Veratridine-Treated Axons.—Observations with this alkaloid were limited, at least partly because the magnitude of the after-impedance approached the limits of sensitivity. In cases in which an after-conductance was not evident, brief repetitive stimulation brought it into view and made measurements possible. Time constants evaluated in this way were included in Table I since data so obtained for cevadine had given time constants of the magnitude obtained with single action potentials. The exponential decay of after-im-

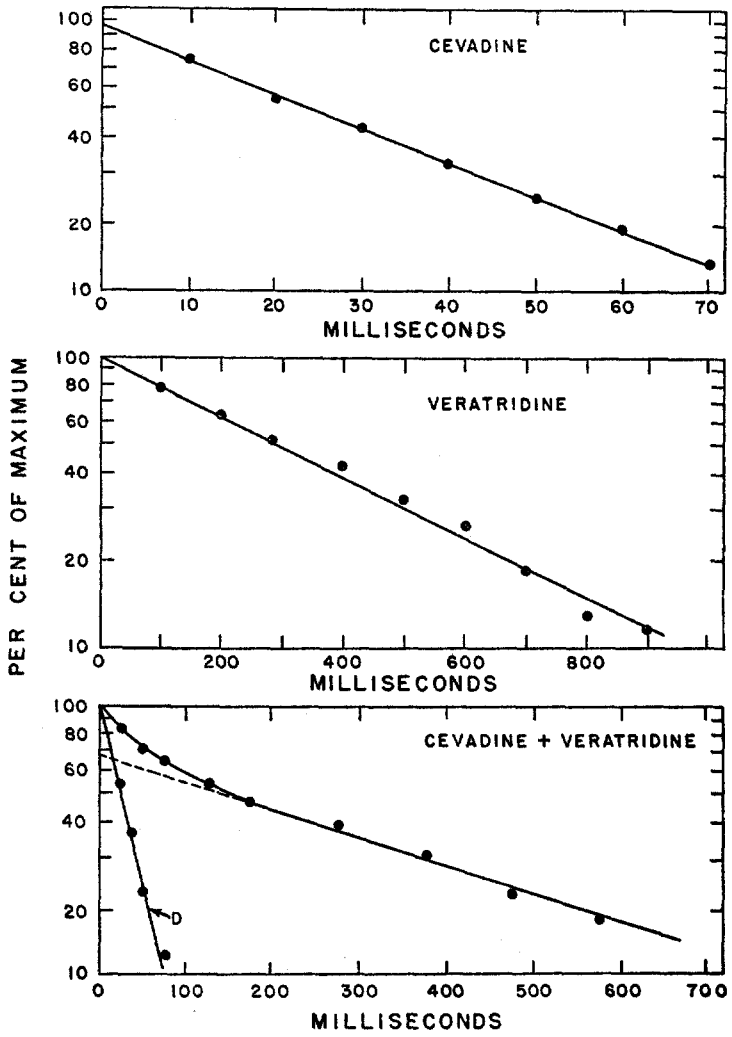

FIG. 3. The time course of the slow return of the bridge balance, plotted relative to the maximum of the negativity after-impedance, following treatment with the individual alkaloids or a 1:1 mixture. *D* is a replot of the difference between the actual curve with the mixture and the indicated extrapolation of the late, linear component to zero time.

pedance with this alkaloid is shown in Fig. 3. The time constant (*ca.* 400 milliseconds) again agrees closely with that for the after-potential (14).

The additive effect of cevadine and veratridine is shown in Fig. 3. It represents the only observation made, but is of interest in demonstrating the ap-

parent independence of action of the two alkaloids, a result consistent with that obtained for the after-potential (14).

DISCUSSION

The Initial After-Impedance.—The recent analysis of the action potential in terms of sodium and potassium conductance changes (9) provides an explanation for the increased conductance associated with the spike. The preceding paper (5) furnishes data which appear to be in good agreement with this approach. However, the initial after-impedance, which the present study has demonstrated succeeds the spike impedance, does not appear in the analysis published by Hodgkin and Huxley (9).[2] No explanation of its nature can be offered at present except in general terms. Thus, since the conductance of a phase is governed by the mobilities and concentration gradients of the ions present, the initial after-impedance may represent a decreased mobility of ions or a steepened ionic gradient within the membrane. The usual disappearance of this phenomenon in the presence of the alkaloids, which render the system leaky to ions, is in accord with both possibilities. A more specific interpretation must await additional data.

Oscillatory Phenomena.—A strict comparison of the temporal relation between the recurrent conductance and potential fluctuations was not attempted. But inspection of records such as Fig. 1 C and D reveals that, contrary to the relationship between spike and spike-conductance, the contour of the conductance change, particularly for the first oscillation, is less rounded than that of potential. The transition from a rising to a falling conductance is sudden. Moreover, the notch in the negativity after-conductance at the end of the fall in conductance indicates a transitory decrease of conductance below the level set by the after-conductance. These characteristics appear to be contrary to the behavior expected from Hodgkin and Huxley's explanation of the oscillatory phenomena (9). Thus, both the rising and falling phases of a depolarizing oscillatory wave are interpreted as the result of an increase in conductance, the former being caused by an increase in sodium conductance, the latter by an increase in potassium conductance. Therefore, as in the spike-conductance, the sum of the two conductance changes, which is what is measured, should lead to a more gradual decline than for the corresponding electrical phenomenon. This is not the case. The theoretical approach also provides no basis for the lowered conductance seen between successive depolarization waves.

[2] A personal communication from Professor Hodgkin informs us that a small decrease in "potassium conductance," amounting to approximately 0.4 per cent of the total conductance change, does appear in their calculations, but cannot be seen on the scale of their Fig. 17 (9). The computed change is therefore much smaller than the observed initial after-impedance.

The Negativity After-Impedance.—The negative after-potential is not included in the analysis by Hodgkin and Huxley (9). The correlated conductance change may originate in at least two ways: (*a*) as the consequence of an increased permeability to ions and (*b*) as the direct effect of an excess of extracellular potassium, liberated during or immediately following the spike, on membrane conductance.

Studies based on prolonged repetitive stimulation have provided evidence that the cumulative depolarization (12–14) and increase in conductance (6, 8, 17) are due solely to the increase in the potassium concentration in the restricted volume of the extracellular space. However, a prohibitively high figure is obtained when a calculation is made of the amount of potassium which must be liberated during a *single* impulse to give the amplitude of the negative after-potential obtained with alkaloid-treated axons. For example, in cevadine the amplitude is 3 mv., for it is 3 per cent of spike height (11, 14), which is about 100 mv. (7). According to the membrane potential–log potassium concentration curve (4), this potential change corresponds to a doubling of the extracellular concentration. From the data of Weidmann (17), the thickness of extracellular fluid clinging to the fiber is probably no less than about 10 micra. In the case of a fiber 400 micra in diameter, this thickness of fluid, 1 cm. in length, would contain 1.5×10^{-3} μM potassium, which is therefore the amount that would have to be liberated per impulse over this length. This corresponds to 1.2×10^{-2} μM/cm^2. for a single impulse, or 4000 times that released by untreated axons (see reference 7). The order of increase actually observed in crab nerve is about equal to the loss from untreated tissue (13, 14). It is therefore unlikely that amounts as large as the calculations indicate could have been released by the alkaloid-treated squid axons.

It must be emphasized that the present conclusion does not alter the earlier one that the polarization and conductance changes during long periods of stimulation are the direct consequence of membrane changes induced by elevated extracellular potassium. Indeed, it would be most difficult to account otherwise for the finding that an increase in the extracellular fluid volume hastens the decline of the after-potential (12), apparently by adding the diffusion of potassium to active reabsorption in the removal of the extracellular potassium (13). Similarly, the precision with which the potassium liberated per impulse in untreated axons can be computed from the increase in conductance during repetitive stimulation (8, 17), when based solely on the measured effect of an increase in external potassium concentration on resting conductance (6, 17), leaves no room for alternative mechanisms.

Obviously, a distinction must therefore be drawn between the factors involved in long term changes and that in the short term process; *viz.*, the after-potential and after-conductance during a single action potential. This clarifies the earlier finding that in veratrine the depolarization resulting from brief

repetitive activity subsides in two stages, an initial, rapid one corresponding to the rate of decline of the after-potential following single spikes, and a secondary, much slower one which corresponds to the only type of recovery seen in untreated nerves (12). The slow phase apparently is due to potassium and its removal, while the rapid phase in the alkaloids may now be regarded as the subsidence of another process, but one during which additional potassium liberation has occurred since (*a*) the initial amplitude of the slow stage is greater than in untreated nerve for the same number of impulses (12) and (*b*) this increased amplitude corresponds to the greater liberation of potassium actually found in the alkaloids (13).

The alternative possibility that the early negative after-potential and negativity after-impedance of single action potentials reflect an increase in ion permeability will now be considered. The formulations of membrane potential and conductance, as derived by Hodgkin and Katz (10), provide a possible means of evaluating the relative importance of the different ion permeabilities in the production of the negative after-potential. On this basis, the membrane potential, E, is a function (*a*) of the permeability to sodium and chloride, P_{Na} and P_{Cl}, relative to potassium permeability, P_K, and (*b*) of the axoplasmic (subscript i) and medium concentrations (subscript o) of the ions:

$$E = (RT/F) \ln (y/w), \tag{1}$$

in which

$$w = (K)_o + (P_{Na}/P_K)(Na)_o + (P_{Cl}/P_K)(Cl)_i$$
$$y = (K)_i + (P_{Na}/P_K)(Na)_i + (P_{Cl}/P_K)(Cl)_o.$$

The membrane conductance, G, is given by

$$G = (F^2/R^2T^2)EP_K wy/(y - w). \tag{2}$$

Using the concentrations in *Loligo* axoplasm 1.5 hours after decapitation (7), a resting conductance of 0.7 mmho/cm^2. (3), and relative mobilities found to describe the membrane potential at normal potassium levels (10), one finds from Equations 1 and 2 that P_K is 1.1×10^{-6}, P_{Na} 4.4×10^{-8}, and P_{Cl} 0.5×10^{-6} cm./sec. The computed value for P_K is about double that indicated by the flux rates found by Keynes for *Sepia* axons (7).

We can now determine the change in P_K, P_{Na}, or P_{Cl} required to lower E by 3 mv., the greatest depolarization achieved by cevadine. According to Equation 1, an increase in P_K cannot be involved since this would make the fiber membrane a better potassium electrode and thereby cause a positive rather than a negative after-potential. A 50 per cent increase in P_{Na}, or a 120 per cent increase in P_{Cl}, could produce the required depolarization, but these changes in permeability differ greatly in their relative effects on membrane conductance. Because of the small value taken for the sodium permeability at rest, its 50 per cent increase would cause G to rise only to 0.77 mmho/cm^2.,

representing an increment of 0.07 mmho/cm². The increase in P_{Cl}, on the other hand, would lead to a membrane conductance of 1.13 mmho/cm²., the increment being over sixfold greater than for the sodium permeability change. These figures may now be compared with the spike-conductance; this may be as high as 40 mmho/cm². (3), although data obtained by Hodgkin and Huxley (see discussion in reference 5) are as low as 23 mmho/cm². Taking these values as the possible range of spike-conductance, we find that an increment of sodium permeability alone, sufficient to produce a 3 mv. negative after-potential, would produce a conductance change 0.2 to 0.3 per cent of the spike-conductance. The increase in chloride permeability, however, would give a change 1 to 2 per cent of the spike-conductance, which is of the order of magnitude of the experimental data. On these grounds, the negative after-potential and its concurrent impedance change appear accounted for as signs of an increased chloride permeability.

This view is also consistent with the similar, exponential time courses of the after-phenomena. Thus, P_{Cl} may be introduced as a variable in Equations 1 and 2. When it is assumed to decline exponentially between its maximum at the peak of the negative after-potential and its minimum at rest, the calculated changes in the membrane potential E and conductance G are found to decline exponentially with time for 80 and 90 per cent of their maximum amplitudes, respectively. Moreover, the time constants of the ΔE-t and ΔG-t curves differ by only 25 per cent. The time constant with which P_{Cl} declines is considerably less than for the potential and conductance. For example, in cevadine it would be about 15 msec. as compared with the 50 msec. for the after-phenomena.

Additional relationships, provided by Hodgkin and Katz (10), permit calculation of the changes in the net flux of sodium, potassium, and chloride ions as a consequence of the increase in P_{Cl}. These indicate that chloride will move outward, accompanied by slightly more than an equivalent amount of potassium because of a decrease in the resting inward leakage of sodium. Therefore, the demonstrated increase in the potassium lost per impulse by alkaloid-treated nerves (13, 14) is at least qualitatively also accounted for in terms of an elevated chloride permeability. The quantity released may be estimated by integrating the chloride flux equation, with chloride permeability as the major variable. The solution is the product of the time constant and the maximum increment in chloride flux, *viz.* 15 msec. $\times$ *ca.* 2×10^{-11} mol/cm.² sec., or 0.3 $\mu\mu$M/impulse cm.² This is about 10 per cent of the amount liberated by an untreated stimulated fiber (7). No analytical data are available to check this calculation for the squid axon. However, in crab nerve the additional amount of potassium released is 60 per cent of that escaping per impulse of untreated nerve (13), which indicates that the order of magnitude is correct.

The characteristics of the negative after-potential and of the negativity

after-impedance therefore appear to be accounted for to a surprising degree in terms of an elevated chloride permeability. However, this conclusion can at best be regarded as tentative until analytical data become available regarding the behavior of sodium and chloride during stimulation of alkaloid-treated nerve. Numerous observations have demonstrated that sodium entry generally accompanies potassium exit, which has also been established for the direct effect of veratrine in causing depolarization and potassium release (15). Two additional difficulties are (*a*) the chloride content of vertebrate nerve fibers is negligible, yet the action of the alkaloids is essentially similar in this tissue, and (*b*) the marked depression of the negative after-potential observed in solutions with choline replacing sodium (Shanes, unpublished) cannot be accounted for by this mechanism.

The authors are indebted to Mr. Ernest Amatniek for his capable assistance with the electronic equipment and to Mrs. Lillian Berkowitz for her help with dissection.

SUMMARY

The increase in conductance, which accompanies the spike in the presence of sea water, is followed by a decrease to below the resting level, here designated as the "initial after-impedance," which lasts 3 msec. and is 3 per cent as great as the increase. Treatment with cevadine usually obliterates the latter but leaves the former essentially unaltered. In addition, the alkaloid gives rise to periodic conductance increases followed by a prolonged, exponentially decaying elevated conductance (the "negativity after-impedance") which correspond closely to potential oscillations and to the negative after-potential. These are also only a few per cent of the major conductance change. Veratridine causes a conductance increase which lasts longer and which also conforms closely with earlier after-potential results.

Preliminary calculations indicate that the negativity after-impedance and the negative after-potential may be due to the subsidence of an elevated chloride permeability. However, no satisfactory explanation is available for the initial after-impedance or for the temporal course of the conductance changes associated with oscillations in membrane potential.

REFERENCES

1. Cole, K. S., and Baker, R. F., Transverse impedance of the squid giant axon during current flow, *J. Gen. Physiol.*, 1941, **24**, 535.
2. Cole, K. S., and Curtis, H. J., Electric impedance of the squid giant axon during activity, *J. Gen. Physiol.*, 1939, **22**, 649.
3. 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.
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**, 1935.

5. Grundfest, H., Shanes, A. M., and Freygang, W., The effect of sodium and potassium ions on the impedance change accompanying the spike in the squid giant axon, *J. Gen. Physiol.*, 1953, **37**, 25.
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., The ionic basis of electrical activity in nerve and muscle, *Biol. Rev.*, 1951, **26**, 339.
8. Hodgkin, A. L., and Huxley, A. F., Potassium leakage from an active nerve fibre, *J. Physiol.*, 1947, **106**, 341.
9. 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.
10. 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.
11. Shanes, A. M., Electrical phenomena in nerve. I. Squid giant axon, *J. Gen. Physiol.*, 1949, **33**, 57.
12. Shanes, A. M., Electrical phenomena in nerve. II. Crab nerve, *J. Gen. Physiol.*, 1949, **33**, 75.
13. Shanes, A. M., Potassium movement in relation to nerve activity, *J. Gen. Physiol.*, 1951, **34**, 795.
14. Shanes, A. M., The ultraviolet spectra and neurophysiological effects of "veratrine" alkaloids, *J. Pharmacol. and Exp. Therap.*, 1952, **105**, 216.
15. Shanes, A. M., Ionic transfer in nerve in relation to bio-electrical phenomena, *New York Acad. Sc.*, 1952, **55**, 1.
16. Tasaki, I., and Mizuguchi, K., The changes in the electric impedance during activity and the effect of alkaloids and polarization upon the bioelectric processes in the myelinated nerve fibre, *Biochim. et Biophysic. Acta*, 1949, **3**, 484.
17. Weidmann, S., Electrical characteristics of *Sepia* axons, *J. Physiol.*, 1951, **114**, 372.