EFFECTS OF EXTERNAL IONS ON MEMBRANE POTENTIALS OF A CRAYFISH GIANT AXON*

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ABSTRACT

Transmembrane potentials in the crayfish giant axon have been investigated as a function of the concentration of normally occurring external cations. Results have been compared with data already available for the lobster and squid giant axons. The magnitude of the action potential was shown to be a linear function of the log of the external sodium concentration, as would be predicted for an ideal sodium electrode. The resting potential is an inverse function of the external potassium concentration, but behaves as an ideal potassium electrode only at the higher external concentrations of potassium. Decrease in external calcium results in a decrease in both resting potential and action potential; an increase in external calcium above normal has no effect on magnitude of transmembrane potentials. Magnesium can partially substitute for calcium in the maintenance of normal action potential magnitude, but appears to have very little effect on resting potential. All ionic effects studied are completely reversible. The results are in generally good agreement with data presently available for the lobster giant axon and for the squid giant axon.

In a previous paper (Dalton, 1958) intracellularly recorded resting and action potentials from a lobster giant axon were investigated as a function of the external concentration of normally occurring cations (sodium, potassium, calcium, and magnesium). Investigations of the lobster giant axon were undertaken in part to compare the resting and action potentials with data already available for the squid giant axon. A logical next step in a comparative study of transmembrane potentials in giant axons was an investigation of the crayfish giant axon, which makes an interesting comparison with the lobster axon for several reasons. Although the crayfish and lobster are, of course, rather closely related organisms, their habitats are quite different. Thus the opportunity is afforded for comparing axons from related animals

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which have quite different blood ion concentrations: the normal blood ion concentration of the American lobster is approximately twice that of the crayfish (van Harreveld, 1936; Cole, 1941). Another important advantage of such a comparative study is concerned with the morphological similarity of the lobster and crayfish. The same giant axon preparation may be obtained from both animals, and experimental techniques found to be suitable for the lobster are adaptable to the crayfish with only minor modifications. These investigations were planned in an attempt to compare the responses of transmembrane potentials to variations in the external concentrations of normally occurring cations in the crayfish giant axon with the existing data for squid and lobster giant axons.

Materials and Methods

Most of the experiments were performed on *Orconectes virilis* obtained from the vicinity of Cambridge, Massachusetts, and from Oshkosh, Wisconsin. Some additional experiments utilized specimens of *Procambarus clarkii* supplied by Carolina Biological Supply Company, Elon College, North Carolina. No differences were distinguished in the responses of the axons from these two crayfish.

The details of the dissection of the circumesophageal connectives from the ventral nerve cord of the crayfish are virtually identical with those already described for the lobster (Dalton, 1958). The minor differences in the details of dissection are related to size difference: the crayfish is somewhat more difficult to dissect, and it is only possible to obtain about 1 cm. of nerve from a crayfish in contrast to about 3 cm. which can be dissected from an average sized lobster. Each circumesophageal connective contains one large (about 100 microns in diameter) giant axon, and two or three more axons which are about one-half the diameter of the largest giant. Penetration of the largest giant is more easily accomplished and this axon was used for the majority of the studies. However, some experiments were run on some of the smaller axons, but no differences in their responses were noted. Before penetration with a glass capillary microelectrode the nerves were desheathed (except for the region of the nerve which was placed over the external stimulating electrodes). No attempt was made to isolate the giants from the rest of the nerve bundle, since they lie on the surface of the nerve bundle, and are easily accessible to the microelectrode.

Experimental techniques were similar to those described for the lobster giant axon. The same nerve chamber was used, and the system for circulating experimental solutions was similar to that already described. In this system about 2 minutes are required for the experimental solution to reach the nerve chamber after switching, and another 2 to 4 minutes for the previous solution to be washed out of the chamber and for an equilibrium to be reached. It seems reasonable to suppose that the presence of the entire nerve bundle in the chamber contributes to a delay in reaching an equilibrium with a change in the external ion concentration. For changes in external sodium and potassium, with the system and rate of circulation being used (about 2 to 3 cc./minute) a steady-state value was reached in 6 to 8 minutes after switching solutions. In such cases the nerve was immediately returned to the "normal" or reference solution after a steady-state had been reached. In the case of

changes in external calcium and magnesium, a steady-state was not reached in 14 to 16 minutes, the usual upper limits for experimental solution treatment in these studies. The "normal" or reference solution used was that of van Harreveld (1936), except that the standard biochemical buffer, tris(hydroxymethyl)aminomethane was used, to bring the pH of the solutions to approximately 7.2. Temperature of the circulating solutions in the nerve chamber was maintained at 9–12°C., and was monitored continuously by a thermistor thermometer bridge (Cole, 1957).

Techniques for recording intracellular resting and action potentials did not differ significantly from those described for the lobster giant axon.

RESULTS

The percentage of successful penetrations of the crayfish giant axon preparation was somewhat less than that of the lobster giant axon. This was apparently due to the smaller size of the crayfish and the necessity of miniaturization of some of the techniques. After penetration, there was a period of approximately 15 minutes during which both resting and action potentials increased in magnitude, possibly due to a sealing in of the microelectrode. After such a 15 minute waiting period before measurements were made, all axons which did not show a resting potential greater than 70 mv. or which did not show an overshoot in the action potential were discarded. Thus, it is impossible to make a very meaningful statement about mean values of resting potentials and of action potentials. However, in the 30 giant axons which were used for the experiments reported in this paper, the mean resting potential was 85 mv. and the mean action potential was 114 mv. Resting potentials as high as 95 mv. and action potentials as high as 138 mv. were seen on occasion. The shape of the action potential resembled that of the lobster rather than that of the squid; there was no undershoot (transient hyperpolarization after the spike) in a "normal" crayfish action potential.

Sodium.—As has been pointed out previously (see Dalton, 1958; Shanes, 1958), sodium is a difficult ion to work with in terms of changes in its concentration in the external medium. Because it is the predominant cation, an appreciable percentage change in external sodium can lead to large changes in osmotic pressure. In reduced sodium solutions, another ion or molecule may be substituted. In most of the experiments reported here, dextrose was used as a substitute for sodium chloride to maintain the solutions isosmotic. The effects of substitution with choline chloride instead of dextrose (to maintain the external chloride concentration constant) were tried on 3 additional axons. Results from these axons are not included in the graphs showing the effects of changes in external sodium concentration; however, the results obtained with choline substitution were essentially the same as with dextrose substitution. For increases in external sodium concentration, the osmotic pressure cannot be kept at its normal value. The following evidence may be cited to show that the effects reported for sodium in excess of normal are not

attributable merely to osmotic effects: (*a*) control experiments with dextrose added to normal perfusion solution to bring the osmotic pressure to an equivalent of one and one-quarter times normal sodium showed no appreciable change in membrane potentials in 8 minutes' treatment (3 experiments) and (*b*) no irreversible changes were noted in either the control experiments just cited or in the experimental elevation of sodium to as much as one and one-half times normal sodium.

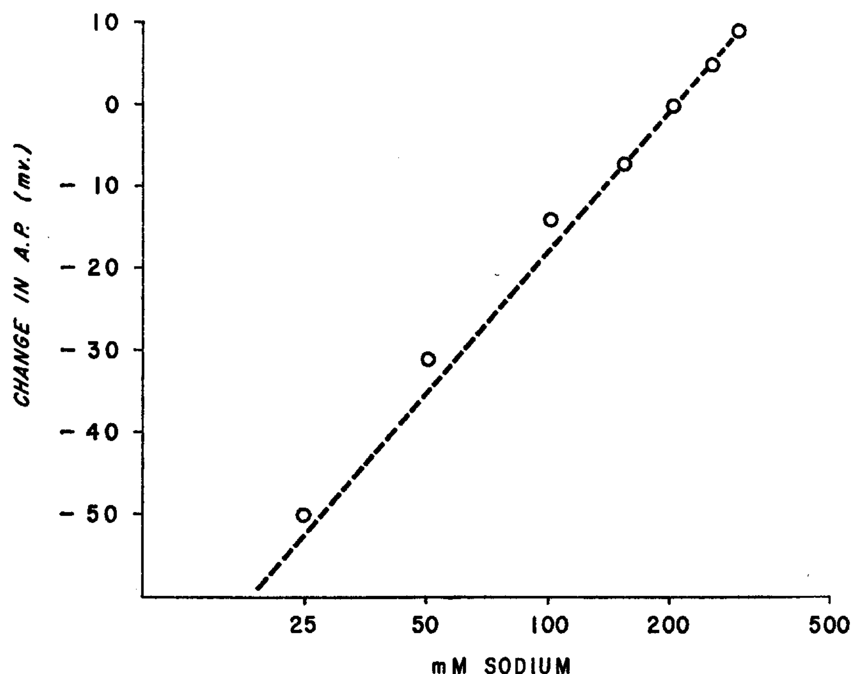

FIG. 1. Change in action potential magnitude as a function of external sodium concentration (log scale). The dashed line has a slope of 58 mv./tenfold change in sodium concentration. Data from a single axon.

Figs. 1 to 3 show the effects of changes in external concentration of sodium ion on the action potential of the crayfish giant axon. Fig. 1 shows a single experiment, with a concentration range of one-eighth to one and one-half times normal sodium. In each case the axon was switched from normal solution to the experimental solution, allowed to reach a steady-state (6 to 8 minutes), and immediately returned to normal solution. Note that the external concentration of sodium is plotted on a log scale. The dashed line is not intended to be the best line through the points given, but rather is the plot of the Nernst equation (a slope of 58 mv./tenfold change in external

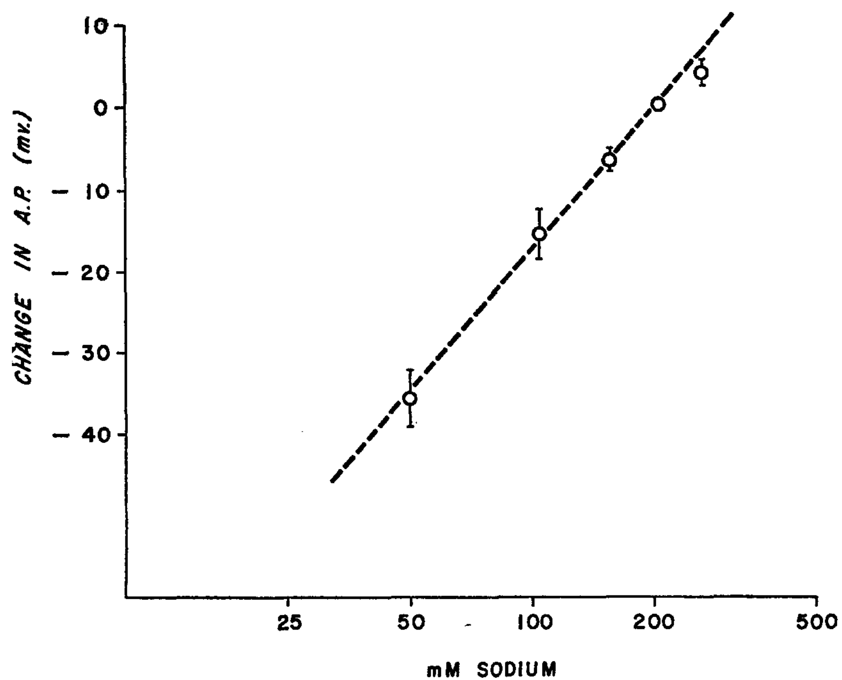

FIG. 2. Change in action potential magnitude as a function of external sodium concentration (log scale). The dashed line has a slope of 58 mv./tenfold change in sodium concentration. Mean data from 6 axons, showing one standard deviation on either side of each experimental point.

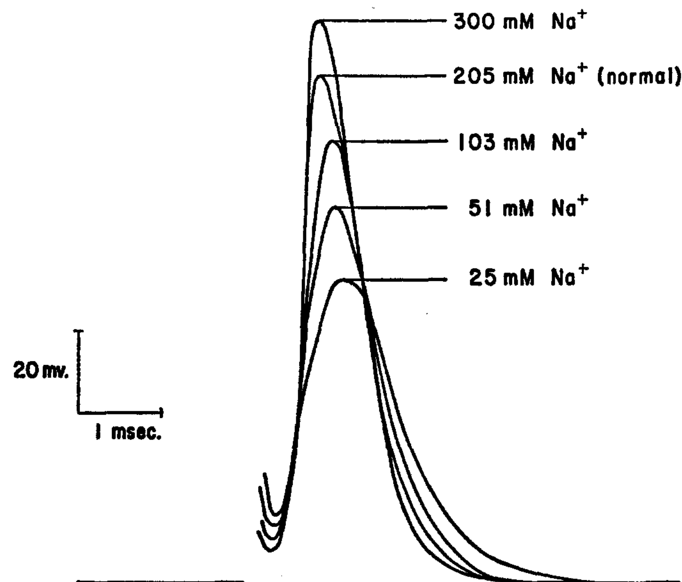

FIG. 3. Superimposed tracings of action potentials after reaching steady-state values in the external sodium concentration shown for each spike.

sodium concentration), drawn through the normal point (at 205 mM external sodium). Fig. 2 shows the same information, but here mean data for 6 giant axons are shown, with one standard deviation indicated on each side of the experimental points. The dashed line is again the line of slope 58 mv./decade concentration change. Fig. 3 shows a series of superimposed tracings of action potentials from a single giant axon at the external sodium concentration indicated in each case. It will be noted that the rate of rise of the action po-

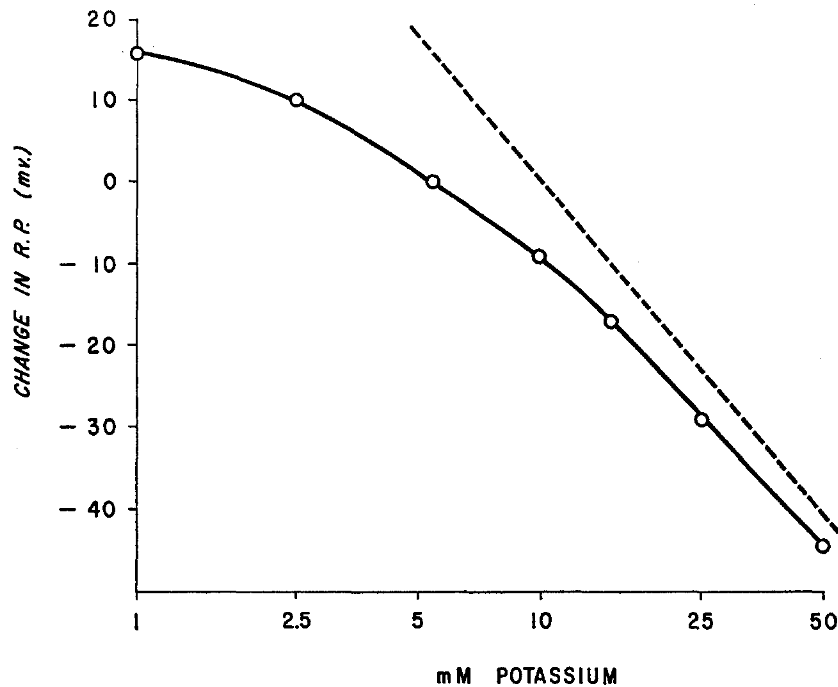

FIG. 4. Change in resting potential magnitude as a function of external potassium concentration (log scale). The dashed line has a slope of 58 mv./tenfold change in potassium concentration. Data from a single axon.

tential is also a function of the external sodium concentration. This result is typical of the experiments involving change in external sodium concentration, but no attempt was made to quantitate the changes in rate of rise (and of fall) of the action potential.

In some excitable tissues, decrease in external sodium has been shown to result in an increase in resting potential (for references, see Shanes, 1958). In the crayfish giant axon, it did not appear that any appreciable change in resting potential occurred with change in external sodium. Measurements were complicated by the fact that although an apparent change in resting poten-

tial occurred in low sodium–high dextrose solutions, much or all of this could be attributed to a change in potential between the measuring and reference electrodes themselves in such solutions. Because of some variability in such measurements, it is impossible with the data at hand to rule out the possibility of some small changes in resting potential with changes in external sodium.

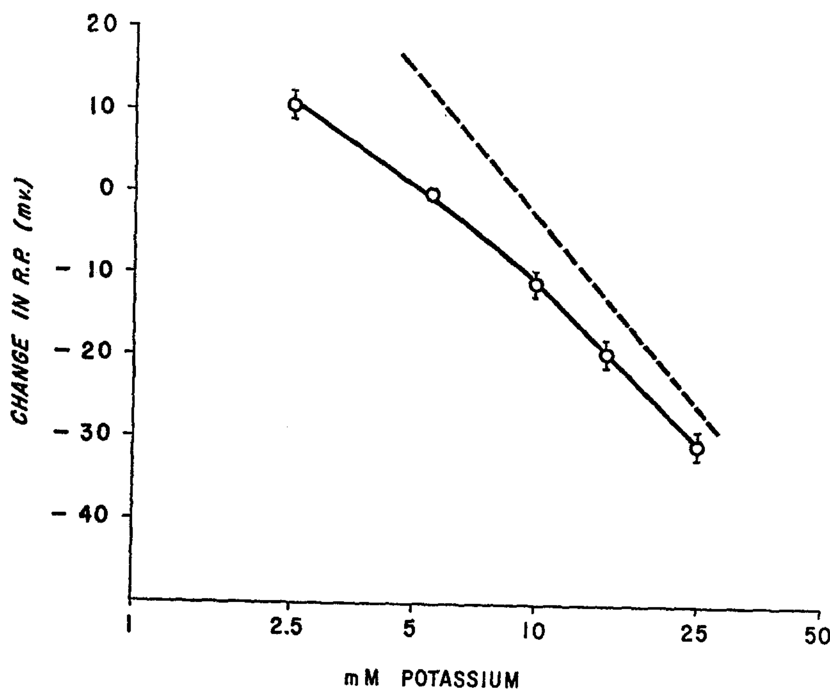

FIG. 5. Change in resting potential magnitude as a function of external potassium concentration (log scale). The dashed line has a slope of 58 mv./tenfold change in potassium concentration. Mean data from 6 axons, showing one standard deviation on either side of each experimental point.

All changes attributable to variations in the external concentration of sodium were completely reversible upon return to a solution of normal concentration.

Potassium.—Changes in potassium ion concentration in the external solution were approximately compensated for (osmotically) by changes in sodium, inasmuch as the corresponding percentage change of sodium is very small for a larger percentage change of potassium. External potassium was usually varied from 2.5 to 25 millimolar (5.4 mM being the "normal" concentration), but in some experiments a range of 1 to 50 mM potassium was used.

Figs. 4 and 5 show the effect of changes in external concentration of po-

tassium ion on the resting potential of this preparation. Fig. 4 shows a single experiment, with a concentration range of 1 to 50 mM potassium. In each case the axon was switched from normal solution to the experimental solution, allowed to reach a steady-state (6 to 8 minutes), and immediately returned to normal solution. The change in resting potential from the value in the reference solution to a new value in an experimental solution is plotted against the log of the external potassium concentration. The dashed line is a line of slope 58 mv./decade change, for comparison. It will be seen that this 58 mv. slope is approached only at high external potassium concentrations. Mean data for 6 giant axon preparations are shown in Fig. 5, with one standard deviation being indicated for each experimental point. Again the 58 mv. line is shown for comparison.

A decrease in action potential also occurred in high potassium solutions. This reduction in action potential was greater than the reduction in resting potential; *i.e.*, the overshoot of the action potential showed a decrease as well. In potassium solutions of concentration 10 to 15 mM or greater, with the resting potential being reduced some 10 to 15 mv., the action potential was progressively reduced to zero. An attempt to determine the exact relationship between the resting potential and the action potential with changes of external potassium has not yet been made, but further experiments to investitate this problem are contemplated.

All changes attributable to variations in the external concentration of potassium ion were completely reversible upon return to a solution of normal potassium concentration.

Calcium.—With variations in external calcium concentration the sodium concentration was varied reciprocally, to maintain isotonic solutions.

The effects of changes in external calcium were tested on 13 giant axon preparations. This number of preparations was investigated in an attempt to get consistent results which could be quantitated, but the attempt was unsuccessful. Quantitation of the results with changes in external calcium was not feasible for 2 reasons: (*a*) the changes observed did not reach a steady-state after 15 to 20 minutes' treatment, but rather continued to change at a slow rate throughout observation, and (*b*) there was great variability in magnitude of results from axon to axon.

The following concentrations of external calcium were tested: 25 mM, 13.5 mM ("normal" concentration), 5 mM, 2.5 mM, and a nominal zero mM. For a given giant axon preparation, the following pattern was seen: in 25 mM calcium, which is almost twice the normal concentration, there was no change in resting potential or in action potential from the normal situation. The 3 concentrations below normal all showed reduction in resting potential and in action potential, the greater effect being seen in the lower concentration. The typical effect of a nominal zero mM calcium solution with normal magnesium (2.6 mM) may be seen in Figs. 6 and 7, which will be discussed more fully in

the next section. Although they do not represent mean values or final effects, the following are typical figures after 12 minutes' treatment in reduced calcium: for 2.5 mM, a reduction in resting potential of 3 to 5 mv., and in action potential of about 15 to 20 mv.; for nominal zero calcium, a reduction in resting potential of 5 to 10 mv., and a reduction in action potential of 50 to 60 mv. or more.

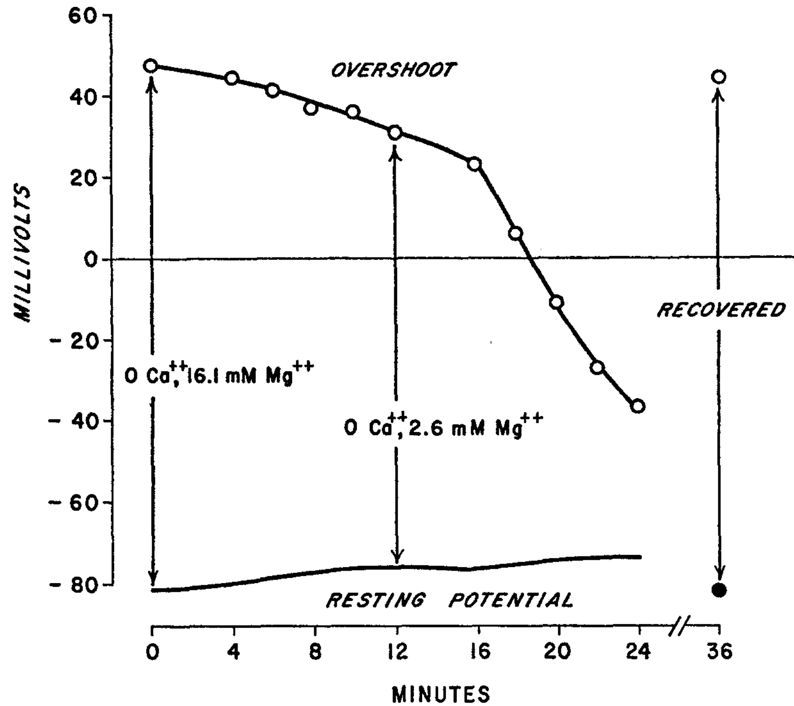

FIG. 6. Effects of divalent ions on membrane potentials. The distance between the curves marked "resting potential" and "overshoot" represents the height of the action potential. Normal external calcium concentration is 13.5 mM; normal magnesium is 2.6 mM (total 16.1 mM). Data from a single axon.

Low calcium solutions had no special marked effects on the shape of the action potential in this preparation; no undershoot developed, as did occur in the lobster giant axon. In general, the duration of the action potential remained about the same in low calcium as in normal solution, with a reduction in rates of rise and fall of the action potential.

No spontaneous repetitive activity was seen in this preparation in reduced external calcium.

All effects observed in reduced calcium solutions were completely reversible.

Magnesium.—The magnesium ion was investigated in terms of its ability to substitute for calcium in the maintenance of "normal" membrane potentials. In the lobster, no function for magnesium could be demonstrated in this respect. A typical experiment to test for the possibility of magnesium substitution for calcium is shown in Figs. 6 and 7. As shown in Fig. 6, if the external calcium concentration is reduced to zero and the external magnesium concentration is increased until its concentration represents the total divalent cation concentration of the normal solution (16.1 mM), the rate of decline of

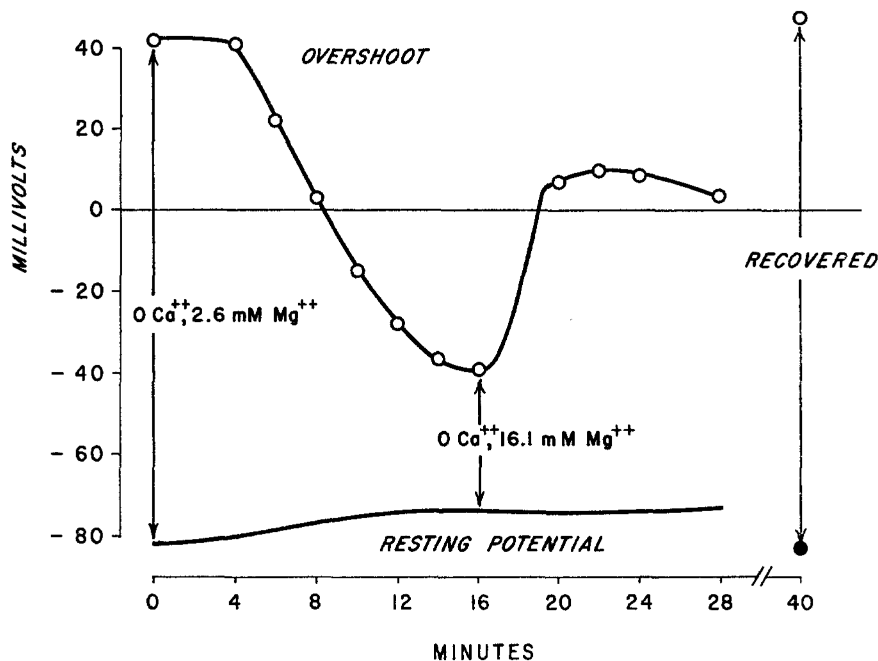

FIG. 7. Same experiment as in Fig. 6, but with sequence of solutions reversed.

resting and action potentials as seen in zero calcium–normal magnesium solution is decreased, but not stopped entirely. In Fig. 7, it will be seen that a sudden increase in magnesium in a nominal zero calcium solution causes a partial recovery of the reduced action potential, but that the resting potential is largely unaffected.

Such results are typical of the results from 5 giant axon preparations. For the same reasons given for studies of the calcium ion alone, no attempt has been made to present mean data.

As shown in Figs. 6 and 7, such effects are completely reversible on return to a solution containing the "normal" concentrations of calcium and magnesium.

DISCUSSION

The values reported for resting and action potentials in the crayfish giant axon are in generally good agreement with those reported by Kao and Grundfest (1956) for other studies on the crayfish giant axon. Resting potential values for the crayfish average about 15 mv. more than values reported for the lobster (Dalton, 1958), which in turn has values higher than those reported for the squid (Curtis and Cole, 1942). The shape of the action potential, with special reference to after potentials, is very similar to that of the lobster, and differs from the squid giant axon action potential in not typically showing an undershoot. Undershoots were occasionally observed in the crayfish, but only when both resting and action potentials were small; this also agrees with results from the lobster axon.

In general, results obtained with changes in external cation concentration are of the same kind and order of magnitude for the crayfish as for the lobster (exception: partial substitution of magnesium for calcium), taking into consideration the differences in total ion concentration in the normal perfusion solution. The ionic proportions in crayfish solution are very similar to those of the lobster, and the crayfish axon can be maintained with little change in membrane potentials in normal lobster solution which has been diluted by a factor of two.

Effects with changes of external sodium concentration for the crayfish were in slightly better agreement with the Nernst equation slope of 58 mv./tenfold change in external concentration than for the squid (Hodgkin and Katz, 1949), and quite a bit better than the lobster, which did not tend to reach a steady-state in reduced external sodium as rapidly or conclusively as the crayfish.

The effects seen with changes in external potassium are similar to those reported for a variety of excitable tissues (for references, see Shanes, 1958), with an approximation to the slope of 58 mv./decade change only at the higher external potassium concentrations. No attempt was made to fit the crayfish data to the Goldman constant field equation (Goldman, 1944), inasmuch as the internal concentrations of ions in the crayfish giant axon are unknown, and there is no real basis for arriving at approximations to the correct concentrations.

The fact that the effects of changes in external calcium did not reach a steady-state, even with extended treatment, is consistent with the hypothesis that the effects of calcium are not exerted merely by means of a concentration gradient between the inside and outside, but that some sort of "washing-out" phenomenon occurs. The fact that increases in external calcium above normal had no effect on membrane potentials (a saturation effect) is consistent with observations for the lobster axon and certain other cells (for references, see Brink, 1954). The decrease in rates of rise and fall of the action potential

in low calcium agrees with results obtained on lobster limb motor axons (Adelman, 1959).

Results with the magnesium ion in this study do not agree with those reported for the lobster giant axon. However, it has been shown for the lobster limb axon (Adelman, 1956) that spike blockade is more rapidly produced if both magnesium and calcium are removed from the external solution than if only calcium is removed. The fact that magnesium partially substitutes for calcium in maintenance of the action potential in the crayfish giant, but has little or no effect on maintenance of the resting potential, suggests that calcium may have at least a dual role in the maintenance and production of transmembrane potentials. One of these functions could be associated with action potential production (with partial substitution by magnesium), and the other associated with resting potential maintenance (with little or no substitution by magnesium). Further experiments to test such a hypothesis are indicated.

Results of these comparative studies suggest that any comprehensive theory to explain membrane phenomena in the squid giant axon would likely be applicable to both the lobster and crayfish giant axons with only minor modifications.

REFERENCES

Adelman, W. J., Jr., The effect of external calcium and magnesium depletion on single nerve fibers, *J. Gen. Physiol.*, 1956, **39**, 753.

Adelman, W. J., Jr., and Adams, J., Effects of calcium lack on action potential of motor axons of the lobster limb, *J. Gen. Physiol.*, 1959, **42**, 655.

Brink, F., The role of calcium ions in neural processes, *Pharmacol. Rev.*, 1954, **6**, 243.

Cole, K. S., Thermistor thermometer bridge: linearity and sensitivity for a range of temperature, *Rev. Sc. Instr.*, 1957, **28**, 326.

Cole, W. H., A perfusing solution for the lobster (*Homarus*) heart and the effects of its constituent ions on the heart, *J. Gen. Physiol.*, 1941, **25**, 1.

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.

Dalton, J. C., Effects of external ions on membrane potentials of a lobster giant axon, *J. Gen. Physiol.*, 1958, **41**, 529.

Goldman, D. E., Potential, impedance, and rectification in membranes, *J. Gen. Physiol.*, 1944, **27**, 37.

van Harreveld, A., A physiological solution for fresh water crustaceans, *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 428.

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.

Kao, C. Y., and Grundfest, H., Conductile and integrative functions of the crayfish giant axons, *Fed. Proc.*, 1956, **15**, 104.

Shanes, A. M., Electrochemical aspects of physiological and pharmacological action in excitable cells, *Pharmacol. Rev.*, 1958, **10**, 59.