EFFECTS OF POTASSIUM, SODIUM, AND AZIDE ON THE IONIC MOVEMENTS THAT ACCOMPANY ACTIVITY IN FROG NERVES*

By TOMOAKI ASANO‡ AND W. P. HURLBUT

(From The Rockefeller Institute for Medical Research)

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ABSTRACT

Stimulation of intact or desheathed frog sciatic nerves produced an increase in the sodium content and a decrease in the potassium content of this tissue. In desheathed preparations the magnitudes of the changes in ionic contents decreased as the concentration of the potassium in the bathing solution was increased, while changing the external sodium concentration produced small effects on the ionic shifts. During tetanization, the rate of decline of the compound action potential also decreased as the external potassium concentration increased. Eliminating the activity respiration with 0.2 mM azide did not greatly modify the changes in sodium and potassium distribution that accompanied activity in either intact or desheathed nerves.

INTRODUCTION

Prolonged stimulation of peripheral nerve leads to changes in the sodium and potassium contents of this tissue (Arnett and Wilde, 1941; Keynes and Lewis, 1951; Shanes, 1951 *a* and *b*). Presumably, these changes in ionic contents represent the cumulative effect of the ionic movements that accompany each volley of impulses (Hodgkin, 1951). Frog peripheral nerve also increases its rate of respiration during activity (Brink *et al.*, 1952), and it has been suggested (Hodgkin, 1951) that this activity respiration might serve to reverse the ionic movements that occur during each impulse and thereby maintain the ionic distribution of stimulated frog nerves close to the normal level. Hodgkin (1951) showed that such a function for the activity respiration was feasible on energetic grounds. Brink *et al.* (1952) and Doty and Gerard (1950) have shown that 0.2 mM azide eliminates almost completely the extra oxygen uptake that accompanies activity in frog nerve, while it only slightly interferes with the resting

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‡ Present address: Department of Physiology, School of Medicine, Kanazawa University, Kanazawa, Japan.

respiration or the ability of a nerve to conduct. This phenomenon provides a tool for testing the suggestion of Hodgkin, and a series of experiments was undertaken to determine whether 0.2 mM azide would modify the changes in the sodium and potassium contents of frog nerves that accompany activity.

To facilitate the exchange of ions between the extracellular spaces of a nerve and the surrounding medium, the perineurium was removed from most of the nerves that were used in this investigation. The potassium concentration of the bathing solution affects the changes that occur in several properties of these preparations during and after prolonged stimulation (Connelly *et al.*, 1956). In the present investigation potassium was found to affect the ionic exchange that occurred during stimulation of such desheathed nerves. The first portion of this report will be concerned with the effects of the external concentrations of sodium and potassium on the changes in ionic contents that accompany activity in frog nerves. The influence of 0.2 mM azide on these changes in ionic distribution will be considered thereafter.

METHODS

Sciatic nerves from the frog *Rana pipiens* were used throughout this investigation. Preparation of the tissue and the procedures followed to determine sodium and potassium have been described elsewhere (Hurlbut, 1958). Before the nerves were weighed and the ions extracted, the tissue was washed for 1 hour at 0–2°C. in a solution of 118 mM choline chloride and 1.8 mM calcium chloride. This procedure eliminates sodium and potassium from the extracellular spaces of a nerve and permits reliable estimates to be made of the cellular content of these ions (Hurlbut, 1958). In those cases in which experiments had been carried out in hypertonic Ringer's solution (220 mM sodium chloride or 110 mM sodium chloride plus 110 mM choline chloride) the washing solution contained 230 mM choline chloride and 1.8 mM calcium chloride.

Electrical stimulation and recording were carried out in the lucite chamber depicted in Fig. 1. The nerve was slipped into the 1 mm. capillary drilled into the lucite block and was positioned so that its peripheral end lay on the more distal of two platinum recording electrodes that penetrated into the capillary. Two platinum stimulating electrodes were mounted in the reservoir; the anode was in air, while the cathode dipped into the solution. A steady flow of fluid was maintained through the capillary by means of a motor-driven syringe. The top of the reservoir was sealed with a rubber stopper through which gas lines passed to provide a supply of oxygen and carbon dioxide, and the chamber was placed in a box in which the temperature was maintained at 20 ± 0.5°C. The strength of the electrical stimulus was supramaximal for both alpha and beta fibers. Tetanization was usually carried out at a rate of 50 shocks/sec. During rest periods the tissue was occasionally stimulated at a rate of 1 shock/sec.

The standard Ringer's solution contained 110 mM NaCl, 2.0 mM KCl, 1.8 mM $CaCl_2$, and 4.8 mM $NaHCO_3$. It was buffered to pH 7.0 by equilibration with a gas mixture that contained 2 per cent CO_2, 20 per cent O_2, and 78 per cent N_2. The con-

centration of potassium was altered at the expense of sodium so that the sum of the concentrations of potassium and sodium chlorides was constant. The sodium concentration was reduced by replacing sodium chloride with an equivalent amount of choline chloride. Ringer's solution containing sodium azide was prepared by substituting sodium azide for an equivalent amount of sodium chloride. All nerves were equilibrated in the experimental solutions for 2 hours before stimulation was begun.

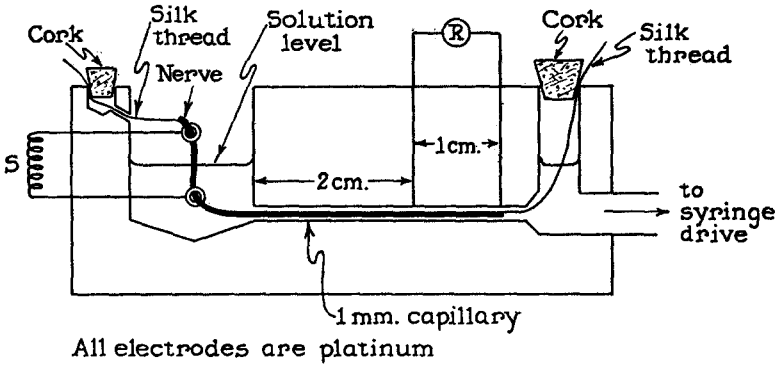

FIG. 1. Schematic diagram of lucite stimulating chamber. The right side of the chamber was connected to a motor-driven syringe which maintained a constant flow of solution through the capillary from the large reservoir on the left. The vertical side arm on the right of the chamber was closed with a stopper so that during the course of an experiment the solution level in this column remained at approximately the height to which it had been adjusted at the beginning of the run.

RESULTS

Effects of Different External Concentrations of Sodium and Potassium

Figs. 2 and 3 illustrate the ionic exchange that occurred in frog nerves during activity in Ringer's solutions that contained various concentrations of sodium and potassium. To obtain these data both sciatic nerves from one animal were soaked in the same solution; one nerve was at rest while the second was stimulated. The differences between the sodium and potassium contents of the two nerves were taken as a measure of the changes in ionic distribution that were produced by the activity. The most striking feature of these data is the fact that the net changes in ionic distribution were reduced by increasing the potassium concentration from 0 to 8.5 mM, while varying the sodium concentration between 55 and 220 mM had only slight effects.

Fig. 4 depicts the time course of the decline of the amplitude of the compound action potential during stimulation in Ringer's solutions that contained various concentrations of sodium, potassium, and choline chlorides. With the sodium chloride concentration near its normal value of 110 mM (upper portion

of Fig. 4), the decline of the action potential was greater the lower was the concentration of potassium. With potassium held fixed at a concentration of 2 mM, replacing half of the sodium chloride with choline chloride had little effect on the failure of the compound action potential (compare the curve for 2 mM potassium in the upper part of this figure with that for 55 mM sodium chloride

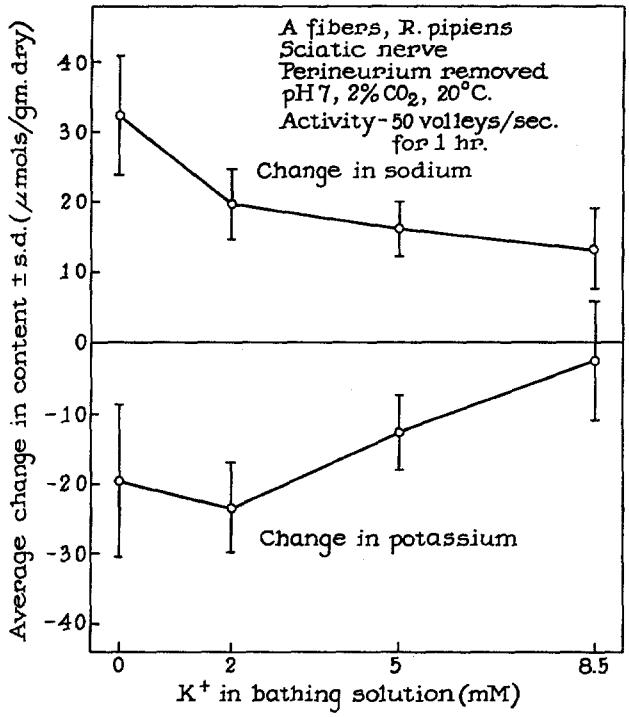

FIG. 2. Effect of the potassium concentration of the bathing solution on the changes in the ionic contents of desheathed nerves stimulated for 1 hour at 50 volleys/sec. Changes in ionic contents are calculated as (active nerve)–(resting control). The bar indicates ± the standard deviation. Each point is the average of five experiments.

in the lower half), while increasing the sodium chloride concentration to 220 mM or adding 110 mM choline chloride increased the decline of the electrical response. It is not known whether the deleterious effects of the two latter solutions were due to the sodium or choline ions *per se* or were the result of the hypertonicity of the solutions. Raising the potassium concentration to 8.5 mM counteracted the effects of 220 mM sodium. In general the effects of these ions on the action potential paralleled the effects they exerted on the changes in the sodium and potassium contents of the stimulated nerves.

Since only small changes in ionic contents occurred during 1 hour of stimulation in 8.5 mM potassium, and the compound action potential held up well, a series of tests was carried out to determine whether a nerve would maintain itself in a steady state of ionic distribution during longer periods of activity in this concentration of potassium. In Fig. 5 are shown the temporal courses of

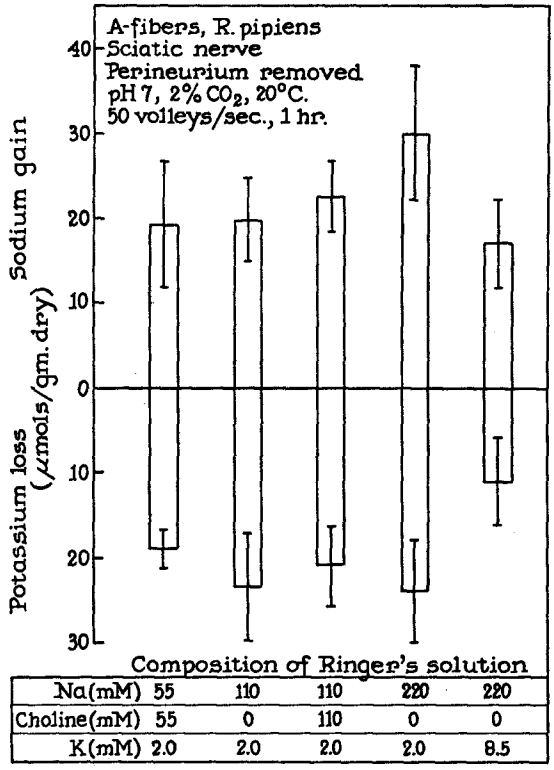

FIG. 3. Changes in ionic contents of desheathed nerves stimulated for 1 hour at 50 volleys/sec. in Ringer's solutions of various compositions. The changes in ionic contents are calculated as (active nerve)–(resting control). The bar indicates ± the standard deviation. Each block is the average of five experiments.

the decline of the amplitude of the compound action potential and of the changes in the sodium and potassium contents of nerves stimulated in Ringer's solution containing 8.5 mM potassium. It is clear that the changes in ionic contents continued to develop for as long as 5 hours, and there were no indications that a new steady state was being approached in this time. The amplitude of the action potential remained nearly constant for 2½ hours and thereafter declined at a rate of approximately 10 per cent per hour.

Fig. 6 illustrates the reversibility of the changes that occurred in the ampli-

tude of the compound action potential and in the sodium and potassium contents of stimulated frog nerves. To measure the recovery of the ionic distribution, both nerves of a pair were stimulated in 2.0 mM potassium Ringer's for 1 hour at a rate of 50 volleys/sec. One nerve was analyzed at the end of this

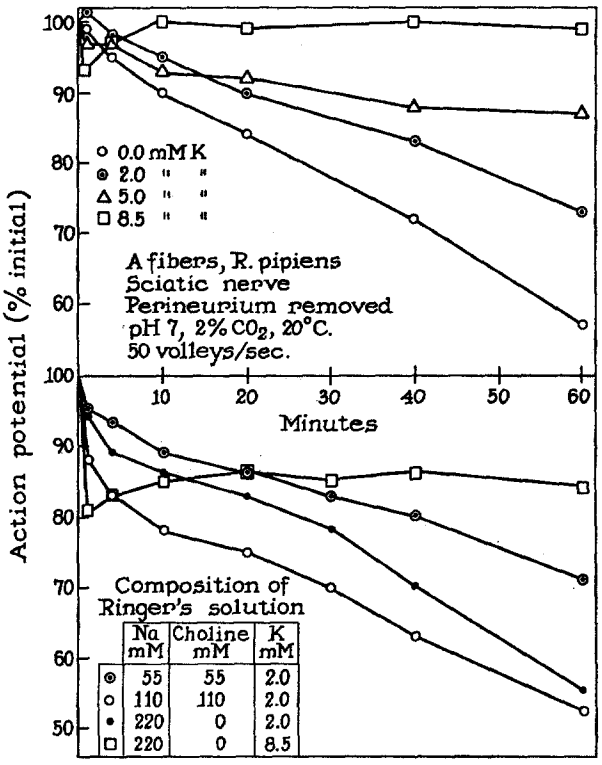

FIG. 4. Decline of the amplitude of the compound action potential of desheathed nerves during tetanization at a rate of 50 volleys/sec. in Ringer's solutions of various compositions. Data were normalized with respect to the amplitude measured immediately before beginning the tetanization. Each curve is the average of five experiments. For the experiments represented in the upper portion of this figure potassium was varied at the expense of sodium so that the sum of the concentrations of potassium and sodium chlorides was equal to 112 mM.

period of activity, while the second was allowed to rest in the solution for several additional hours. The differences between the sodium and potassium contents of these nerves were taken as a measure of the recovery that occurred in the second nerve during the rest period. The changes in ionic contents appeared to be fully reversible, although recovery proceeded slowly with a half-time of approximately 2 hours. The action potential recovered somewhat more rapidly.

Effects of 0.2 mM Azide

Nerves with the Perineurium Removed.—Two techniques were employed to test the effect of 0.2 mM azide on the ionic shifts that occur during activity in desheathed nerves. In the first method both members of a pair of nerves were bathed in a Ringer's solution that contained the inhibitor. One of the nerves was stimulated, while the second remained at rest, and the differences between

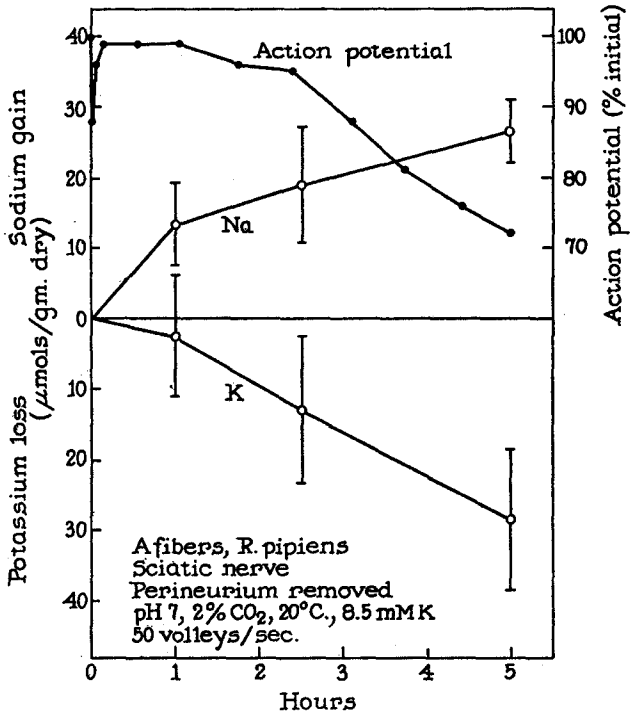

FIG. 5. Time course of the decline of the amplitude of the compound action potential and of the changes in ionic contents of desheathed nerves during tetanization at 50 volleys/sec. in Ringer's solution with 8.5 mM potassium. Each point on the curve describing the changes in ionic contents is the average of five experiments ± the standard deviation. Action potential readings were normalized as in Fig. 4.

their sodium and potassium contents were used to measure the net ionic change that occurred during the activity. Table I-A compares the results of these experiments with the results that were obtained in the absence of azide, and which have already been presented in Figs. 2 and 5. From Table I-A it can be seen that 0.2 mM azide produced a small increase in the ionic exchange that occurred during an hour of activity in Ringer's solution containing 2.0 mM potassium. However, this concentration of the poison had no effect when the tests were conducted in 8.5 mM potassium Ringer's.

To secure the data of Table I-B both nerves of a pair were stimulated, one being washed in Ringer's solution containing azide while the second was bathed in azide-free Ringer's solution. The table shows that when these experiments were performed in Ringer's solution that contained 2.0 mM potassium, those

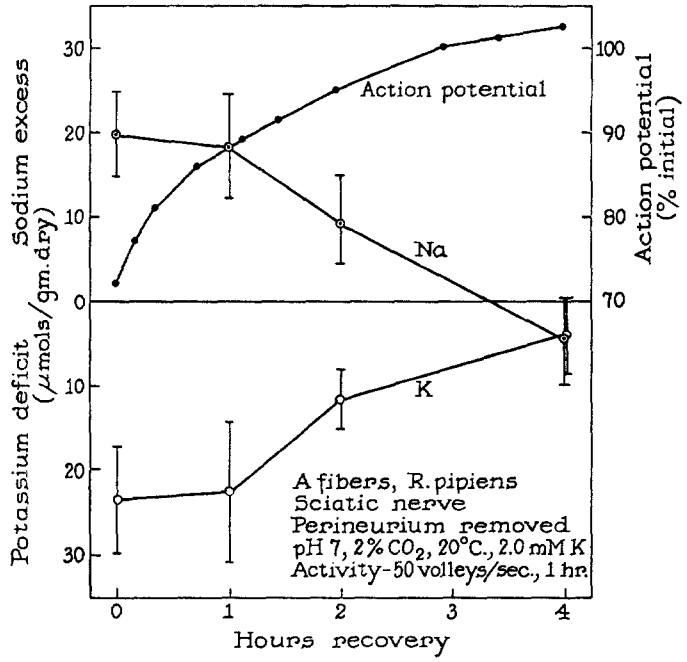

FIG. 6. Time course of the recovery of the amplitude of the compound action potential and of the ionic contents of desheathed nerves after 1 hour of activity at 50 volleys/sec. in Ringer's solution containing 2.0 mM potassium chloride. The initial points of the curves describing the recovery of the ionic contents are the average changes in ionic contents that occurred during the activity. These points were taken from Fig. 2. Recovery is plotted with respect to these points, the quantity that was determined experimentally being the difference between these initial points and those at later times. Each point on the curves describing the changes in ionic contents is the mean of five experiments ± the standard deviation. The action potential data were normalized as in Fig. 4.

nerves that had been stimulated in the presence of azide contained slightly less potassium and a little more sodium than their mates. In Ringer's solution that contained 5.0 mM potassium, however, no difference was detected between the two groups of nerves. Part of the effects observed at the lower potassium concentration may have been unrelated to activity, since 0.2 mM azide may produce small changes in the sodium and potassium contents of resting nerves that are bathed in Ringer's solution containing 2.0 mM potassium (Hurlbut, 1958).

When the potassium concentration of the Ringer's solution was 2.0 or 5.0 mM, the rate of decline of the amplitude of the compound action potential was not greatly affected by 0.2 mM azide. When the Ringer's solution contained 8.5 mM potassium and 0.2 mM azide, however, the spike height fell to 65 per cent of its initial amplitude after 2½ hours of activity at 50 volleys/sec. As can be

TABLE I

Effects of 0.2 mM Azide on the Changes That Occur in the Sodium and Potassium Contents of Desheathed Nerves during Activity in Ringer's Solutions with Various Concentrations of Potassium

A fibers. 50 volleys/sec. Each result is the mean value obtained from five pairs of nerves. Figures in μmoles/gm. dry ± S.D.

A

Ringer K concentration, mM	2.0		8.5			
Duration of activity, hrs.	1		1		2½	
Azide, mM	0	0.2	0	0.2	0	0.2
K loss	23.4 ± 6.3	34.0 ± 6.0	2.5 ± 8.5	5.8 ± 3.4	13.0 ± 10.3	11.6 ± 4.8
Na gain	19.9 ± 4.9	32.2 ± 5.9	13.4 ± 5.8	12.1 ± 6.4	19.0 ± 8.2	20.7 ± 4.3

B

Ringer K concentration, mM	2.0			5.0		
Duration of activity, hrs.	1			2½		
Azide, mM	0	0.2	Δ	0	0.2	Δ
K content	132 ± 13.9	123 ± 12.2	−9.0 ± 5.9	137 ± 8.5	136 ± 4.2	−1.8 ± 7.4
Na content	63 ± 7.5	69 ± 5.3	6.7 ± 6.7	76 ± 13.5	78 ± 9.1	3.5 ± 14.6

seen from Fig. 5, the action potential declined only slightly in this time in the absence of azide.

Intact Nerves.—A series of experiments was also undertaken with intact nerves to determine the effects of 0.2 mM sodium azide on the changes in the ionic contents that accompany activity. All these experiments were carried out in a Ringer's solution that contained 2.0 mM potassium chloride. These nerves were placed overnight (16 hours) in a furnace maintained at approximately 550°C., and the resulting ash was taken up in distilled water for a determination of the sodium and potassium contents of the tissue. The sodium content of these preparations, when referred to the dry weight, showed considerable

variation and was dependent upon the water content of the tissue. The sodium and water contents were correlated so as to suggest that the variations in these parameters may have been a reflection of variations in the size of the extracellular spaces of the nerves. The data were found to be more uniform when referred to the wet weight, and for this reason they are presented here in these units.

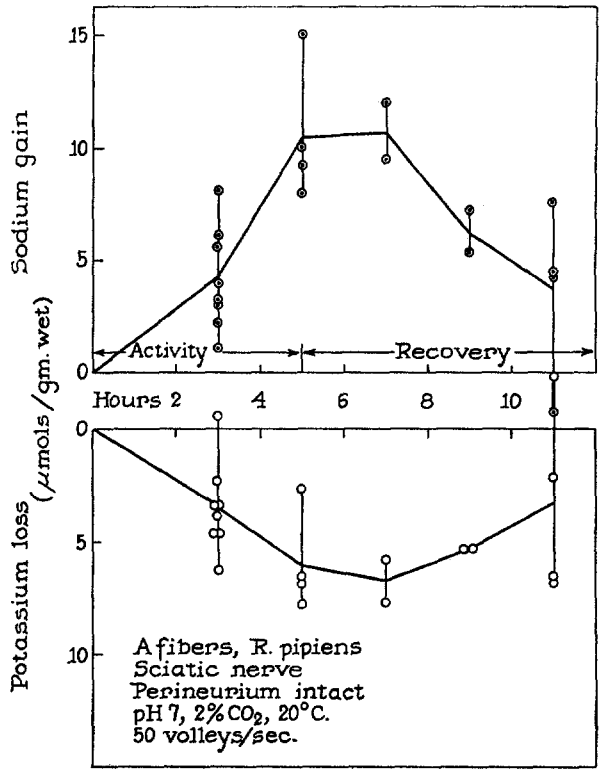

FIG. 7. Time course of the changes in the ionic contents of intact nerves during and after stimulation at a rate of 50 volleys/sec. Recovery is plotted in the same manner as in Fig. 6.

Since intact frog nerves are approximately 75 per cent water (Fenn *et al.*, 1934; Hurlbut, 1958), the wet weight data may be converted to dry weight data by multiplying by four.

Fig. 7 illustrates the temporal course of the changes that occurred in the ionic contents of these nerves during long bouts of activity and during subsequent periods of rest. The changes in ionic contents that occurred during the recovery were determined by the technique used for the desheathed nerves. The ionic exchange produced by 5 hours of activity appears to be at least partially

reversible, although this is not certain in the case of potassium. It can be seen that to produce ionic shifts of similar magnitude in intact and desheathed preparations, activity must be maintained for considerably longer periods in the former case. The small size of the changes observed in intact nerves may be due to two effects: (1) Slowing of the exchange of ions between the extracellular

TABLE II

Effects of 0.2 mM Azide on the Changes That Occur in the Sodium and Potassium Contents of Intact Nerves during Activity

A fibers. 50 or 100 volleys/sec. 2 to 3 hours. Each result is the mean value obtained from five pairs of nerves.

Azide	K content μmoles/gm. wet ± S.D.			Na content μmoles/gm. wet ± S.D.			H_2O content per cent wet weight ± S.D.		
	Resting	Stimulated	Δ	Resting	Stimulated	Δ	Resting	Stimulated	Δ
mM									
0	38.7 ± 3.1	34.3 ± 2.6	4.5 ± 1.7	66.5 ± 1.9	71.8 ± 2.3	5.3 ± 1.8	75.1 ± 0.3	75.9 ± 1.0	0.8 ± 0.8
0.2	36.9 ± 1.1	33.5 ± 3.2	3.4 ± 2.8	67.0 ± 3.4	70.5 ± 4.2	3.5 ± 2.7	75.7 ± 1.2	75.7 ± 0.5	0.0 ± 1.3

TABLE III

Sodium and Potassium Contents of Resting Desheathed Nerves Soaked in Ringer's Solutions of Various Compositions

Ringer's concentration (mM) of				Mean soaking time	Ion content μmoles/gm. dry ± S.D.		No. of nerves
KCl	NaCl	Choline chloride	Azide		K	Na	
				hrs.			
0	112	0	0	3	128 ± 17.1	53 ± 7.5	5
2.0	110	0	0	3	175 ± 17.6	40 ± 9.4	5
5.0	107	0	0	3	172 ± 5.1	31 ± 3.2	5
8.5	103.5	0	0	4 ½	188 ± 11.0	30 ± 5.2	19
2.0	55.0	55.0	0	3	160 ± 5.3	24 ± 2.1	5
2.0	110	110	0	3	143 ± 13.6	40 ± 4.5	5
2.0	220	0	0	3	135 ± 4.3	67 ± 15.6	5
8.5	220	0	0	3	153 ± 7.3	53 ± 5.9	5
8.5	103.5	0	0.2	3 ¾	175 ± 9.8	34 ± 6.8	10
2.0	110	0	0.2	3	165 ± 13.5	36 ± 5.4	5

spaces of the nerve and Ringer's solution because of the presence of the perineurium. (2) Increase in the concentration of potassium in the extracellular space reducing the ionic exchange as was observed with desheathed preparations.

Table II presents the results of the experiments that were performed to determine the effect of 0.2 mM azide on the ionic exchange of activity. In these experiments both members of a pair of nerves were bathed either in unmodified

Ringer's solution or in Ringer's solution that contained the inhibitor, and one nerve was stimulated while the second remained at rest. The azide had little effect on the changes in the ionic contents that were produced by activity in these preparations.

Ionic Contents of Resting Nerves with the Perineurium Removed

Table III summarizes the data that were obtained on the sodium and potassium contents of the resting desheathed nerves that had been used as the controls in the experiments reported here. It can be seen that appreciable changes in the ionic distribution of these nerves occurred in potassium-free Ringer's solution and in Ringer's solution that contained 220 mM sodium chloride. Raising the potassium concentration to 8.5 mM reduced the ionic shifts produced by 220 mM NaCl in resting preparations. The other solutions produced small changes of questionable significance.

DISCUSSION

The most interesting feature of the present data is the fact that the magnitudes of the changes in the sodium and potassium contents of stimulated frog nerves are dependent on the concentration of potassium in the bathing solution, while they are only slightly affected by changes in the external sodium concentration or by suppression of the activity respiration. These findings may be reasonably interpreted in terms of the ionic theory of the nerve impulse (Hodgkin, 1951). According to this hypothesis, the minimum change in the ionic contents that is produced by the passage of a single impulse along a nerve fiber is C_wV/F, in which C_w = capacitance/unit weight; V = amplitude of the action potential in volts; F = faraday. Hodgkin (1951) has estimated C_w for frog nerves to be 8 μ fd./gm. wet. Since the dry weight of intact frog nerves is approximately 25 per cent of the wet weight, C_w = 32 μ fd./gm. dry. The amplitude of the action potential is approximately 120 mv. (Huxley and Stämpfli, 1951 *a*). Therefore, after 1 hour of activity at 50 volleys/sec., the minimum change in the sodium and potassium contents should be:

$$\Delta K = \Delta Na = \frac{32 \times 10^{-6} \times 0.12}{9.65 \times 10^4} \times 50 \times 60 \times 60 = 7.2 \ \mu\text{moles/gm. dry.}$$

If Tasaki's (1955) measurements of the capacitance of single frog fibers are used, this figure is approximately doubled. The measured changes in the sodium and potassium contents of stimulated nerves are of the same order of magnitude as is predicted by these estimates. Therefore, it is not unreasonable to assume that the primary cause of these changes is the ionic exchange that accompanies each volley of impulses. Under any given set of conditions the net changes in ionic contents should represent the summation of the ion shifts that occur during each volley of action potentials minus any recovery of the ionic distribu-

tion that may occur during the interval between successive volleys. If this interpretation is correct, then the effects of various agents on the magnitudes of the changes in ionic contents may be explained in terms of changes in the quantities of ions that exchange during the action potentials or in terms of changes in the amount of recovery hypothesized to occur during the interval between successive volleys of action potentials.

The ionic theory predicts that under various experimental conditions the quantities of ions that are transferred across the nerve membrane during an action potential are roughly determined by the amplitude of the action potential, provided that the experimental treatments produce no large changes in the magnitudes or time courses of the accompanying permeability changes. The results of Tasaki and Freygang (1955) and Tasaki and Mizuguchi (1949) indicate that at a node the action potential and impedance changes follow parallel time courses and are affected to approximately the same extent by various experimental treatments. In view of this parallelism between the two variables, it will be assumed that the effects of various solutions on the permeability changes are small as long as the effects on the action potential are small.

If the active nodal membrane behaved as an ideal sodium electrode, then doubling or halving the external sodium concentration would change the magnitude of the overshoot by ± 30 per cent. The data of Huxley and Stämpfli (1951 *a*) indicate that under normal conditions the overshoot comprises only about 40 per cent of the amplitude of the action potential, so that these variations in the external sodium concentration would change the action potential by only ± 12 per cent. The experimental data of Huxley and Stämpfli (1951 *b*) support this argument since they show that the magnitude of the nodal action potential is changed by only ± 10 per cent when the external sodium concentration is increased 25 per cent or reduced by 50 per cent. If it is assumed also that the magnitude and time course of the permeability changes are not greatly influenced by changes in external sodium, then the quantity of sodium entering a nerve fiber during an impulse should be relatively insensitive to variations of the external concentration of this ion.

Shanes (1956) has shown that the influx of potassium into desheathed toad nerves is reduced by 30 per cent when the external sodium concentration is reduced by 90 per cent, a rather small effect. It has also been demonstrated in giant axons of invertebrates (Hodgkin and Keynes, 1955) that the extrusion of sodium is not markedly affected by changes in the external concentration of this ion. This evidence suggests that variations in the external concentration of sodium should have little effect on any recovery processes that may operate to reverse the ionic movements that occur during an impulse. Our finding that the external concentration of sodium has little effect on the changes in ionic content that accompany activity in frog nerves is consistent with the observations on the effect of this ion on the action potential and ion transport processes.

At low potassium concentrations the resting nerve membrane deviates considerably from the ideal behavior represented by the logarithmic relation between membrane potential and external potassium concentration. The data of Huxley and Stämpfli (1951 *b*) show that potassium at concentrations up to 5 mM has little effect on the action potential of single myelinated fibers. However, at a concentration of 10 mM this ion may reduce the amplitude of a response by 40 per cent. Spyropoulos (1956) has shown that the duration of the action potential of single toad fibers increases several fold during tetanization at frequencies from 50 to 150 shocks/sec. It is conceivable that such effects become smaller at higher potassium concentrations, since we have observed that raising the potassium content of the Ringer's fluid usually has a beneficial effect on a tetanized nerve trunk. Thus, it is possible that the effect of 8.5 mM potassium on the ionic exchange accompanying activity is due to a reduction in the amount of sodium and potassium that exchange during each impulse.

Another possibility is that potassium affects the magnitudes of the changes in ionic contents by influencing the rates of recovery processes that may operate to restore the electrolyte distribution during the interval between successive impulses. The rate of extrusion of sodium from frog muscle (Keynes, 1954) and invertebrate giant axons (Hodgkin and Keynes, 1955) is related to the external concentration of potassium, the extrusion rate increasing with increases in the potassium concentration. Shanes (1957) has shown that the post-anoxic increase in the rate of sodium extrusion from toad nerve is dependent on the potassium content of the bathing solution. These findings suggest that the rates of any ionic recovery processes may be small in a potassium-free medium. Consequently, the changes in ionic distribution that are measured during activity in potassium-free Ringer's solution should provide the best estimate of the ionic shifts that occur during an action potential. In the present case, the changes in the sodium and potassium contents that occurred during 1 hour of activity at 50 volleys/sec. in a potassium-free medium were 20 to 30 μmoles/gm. dry, or 3 to 4 times those predicted by the calculations based on the value of the nerve capacitance. The changes in the sodium and potassium contents that occurred during an hour's activity at 50 volleys/sec. in 8.5 mM potassium Ringer's solution were 0 to 10 μmoles/gm. dry. If the small effect that was obtained at the higher potassium concentration were due to the acceleration of recovery processes that operate during the rest periods between successive volleys, then in Ringer's solution with 8.5 mM potassium this recovery proceeds at an average rate of approximately 20 μmoles/gm. dry/hr.

Similar results are obtained using the data from the experiments in which the nerves were stimulated for 5 hours in Ringer's solution containing 8.5 mM potassium. Thus, assuming that 25 μmoles of sodium and potassium exchange during each hour of stimulation at 50 volleys/sec., the net changes in ionic contents after 5 hours of activity would be 125 μmoles/gm. dry. In 8.5 mM

potassium Ringer's solution, the observed changes in ionic contents after this time were approximately 30 μmoles/gm. dry. Assuming the difference between these figures to be due to recovery, this recovery amounts to about 19 μmoles/gm. dry/hr.

According to the measurements of Shanes and Berman (1955) and Shanes (1956) the sodium and potassium fluxes in resting desheathed toad nerves in 1.7 mM potassium are 10 to 20 μmoles/gm. dry/hr. The previous calculations indicate, then, that during tetanization at a rate of 50 volleys/sec. in Ringer's solution containing 8.5 mM potassium, a nerve may absorb potassium at 2 to 3 times the rate characteristic of the resting tissue in 1.7 mM potassium.

It is possible that these explanations of the sparing action of potassium on the ionic shifts that accompany activity are not correct. There is no proof that the changes in ionic contents were due to the movements of sodium and potassium ions during each impulse, and there is no proof that any recovery occurs during the activity. However, the discussion shows that the present results may be reasonably interpreted in these terms.

From the fact that 0.2 mM azide has little effect on the magnitudes of the ionic shifts that occur during activity it cannot be definitely concluded that the activity respiration of frog nerve does not normally serve to reverse the ionic shifts that accompany each volley of impulses. It is possible that non-oxidative chemical reactions substitute for the inhibited respiration. Unfortunately, the available data on lactic acid production and stores of ATP and creatine phosphate in frog nerve do not permit a definite decision as to whether or not these energy sources could take the place of the suppressed respiration.

The work required to exchange 1 μmole of sodium for 1 μmole of potassium may be estimated from the formula (Levi and Ussing, 1949):

$$W = 2.3RT\left[\log\frac{K_i}{K_o} + \log\frac{Na_o}{Na_i}\right] \times 10^{-6}$$

in which

R = gas contant in calories/mole-degree,
T = absolute temperature.

Subscripts i and o refer to inside and outside concentrations of sodium and potassium, respectively.

Using the estimates for K_i and Na_i obtained by Hurlbut (1958) and solving this equation for an external potassium concentration of 8.5 mM, the result is: $W = 2.2 \times 10^{-3}$ cal./μmole. The present data indicate that in Ringer's solution with 8.5 mM potassium, 0.2 mM azide does not increase the ionic exchange that occurs during 2½ hours of activity at 50 volleys/sec. In this time the postulated ionic recovery has amounted to 50 μmoles/gm. dry, requiring 0.11 cal./gm. dry.

According to unpublished measurements by Dr. S. C. Cheng, intact frog sciatic nerves in Ringer's solution containing 2.0 mM potassium increase their rate of production of lactic acid by 3.3 μmoles/gm. dry/hr. when stimulated at a rate of 50 volleys/sec. in the presence of 0.2 mM azide (increase measured with respect to resting nerves in 0.2 mM azide). Assuming that the lactate was derived from glucose and assuming that the free-energy change accompanying the conversion of 1 mole of glucose to 2 moles of lactic acid is 58 kcal., this rate of glycolysis provides, in 2½ hours, a free-energy yield of 0.24 cal./gm. dry. If it is assumed that 2 moles of ATP are synthesized for each mole of glucose utilized,[1] and if the free energy of hydrolysis of the terminal phosphate bond of ATP is taken to be 8 kcal./mole (Kitzinger *et al.*, 1957), the energy yield of the increased lactic acid production is 0.06 cal./gm. dry.

The energy reserve represented by creatine phosphate and ATP may be computed to be 0.19 cal./gm. dry, if one uses the estimates of Gerard and Tupikova (1938) and Greengard *et al.* (1954) for the nerve content of these substances and employs the previous figure for the free energy of hydrolysis of the terminal phosphate bonds of these compounds.

If one uses the smaller estimate for the energy provided by the extra lactic acid production, and assumes the efficiency of the ion transport processes to be 50 per cent, then all the energy derived from the increase in the rate of glycolysis and the breakdown of nearly all the ATP and creatine phosphate would be required to account for the postulated recovery of the ionic distribution. However, if the higher estimate of the glycolytic energy is applicable, then sufficient energy might be available to account for the hypothesized recovery. More information on the efficiency of glycolysis in living axons is required before a conclusion can be reached as to whether or not the lactic acid production and stores of ATP and creatine phosphate can substitute for the inhibited activity respiration.

It should be stressed again that there is no proof that any appreciable recovery of the ionic distribution occurs during the intervals between successive stimuli. If no resorting of ions occurs normally during these intervals, then one would not expect that the ionic shifts that accompany activity would be increased by suppression of the activity respiration.

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[1] Handbook of Biological Data, (W. Spector, editor), Philadelphia, W. B. Saunders Co., 1956, 233.

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