POTASSIUM MOVEMENT IN RELATION TO NERVE ACTIVITY*

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INTRODUCTION

A tentative generalization, proposed on the basis of a number of bioelectrical studies, is that changes of potassium concentration at the nerve fiber surfaces—resulting from imbalance between conditions tending to cause intracellular potassium escape and metabolic reactions leading to uptake—are a major factor in the fluctuations of potential level (7–9, 13). This has been confirmed for anoxia and postanoxic recovery through establishment of predicted correlations with potassium movement by direct analytical methods in crab (10) and in frog nerve (3, 11, 12).

The primary purpose of the present study has been to test in several ways the validity of the proposal that the polarization changes during and following repetitive activity (*viz.*, the negative and positive after-potentials) are likewise the consequence of potassium release and reabsorption (9). To this end, electrical changes and potassium shifts have been studied under conditions as similar as practicable. Crab nerve was selected for experiment because its very long after-potentials (1, 4, 6, 9) gave promise of permitting the detection of related potassium transfer if such should occur. The specificity of veratrine with respect to the after-potentials is well known; therefore, its effect on ionic displacement as well as the electrical changes of activity also has been examined. Still another test of the hypothesis has been sought in observations of the effect on potassium movement of large and small volumes of medium in contact with the fibers; this will be compared with corresponding effects on electrical behavior (9).

A corollary of the working hypothesis, which has served as a basis for experimental design, is that functional changes may be a result in part of the postulated extracellular accumulation of potassium. The limited nature of the

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recovery from anoxia obtainable in vertebrate nerve with washing[1] may be due to diffusion barriers. Consequently the possibility of restoring by washing the functioning of anoxic squid giant axon and crab nerve, where the sheaths are more permeable, has been explored; these results will be described also.

Method

The general procedure for following the polarization changes induced by and following activity was the same as that described previously (9), *viz.*, individual *Libinia emarginata* leg nerves were mounted in a moist chamber in the usual way employed for measuring potassium-induced "injury" potentials, this potential difference was balanced out potentiometrically, stimulation was applied *via* silver wires to the ends of the nerves not in KCl, and the deflections of a calibrated high sensitivity galvanometer measured the polarization fluctuations of the central regions of the nerves relative to the ends in KCl. Action potentials were checked directly with an oscillograph. An adequate period—at least 1 hour—was permitted for equilibration prior to and between successive observations on a given nerve. The tetanus, at a rate of 2 shocks (condenser discharges) per second unless noted otherwise, was so low in frequency that spikes *did not* contribute to the deflection of the galvanometer; it was applied for 5 minutes since this time sufficed for a nearly maximal potential change. Recovery was followed for 20 minutes, during which time the repolarization process was practically maximal. Typically, three runs were made on each nerve. The first was a control with the central area of the nerve in contact with an artificial sea water (30 mM $CaCl_2$, 15 mM KCl, a pH 7.4 phosphate buffer equivalent to 0.05 M NaCl, and NaCl to bring the osmotic pressure to an equivalent of 520 mM NaCl); the second was with the same region in artificial sea water containing the experimental agent and with which it had been equilibrated for at least an hour; and the third was another control.

The study of potassium movement, with certain modifications, was carried out as described earlier (10). Thus, at half hour intervals, small volumes (0.5 to 0.8 ml.) of sea water were placed successively in contact with each of 2 paired sets of 4 leg nerves weighing approximately 200 mg., each set being mounted in identical perfusion chambers. Stimulation was usually applied to each of the sets, samples being collected before, during, and after the stimulation periods. The same reservoir units discussed earlier (10) were employed for oxygenation and for advancing fresh samples of medium. Improved perfusion chambers, like that shown diagrammatically in Fig. 1, served for stimulation of the nerves,[2] for observation of the spikes, and for recirculation of the solution which was finally collected by way of stopcock *E*. Entry of gas through *A* (after suitable humidification), with stopcocks *F* and *G* open and *E* appropriately

[1] Feng, T. P., and Gerard, R. W., Mechanism of nerve asphyxiation: With a note on the nerve sheath as a diffusion barrier. *Proc. Soc. Exp. Biol. and Med.*, 1930, **27**, 1073.

[2] Electrodes inserted by way of the stopper shown in Fig. 1 can be used to stimulate a length of nerve not in contact with the medium; this procedure does not alter the amount of potassium released by frog nerve.

set, caused the contained solution to circulate in the direction shown by the arrow. Analyses were carried out on 20-fold diluted samples with a Beckman flame spectrophotometer as previously described (10). Spikes were observable whether or not solution was present in section *P*; these were followed during stimulation. The same perfusion and reservoir units also served for the experiments on the effects of washing on anoxic failure; in addition to *Libinia* nerves, the giant axons of *Loligo pealii* were

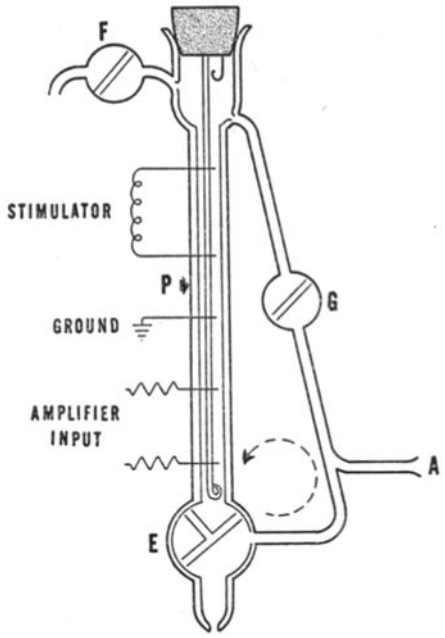

FIG. 1. Pyrex perfusion and stimulation unit showing the connections to the platinum electrodes.

studied. Every possible precaution, including continuous nitrogen flow, was taken to assure that oxygen was not trapped or did not penetrate anywhere in the various parts of the system. All experiments were carried out at room temperature (20–25°C.).

RESULTS

After-Potentials.—Table I is a tabulation of (*a*) the amplitude and time constant of development of the depolarization during repetitive stimulation and (*b*) the corresponding data for the succeeding repolarization process, as modified by veratrine, calcium, and glucose. The actual data are given rather than means and their standard deviations because the latter would give an excessively low estimate of the true order of significance of the experimental effects. Each nerve serves as its control twice, hence variations from nerve to nerve are minimized as a complicating factor.

TABLE I

The Maximal Depolarization and Repolarization of Individual Nerves during and Following Stimulation at the Rate of 2 Shocks per Second, and the Corresponding Time Constants (T_D and T_R), in Experimental Solutions (X) and in Control Solutions Which Preceded (I) and Followed (F) the Experimental Solutions

Column 1 gives any components present in all 3 solutions in addition to the usual constituents in the artificial sea water, column 2 the agent present only in the experimental solution. V = veratrine, Ca = calcium, G = glucose; the associated figures give the concentrations: mg. per cent for veratrine and mM/liter for calcium and glucose.

1	2	Depolarization			T_D			Repolarization			T_R		
		I	X	F	I	X	F	I	X	F	I	X	F
		mv.	*mv.*	*mv.*	*sec.*	*sec.*	*sec.*	*mv.*	*mv.*	*mv.*	*sec.*	*sec.*	*sec.*
—	0.025V	0.9	4.8	3.0	68	49	65	1.2	5.5	4.0	162	216	180
		2.4	3.5	2.6	85	48	60	2.8	4.3	3.2	154	170	170
—	0.050V	3.7	7.1	4.8	94	29	48	5.0	8.6	5.7	198	234	177
		2.2	5.9	4.0	74	17	30	2.9	5.5	4.3	138	210	174
		2.5	6.3	4.4	79	21	41	3.0	5.7	4.1	190	270	200
		2.6	5.1	3.2	104	59	80	3.8	6.4	3.9	246	243	201
—	90Ca	3.1	2.7	1.9	50	45	32	3.8	2.9	1.5	228	140	153
		2.3	1.8	1.5	95	91	89	3.1	2.0	1.7	260	141	168
		3.9	3.1	3.4	80	81	82	4.6	4.0	4.2	193	127	195
		3.2	3.0	3.2	69	68	80	4.4	3.6	4.0	206	160	190
0.025V	60Ca	6.4	6.1	5.5	39	30	41	8.2	6.8	6.7	234	171	271
	120Ca	5.8	5.1	4.9	66	22	52	6.8	5.8	5.9	321	144	232
0.050V	60Ca	4.7	4.2	3.0	51	29	35	5.3	4.4	2.8	280	136	144
	90Ca	4.9	5.1	3.9	24	13	24	5.7	5.4	4.4	198	123	174
		5.4	4.8	4.3	53	20	33	7.2	5.0	4.2	263	124	174
—	50G	4.6	3.6	2.8	84	88	89	5.3	3.8	2.7	276	216	175
		3.3	2.9	2.5	106	87	96	4.1	3.2	2.6	312	248	195
	200G	3.2	2.3	2.3	101	92	92	3.8	2.9	2.5	276	212	206
		5.2	4.6	3.4	79	77	78	6.5	4.7	3.4	280	189	183
0.025V	100G	5.2	4.7	4.7	42	41	41	6.5	5.0	5.6	299	236	250
		5.5	4.4	6.1	35	35	26	6.7	5.6	6.4	218	213	219
		3.6	4.3	4.5	40	37	41	4.5	4.6	5.1	348	285	270
		2.7	4.2	4.7	39	45	45	3.3	4.8	5.7	198	201	225
—	—	2.7	2.3	2.1	68	66	68	3.8	2.9	2.4	168	140	126
		2.2	1.7	1.2	74	70	74	2.6	1.6	0.9	147	120	90
		2.3	1.8	1.1	75	80	61	2.6	1.9	1.2	174	153	113
		1.8	1.0	0.6	73	50	34	1.6	1.1	0.8	120	96	120
0.025V	0.025V	5.0	5.6	5.3	41	41	42	7.2	6.7	6.2	222	207	204
		5.2	3.9	3.1	61	58	57	6.1	4.4	3.3	207	192	168

It is apparent that veratrine consistently increases both the rate and magnitude of the initial depolarization[3] as well as the amplitude of the repolarization and prolongs the recovery process. A comparison of the absolute magnitudes of the depolarization and repolarization changes demonstrates that the latter is almost invariably larger; as previously pointed out, the excess of repolarization, which was missed by earlier investigators owing to the limited volume of solution usually in contact with the region used for recording (see reference 9), is comparable to and may be designated as a positive after-potential. The amplitude of the excess polarization averages between 0.5 and 2.0 mv. and is not significantly different in veratrine. Removal of veratrine under the experimental conditions employed does not completely eliminate its effect on the amplitude of the polarization changes. This is particularly clear from a consideration of the behavior of nerves subjected continually to the same solutions, *viz.*, the amplitude of the potential changes generally declines with time, as may be seen from the set of data at the bottom of Table I.

The action of excess calcium on the negative after-potential was of interest in view of the dependence of the direction of its effect in the squid giant axon on the presence or absence of veratrine (8). Table I indicates that its only consistent effect in artificial sea water is a hastening of the recovery process. In veratrine the improved recovery in elevated calcium still is evident; in addition, the rate of depolarization during stimulation is markedly increased, as expected from the increase of the negative after-potential following each spike (9), but again the amplitude of the potential change is not significantly affected.

The possibility that glucose may reduce the depolarization resulting from stimulation, as it does during anoxia (7, 9), was also explored. The data indicate no such effect.

Potassium Shifts.—Table II gives the averaged data from six experiments with paired nerves, all of which behaved essentially as indicated by the averages. Potassium may or may not be taken up from the medium when the fibers are at rest. Stimulation, however, causes a marked loss of potassium, and its cessation is followed by a considerable uptake which tends to decline with time. The amplitude of these changes is consistently augmented by veratrine.[3]

It may be seen from Table II that individual experiments were conducted on paired nerves in such a way as to provide the equivalent of 3 independent controlled experiments. Thus, the pairs served as mutual controls inasmuch as one was first stimulated in the absence and the other in the presence of

[3] It is important to note that under the conditions employed veratrine does *not* induce a repetitive response to stimulation (9) so that unlike the situation in a number of earlier studies on other properties of nerve, its effects can be related specifically to the negative after-potential rather than to multiple firing.

veratrine; moreover, each of the pair served as its own control and, because the control, experimental sequence differed in the two, the possibility of error from a time trend was eliminated. The standard deviations of the means given in Table II express the variability of nerves taken from different animals on different days and therefore underestimate the precision with which an experimental effect—for example of stimulation or of veratrine—was actually detectable. The individual data are therefore given in Table III to indicate the reliability of the observations. The experiments shown are equivalent to a total of 17, of which only one failed to demonstrate that veratrine caused an increase in

TABLE II

The Average Changes in the Potassium Content of 1.0 Ml. Sea Water Surrounding Paired 1.0 Gm. Wet Weight Crab Nerves after Exposure for the Indicated Periods to the Successive Conditions Shown

S.W. = sea water, V. = veratrine.

Conditions of nerves in unit 1	Potassium change		Conditions of mates in unit 2
	1	2	
	μM	μM	
½ hr. rest in S.W.	−1.4 ± 0.2	+0.3 ± 0.5	½ hr. rest in S.W.
½ hr. rest in S.W.	−1.5 ± 0.8	+0.1 ± 0.5	½ hr. rest in S.W. + V.
Ca. 5 min. stimulation in S.W.	+2.6 ± 1.1	+5.9 ± 0.5	*Ca.* 5 min. stimulation in S.W. + V.
½ hr. recovery in S.W.	−2.9 ± 0.4	−4.9 ± 1.0	½ hr. recovery in S.W.
½ hr. recovery in S.W. + V.	−1.9 ± 0.5	−4.0 ± 0.5	½ hr. recovery in S.W.
Ca. 5 min. stimulation in S.W. + V.	+4.3 ± 0.6	+2.8 ± 0.5	*Ca.* 5 min. stimulation in S.W.
½ hr. recovery in S.W. + V.	−3.5 ± 0.4	−3.6 ± 0.7	½ hr. recovery in S.W.
½ hr. recovery in S.W. + V.	−3.1 ± 0.7	−2.1 ± 0.6	½ hr. recovery in S.W.

potassium release during stimulation; in the one exception the spike declined far more in veratrine than in its absence, which would account for the lesser potassium release. An almost equally consistent effect is seen for the increased uptake of potassium in veratrine. One exception is the same as before; the other is a nerve in the very first set which had been stimulated to complete failure in veratrine. In the latter a greater potassium loss had occurred during the stimulation period, but a large escape still continued during the first half hour following, and this slightly exceeded the uptake which occurred in the next half hour, hence the small net loss shown for 1 hour. If the potassium had been followed longer, a definite absorption would probably have been obtained. Greater uptakes could probably have been demonstrated in the other experiments as well, for in these too the recovery of potassium was limited to 1 hour. In view of the large number of preparations which were nevertheless observed

to extract more potassium than was lost, it appears to be a general rule that the uptake of potassium following stimulation exceeds that lost during activity.

Another series of experiments was conducted to determine the extent to which removal for analysis of potassium lost during stimulation modified the subsequent recovery of potassium compared, for example, with the more usual moist chamber situation in which the potassium lost to the extracellular spaces is permitted to remain for reabsorption. The averaged results of 5 paired experiments are given in Table IV. Here again the standard deviations of the means do not adequately express the reliability of the experimental effects. In

TABLE III

Potassium Loss during Stimulation, and Uptake during the Following Hour of Recovery

S_1 and V_1 are the losses observed in one of two paired sets of nerves, first in sea water and then in veratrine and V_2 and S_2 are the same observed in the second set, first in veratrine and then an hour after the removal of the alkaloid mixture. RS_1 and RV_1 are the uptakes of potassium during the hour following S_1 and V_1, and RV_2 and RS_2 are the corresponding values for the other nerve. S and V values are corrected for loss or gain noted prior to stimulation.

Stimulation		Potassium loss by nerve				Potassium uptake by nerve				Veratrine concentration
Rate per sec.	Duration	S_1	V_1	V_2	S_2	RS_1	RV_1	RV_2	RS_2	
	min.	μM/gm.	μM/gm.	μM/gm.	μM/gm.	μM/gm.	μM/gm.	μM/gm.	μM/gm.	*mg. per cent*
5	10	5.8	11.1*	9.4	8.3	7.8	−0.3	12.2	5.5	0.025‡
5	5	5.5	6.9	6.7	4.3	4.2	6.1	6.9	3.6	0.013
5	5	3.6	6.1	6.8	4.8	2.1	4.3	10.6	10.0	0.013
4	3	4.1	3.8§	5.8	2.7	5.9	4.8	8.7	3.6	0.025
4	4	—	3.9	5.7	2.3	—	6.2	5.8	4.2	0.025
4	4	0.5	3.5	—	—	5.9	8.5	—	—	0.025
4	4	0.6	3.2	4.6	2.9	6.2	9.7	12.2	8.0	0.025

* Stimulated to complete failure.

‡ This row of figures not included in averages given in Table II.

§ Spike declined to ½ the original amplitude.

every case potassium uptake during recovery was significantly less when the potassium released during activity remained in the extracellular spaces and was allowed to be taken up again. Also of interest is the intense uptake of potassium, by fibers which had been washed during stimulation, even after an hour in the absence of a large source of potassium.

Potassium Release and Activity.—An important question arising from the demonstrated release of potassium during anoxia and stimulation is the extent to which changes in functional activity may be a consequence of (*a*) a depletion of intracellular potassium, (*b*) an elevation in the extracellular potassium level, and (*c*) an alteration in the metabolic state of the fibers. The relative importance of these, and a possible role of enveloping sheaths, are indicated by preliminary

observations of the recovery of anoxic fibers in oxygen gas and in oxygen-free solution.

Four prolonged experiments were conducted on squid giant fibers, two with the other small fibers present and two with carefully cleaned axons. The preparations were mounted in *P* (Fig. 1) without circulating solution but with a flow of nitrogen which first passed by way of the sea water–containing reservoir. Single test shocks were applied at 1 to 5 minute intervals. A typical experimental series is shown in Fig. 2.

Within 20 to 30 minutes the action potential was observed to fail; this was usually preceded by a decline in amplitude and by an increase in the delay in spike arrival at the recording electrode. The threshold rose only a few minutes

TABLE IV

Average Potassium Changes of 1.0 Ml. Sea Water (S.W.) Samples Brought in Contact with Paired 1.0 Gm. Nerve Sets Subjected Successively to the Indicated Conditions

Stimulation rate 4 shocks per second.

Conditions of nerves in unit 1	Potassium change		Conditions of mates in unit 2
	1	2	
	μM	μM	
10 min. stimulation in gas.....	—	+4.2 ± 1.2	10 min. stimulation in S.W.
30 or 60 min. rest in gas.....	—	—	30 or 60 min. rest in gas
½ hr. recovery in S.W.....	−1.1 ± 0.7	−4.6 ± 0.8	½ hr. recovery in S.W.
½ hr. recovery in S.W.....	−1.2 ± 0.6	−3.9 ± 0.4	½ hr. recovery in S.W.
10 min. stimulation in S.W.....	+4.5 ± 0.8	+5.8 ± 1.1	10 min. stimulation in S.W.
30 or 60 min. rest in S.W.....	—	−3.2 ± 0.9	½ hr. recovery in S.W.
½ hr. recovery in S.W.....	−4.2 ± 0.3	−3.3 ± 0.9	½ hr. recovery in S.W.
½ hr. recovery in S.W.....	−2.3 ± 0.5		

before failure. The stopcocks were then flushed with nitrogen to eliminate trapped oxygen, and the nitrogenated sea water was introduced into *P*. The action potential reappeared within a second or two. The solution was then removed (by displacement with nitrogen) and nitrogen flow continued. This cycle was repeated several times each time with the same result. Oxygen was then introduced without solution following one of the failures. Recovery was strikingly slower: 3 minutes were required for the first appearance of the spike, and another 10 for the full return to the original amplitude and conduction velocity. Several cycles of failure in nitrogen and rapid complete recovery in oxygen-free sea water were again repeated prior to another return to oxygen gas. Again the spike required minutes to reappear in oxygen; at this stage 12 minutes were still insufficient for return of the full amplitude of the spike, but 1 minute of washing achieved full recovery. In one squid nerve the cycles of anoxic failure and restoration by washing or oxygen were continued further, so that a total of 16 failures and recoveries was observed over a period of $10\frac{1}{2}$

hours. In this case a definite decline in spike height became apparent only after the eleventh recovery and was increasingly marked with further anoxic failures. Depletion of intracellular potassium was probably a factor here, for in the single axons which had just failed permanently the potassium content was found to be only 26μ M/gm. Recovery in oxygen at the later times was prolonged and tended to be incomplete unless the fiber was washed. A fiber made functional by oxygen-free solution was not improved when oxygen was made available. The last 2 observations, together with the difference in recovery in solution

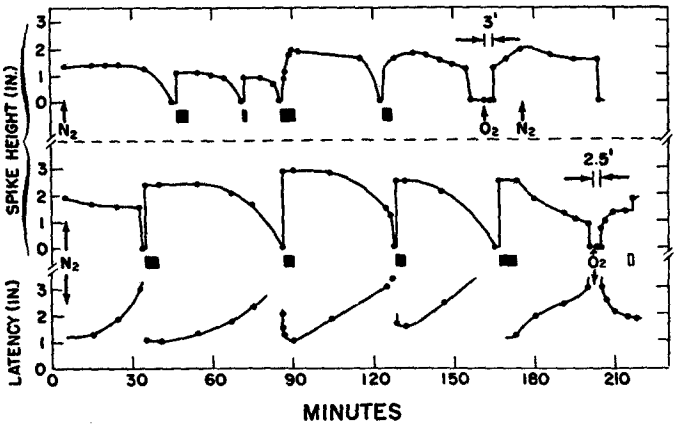

FIG. 2. Temporal characteristics of the functional changes of a squid axon, only partly cleaned, (*a*) in nitrogen gas, (*b*) upon washing with nitrogenated sea water ▬, (*c*) upon return to oxygen gas, and (*d*) upon washing with oxygenated sea water □. Spike height and latency (*i.e.*, from initiation of the sweep by the stimulus to time of arrival of the spike) are given as inches deflection on the cathode ray tube screen. Single supramaximal test shocks were sent into the nerve only at the indicated points except following failure, when a rate of 1/sec. was maintained until recovery was noted. The lower series is a continuation of the upper one and presents in addition the concomitant effects on the latent period and conduction time combined.

and oxygen, definitely indicate that washing restores activity by removal of an accumulated product like potassium rather than because of oxygen contamination.

Two sets of crab nerves, each composed of 3 nerves, treated in the same way behaved somewhat differently from the axons; again recovery in solution and oxygen was sufficiently different to demonstrate that oxygen contamination was not involved in the washing effects. The first anoxic failure required 10 to 15 minutes. Washing gradually restored the spike, which attained a maximum in about 5 minutes. Successive failures in nitrogen usually occurred more rapidly—within 3 to 7 minutes following removal of the oxygen-free sea water. Reappearance of the spike in oxygen gas occurred in 2 to 5 minutes, 10 to 15

minutes being required for the optimum, which was appreciably larger than that obtained with washing alone or before nitrogen was introduced. During the earlier anoxic failures, prolonged washing gave spikes about 60 per cent of those obtainable following recovery in oxygen. As the number of cycles of anoxia was increased, the maximum spike produced with washing became smaller and that in oxygen gas became larger than that of freshly mounted preparations, with the result that spikes observed in oxygen finally attained values 4 to 5 times greater than those produced with washing alone. In view of the evidence for reabsorption following anoxia (10), the large spikes obtained with recovery in oxygen gas were probably the result of a substantial lowering of the potassium content of the extracellular spaces (*cf.* reference 9). The poorer recovery with washing, compared to that of the giant axon, may have been due to interference with diffusion by the large amount of crab tissue used—a procedure made necessary to assure measurable spikes in the perfusion units.

DISCUSSION

The potassium shifts are in the direction predicted from the hypothesis (9) that changes in the potassium level at the fiber surfaces represent an important factor underlying (*a*) the depolarization caused by repetitive activity, (*b*) the enhancement of this depolarization by veratrine, and (*c*) the repolarization and hyperpolarization following the cessation of activity. The escape of potassium with stimulation has of course been known for some time. The additional observations on the uptake of potassium following activity and on the action of veratrine are new and a direct consequence of the application of techniques designed to test the hypothesis. Qualitatively, therefore, insofar as predictability constitutes proof of a causal relationship, the results may be considered evidence for the proposed mechanism of after-potential production.[4]

The finding that stimulation of the nerves in a large volume of solution causes a greater subsequent rate of potassium uptake than when the volume is small (Table IV) is also in keeping with the more perceptible positive after-potential under these conditions (9). The reduction of the potassium content of the medium under these conditions amounted to 5 to 20 per cent—sufficient to account for the observed magnitudes of the positive after-potential as calculated by the procedure described below. Apparently when the nerves are stimulated on filter paper strips or on metal electrodes the released potassium remains immediately available for reabsorption in the extracellular space and thereby

[4] It may be pointed out that the loss of potassium during anoxia was predicted (7, 13) while only evidence to the contrary was available (reference 2; see also Fenn, W. O., Occasional Publications of the American Association for the Advancement of Science, No. 2, April, 1934, suppl., **79,** 16) and the reabsorption of recovery was concluded (7, 13) considerably before analytical data to substantiate it became available (3, 10).

minimizes the depletion of potassium from the extracellular solution during recovery.

The completeness and rapidity with which washing can restore the functional activity of anoxic fibers, where potassium release is known to occur and where the functional decline is similar to that caused by potassium, provide an even more direct indication that surface potassium rather than any other factor or condition suffices to account for the electrical and related functional changes. The extent to which this view will actually prove sufficient as well as necessary to account for the phenomena studied hinges on the establishment of quantitatively precise relationships and on the further application of pharmacological agents with measurable effects on potassium movement.

Analysis of the kinetics of potassium diffusion in the extracellular spaces is required for an exact evaluation of the potassium at the fiber surfaces from available data. This is beyond the scope of the present report and will be described elsewhere. Nevertheless, it is desirable to determine in a preliminary way whether the actual potassium shifts are at least of the correct order of magnitude to account for the polarization changes. Such a preliminary comparison is possible with suitable simplifying assumptions for purposes of calculation. Thus, from the data in Table II it may be stated that the control nerves actually release at least 3.0×10^{-3} μM/(gm. impulse)[5] whereas veratrine-treated nerves lose 4.9×10^{-3} μM/(gm. impulse). The figure for the control is slightly smaller than that indirectly obtained by Hodgkin and Huxley (5), but larger than that reported by Cowan (2); as pointed out by Hodgkin and Huxley, the larger value is to be anticipated when stimulation to fatigue is avoided, as in the present experiments. These analytical data are probably on the low side since it is unlikely that all the potassium which escaped into the extracellular space succeeded in reaching the circulating sea water collected for analysis upon cessation of stimulation.

From Table I the averaged depolarizations are 3.0 mv. for the control and 5.5 mv. in veratrine for 600 impulses. Equation I in reference 13 permits the calculation of the equivalent increase in the potassium concentration; if as a rough approximation the assumption is made that the potassium released is homogeneously distributed within an extracellular space constituting half the total weight of the nerve, one obtains 5.0×10^{-3} and 11×10^{-3} μM/(gm. impulse) for the potassium liberated by stimulated controls and veratrinized nerves. These values are of the correct order of magnitude. Little more can be said because of uncertainty regarding the restraint on diffusion imposed by sheaths and the error in the estimate of the extracellular space. It may be noted that an extracellular space no greater than 0.25 ml./gm. wet weight of nerve is

[5] This designation refers to the micromols of potassium released by 1 gm. wet weight of nerve as calculated for a single impulse from the analytical data for a tetanus.

indicated by chloride analyses,[6] which would give potassium figures practically identical with the analytical ones.

A comparison of the *relative* increase produced by veratrine on the measured and calculated potassium losses may be expected to give better agreement since kinetic factors would tend to cancel out. Veratrine increased the measured potassium loss 1.6 times and the loss calculated from the negative after-potential 2.2 times. Thus, the agreement is good, especially since the difference is in the direction to be anticipated from the somewhat higher veratrine concentrations used for the electrical measurements.

The data therefore do support the possibility that the released potassium is both necessary and sufficient to account for the negative after-potential.

The author is indebted to Miss Tess Abramsky for her competent assistance in collecting the crab nerve data.

SUMMARY

The depolarization of crab nerve during repetitive stimulation is unaffected by the presence of glucose or by an increase in the calcium content of the medium. It is increased in both amplitude and rate by veratrine; in the presence of this alkaloid mixture the rate but not the magnitude of the depolarization is increased by an elevation in the calcium concentration. Repolarization following stimulation is unaltered by glucose and accelerated by a greater calcium concentration. Veratrine increases both the amplitude and the time constant of repolarization; its effect on the time constant is counteracted by an elevation of the calcium in the medium.

Potassium released during stimulation and its reabsorption following activity have been observed by analyses of small volumes of sea water in contact with crab nerve. Under the conditions employed 3×10^{-3} μM potassium is liberated per impulse per gm. wet weight of nerve. This loss is increased by low concentrations of veratrine, which also increase the amount reabsorbed during recovery. The depletion of potassium from the medium is appreciably less if the potassium previously released during activity has not been removed.

Inexcitability resulting from anoxia can be washed away with oxygen-free solution—rapidly and completely in the case of the squid axon, slowly and incompletely in crab nerve.

The potassium shifts are in the proper direction and of the correct order of magnitude to account for the negative and positive after-potentials in terms of potassium accumulation or depletion in the extracellular space.

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