STUDIES ON PERMEABILITY IN RELATION TO NERVE FUNCTION

II. IONIC MOVEMENTS ACROSS AXONAL MEMBRANES*

by

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INTRODUCTION

The ionic concentration gradients which exist between the inside and the outside of nerve fibres and their possible role in nerve function have been discussed in the preceding paper. In spite of the importance of this question very little information is available as to the ionic movements across axonal surface membranes in rest and during activity. The investigations on the giant axon of Squid have demonstrated that this material is most suitable for permeability studies. With the increased availability of radioactive ions from the Oak Ridge pile a more direct approach to the problem became feasible. It was thought that precise and more quantitative data might be obtained by subjecting the giant axon of Squid, *Loligo peallii*, to artificial environments in which all or part of a given ionic constituent was replaced in isomolar concentration with its radioactive isotope.

METHODS

Chemical. Na^{24} and K^{42}, available from the Oak Ridge pile in the form of the carbonates, were dissolved in the smallest possible volume of distilled water and then converted to the chlorides by the addition of equivalent quantities of dilute HCl. Aliquots of the neutral solution were then transferred to tared vials and evaporated to dryness under infra-red heating lamps. The quantity of salt per vial was determined by weighing and artificial sea water was prepared from these as described below. All necessary precautions were maintained (*i.e.*, remote control pipetting behind thick lead shields, etc.) in carrying out the conversions of carbonates to chlorides***.

The Ca^{45} employed in our earliest experiments was that obtained from the Oak Ridge pile in the form of $CaCO_3$ (AEC Catalog Item # 13 A). Since this material contained A^{37} in addition to Ca^{45}, it was deemed necessary to pump out the A^{37} under high vacuum before carrying out the conversion of the carbonate to chloride. In general, the latter conversion was carried out in a manner similar to that for Na^{24} and K^{42} above. In later experiments, high specific acitivity Ca^{45} was employed

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(AEC Catalog Item # S–5)*. Aliquots of the Ca^{45} solution were pipetted into the appropriate volumes of Ca-free artificial sea water to give the correct Ca concentration (0.012 M).

Preparation of biological material. The last stellar nerves (containing a giant axon) were excised from specimens of *Loligo peallii*, after first tying both ends of the portion desired. Nerve sections were then kept in fresh natural sea water for 1½ to 2 hours before use. The results of STEINBACH AND SPIEGELMAN[1] had indicated that during the first 2 hours after excision of stellar nerves, the chemically determined values for Na vary considerably and it is only after this time has elapsed that the axoplasm comes into equilibrium with its outer environment. The value for Na reaches its maximum value of 10 meq. per cent within this period.

The nerves were then exposed to artificial sea water prepared according to PANTIN[2] in which all or part of a given ion species had been replaced in isomolar concentration with radioactive material. The sea water contained 0.52 M NaCl, 0.013 M KCl, 0.012 M $CaCl_2$, and 0.024 M $MgCl_2$. The p_H was adjusted to 7.7–8.0 by the addition of a small volume of bicarbonate or NaOH, the latter in those cases where the adjustment required considerable amounts of alkali. After the desired period of exposure, the nerves were removed and rinsed several times in a few changes of fresh natural sea water. After blotting of filter paper, the proximal end was cut off. The axoplasm (nerve cytoplasm) was extruded by the application of gentle but gradually increasing pressure with a pair of forceps in the direction of the cut end. The extruded axoplasm was collected on a tared aluminum planchet (130–150 mg each and about one inch in diameter) and weighed quickly with a torsion balance. One ml of distilled water was then added to each planchet to insure even distribution of the radioactive substance over the entire area of the planchet.

Determination of radioactivity. Samples were then evaporated to dryness under infra-red lamps and the radioactivity measured with a Tracerlab 64 Scaler**. Measured radioactivities were recalculated to zero time from the decay curve of the individual ion under investigation in order to correct for the decomposition which occurred during the measurement of sample activities. This correction becomes appreciably large, when using Na^{24} and K^{42} which have half-lives of 14.8 and 12.4 hours respectively. Comparison of the activities of the samples with standards prepared from aliquots of the radioactive artificial sea water (and analysed at the same level in the counting chamber) enabled the calculation of the ion content of the axoplasm samples.

The method of preparation of the standards for Tables I, II, and III are given at the top of each of these tables. The Na standards for the data given in Tables IV, VI, IX, and X were prepared by diluting the sea water (containing 0.39 M $Na^{23}Cl$ + 0.13 M $Na^{24}Cl$) 250 times with distilled water. 0.5 ml aliquots were then evaporated to dryness in duplicate on aluminum planchets (1.04 micromoles Na/0.5 ml). For Tables VII and VIII, Na standards were prepared by this same method. However, since a reduction in the total NaCl concentration had been made in order to maintain the isotonicity in the presence of added inhibitors of cholinesterase, the 0.5 ml aliquots contained only 1.00 micromole Na/0.5 ml. The K standards for the data given in Tables V and VII were prepared by diluting the sea water (containing 0.013 M $K^{42}Cl$) 100 times and then evaporating 1.0 ml aliquots in duplicate as above (0.13 micromole K/1.0 ml). Radioactivities recorded in Tables IV through X have all been corrected to zero time.

Electrical. Nerves were tested for normality of conduction both before and after exposure to radioisotope containing sea water. The nerves were stimulated through a pair of silver wire electrodes by condenser discharge shocks of a time constant less than 0.2 milliseconds. Action potentials were led off by means of a second pair of silver wire electrodes to a condenser coupled amplifier of a modified Toeney differential type circuit and then recorded on a DuMont No. 279 Dual Beam Oscilloscope. Only those nerves were used which still exhibited normal conduction at the end of the experiment.

Studies of the rates of ion exchange during electrical activity of the nerves were carried out in the following manner: Nerve chambers were used of narrow bore polystyrene tubing (2 mm i.d.) into which were sealed, at right angles to the length and at 5 mm intervals, 0.0156″ diameter Pt wire as described previously (II). Nerves were mounted in the chamber by threading a long thin wire through the polystyrene tube (one end of the wire having previously been tied to the thread attached to the nerve). The nerve was then carefully drawn into the tube. By slipping a piece of narrow bore rubber tubing over that end of the polystyrene tube from which the thread issued, the thread—and thereby the nerve—was fixed in position. The rubber tubing was then connected to a perfusion bottle filled with sea water containing the radioactive ions. Perfusion of the nerve preparation was carried out by means of gravity. The diameter of the plastic tubing chosen was such that only a very thin layer of sea water remained between the nerve and the wall of the polystyrene tube. Thus, the difficulty of excessive shunting by the sea water was largely eliminated and stimulation of, and recording from, the nerve was possible throughout the period of exposure to the isotope containing sea water.

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** We are indebted to Dr G. FAILLA and the MARINE BIOLOGICAL LABORATORY, WOODS HOLE, MASS., for making the Scaler available to us.

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RESULTS

A. ION EXCHANGES AT REST

1. *Potassium.* In one series of experiments the stellar nerves were exposed to artifical sea water in which the K^{39} had been replaced by K^{42} in the usual sea water concentration (0.013 M). Analysis of axoplasm samples indicated that there was a rapid exchange of potassium under these conditions. Table I gives a few examples illustrating the size of the axoplasm samples, the magnitude of the radiation measured and the manner in which the standards were prepared. All of the data obtained in this way are presented in Fig. 1. Each point on the graph represents a single experiment. The number of millimoles (mM) of K^{42} which penetrated per 100 gm axoplasm (wet weight) is plotted against time of exposure of the nerve fibre to the radioisotopic sea water. It will be noted from Fig. 1 that the rate of penetration of K^{42} through the nerve membrane is initially quite high but it then slows markedly and within 60 min, analyses indicate an approach to a maximal value or 2.5 millimoles/100 g asymptotically. If one accepts the values for the potassium content of the axoplasm found in the literature (STEINBACH AND SPIEGELMAN, 32.1 meq. per cent[1]; BAER AND SCHMITT, 27 meq. per cent[3]; WEBB AND YOUNG 25.3 meq. per cent[4]) it can be seen that the maximum exchange obtainable under these conditions is approximately one tenth of the total K concentration of the axoplasm.

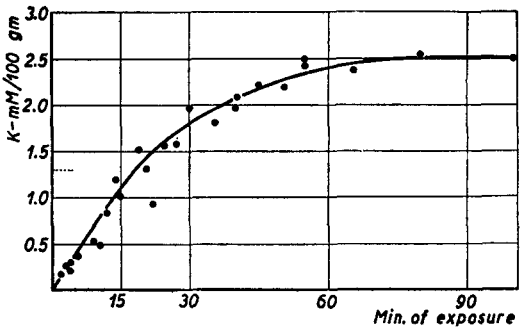

Fig. 1. K penetration across the membrane of the giant axon of Squid when exposed to 0.013 $K^{42}Cl$ in artificial sea water. The horizontal broken line on the ordinate indicates the K^{42} concentration outside. The penetration of K^{42} in millimoles (mM)/100 g axoplasm (wet weight) is plotted against time in minutes.

In all probability, the curve in Fig. 1 is a composite of at least two, or possibly more, distinct reactions. The first part of the curve, with the steepest slope, is, in all probability, a true measure of the rate of exchange of K across the nerve membrane. The second phase in which the rate of exchange has slowed down may possibly be ascribed to a movement of the radioactive ions from the inside to the outside after having reached a certain level. Finally, when the inside concentration is about twice that of the outside, there appears to be an equilibrium of the movements in the two directions.

The experiments show that even at rest, there is a dynamic equilibrium between the K inside the fibre and that in its outer environment[4a]. Within 50 min an equilibrium is established. Under such conditions only about one tenth of the total K inside the fibre has exchanged for K^{42} in the bathing medium. The K^{42} concentration inside the fibre is 2.5 millimoles/100 g axoplasm against 1.3 millimoles/100 ml for the sea water. When a steady state of exchange has been attained, it is possible to calculate the permeability constant for this exchange of K at rest by means of COLLANDER's equation as modified by KROGH[5]. According to KROGH where d is the diameter of the cell (cm), t is

$$P = 0.576 \frac{d}{t} \log_{10} \frac{C_s}{C_s - C_o \frac{a_s}{a_o}}$$

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time (hours), C_s and C_o concentrations of the ion inside and outside respectively, and a_s and a_o are the corresponding activities. d may be assumed to be = 0.05 cm, C_s = 0.32 M (STEINBACH AND SPIEGELMAN) and C_o = 0.013 M. Substituting 40400 cts/min/ml for a_o (from Table I) and 77700 cts/min/g for a_s (from Fig. 1) when t = 0.83 h, one obtains a value of $1.25 \cdot 10^{-3}$ cm/h for P, the permeability constant, from the equation above.

TABLE I

K^{42} PENETRATION

Nerves exposed to sea water containing 0.013 M $K^{42}Cl$ for varying periods of time. Standards (S_1 and S_2): sea water diluted 1:10 and then 0.5 ml evaporated to dryness in duplicate (0.65 micromole K^{42}/0.5 ml). Counts per min indicate the actual count, uncorrected for time decay of radioactivity.

Time of exposure (min)	Axoplasm (mg)	Counts per min	Millimoles per 100 g	Micromoles per 100 g per min
4	9.2	90	0.30	75
9	7.6	135	0.52	58
14	11.7	466	1.19	85
19	11.6	570	1.51	80
24	8.3	430	1.60	67
30	9.2	570	1.91	64
45	12.9	930	2.22	49
55	16.0	1247	2.49	45
65	13.6	1007	2.38	37
80	18.8	1490	2.54	25
S_1		2017 } average		
S_2		2022 } 2020		

Fig. 2 shows the rates of exchange of K against time. It will be noted that the rate is initially high but then drops to a value which is only about one fourth of that of the initial rate. The rate of penetration approaches a limiting value of 20 millimoles/100 g/min (or $2.5 \cdot 10^{-6}$ mole/cm²/min assuming an average diameter of 500 μ).

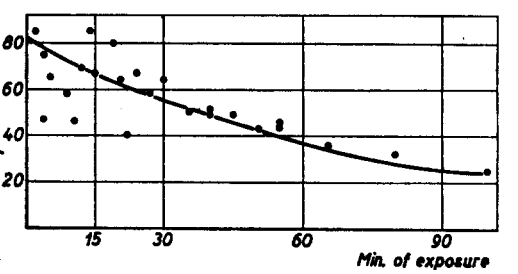

Fig. 2. Rate of K penetration across the membrane of the giant axon of Squid when exposed to 0.013 M $K^{42}Cl$ in artificial sea water. The rate of penetration of K^{42} in micromoles (μM)/100 g/min is plotted against time of exposure in min.

In a second series of experiments, the nerves were exposed to 0.026 M K^{42} Cl in the bathing sea water (twice the normal K concentration). In carrying out these experiments, a decrease in NaCl concentration was made equivalent to the increase in KCl in order to maintain the isotonicity of the sea water. The data obtained are plotted in Fig. 3.

It is evident from a comparison of Figs 1 and 3 that the shapes of the curves obtained for 0.013 M and 0.026 M KCl are very much alike. However, since the ordinate in Fig. 3 is greater by a factor of two, it can be seen that in the latter case the penetration of K^{42} into the fibre reaches a maximal value of 5.3 millimoles/100 g axpolasm. As in the case of the experiments with 0.013 M KCl, exchange of K^{39} inside for K^{42} outside reaches an equilibrium when the inside concentration of K^{42} is twice that of the outside.

As in the case of Fig. 1, Fig. 3 should probably have been resolved into three distinct phases. The considerations applied to the segments of Fig. 1 are also applicable

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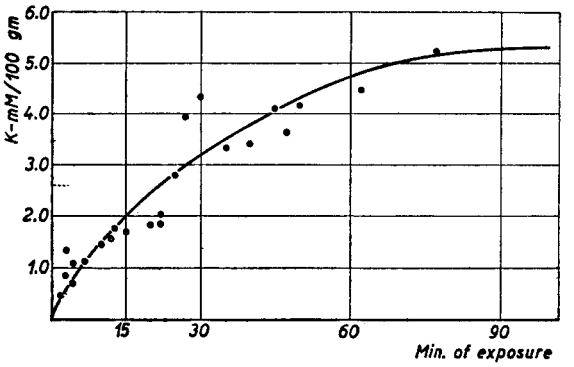

Fig. 3. K penetration across the membrane of the giant axon of Squid when exposed to 0.026 M $K^{42}Cl$ in artificial sea water (twice the normal K concentration). The horizontal broken line on the ordinate indicates the K^{42} concentration outside. The penetration of K^{42} in millimoles (mM/100 g axoplasm (wet weight) is plotted against time in minutes.

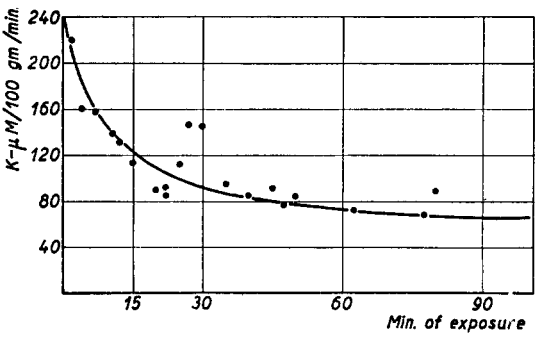

Fig. 4. Rate of K penetration across the membrane of the giant axon of Squid when exposed to 0.026 M $K^{42}Cl$ in the artificial sea water (twice the normal K concentration). The rate of penetration of K^{42} in micromoles (μM)/100 g/min is plotted against time of exposure in minutes.

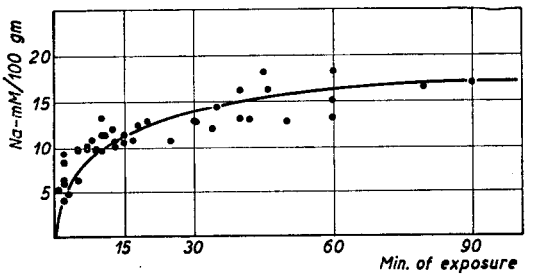

Fig. 5. Na penetration across the membrane of the giant axon of Squid when exposed to artificial sea water containing either 0.13 M or 0.065 M $Na^{24}Cl$. Total NaCl concentration is 0.52 M. The penetration of Na in millimoles (mM)/100 g axoplasm (wet weight) is plotted against time of exposure in minutes.

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to those of Fig. 3. The rates of K^{42} penetration against time with 0.026 M KCl outside are given Fig. 4. From a comparison of Figs 2 and 4, it is evident that the initial rate of K^{42} penetration, using 0.026 M KCl outside, is greater than that of the initial penetration rate obtained with 0.013 M KCl outside. Also, in the case of 0.026 M KCl outside, the rate of penetration falls more rapidly than in Fig. 1. However, the limiting rate of penetration finally attained is twice that of Fig. 2.

2. *Sodium.* The problem of Na penetration into the giant axons of Squid was investigated in a manner similar to that employed for K^{42}. In this case, however, either one fourth or one eighth of the Na^{23} in the sea water (normally 0.52 M) was replaced by Na^{24}. The remainder of the Na, necessary for maintainence of isotonicity of the sea water, was made up with ordinary Na^{23}. All other ions were maintained in their normal concentrations. Calculation of the Na penetrating the fiber was made on the assumption that there was no inherent difference in the case of Na^{23} and Na^{24} penetrations. Some typical data obtained are illustrated in Table II.

Fig. 5 represents all of the Na penetration data accumulated. It will be noted that Na enters the fibres at a rather high initial rate which falls markedly quite quickly. The Na penetration reaches a maximum of approximately 17.0 millimoles/100 g. This value is in good agreement with the value of 16.2 meq. per cent (16.2 millimoles/100 g) calculated by STEINBACH AND SPIEGELMAN[11] from the data of WEBB AND YOUNG. Our value for the Na penetrating would, therefore, seem to indicate that exchange of Na across the nerve membrane is

complete within about 30 min. Attainment of the steady state is accomplished when all of the Na inside the nerve has been exchanged for Na^{24}. Under such conditions, substituting in the permeability equation, the values of 0.162 M for C_s (WEBB AND YOUNG), 0.52 M for C_o, 934.3 cts/min/μl for a_o (Table II) and 293.6 cts/min/μg for a_s (Fig. 3) with t = 0.5 h and d = 0.05 cm, gives a value for the permeability constant of $5.76 \cdot 10^{-2}$ cm/h.

TABLE II

Na^{24} PENETRATION

Nerves exposed to sea water containing 0.39 M $Na^{23}Cl$ + 0.13 $Na^{24}Cl$ for varying periods of time. Standards (S_1 and S_2): sea water diluted 1:100 and then 0.4 ml evaporated in duplicate (2.1 micromoles/0.4 ml). Counts per min indicate the actual count, uncorrected for time decay of radioactivity.

Time of exposure (min)	Axoplasm (mg)	Counts per min	Millimoles per 100 g	Micromoles per 100 g per min
3	11.4	1014	4.7	1.57
9	12.0	2090	9.7	1.08
11	15.2	3550	12.4	1.13
20	13.8	3234	12.8	0.64
35	15.2	3924	14.3	0.41
42	14.6	3420	13.0	0.31
50	12.1	2770	12.7	0.25
55	10.2	4834	26.8	0.49
60	10.4	2160	11.7	0.20
80	11.1	3464	17.9	0.23
S_1		3720 average		
S_2		3754 3737		

The degree of scattering appears to be slightly larger in the case of Na than of K. This could, to some extent, be due to a slight contamination of the samples with radioactive sea water since the sea water contained such a high concentration of radioactive Na. Another factor may be the individual variations in Na content of these nerves. The data of STEINBACH AND SPIEGELMAN indicate that the values vary considerably from one nerve to the next: 3 to 4 hour exposure of axons to sea water gave Na values varying from 7.8 to 17.4 meq. per cent. No apparent effort was made in their work to determine whether or not all of these nerves maintained conduction. It is, therefore, not certain that such large deviations are actually within the normal range of variation. Nevertheless, it is quite conceivable that marked individual deviations occur.

The rates of penetration of Na into Squid nerves are plotted against time in Fig. 6. It will be noted that the initial rate of penetration of Na into fibres is extremely high but falls to a very low level within 15 to 20 min. The rate of penetration after 40 min of exposure has fallen to a value about one twen-

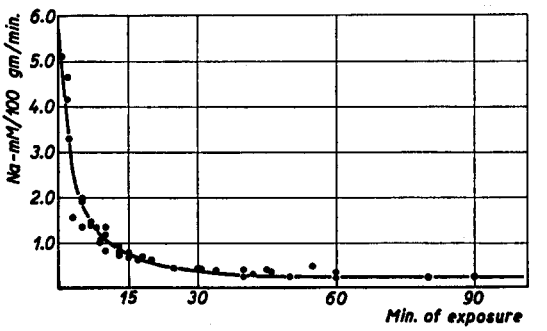

Fig. 6. Rate of Na penetration across the membrane of the giant of Squid when exposed to artificial sea water containing either 0.13 M or 0.065 M $Na^{24}Cl$. Total NaCl concentration is 0.52 M. The rate of penetration of Na^{24} in millimoles (mM)/100 g/min is plotted against time of exposure in min.

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tieth of that of the initial rate. This rapid fall in the rate of penetration is further support for the assumption that complete exchange of Na across the membrane occurs within a short period of time.

Extrapolation of the curve in Fig. 6 to zero time gives a value of 5.8 millimoles/100 g/min for the initial rate of Na exchange in these nerves. If one carries out a similar operation for the curve of Fig. 2, a value of 0.082 millimole/100 g/min for K is obtained. These results seem to indicate that the initial rate of exchange of Na is many times greater than of K. These findings do not support the concepts of CONWAY[6] that nerve membranes are impervious to Na although it has to be kept in mind that the observations are limited to the giant axons of Squid. The observations presented are consistent with those of STEINBACH AND SPIEGELMAN who have been able to demonstrate that Na enters these nerves.

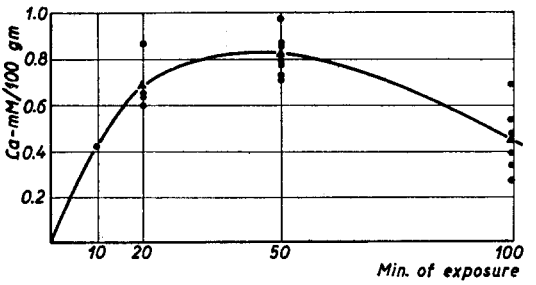

Fig. 7. Ca penetration across the membrane of the giant axon of Squid when exposed to artifical sea water containing 0.012 M $Ca^{45}Cl_2$. The penetration of Ca^{45} in millimoles (mM)/100 g axoplasm (wet weight) is plotted against time in minutes.

3. *Calcium.* Table III gives some of the date obtained when nerves were exposed to high specific activity of Ca^{45} (0.012 M) in artificial sea water for varying periods of time. All of the date obtained are plotted in the curve of Fig. 7. As in the cases of Na and K, each point on the curve represents a single nerve. The curve has been drawn through the mean of the several values at a given time of exposure. The data obtained were the same when low specific activity Ca^{45} was used.

TABLE III

Ca^{45} PENETRATION

Nerves exposed to sea water containing 0.012 M $Ca^{45}Cl_2$ (high specific activity) for varying periods of time. Standards (S_1 and S_2): sea water diluted 1:200 and then 0.5 ml evaporated in duplicate (0.03 micromole Ca^{45}/0.5 ml).

Time of exposure (min)	Axoplasm (mg)	Counts per min	Millimoles per 100 g	Micromoles per 100 g per min
50	19.2	10167	0.79	15.6
50	8.2	4762	0.87	17.2
50	6.0	2829	0.71	14.2
50	6.6	3271	0.74	14.6
100	9.4	1607	0.26	2.7
100	4.6	1139	0.37	3.8
S_1		1997 } average		
S_2		2010 } 2004		

It will be noted from Fig. 7 that the Ca^{45} inside the nerve seems to reach a maximum value of 0.82 millimole/100 g within 45 min and then decreases to a value of 0.45 millimole/100 g at 100 min of exposure. It is evident, therefore, that the Ca penetrates into these nerve fibres. The values obtained seem to indicate that the concentration of Ca^{45} at 100 min is lower than at 50 min. Further investigations are desirable for an interpretation of this observation.

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Fig. 8 is a curve obtained by plotting the rates of penetration of Ca^{45} into the nerves against time of exposure. It will be noted that the initial rate of exchange, extrapolated to zero time, is quite high and comparable to the initial extrapolated value for K (50 micromoles/100 gm/min and 82 micromoles/100 gm/min respectively).

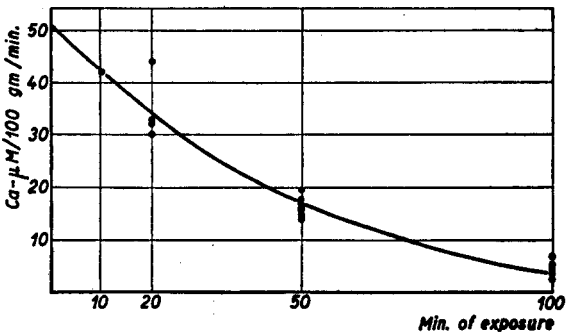

Fig. 8. Rate of Ca penetration across the membrane of the giant axon of Squid when exposed to artificial sea water containing 0.012 M $Ca^{45}Cl$. The rate of penetration of Ca^{45} in micromoles (μM)/100 g/min is plotted against time of exposure in minutes.

B. FACTORS INFLUENCING EXCHANGE OF Na AND K

In view of the considerable individual variations of the ion content of these nerves, it appeared advisable to modify the method of accumulation of data in studying the effects of a number of factors on the ion exchanges across the nerve membrane. Instead of collecting single values at varying periods of exposure, a large number of nerves were exposed simultaneously under identical conditions and for the same period of time. At least five values were obtained for a given condition and only the average values utilized in carrying out comparisons. All exposures were limited to 30 min. They were carried out at room temperature (22° C), except for the cases in which the Q_{10} of Na and K exchange were studied.

1. *Q_{10} of Na and K exchange.* Table IV contains the data obtained when nerves were exposed to 0.39 M Na^{23} Cl + 0.13 M Na^{24} Cl in artificial sea water for 30 min at 22° and 13° C respectively. At 22° C, the average of eight nerves gave a value of 9.5 millimoles/100 g while at 13° C the average of eight nerves was 8.6 millimoles/100 g. This would correspond to a Q_{10} of 1.22.

TABLE IV

EFFECT OF TEMPERATURE ON THE RATE OF PENETRATION OF Na

Nerves exposed for 30 min to sea water at 22° and 13° C containing 0.39 M $Na^{23}Cl$ + 0.13 M $Na^{24}Cl$. S_1 and S_2 = standards.

22° C Axoplasm (mg)	Counts per min	Millimoles per 100 g	13° C Axoplasm (mg)	Counts per min	Millimoles per 100 g
S_1	1324 } average				
S_2	1285 } 1305				
10.2	1273	9.9	11.4	1405	9.8
9.4	1321	11.2	16.6	1964	9.4
5.2	590	9.1	14.6	1624	8.9
8.8	996	9.0	20.4	2100	8.2
5.8	599	8.2	12.6	1199	7.6
12.0	1341	8.9	8.8	1001	9.0
14.2	1681	9.4	16.6	1694	8.1
12.2	1568	10.4	18.8	1864	7.9
Average		9.5	Average		8.6

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The exchange of K was studied under identical conditions (30 min exposure at 22° and 13° C) using 0.013 M $K^{42}Cl$ instead of $K^{39}Cl$ in the sea water. At 22° C the average of seven nerves was 1.31 millimoles/100 g and at 13° C the average of the same number of nerves was 1.09 millimoles/100 g (Table V). This would correspond to a Q_{10} of 1.33.

TABLE V

EFFECT OF TEMPERATURE ON THE RATE OF PENETRATION OF K

Nerves exposed for 30 min to sea water at 22° C and 13° C containing 0.013 M $K^{42}Cl$. S_1 and S_2 = standards.

22° C Axoplasm (mg)	Counts per min	Millimoles per 100 g	13° C Axoplasm (mg)	Counts per min	Millimoles per 100 g
S_1	1014 } average				
S_2	1051 } 1033				
10.8	1230	1.43	6.4	525	1.03
10.4	1152	1.39	8.4	732	1.10
7.2	758	1.33	4.2	395	1.18
			10.4	1030	1.25
S_1	482 } average				
S_2	488 } 485				
7.2	372	1.39	6.4	272	1.14
15.8	659	1.12	13.2	456	0.92
12.4	586	1.27	9.0	328	0.98
22.0	1000	1.22			
Average		1.31	Average		1.09

The values for the Q_{10} obtained above for both Na and K are in good agreement with the theoretical value of 1.25 calculated from ionic conductivity measurements. The ionic velocities increase by about 2 to 2.5% for every degree rise of temperature[7]. It is, therefore, possible that no important energy yielding chemical reactions are involved in the exchange of ions across the nerve membrane under these experimental conditions.

2. *Electrical activity and Na exchange.* Stimulation of nerves by supramaximal shocks while being perfused with sea water containing 0.39 M $Na^{23}Cl$ + 0.13 M $Na^{24}Cl$ produced a marked alteration in the rate of exchange of Na when compared to resting nerves. As described under Methods, nerves were mounted in plastic chambers in which stimulating and recording electrodes were imbedded. The nerves were stimulated at a rate of 100 times per second for 30 min. Only those nerves which exhibited normal responses throughout this period of stimulation were analysed. Analysis of the axoplasm of six of these nerves indicated that 15.9 millimoles Na/100 g (mean value) had exchanged within 30 min as compared with 9.5 millimoles/100 g at rest. This would correspond to an increase in the rate of exchange of approximately 67% above that at rest. The results of the individual analyses are recorded in Table VI.

If the cation molarity (Na plus K) of the Squid axoplasm is a constant, as is suggested by the work of STEINBACH AND SPIEGELMAN, then it is evident that during nerve

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TABLE VI

EFFECT OF ELECTRICAL ACTIVITY OF THE NERVE ON THE RATE OF PENETRATION OF Na

Nerves were stimulated at a rate of 100 times per second for a period of 30 min in sea water containing 0.39 M $Na^{23}Cl$ + 0.13 M $Na^{24}Cl$ at 22° C. S_1 and S_2 = Standards.

Axoplasm (mg)	Counts per min	Millimoles per 100 g
S_1	2045 } average	
S_2	2042 } 2044	
11.2	3420	15.5
20.4	6490	16.2
16.0	5340	17.0
12.2	4380	18.3
6.2	1803	14.8
11.4	3002	13.6
Average		15.9
Control (see Table IV)		9.5

activity, a quantity of K has been lost by the nerve to the sea water equivalent to the Na which penetrated during the same period. In the case under consideration, this would be equivalent to a loss of 6.4 millimoles K/100 g of axoplasm. This loss appears to be very high since, as discussed earlier, at rest a maximum of 2.5 millimoles K/100 g are easily exchangeable.

A few calculations concerning the exchange of ions during activity of the nerve may be of interest. The average diameter of the stellar nerve may be assumed to be of the order of 500 μ. An axoplasm cylinder of r = 0.025 cm and weighing 1 g would have a surface area of 80 cm^2. Since an increased exchange of 6.4 millimoles Na/100 g (or $6.4 \cdot 10^{-5}$ mole/g) has been demonstrated for a nerve which had been stimulated $1.8 \cdot 10^5$ times (100 per second for 30 min), it follows that $6.4 \cdot 10^{-5}$ mole/g divided by $1.8 \cdot 10^5$ or $3.6 \cdot 10^{-10}$ mole/g/impulse of Na penetrated into the axoplasm of the nerve from the sea water. This value corresponds to $4.5 \cdot 10^{-12}$ mole of Na penetrating/cm^2/impulse. It has been reported by PUMPHREY AND YOUNG[8] that the diameters of these giant nerve fibres of Squid usually vary from 280 to 720 μ in diameter and may in some cases by as large as 1000 μ (1 mm). If one calculates the values of Na which would penetrate per cm^2 per impulse for the usual extremes in the size of the fibres under the above conditions, one obtains the values $2.6 \cdot 10^{-12}$ and $6.5 \cdot 10^{-12}$ mole/cm^2/impulse for the smaller and larger diameters respectively. If one assumes that the increased Na penetration during activity is equivalent to the K loss during the same period, as the work of several investigators indicates, then it follows that the transfer of $4.5 \cdot 10^{-12}$ mole/cm^2/impulse of K has occurred during the period of nerve activity. This value is in excellent agreement with that indirectly calculated by HODGKIN AND HUXLEY[9] on the basis of the changes in membrane conductivity which occur in single fibre preparations of *Carcinus maenus* nerves during normal conduction. They obtained a value of $1.7 \cdot 10^{-12}$ mole/cm^2/impulse. The value is also in good agreement with that obtained by KEYNES[10]. This investigator soaked multifibre preparations of *Carcinus* nerves in K^{42}. Upon stimulation he found the leakage of $2.1 \cdot 10^{-12}$ mole/cm^2/impulse. The data with Na^{24}, like those of KEYNES, are direct. The method of HODGKIN AND HUXLEY, although most ingenious, necessitates numerous assumptions and is therefore

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inherently indirect. In spite of the fact that the methods and materials employed are different, the agreement is surprisingly close in the three cases.

3. *Effect of inhibitors of acetylcholine-esterase on the ion exchange.* The effects of two inhibitors of acetylcholine-esterase were studied on the rate of exchange of Na and K in these fibres. In Table VII are given the results obtained when giant axons were exposed for 30 min to 0.022 M diisopropyl fluorophosphate (DFP) in sea water containing 0.013 M $K^{42}Cl$. DFP at this concentration is capable of abolishing nerve conduction within approximately 2 min[11] and the action of this compound can probably be attributed exclusively to the inactivation of the enzyme[12]. The average of five nerves exposed to sea water containing DFP and K^{42} gave a value of 1.08 millimoles K/100 g while exposure to sea water for the same period of time in the absence of DFP gave a value of 1.31 millimoles/100 g. Assuming, as above, that the average diameter of these fibres is 500 μ (area of 1 g cylinder of axoplasm being equal to 80 cm^2), then one obtains a value of $5.5 \cdot 10^{-9}$ mole/cm^2/min as the rate of exchange of K in sea water at rest. In the presence of DFP this rate falls to $4.5 \cdot 10^{-9}$ mole/cm^2/min. This would correspond to a decrease of $1.0 \cdot 10^{-9}$ mole/cm^2/min in the presence of DFP. Although the concentration of K^{42} in the axoplasm is smaller in the presence of DFP than in its absence, this result does not indicate a decreased permeability. In view of the concentration gradient between the inside of the axon and its outer environment an increase in permeability may lead to an increase of the K outflow from the interior. The K^{42} penetrating from the outside may share the same fate and the final inside concentration will eventually be smaller than that under normal conditions.

TABLE VII

EFFECT OF DFP ON THE RATE OF PENETRATION OF K AND Na

Nerves exposed to 0.022 M DFP in sea water containing either 0.013 M $K^{42}Cl$ or 0.37 M $Na^{23}Cl$ + 0.13 M $Na^{24}Cl$. S_1 and S_2 = standards.

K Axoplasm (mg)	Counts per min	Millimoles per 100 g	Na Axoplasm (mg)	Counts per min	Millimoles per 100 g
S_1 1014 } average			S_1 1324 } average		
S_2 1051 } 1033			S_2 1285 } 1305		
5.8	472	1.02	10.6	2319	16.8
8.0	776	1.21	8.6	1589	14.2
4.6	398	1.09	12 0	2535	16.2
6.0	447	0.93	10.2	2480	18.6
5.4	493	1.15	16.0	2990	14.3
			7.0	1649	18.0
Average		1.08	13.8	2970	16.5
			9.6	2055	16.6
			14.8	2975	15.4
			Average		16.4
Control (see Table V)		1.31	Control (see Table IV)		9.5

This view is confirmed by the effect of the DFP on the Na movement. Table VII gives the results obtained when nerves were exposed to DFP in the same concentration as above (0.022 M) in the presence of 0.13 M $Na^{24}Cl$ + 0.37 M $Na^{23}Cl$ in the sea water. The mean of nine nerves exposed to DFP in sea water gave a values of 16.4 millimoles

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Na/100 g as compared to 9.5 millimoles/100 g when exposed to sea water in the absence of DFP. This would correspond to a rate of penetration of Na of $4.0 \cdot 10^{-8}$/cm²/min in the absence of DFP and a penetration of $6.9 \cdot 10^{-8}$ mole/cm²/min in the presence of DFP, assuming the average fibre diameter to be 500 μ. The rate of Na penetration has increased markedly. This could be expected on the basis of the concentration gradient in the event of increased permeability. It may be noted that the Na penetration has increased to a greater extent than the K penetration has decreased. Considering the difference in the rates of entrance of Na and K, it has to be kept in mind that in the experiments described, only the penetration of ions into the interior has been determined. No measurements have been carried out in respect to the leakage of K. If the amount of K actually passing from the inside to the outside were considerably increased, this would not be indicated by the method used.

The effect of eserine, another inhibitor of acetylcholine-esterase, on the rate of Na penetration into the nerve was also studied. The sults are given in Table VIII. It will be noted that 13.2 millimoles Na/100 g enter these nerves in the presence of 0.019 M eserine in the sea water containing 0.13 M $Na^{24}Cl$ + 0.37 M $Na^{23}Cl$. This would correspond to a rate of exchange of Na of $5.5 \cdot 10^{-8}$ mole/cm²/min in the presence of eserine as compared to $4.0 \cdot 10^{-8}$ mole/cm²/min in its absence, again assuming the average fibre diameter to be 500 μ. The above value is the average of ten nerves and, as in the other experiments, nerves were exposed for 30 min to the eserine-containing sea water. Eserine, in the concentration used, abolishes nerve conduction reversibly within 5–15 min. The time required to abolish the action potential of these nerves shows considerable variation in the case of eserine and is closely dependent upon the p_H and other factors[13]. Air oxidation of the eserine proceeds rapidly at the p_H employed (7.7–8.0) and therefore

TABLE VIII

EFFECT OF ESERINE ON THE RATE OF PENETRATION ON Na

Nerves exposed to 0.019 M eserine in sea water (p_H 7.7–8.0) containing 0.37 M $Na^{23}Cl$ + 0.13 M $Na^{24}Cl$. S_1 and S_2 = Standards.

Axoplasm (mg)	Counts per min	Millimoles per 100 g
S_1	2002 | average	
S_2	1910 | 1956	
22.0	5307	12.3
15.0	3705	12.6
19.4	5550	14.7
3.8	1019	13.7
S_1	1820 | average	
S_2	1861 | 1841	
9.2	2458	15.1
22.4	4950	12.0
8.4	1946	12.6
14.8	3660	13.4
20.8	4720	12.3
26.0	6490	13.5
Average		13.2
Control (see Table IV)		9.5

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a given solution cannot be used for a prolonged period of time. The results presented were obtained with fresh eserine solutions. Although there is a marked increase in Na exchange, the effect of eserine is not as large as that obtained with DFP.

4. *Cocaine and Na exchange.* The effects of cocaine in 0.005 M in sea water have been studied using 0.13 M $Na^{24}Cl$ + 0.39 M $Na^{23}Cl$ in the bathing fluid. Nerves were exposed to this solution for 30 min. The results are reported in Table IX. No decrease in membrane permeability is evident from the data. The Na exchange amounted to 11.2 millimoles/100 g (average of six nerves). Again assuming a fibre diameter of 500 μ, this would correspond to a rate of Na exchange of $4.6 \cdot 10^{-8}$ mole/cm^2/min, a slight increase compared with the control.

TABLE IX

EFFECT OF COCAINE ON THE RATE OF PENETRATION OF Na

Nerves exposed to 0.005 M cocaine in sea water containing 0.39 M $Na^{23}Cl$ + 0.13 M $Na^{24}Cl$. S_1 and S_2 = standards.

Axoplasm (mg)	Counts per min	Millimoles per 100 g
S_1	2045 | average	
S_2	2042 | 2044	
6.4	1301	10.5
5.8	1102	9.9
4.8	995	10.8
12.8	2983	12.1
4.8	1142	12.3
7.2	1616	11.6
Average		11.2
Control (see Table IV)		9.5

5. *Effect of X-ray irradiation.* The effects of high intensity X-ray irradiation on the membrane permeability to Na was studied. Nerves were irradiated with 50000 R and 125000 R while immersed in a shallow dish containing natural sea water (water layer about 5 mm thick). Immediately after irradiation, the nerves were transferred to artificial sea water containing 0.39 M $Na^{23}Cl$ + 0.13 M $Na^{24}Cl$. After 30 min exposure to sea water the nerves were analysed. Only those nerves which still exhibited normal conduction upon stimulation were used. The results are given in Table X.

In the axoplasm of nerves irradiated with 125000 R, an average value of 14.1 millimoles/100 g was found (average of seven values). This corresponds to a penetration of $5.9 \cdot 10^{-8}$ mole/cm^2/min. Consequently, the rate of penetration had markedly increased. The findings suggest that irradiation had strongly increased the permeability.

Irradiation with 50000 R gave an average value of 10.9 millimoles Na/100 g (average of eight nerves). This corresponds to a rate of penetration of Na of $4.7 \cdot 10^{-8}$ mole/cm^2/min. The increase in the rate of penetration is relatively small but appears significant, especially in connection with the high increase observed with the larger dose of irradiation. It may be noted that the effect was obtained immediately after irradiation.

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TABLE X

EFFECT OF X-RAY IRRADIATION ON THE RATE OF PENETRATION OF Na

Nerves irradiated with 50000 R and 125000 R respectively in natural sea water and then exposed for 30 min to artificial sea water containing. 0.39 M $Na^{23}Cl$ + 0.13 M $Na^{24}Cl$. S_1 and S_2 = standards.

50000 R Axoplasm (mg)	Counts per min	Millimoles per 100 g	125000 R Axoplasm (mg)	Counts per min	Millimoles per 100 g
S_1	2002 | average				
S_2	1910 / 1956				
10.4	2425	12.4	7.0	1610	12.2
13.4	2620	10.4	5.0	1248	13.3
13.8	2375	9.1	3.8	1033	14.5
			6.2	1700	14.6
S_1	1820 | average		S_1	2045 | average	
S_2	1861 / 1841		S_2	2042 / 2044	
6.6	1198	10.3	5.6	1380	12.5
7.6	1565	11.6	4.0	1225	15.6
8.4	1576	10.6	5.4	1711	16.1
9.8	1696	9.7			
11.8	2725	13.0			
Average		10.9	Average		14.1
Control (see Table IV)					9.5

DISCUSSION

From the results obtained upon exposure of nerves to sea water, at rest, containing radioactive K^{42}, it can be seen that part of the K of the nerve interior is in dynamic equilibrium with that in the outer bathing medium. The lack of exchange of approximately 90% of the K^{39} under these conditions is unexplained. It appears that most of the K inside the nerve is not easily lost by the cell. Once the free, easily diffusible K has been exchanged for K^{42}, the rate of K exchange falls to a very low level. This is in good agreement with the observations of HEVESY AND HAHN on rabbit muscle and red blood cells[14], of STEINBACH on *Thyone briareus* muscle[15], and of HEPPEL on rat muscle[16]. In all of these investigations no more than 10–30% of the total K content of the tissues under investigation was exchangeable at rest.

In an effort to explain the difficulty of incomplete K exchange essentially two theories have been discussed. The one considers the possibility that the K is present in bound form. The idea has been proposed that a K salt of an unknown organic acid with a very low dissociation constant exists. As emphasized by KROGH[5], there is no evidence for the existence of bound K and from a theoretical basis, it appears doubtful that it can exist. HILL AND KUPALOV[17] have shown that all the K inside the muscle cell is required to be in ionic form in order to account for the osmotic pressure. Moreover, its presence in ionic form is necessary to insure the neutral reaction. Another possibility discussed is the presence of K impermeable barriers inside the cell. No such structures are known. The reasons for exchange of only a small fraction of the total K cannot be resolved at present.

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The values for the Q_{10} for K and Na exchange obtained, 1.22 and 1.33 respectively, are in good agreement with the value of 1.25 calculated theoretically from ionic conductively measurements. These figures do not support the assumption that important energy yielding reactions are involved in the transport of ions across these nerve membranes in resting condition. KROGH discusses the possibility that the extrusion of Na from the cell interior is an active process requiring energy. In support of this hypothesis, he cites experiments of HARRIS[18] and DANOWSKI[19] with rabbit and human erythrocytes in which it had been shown that, at low temperature and at body temperature in the absence of glucose, K is lost to the bathing medium and replaced by Na. When glycolysis is restored, the normal K balance is restablished, even *in vitro*, with a resumption of rapid Na extrusion. If the extrusion of Na is an active process in the nerve preparation tested, under resting condition, one would have expected to obtain a larger value for the Q_{10}. Lowering the temperature of these nerves by ten degrees should have produced a marked effect on the glycolytic processes and should have been expected to yield larger Na values than those obtained.

The fact that in resting condition no expenditure of energy seems to be required for the ionic movements does by no means preclude the possibility that under other conditions these movements may require energy. It appears likely that during the early growth stage of these nerves chemical reactions are in operation which are responsible for the establishment of the large concentration gradient between the potassium inside the fibre and that in the outer bathing fluid. The same is true for the disequilibrium observed after activity. The extra oxygen uptake observed after activity indicates that energy yielding reactions are involved in the restoration of the resting condition.

The present studies of the ion exchange occurring in nerve during activity have indicated that the Na content increases markedly. Similar results have been obtained with muscle tissue by FENN *et al.* on frog, and rat[20, 21, 22], WOOD, COLLINS AND MOE on dog gastrocnemius[23], TIPTON on cat muscle[24], HEPPEL on K-deprived rats[25] and HAHN AND HEVESY on rats[14]. All of these investigations show that in contracting muscles the permeability to ions is increased. K is lost from the fibres and is replaced by Na. STEINBACH AND SPIEGELMAN[1] have demonstrated that the cation molarity of the Squid axoplasm is, under a variety of conditions, constant at rest. It appears, therefore, justifiable to assume that during nerve activity K loss is compensated for by the penetration of an equivalent quantity of Na into these fibres.

This idea is supported by the demonstration of the penetration of $4.5 \cdot 10^{-12}$ mole Na/cm^2/impulse, a value which is in close agreement with the value of $1.7 \cdot 10^{-12}$ mole K/cm^2/impulse found by HODGKIN AND HUXLEY[9] and $2.1 \cdot 10^{-12}$ mole K/cm^2/impulse reported by KEYNES[10]. The value reported here indicates that during activity a considerable increase of Na inside takes place. 6.4 millimoles per 100 g were found after 30 min stimulation at 100 per second as compared with 1.3 millimoles per 100 g at rest. If an equivalent amount of K has leaked out, 21% of the total K content has been exchanged during this stimulation period. It should be noted here that the period of stimulation employed is by no means the maximum possible with these nerves. Much more prolonged periods of stimulation at 100 per second are possible and one would expect an even greater ion exchange. It should be borne in mind that the above changes are completely reversible and cessation of stimulation should result in restoration of the normal balance. From the above considerations, it may be concluded that, even though 90% of the K content of the nerve is not exchangeable at rest, during activity

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some reactions have occurred which facilitate the more rapid loss of K by these fibres.

A short discussion of the methods employed in the papers of HODGKIN and HUXLEY AND KEYNES as compared with the present investigations might be of interest. The method used by HODGKIN AND HUXLEY involves measurement of the small changes in the ionic conductivities over small areas of the nerve membranes before and after activity. Both the electrical recording equipment and the electrode assemblies are complex and the method employed necessitates numerous assumptions. The method employed by KEYNES is more direct. However, he has used multifibre preparations. Under such circumstances, one could expect a retarded diffusion of K^{42} away from the nerve preparation because of the possible trapping of K in the intracellular fluids. Since only the radioactivity of the K^{42} remaining in the nerve preparation was measured in these investigations, one would expect that values obtained in this manner would be higher than the actual intracellular K^{42} content of the fibres. The calculated value for the K leakage per cm^2 per impulse would therefore be expected to be smaller than the true value.

The method employed in the present investigation is direct. Since it is possible to analyse directly the axoplasm of the single nerve fibre, the values obtained must be considered to be more precise than those obtained by either of the above methods. The only assumption involved is the exact size of the individual fibres employed. However, since all of the Squid used were of approximately the same size, it is safe to assume that the fibres were all of approximately the same diameters. For medium size Squid this is approximately 500 μ (0.05 cm). It is justifiable to assume that the average value is close to this figure.

The investigation of the effect of inhibitors of acetylcholine-esterase on the rates of the ion exchange across the nerve membrane requires some comment. It has been shown that exposure of nerves to sea water for 30 minutes containing K^{42} plus DFP causes a decrease in the rate of K exchange from 1.31 to 1.08 millimoles per 100 g. The exposure of nerves to DFP has apparently altered the permeability of the nerve membrane. The DFP could conceivably have affected the membrane by decreasing its permeability. However, the effect of DFP on the rate of Na penetration excludes this interpretation. The value for the Na penetration markedly increased from 9.6 millimoles Na per 100 g to 16.4 millimoles upon the addition of 0.022 M DFP. If the DFP had had the effect of decreasing the membrane permeability one would have expected a decreased Na exchange. It might have been expected that with increased ion permeability the K could penetrate into the fibre more readily, However, since the concentration of K inside of these nerves is approximately 20 times that of sea water, it is likely that the easily exchangeable K will rapidly diffuse out into the sea water in an attempt to equalize the adverse concentration gradient across the nerve membrane. The K, in this case, will be replaced by the entrance of Na in order to maintain the electrical neutrality of the axoplasm. In such an event, the exchange of K^{42} would proceed at a decreased rate and this obviously accounts for the decreased K exchange in the presence of DFP. Thus, the Na and K exchange measurements are consistent with the concept that the membrane permeability had been increased by the DFP.

The probability of the exchange of K^{39} for radioactive Na^{24} was discussed before. Another factor to be considered is the constancy of the total cation content of these nerves. It has been demonstrated by STEINBACH AND SPIEGELMAN[1] that under normal resting conditions the cation content (Na + K) of these nerves is a constant. However,

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it is not known whether nerves in which the permeability has been increased still maintain their normal total cation concentration. It is possible that under these conditions Na as well as Cl may diffuse into the cell. This would result in increased total base content. Since the total base content of the axoplasm samples has not been measured, the contribution by the NaCl diffusion into the nerve cannot be evaluated. This problem has to be investigated further.

The effect of eserine, another inhibitor of acetylcholine-esterase had a similar but less marked effect than DFP in increasing the membrane permeability to Na. It may be noted, that in the case of DFP conduction was, on the basis of previous experience, abolished irreversibly. In the case of eserine the effect was almost certainly still reversible.

The result obtained with acetylcholine-esterase inhibitors, suggest that these substances may be capable of altering the membrane permeability. Since the only known action of these compounds is the inhibition of the enzyme acetylcholine-esterase[26] which is known to be closely connected with nerve conduction, it is possible that the effect observed is a manifestation of the inactivation of the enzyme. These experiments do not permit any definite conclusion, especially in view of the irreversible action of DFP during the long exposure period used. However, they may open a new approach to the importance of the acetylcholine-esterase system in the permeability of the surface membrane to ions.

The study of effects of cocaine on the membrane permeability to Na has indicated a small increase in the rate of exchange. The data are inadequate to judge whether or not this increase is significant. Employing the same concentration of cocaine ($5 \cdot 10^{-3}$ M), SHANES[27], from membrane potential measurements, came to the conclusion that a decrease in permeability had been accomplished. The results obtained here fail to confirm his reports.

The study of effects of irradiation of nerves with large doses of X-rays (50000 R and 125000 R) indicates that immediately following exposure, marked alterations in membrane permeability are evident. Exposure to 125000 R caused a large increase in membrane permeability while 50000 R caused only a small but significant increase. It should be noted that these studies were carried out immediately after irradiation. It is possible that a more marked effect would be evident with smaller doses of irradiation if longer periods of time were permitted to elapse between irradiation and exposure to radioactive ions. From our present knowledge, it is clear that the most notable effects of exposure to radiation occur after prolonged periods of time so that a longer time lapse than that used in these experiments might be preferable. It appears significant that it has been possible to demonstrate increased membrane permeability as result of X-ray irradiation.

I wish to express my gratitude to Dr DAVID NACHMANSOHN for suggesting these investigations and for the guidance and encouragement he has given throughout the course of this research. I am indebted to Mrs EMILY FELD-HEDAL and Mrs HEIDI RICHARDS for their assistance in the experiments.

SUMMARY

1. Studies on the permeability of the surface membranes of the giant axon of Squid to K indicate that a dynamic rather than a static equilibrium exists at rest. Approximately 10% of the total K

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in the fibre is replaced by K^{42} from the bathing medium within one hour. When the nerve is bathed in twice the normal K concentration (0.026 M) the K content of the axoplasm reaches a maximum twice that obtained with the normal K concentration outside.

2. Exposure of nerves to sea water containing Na^{24} results in a total exchange of all of the Na in the axoplasm for its radioactive isotope within 20 to 30 minutes.

3. Studies with Ca^{45} in the outer bathing fluid indicate an uptake of Ca^{45} to the extent of 0.85 millimoles per 100 g within 45 minutes and then a decrease to 0.45 millimoles per 100 g at 100 minutes of exposure.

4. The temperature coefficient (Q_{10}) obtained from the rates of exchange of Na and K does not indicate that there are important energy yielding chemical reactions involved in the exchange of ions across the membrane at rest. The values obtained (1.22 for K and 1.33 for Na) are in good agreement with the theoretical value (1.25) calculated from ionic conductivity measurements.

5. Electrical activity causes an increased rate of Na penetration into the fibre. $4.5 \cdot 10^{-12}$ mole of Na enter per cm^2 per impulse.

6. Inhibitors of cholinesterase, *e.g.*, eserine and DFP, seem to produce an increase in membrane permeability. The rate of K^{42} penetration is decreased, that of Na^{24} increased.

7. Exposure to cocaine (0.005 M) does not affect markedly the rate of Na^{24} penetration.

8. X-ray irradiation with 125000 R produces a large and immediate increase in membrane permeability to Na^{24} whereas 50000 R produces a smaller effect but in the same direction.

RÉSUMÉ

1. L'étude de la perméabilité au potassium de la membrane du cordon nerveux principal de Seiche indique l'existence au repos d'un équilibre dynamique plutôt que statique. Environ le 10% du K total de la fibre est remplacé par K^{42} du milieu environnant en une heure. Si le nerf est immergé dans une solution de concentration de K deux fois plus grande que la concentration normale (0.026 M) la teneur en K de l'axoplasme atteint un maximum qui est égal au double de la valeur obtenue avec une concentration externe normale de K.

2. Si l'on expose un nerf à l'eau de mer contenant Na^{24} un échange total a lieu entre le Na de l'axoplasme et son isotope radioactif en 20 à 30 minutes.

3. Si le bain extérieur contient Ca^{45}, celui-ci est absorbé jusqu'à 0.85 millimoles par 100 g en 45 minutes, puis la concentration de Ca^{45} décroît jusqu'à une valeur de 0.45 millimoles par 100 g au bout de 100 minutes.

4. Le coefficient de température (Q_{10}) obtenu à partir des vitesses d'échange de Na et K ne semble pas indiquer que des réactions chimiques dégageant d'importantes quantités d'énergie soient liées à l'échange des ions à travers la membrane. Ses valeurs obtenues (1.22 pour le K et 1.33 pour le Na) sont en accord avec la valeur théorique (1.25) calculée à partir de mesures de conductivité ionique.

5. L'activité électrique augmente la vitesse de pénétration du Na dans la fibre. $4.5 \cdot 10^{-12}$ mols de Na pénétrent par cm^2 et par influx.

6. Les inhibiteurs de l'acétylcholine estérase, p. ex. l'ésérine et le DFP semblent, augmenter la perméabilité de la membrane. La vitesse de pénétration de K^{42} diminue tandis que celle de Na^{24} augmente.

7. Une exposition à la cocaïne (0.005 M) n'affecte pas considérablement la vitesse de pénétration de Na^{24}.

8. L'irradiation aux rayons-X de 125000 R produit une augmentation importante et immédiate de la perméabilité de la membrane au Na^{24}. 50000 R produisent un effet moindre dans le même sens.

ZUSAMMENFASSUNG

1. Die Permeabilität der Membranen des Hauptnervenstranges vom Tintenfisch (*Loligo peallii*) für K wurde untersucht und gefunden, dass in der Ruhe eher ein dynamisches als ein statisches Gleichgewicht zu bestehen scheint. Ungefähr 10% des gesamten K-Gehaltes der Faser werden innerhalb einer Stunde durch K^{42} aus der umgebenden Lösung ersetzt. Ist der K-Gehalt des Bades zweimal so gross wie die normale Konzentration (0.026 M), dann ist auch der maximale K-Gehalt des Nervenstranggewebes zweimal so gross wie bei normaler äusserer Konzentration.

2. In Na^{24}-haltigem Meerwasser findet ein vollkommener Austausch des im Gewebe enthaltenen Na gegen sein radioaktives Isotop innerhalb 20 bis 30 Minutes statt.

3. Enthält das äussere Bad Ca^{45}, so wird dieses bis zu 0.84 Millimol per 100 g in 45 Minuten aufgenommen; dann nimmt der Ca^{45}-Gehalt wieder ab und beträgt noch 100 Minuten 0.45 Millimol per 100 g.

4. Der aus den Austauschgeschwindigkeiten für Na und K errechnete Temperaturkoeffizient

(Q_{10}) weist nicht darauf hin, dass in der Ruhe stark exothermische chemische Reaktionen an dem Ionenaustausch durch die Membrane beteiligt sind. Die erhaltenen Werte (1.22 für K und 1.33 für Na) stimmen gut mit dem aus Messungen der Ionenleitfähigkeit errechneten theoretischen Werte (1.25) überein.

5. Durch elektrische Arbeit wird das Eindringen von Na beschleunigt. $4.5 \cdot 10^{-12}$ Mol Na per cm^2 dringen bei jeder Anregung ein.

6. Hemmstoffe der Acetylcholinesterase, wie Eserin und DFP scheinen die Permeabilität der Membrane zu erhöhen. K^{42} wird langsamer, Na^{24} rascher aufgenommen.

7. Cocaïn (0.005 M) beeinflusst die Aufnahmegeschwindigkeit von Na^{24} nicht merklich.

8. Bestrahlung mit Röntgen-Strahlen (125000) erhöht R die Permeabilität für Na^{24} augenblicklich stark, mit 50000 R ist dieser Effekt gleichgerichtet aber geringer.

REFERENCES

1 H. B. STEINBACH AND S. SPIEGELMAN, *J. Cellular Comp. Physiol.*, 22 (1943) 187.
2 C. F. A. PANTIN, *J. Exptl Biol.*, 11 (1934) 11.
3 R. S. BAER AND F. O. SCHMITT, *J. Cellular Comp. Physiol.*, 14 (1939) 205.
4 D. A. WEBB AND J. Z. YOUNG, *J. Physiol.*, 98 (1940) 299.
4a M. A. ROTHENBERG AND E. A. FELD, *J. Biol. Chem.*, 172 (1948) 345.
5 A. KROGH, *Proc. Roy. Soc.*, B 133 (1946) 140.
6 E. J. CONWAY, *Irish J. Med. Science*, Oct.–Nov. (1947) 593.
7 S. GLASSTONE, *Textbook of Physical Chemistry*, D. van Nostrand Co (1941) 895.
8 R. J. PUMPHREY AND J. Z. YOUNG, *J. Exptl Biol.*, 15 (1938) 453.
9 A. L. HODGKIN AND A. F. HUXLEY, *J. Physiol.*, 106 (1947) 341.
10 R. D. KEYNES, *J. Physiol.*, 107 (1948) 35 P.
11 T. H. BULLOCK, H. GRUNDFEST, D. NACHMANSOHN, AND M. A. ROTHENBERG, *J. Neurophysiol.*, 10 (1947) 63.
12 H. GRUNDFEST, D. NACHMANSOHN, AND M. A. ROTHENBERG, *J. Neurophysiol.*, 10 (1947) 155.
13 T. H. BULLOCK, D. NACHMANSOHN, M. A. ROTHENBERG, AND K. STERLING, *J. Neurophysiol.*, 9 (1946) 253.
14 G. HEVESY AND L. HAHN, *Kgl. Danske. Videnskab. Selskabs Biol. Medd.*, 16 (1941) 1.
15 H. B. STEINBACH, *J. Cellular Comp. Physiol.*, 9 (1937) 429.
16 L. A. HEPPEL, *Am. J. Physiol.*, 127 (1939) 385.
17 A. V. HILL AND P. S. KUPALOV, *Proc. Roy. Soc.*, B 106 (1930) 445.
18 J. HARRIS, *Biol. Bull.*, 79 (1940) 373.
19 T. S. DANOWSKI, *J. Biol. Chem.*, 139 (1941) 693.
20 W. O. FENN, *Physiol. Revs*, 16 (1936) 450.
21 W. O. FENN AND D. M. COBB, *Am. J. Physiol.*, 115 (1936) 345.
22 W. O. FENN, D. M. COBB, J. F. MANERY, AND W. R. BLOOR, *Am. J. Physiol.*, 121 (1937) 595.
23 E. H. WOOD, D. A. COLLINS ,AND G. K. MOE, *Am. J. Physiol.*, 128 (1940) 635.
24 S. R. TIPTON, *Am. J. Physiol.*, 124 (1938) 322.
25 L. A. HEPPEL, *Am. J. Physiol.*, 128 (1939) 440.
26 M. DIXON AND D. M. NEEDHAM, *Nature*, 158 (1946) 432.
27 A. M. SHANES, *Science*, 107 (1948) 679.

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