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THE POTASSIUM PERMEABILITY OF A GIANT NERVE FIBRE

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The experiments described in the preceding paper (Hodgkin & Keynes, 1955) show that metabolic inhibitors like dinitrophenol and cyanide produce large changes in the relative magnitude of the fluxes of potassium moving inwards and outwards across the membranes of giant axons from *Sepia officinalis*. For normal fibres recovering from stimulation the influx is 15–30 pmole/cm^2 sec, while the efflux is 20–40 pmole/cm^2 sec. On poisoning with dinitrophenol, which virtually abolishes the sodium efflux, the potassium influx is reduced to 2–3 pmole/cm^2 sec, while the efflux remains the same or is slightly increased. These facts may be explained by supposing that in normal fibres there is, in addition to the passive potassium movements seen by themselves in poisoned fibres, an active uptake of potassium, coupled to the extrusion of sodium, and amounting to about 20 pmole/cm^2 sec. This hypothesis is attractive in that it provides a reasonable explanation of the further observation that the sodium efflux drops by some 20 pmole/cm^2 sec when external potassium is removed, but it raises one serious difficulty which needs to be resolved.

According to the equation derived by Ussing (1949*b*) for independent passive transport of ions, the influx (M_i) and the efflux (M_o) of a monovalent cation such as potassium should be related in the following way:

$$M_i/M_o = \exp\,(E - E_K)F/RT, \qquad (1)$$

where E is the potential difference across the membrane (here taken as external potential minus internal potential), and E_K is the equilibrium potential for potassium, defined by $E_K = \frac{RT}{F} \ln \frac{[K]_i}{[K]_o}$, where $[K]_i$ and $[K]_o$ are the internal and external concentrations of potassium. This equation has also been derived in different ways by Teorell (1949) and Hodgkin & Huxley (1952*a*). The essential assumption is that with a constant potential difference across the membrane the chance of any individual ion crossing the membrane in a given

time interval is not affected by the other ions that are present. Examples of mechanisms to which such an independence relation should apply are those involving combination with carriers where only a small proportion of the carrier is combined, or systems involving diffusion where the ions are present at fairly low concentrations throughout the membrane, and have no tendency to concentrate in narrow channels.

It seemed at one time that the potassium fluxes in cephalopod axons were in reasonable agreement with equation (1) (Keynes, 1951; Hodgkin, 1951). Thus Keynes found ratios of 0·3–0·4, which is about the value predicted for a potassium concentration ratio of 34 and a resting potential of 62 mV (Weidmann, 1951). This agreement must now be regarded as fortuitous, since poisoned axons—in which the fluxes should be wholly passive—give an average flux ratio of about 0·1, whereas the average values of resting potential and potassium concentration obtained for similar fibres require a ratio of 0·4. The quantities used in applying the equation are, of course, subject to appreciable uncertainty, but the discrepancy is so large that it cannot be removed by any reasonable allowance for experimental error. It therefore appears that the movements of potassium through the surface membrane cannot be independent in the sense required for equation (1) to hold.

It does not follow that active transport must be involved, since it is possible to have passive movements which are not independent. In this case the influx and efflux are equal when $E = E_K$ as predicted by equation (1), but for all other situations the relation no longer applies. An example is provided by a system in which there is a large exchange across a membrane, but little net transport (Ussing, 1949*a*; Mitchell, 1953). However, such behaviour will not account for our results, since it makes the flux ratio closer to unity than the independence relation would predict. The type of hypothesis required to explain a flux ratio which is anomalous in being unduly high is one in which the ions are assumed to move in single file through narrow channels or along chains of sites which stretch through the membrane. In this kind of system the influx is small when the main direction of the ionic movements is outward, because ions travelling inwards have to move against a stream coming in the opposite direction. Such competition between influx and efflux results in exaggerated flux ratios.

These considerations suggest that it is highly desirable to measure the potassium flux ratios as a function of the driving force, $E - E_K$. A certain amount of information can be obtained by varying the external potassium concentration, but the range covered in this way is small, since E and E_K are approximately equal when the external potassium exceeds 50 mM. This paper is therefore mostly concerned with a series of experiments in which E_K was varied by altering the external potassium concentration, and E was controlled by current flow. The measurements were not at all accurate, but it proved

possible to change the flux ratio over a range of 2400, so that errors introduced by imperfections in technique or by variations from one fibre to the next, were not as important as they might seem at first sight.

METHOD

The main new apparatus was a device (Fig. 1) for measuring the potassium fluxes in a short length of a 200 μ *Sepia* axon whose membrane potential could be altered by applying current, or by depolarizing the adjacent regions with potassium-rich solutions. Two very similar versions of the apparatus were built, and were used for parallel determinations of influx and efflux on different fibres. The essential feature of the device was a thin-walled glass tube (2·0 mm outside diam., 1·6 mm inside diam.) which could be connected with a pair of motor-driven syringes operating in push-pull. Two holes about 300 μ in diameter were made on opposite sides of the tube, by drilling

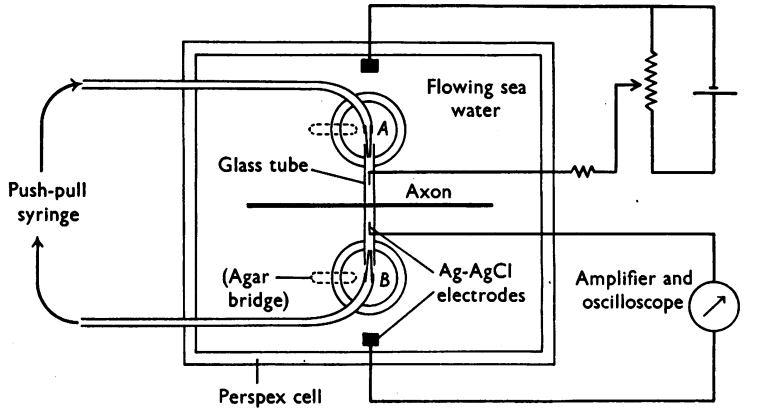

Fig. 1. Diagram of apparatus. When the agar bridge was used, the circular pot *A* or *B* in which it was placed was filled with solution.

with a needle and diamond paste and subsequently rounding the sharp edges in a microflame. The fibre was drawn through the holes with a hair, and held in position by two pairs of forceps. For measurements of influx, a solution containing ^{42}K was placed in one of the push-pull syringes, and was made to flow past the centre of the fibre at 0·25 ml./min for periods up to 40 min. During the whole of this time the parts of the fibre outside the tube were washed with a constantly renewed supply of inactive solution, so that the radioactive ions could only come into contact with the central portion of the fibre inside the tube. Tests made with dyes showed that there was little mixing between the inner and outer solutions, and that the boundary between them was sharp. After 20–40 min the flow of ^{42}K sea water was stopped, both syringes were removed, and the tube was thoroughly flushed through with an inactive solution. The fibre was then taken out of the apparatus, washed in sea water, and mounted above a Geiger counter in flowing sea water (see Keynes, 1951, Fig. 1) in order to determine the quantity of radioactivity in the axis cylinder. The influx was calculated from the rate of gain of labelled potassium, and the area of membrane exposed to tracer in the tube, taken as $\pi \times$ axon diameter $\times l$, where l was the mean of the internal and external diameters of the tube. In some cases, where the time spent in ^{42}K was not short compared with the time constant for loss of radioactivity, it was necessary to make an allowance for the efflux of ^{42}K during the period in the tracer solution. This was done by calculating the rate constant for loss of activity (k) from the observed axon diameter and $[K]_i$, and from a probable value for the efflux under similar conditions taken from Fig. 6, and correcting the influx by the factor $kT/(1 - e^{-kT})$ as described by Keynes (1954).

For measurements of efflux the fibre was first loaded with ^{42}K and was then placed in the apparatus with inactive solutions both in the tube and in the outer compartment. The push-pull syringes were run for 20 min and the quantity of radioactivity collected in the receiving syringe was determined with a liquid counter as described in the previous paper (Hodgkin & Keynes, 1955). While the receiving syringe was being emptied and washed out, a pair of stand-by syringes was in operation. After making several determinations of efflux under different conditions, the fibre was removed, washed in K-free choline sea water, and the central part dried on a thread of quartz. The quantity of ^{42}K in this fragment was determined by counting with a solid counter, and the total K by activation analysis (Keynes & Lewis, 1951*b*). The efflux was worked out in the usual way from the membrane area, the rate of loss of labelled potassium, and the proportion of labelled to total potassium in the axoplasm.

One way of altering the membrane potential in the central part of the fibre was to depolarize the two ends outside the tube with a potassium-rich solution, and to rely on local circuits to keep the resting potential in the central region at about the same level as in the rest of the fibre. When this method was employed the central tube was usually connected with the outer compartment by

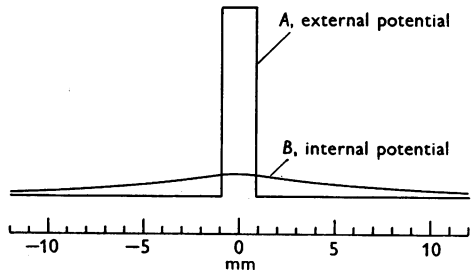

Fig. 2. Theoretical distribution of potential in a cable-like system. *A* is the external potential applied to the axon. *B* is the displacement of the internal potential from its resting value, calculated for an axon with a space constant of 7 mm. Note that the potential difference across the membrane is nearly constant in the central region, and is about 90% of the applied potential.

an agar bridge. For efflux measurements this was placed between pot *A* and the external solution, in order to avoid loss of radioactive ions by diffusion into the agar. For influx measurements it was placed between pot *B* and the external solution in order to avoid dilution of the radioactive solution by diffusion from the agar. The agar bridge was not used in all experiments, and was not really essential, since the axon did not fit tightly into the holes drilled through the tube, and there was always a fairly low resistance path between the inside of the tube and the outer compartment.

The other way of using the apparatus was to have a solution containing 10·4 mM-K in the outer compartment, and to alter the potential of the tube by applying current. Under these conditions the change in membrane potential in the central region will be nearly equal to the applied potential provided that the space constant of the fibre is large compared to the width of the tube. This is illustrated by Fig. 2, which shows *A*, the external potential and *B*, the internal potential calculated by linear equations on the assumption that the membrane resistance is constant and that the space constant is 7 mm. Weidmann (1951) gives data which suggest that the space constant for resting unpoisoned *Sepia* axons in a large volume is about 10 mm; we have used a lower value because our fibres were always stimulated and soaked for an hour in dinitrophenol at the start of the experiment. The error will be less if Weidmann's figure applies. For the conditions assumed in Fig. 2, the mean change in membrane potential for the region inside the tube is 88% of the applied potential. This figure is only valid when the applied potential is small. For displacements greater than 5 mV it is necessary to allow for rectification in the membrane, which will reduce the error when the inside of the tube is anodal, and increase it markedly when the tube is cathodal. Our

procedure was therefore to regard the applied potential as an upper limit, and to estimate the errors as well as we could by calculations based on electrical data obtained in other experiments.

Axons from *S. officinalis* were used throughout the investigation. They were almost always stimulated for 3–10 min at 50/sec and poisoned with 0·2 mM-dinitrophenol for at least an hour before any tests were made. Samples of ^{42}K of about twice the usual specific activity were provided by the Atomic Energy Research Establishment, Harwell. For efflux experiments the ^{42}K was usually introduced by soaking in 50 mM-K for 1–3 hr at about 4° C. Unless otherwise stated, electrical and chemical methods were similar to those described in the previous paper (Hodgkin & Keynes, 1955).

RESULTS

Electrical measurements

In order to interpret the tracer experiments it was essential to have good data for the relation between membrane potential and external potassium concentration in the absence of applied currents. This was obtained by impaling the axons with 0·5 μ micro-electrodes filled with 3 M-KCl (Ling & Gerard, 1949; Nastuk & Hodgkin, 1950). The thin layer of connective tissue surrounding *Sepia* axons is not always easy to penetrate, and it proved vital to anchor the fibres properly before attempting to impale them. In the first experiments we left fine strands of the nerve trunk adhering to the otherwise clean axon, and fixed them with pins to the bottom of a Perspex cell. Later we pulled the axons into a 400 μ glass capillary, one wall of which had been ground away over the central region for a distance of 10 mm, in order to allow the micro-electrode to be applied to the axon. The fibres were impaled two or three times in each solution, and frequent checks were made of the resting potential in the normal sea water containing 10·4 mM-K. The membrane potential during the positive phase which follows the spike in cephalopod axons was determined in some of the experiments. Axons are normally inexcitable in 52 mM-K, but a good anode break excitation could still be obtained by polarizing the membrane with an anodal current, and then switching off the current. The changes brought about by altering the potassium concentration were usually reversible, except with 490 mM-K, which tended to leave the membrane potential depressed by a few mV on returning to 10·4 mM-K. The results of successive impalements showed a scatter which we attribute to slight alterations in the way in which the membrane was penetrated, and to slight differences between micro-electrodes. (These were often broken by the connective tissue, and had to be replaced at frequent intervals.) In a representative experiment, nine impalements in 10·4 mM-K gave a mean resting potential of 62 mV, with a standard deviation of 2·6 mV (S.E. of mean 0·9 mV).

The result in which we were primarily interested was the relation between potassium concentration and resting potential in fibres treated with 0·2 mM-dinitrophenol. The values obtained in four experiments are given in Table 1, and averages are plotted in Fig. 3. Table 2 and Fig. 4 show the very similar behaviour of fibres which had not been poisoned. It can be seen that as in

other excitable tissues (Hodgkin, 1951) the relation between membrane potential and log $[K]_o$ has a slope of about 50 mV at high potassium concentrations, but that the slope falls off considerably at low potassium concentrations.

TABLE 1. Effect of external potassium concentration $[K]_o$, on the membrane potential in fibres poisoned with 0·2 mM-dinitrophenol

	Resting potential (mV)					Membrane potential (mV) at peak of positive phase				
	Axon					Axon				
$[K]_o$ (mM)	1	2	3	4	Mean	1	2	3	4	Mean
3·5	—	—	—	66	66	—	—	—	92	92
10·4	63	61	63	62	62	—	—	76	75	76
20·7	—	57	61	56	58	—	—	65	64	65
52	41	42	45	43	43	—	—	—	44	44
104	25	29	28	26	27	—	—	—	—	—
207	8	14	13	13	12	—	—	—	—	—
490	−10	0	—	−11	−7	—	—	—	—	—

Experimental details

Axon no.	Diameter (μ)	$[K]_i$ (mM)	$[Na]_i$ (mM)
1	220	274	68
2	195	255	58
3	254	242	45
4	276	307	61
Mean	236	270	58

Axons were stimulated for 5 min at 50/sec and then soaked in 0·2 mM-DNP for 1 hr before use. The results given for 10·4 mM-K are the average of 10–20 separate impalements; in the other solutions two or three impalements were made in each case. The concentrations of Ca and Mg were kept as in sea water throughout. $[Na]_o$ was altered with $[K]_o$ so that their sum was always 490 mM. All solutions contained 0·2 mM-dinitrophenol. Temperature 17–19° C. Potentials are here given as external minus internal potential; concentrations are in m-mole/l. (axoplasm or solution).

TABLE 2. Effect of external potassium concentration on membrane potential in unpoisoned fibres

	Resting potential (mV)				Membrane potential (mV) at peak of positive phase			
	Axon				Axon			
$[K]_o$ (mM)	1	2	3	Mean	1	2	3	Mean
0	70	83	65	73	94	91	94	93
3·5	—	—	63	63	—	—	84	84
10·4	63	69	60	64	76	76	74	75
20·7	—	—	53	53	—	—	62	62
52	46	43	40	43	—	—	—	—
207	20	18	14	17	—	—	—	—
490	−1	1	−4	−1	—	—	—	—

Potentials are given as external potential minus internal potential.

Experimental procedure as for Table 1 except that fibres were not poisoned with DNP and were not stored for analysis.

This suggests that K^+ ions carry most of the membrane current in depolarized axons, as found by Hodgkin & Huxley (1953), but that other ions make a significant contribution when $[K]_o$ is small. The relation between potential and

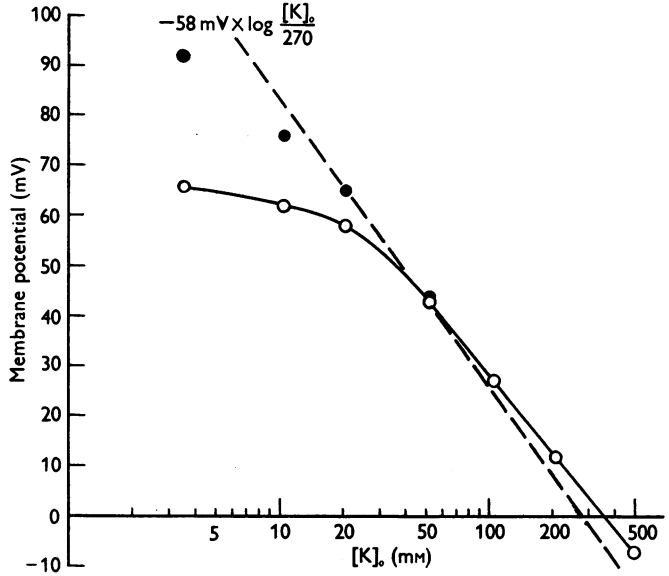

Fig. 3. Effect of external potassium concentration on membrane potential in *Sepia* axons poisoned with 0·2 mM-dinitrophenol. Abscissa: potassium concentration in mM (logarithmic scale). Ordinate: average membrane potential from Table 1 (external potential minus internal potential). ○, resting membrane potential; ●, membrane potential during positive phase. The average internal potassium concentration was 270 m-mole/l. axoplasm. Temperature 17–19° C.

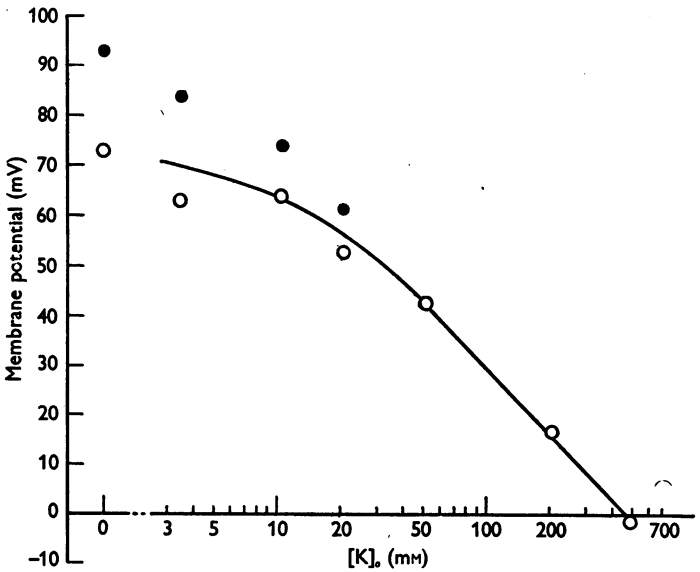

Fig. 4. Effect of external potassium concentration on membrane potential of unpoisoned *Sepia* axons. Average results from Table 2. Symbols as for Fig. 3. Temperature 17–19° C.

log $[K]_o$ is steeper during the positive phase, indicating that the permeability to potassium increases during the spike and does not return to its normal level until a few milliseconds afterwards (cf. Hodgkin & Katz, 1949; Hodgkin & Huxley, 1952*a*). With 52 mM-K the positive phase associated with an anode break spike was only about 1 mV, showing that the membrane potential was then close to the equilibrium potential for potassium. Further evidence for this conclusion comes from the approximate equality of potassium influx and efflux at this external concentration (see Table 4), and from the agreement between the observed membrane potential and that calculated from the measured internal potassium concentration.

The broken line in Fig. 3 was drawn without introducing any arbitrary constants according to the equation

$$E_K = -58 \text{ mV} \log \frac{[K]_o}{[K]_i},$$

where $[K]_i$ was the measured internal potassium concentration at the end of the experiment (in m-mole/l. axoplasm). The fibres used for Fig. 4 were not analysed, so that no comparable line could be drawn here. The agreement between the observed and theoretical membrane potentials, over a range of potassium concentrations in which the fibres seem to be nearly in passive equilibrium with respect to K^+ ions, justifies the method of calculating E_K and allows the electrochemical potential difference acting on K^+ ions to be calculated when the system is not in equilibrium. Thus with 10·4 mM-K in the external solution the average membrane potential was 20 mV below the equilibrium potential, so that $E - E_K$ is taken as -20 mV.

Potassium fluxes without applied current

During the course of the work described in the previous paper we accumulated data for the potassium fluxes in sea water containing 0·2 mM-dinitrophenol and 10·4 mM-K. The average values obtained in these and other experiments are given in Table 3. In addition, we wished to obtain figures for the potassium fluxes without applied current in solutions containing higher potassium concentrations. The method of following the changes in radioactivity of the nerve itself (Keynes, 1951) was adopted, since this gave both influx and efflux in the same experiment. A typical experiment like that illustrated in Fig. 5 consisted of (1) a dip into the radioactive solution, (2) a period of counting in an inactive solution, (3) a second dip in the radioactive solution, (4) a short period of counting, (5) measurement of axon diameter, and (6) determination of potassium content by activation analysis of the length of axon used for the experiment. The influx was calculated from the rate of gain of radioactivity with a correction (always under 10%) for the concurrent loss of ^{42}K, as described by Keynes (1954), and the efflux from the rate constant for loss of radioactivity and an estimate of the internal potassium concentration (column *e*,

Table 4) based on the final analysis. The two measurements of potassium influx agreed closely for $[K]_o \leqslant 50$ mM, but at higher concentrations the second dip always gave a smaller influx, indicating that the permeability mechanism had been impaired by the long exposure to the potassium-rich solution.

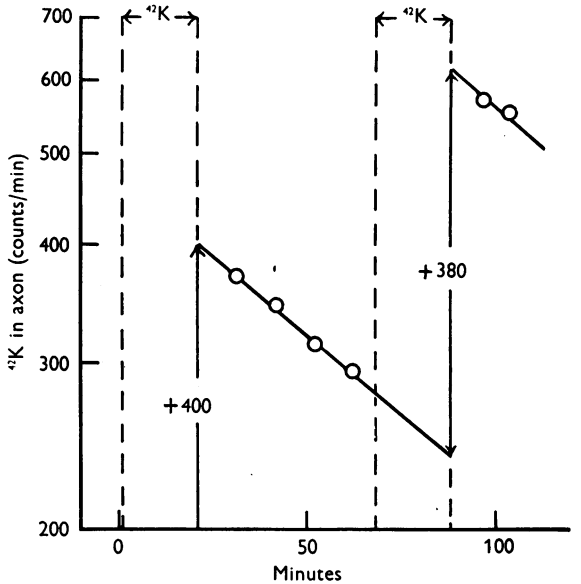

Fig. 5. Determination of potassium flux ratio in 0·2 mM-DNP sea water containing 52 mM-K. The axon was immersed in a ^{42}K solution between the dotted lines, and during the rest of the experiment was in a flowing inactive solution of identical composition. The axon diameter (174 μ) and the potassium concentration in the axoplasm (243 mM) were measured at the end of the experiment. Temperature 20° C.

The results in Tables 3 and 4 suggest that the potassium movements in poisoned axons are entirely passive. Thus the influx is greater than the efflux for $[K]_o \geqslant 100$ mM, and is less for $[K]_o \leqslant 20$ mM. This is consistent with Fig. 3, which shows that $E > E_K$ for $[K]_o > 50$ mM, and $E < E_K$ for $[K]_o < 50$ mM. On the other hand, the flux ratios are plainly not in agreement with equation (1). This is illustrated in Fig. 7, which shows that the ratios are much larger than those calculated from the equation. However, the evidence from experiments with no current is not very strong, since E is too close to E_K over most of the range of potassium concentrations for large deviations to be expected.

Potassium fluxes with membrane current: the efflux in a depolarized axon

Before discussing the main results it may be helpful to consider a rather simple experiment which shows that the potassium movements are not independent. The apparatus shown in Fig. 1 was employed, with the agar bridge connecting pot *A* to the external solution. The experiment consisted in measuring the efflux from a short length of fibre which was depolarized by

TABLE 3. Potassium fluxes in *Sepia* axons poisoned with 0·2 mM-dinitrophenol, at an external potassium concentration of 10·4 mM

	Method	No. of expts.	Mean flux (pmole/cm² sec)	Range (pmole/cm² sec)
K influx	A	11	3·0	2·0–4·3
K influx	B	2	3·1	2·6–3·8
Value taken for influx			3·0	
K efflux	C	4	25	13–35
K efflux	D	5	39	29–67
K efflux	E	6	18	15–20
Value taken for efflux			27	

A was the standard influx method (Keynes, 1951, Fig. 1), using an 18 mm window over the Geiger counter.

B was similar, but used the apparatus of Fig. 1 for measuring the uptake of ^{42}K by 1·8 mm of axon.

C depended on determining the rate constant for loss of radioactivity by an 18 mm length of axon.

D depended on counting the radioactivity in the effluent from a 17 or 29 mm length of axon.

E depended on counting the radioactivity in the effluent from a 1·8 mm length of axon (using apparatus of Fig. 1).

TABLE 4. Potassium fluxes in *Sepia* axons poisoned with 0·2 mM-dinitrophenol, in potassium-rich solutions

a Expt.	*b* Axon diam. (μ)	*c* $[K]_o$ (mM)	*d* $[K]_i$ (mM)	*e* $[K]_i$ (mM)	*f* Influx (pmole/cm² sec)	*g* Efflux (pmole/cm² sec)	*h* Influx/Efflux	
1	249	20·7	178	243	15·1 25·2	42	0·46	0·46
2	207	20·7	207	250	11·3 21·6	34	0·46	
3	174	52	243	278	135 128	151	0·87	1·05
4	204	52	246	256	109 109	87	1·25	
5	213	104	271	294	232 197	178	1·20	1·36
6	185	104	259	273	212 156	119	1·53	
7	175	207	242	277	374 222	155	1·86	1·90
8	191	207	277	277	465 221	165	1·94	

Temperature 19–21° C.

All fibres had been stimulated for 5–10 min at 50/sec.

Column *d* was obtained from the potassium concentration determined by activation analysis at the end of the experiment.

Column *e* was obtained by interpolation between column *d* and an initial value derived from the figures of Keynes & Lewis (1951*b*).

Axon diameters were measured at the end of the experiment, and no allowance has been made for possible swelling in the potassium-rich solutions.

immersing all but the central 2 mm in sea water containing 104 mM-K. The solution in the tube was either (*a*) the same as that outside (104 mM-K) or (*b*) a similar solution with only 10·4 mM-K. It was thus possible to examine the effect of a tenfold decrease in external potassium on the potassium efflux, at a roughly constant membrane potential. If K^+ ions moved through the membrane independently of one another, and if the method of maintaining the constancy of the resting potential was perfect, one would expect the efflux to remain unaltered when the external potassium concentration was changed. Since the resting potential would, in practice, be somewhat greater in the 10·4 mM-K sea water, lower fluxes into this solution might be

TABLE 5. Effect on potassium efflux of changing external potassium concentration at 'constant' membrane potential in a depolarized axon

			Expt. 3	
$[K]_o$ within tube (mM)	Expt. 1 efflux of K* (counts/min per min)	Expt. 2 efflux of K* (counts/min per min)	Efflux of K* (counts/min per min)	Efflux of K (pmole/cm² sec)
104	2·12	2·98	19·1	172
10·4	4·83	8·31	39·4	451
104	1·99	3·10	10·6	149
10·4	3·98	7·36	28·0	494
104	1·43	—	8·2	180

The apparatus of Fig. 1 was used, and the solution in the outer compartment contained 104 mM-K throughout the experiments.

Expts. 1 and 2 were made with unpoisoned fibres, and ^{42}K was introduced by soaking in 20·7 mM-K sea water.

Expt. 3 was made with a fibre poisoned with 0·2 mM-DNP, and ^{42}K was introduced by soaking in 52 mM-K sea water.

Temperature 15–20° C.

Radioactivity was collected over periods of 18–20 min, with intervals of about 3 min for changing solutions.

predicted. Table 5 shows that this expectation was not fulfilled, for the efflux into 10·4 mM-K was consistently *greater* than that into 104 mM-K. This is a striking result, because an increase in external potassium is normally associated with an increase in efflux. For example, in the experiments of Tables 3 and 4, in which the membrane potential was allowed to take up a value appropriate to the potassium concentration, a tenfold increase of external potassium caused a fivefold increase in efflux. Yet in Table 5 a tenfold increase in $[K]_o$ was followed by a reduction in efflux to 1/2·6. This observation suggests that it may be easier for potassium ions to leave the fibre when the number travelling in the opposite direction is reduced, and that the potassium movements across the membrane are not independent.

The first two experiments in Table 5 were made with unpoisoned axons in sea water, and were not worked out in full because the fibres were not stored for activation analysis. The third axon had been poisoned with 0·2 mM-dinitrophenol, and was analysed for labelled and total potassium at the end of the

experiment. The fluxes shown in the right-hand column were worked out on the assumption that the internal potassium concentration remained the same throughout the experiment.

In order to compare this experiment with others, it is necessary to decide on a likely figure for the membrane potential. A lower limit can be obtained by assuming that the space constant was large compared with the width of the tube. In this case the whole fibre would have the membrane potential established by 104 mM-K; from Table 1 this is seen to be 27 mV. A better estimate can be made with the help of linear cable equations, taking the space constant as 2 mm from the results of other experiments with 104 mM-K. Such an analysis indicates that the membrane potential at the edge of the tube was 37·4 mV, and that at the centre it was 39·7 mV; the average potential was 38·9 mV. An alternative method was to adjust the value of the space constant until the calculated membrane current agreed with that worked out from the transport of K^+ ions (see Hodgkin & Huxley, 1953). This gave an apparent space constant of 2·7 mm, and a mean potential of 37 mV.

The influx in a depolarized axon

The results obtained when the influx was measured under conditions similar to those for Table 5 are given in Expts. 1–3 of Table 7. They show that a rise in external potassium concentration from 10·4 to 104 mM at an approximately constant membrane potential, increases the influx 20–40 times. If the ions moved independently the influx would only be increased 10 times. It therefore appears that the chance of any one K^+ ion entering the fibre is improved by increasing the net inward movement of potassium.

The flux ratio in a depolarized axon

The experiments just described show that when $[K]_o$ is changed from 10·4 to 104 mM at a more or less constant membrane potential, the influx is increased 30 times and the efflux is decreased 2·6 times. The tenfold rise in external potassium therefore alters the flux ratio by a factor of about 80. It cannot be argued that this effect is merely the consequence of a change in potassium permeability, since this would not alter the flux ratio. Nor can the result be attributed to changes in membrane potential, since these will be in the direction to reduce, not to increase, the change in flux ratio. Apart from experimental error, the only remaining conclusion is that the independence relation does not apply in depolarized axons, and that K^+ ions interact with one another in crossing the membrane.

Collected results

We also made a number of measurements in which the membrane potential was altered by externally applied currents. These results are collected in Tables 6 and 7, together with some further short-circuit experiments. In

TABLE 6. Effect on potassium efflux of membrane potential and external potassium concentration in fibres poisoned with 0·2 mM-dinitrophenol

Expt.	Axon diam. (μ)	$[K]_i$ at end of expt. (mM)	$[K]_o$ within tube (mM)	$[K]_o$ in outer compartment (mM)	p.d. between tube and outer compartment (mV)	Efflux (pmole/cm² sec)	Estimated membrane potential (mV)
1	250	207	104	104	0	172	27
			10·4	104	0	451	27 (37)
			104	104	0	149	27
			10·4	104	0	494	27 (37)
			104	104	0	180	27
2	274	269	10·4	10·4	0	21	62
			10·4	10·4	+10	5·1	72
			10·4	10·4	0	19	62
			10·4	10·4	−20	222	42 (52)
			10·4	10·4	0	21	62
3	195	284	10·4	10·4	0	19	62
			10·4	10·4	−10	73	52 (55)
			10·4	10·4	0	21	62
4	245	216	10·4	10·4	0	13	62
			10·4	10·4	+19	2·4	81
			10·4	10·4	0	17	62
5	218	171	10·4	10·4	0	15	62
			10·4	10·4	+20	2·2	82
			10·4	10·4	0	19	62
6	287	246	20·7	10·4	0	10	62
			20·7	10·4	+10	3·4	72
			20·7	10·4	0	10	62
7	256	226	20·7	10·4	0	16	62
			20·7	10·4	+10	6	72
			20·7	10·4	0	13	62
8	218	211	10·4	10·4	0	14	62
			52	10·4	0	16	62 (59)
			10·4	10·4	0	17	62
			52	10·4	0	15	62 (59)
			10·4	10·4	0	15	62
9	241	271	52	10·4	0	17	62 (59)
			52	10·4	+ 5	7·4	67 (64)
			52	10·4	0	7·5	62 (59)
10	284	170	52	10·4	0	31	62 (59)
			52	10·4	+ 6	11·4	68 (65)
			52	10·4	0	41	62 (59)
11	226	149	52	10·4	0	18	62 (59)
			52	10·4	+10	1·5	72 (69)
12	200	193	52	10·4	0	21	62 (59)
			52	10·4	+10	1·3	72 (69)
			52	10·4	0	26	62 (59)

Membrane potentials were obtained by adding the externally applied potential difference to the resting potential expected from the potassium concentration in the outer compartment.

Figures in brackets have been corrected by the methods indicated in the text.

Temperature 15–18° C.

TABLE 7. Effect on potassium influx of membrane potential and external potassium concentration in fibres poisoned with 0·2 mM-dinitrophenol

Expt.	Axon diam. (μ)	$[K]_i$ at end of expt. (mM)	$[K]_o$ within tube (mM)	$[K]_o$ in outer compartment (mM)	p.d. between tube and outer compartment (mV)	Influx (pmole/cm² sec)	Estimated membrane potential (mV)
1	207	212	10·4	104	0	5·0	27 (37)
			104	104	0	195	27
2	170	—	10·4	104	0	10·9	27 (37)
3	234	296	104	104	0	216	27
4	179	161	10·4	52	0	6·6	43 (47)
			52	52	0	102	43
5	224	281	10·4	10·4	0	2·6	62
			52	10·4	0	59	62 (59)
6	191	226	10·4	10·4	0	3·8	62
7	190	239	10·4	10·4	+10·5	5·0	72·5
8	231	224	10·4	10·4	+18	5·3	80
9	265	282	20·7	10·4	+10	13·0	72
10	231	281	20·7	10·4	+10	11·5	72
11	190	222	52	10·4	+10	57	72 (69)
12	187	236	52	10·4	+10	53	72 (69)

For methods of estimating membrane potentials see Table 6 or text.
Temperature 16–20° C.

TABLE 8. Collected average results of effect on potassium fluxes of membrane potential and external potassium concentration

a Row	*b* $[K]_o$ (mM)	*c* Estimated membrane potential (*E*) (mV)	*d* Estimated driving force ($E - E_K$) (mV)	*e* Influx (pmole/cm² sec)	*f* Efflux (pmole/cm² sec)	*g* Influx/Efflux	
1	10·4	27 (37)	−55 (−45)	7·4	471	0·016	
2	10·4	42 (52)	−40 (−30)	6·6	222	0·030	
3	10·4	52 (55)	−30 (−27)	(4·6)	73	0·063	
4	10·4	62	−20	3·1	18	0·17	0·12
5	10·4	62	−20	3·0	32	0·09	
6	10·4	72	−10	5·0	5·1	0·98	
7	10·4	81	− 1	5·3	2·3	2·3	
8	20·7	58	− 7	18	38	0·46	
9	20·7	72	+ 7	12·2	4·6	2·7	
10	52	43	+ 1	120	115	1·05	
11	52	62 (59)	+20 (+17)	59	18	3·3	
12	52	68 (65)	+26 (+23)	(57)	9·2	6·2	
13	52	72 (69)	+30 (+27)	55	1·4	39	
14	104	27	+ 3	206	167	1·23	1·30
15	104	27	+ 3	197	146	1·36	
16	207	12	+ 5	304	160	1·90	

Column *c* was obtained as in Tables 6 and 7 from data in Table 1; the first figure is the nominal membrane potential, and the figure in brackets has been roughly corrected for cable effects. E_K is calculated as $58 \log_{10} (270/[K]_o)$; this is the straight line in Fig. 2.

Columns *e* and *f* are from Tables 3, 4, 6 and 7. Sources of information are: row 1 from Table 6, Expt. 1, and Table 7, Expts. 1 and 2, abbreviated to *R*1; 6/1; 7/1, 2. *R*2; 6/2; 7/4. *R*3; 6/3; influx interpolated. *R*4; 3/B; 3/E. *R*5; 3/A; 3/C, D. *R*6; 6/2; 7/7. *R*7; 6/4, 5; 7/8. *R*8; 4/1, 2. *R*9; 6/6, 7; 7/9, 10. *R*10; 4/3, 4. *R*11; 6/8, 9, 10, 11, 12; 7/5. *R*12; 6/9, 10; influx interpolated. *R*13; 6/11, 12; 7/11, 12. *R*14; 6/1; 7/1, 3. *R*15; 4/5, 6. *R*16; 4/7, 8.

Geometric means have been employed.

Table 8 the same results (and others from Tables 3 and 4) are presented in a less direct but more convenient form. When the agar bridge was used as a short circuit, the membrane potential was estimated in the manner described on p. 72. With externally applied currents the value given is that appropriate to the potassium concentration in the outer compartment plus the potential difference produced by an external circuit connected between the tube and the outer compartment. This should be fairly reliable for membrane potentials greater than 55 mV, but becomes inaccurate for lower potentials. Corrected values were calculated by the type of method used on p. 64, but must be regarded as very tentative estimates.

The results in Table 8 provide evidence that (1) the potassium permeability or conductance increases on depolarization, (2) potassium movements in poisoned fibres are passive, and (3) potassium movements in poisoned fibres are not independent. These points are considered in turn in the following sections.

Potassium fluxes and membrane potential

Fig. 6 illustrates the relation between membrane potential and the potassium fluxes with a fixed external potassium concentration of 10·4 mM. It shows that the efflux increases steeply when the membrane is depolarized, and varies over a range of 200 times. In the steepest part of the curve the efflux is doubled by a depolarization of 4 mV.

The influx did not alter by a factor of more than 3 over the whole range, but owing to the scatter of the results it was impossible to determine the exact shape of the curve. The relative constancy of the influx is presumably to be explained by the opposed actions of (1) an increase of the electrochemical gradient against which the K^+ ions are moving, and (2) an increase in the permeability of the membrane to potassium. For the efflux these two factors operate in the same direction.

The influence of membrane potential on the potassium fluxes with higher external potassium concentrations (e.g. 52 mM) is similar to that in Fig. 6, except that at a given membrane potential the effluxes are usually smaller and the influxes always much larger than with 10·4 mM-K. The results also suggest that the influx from 52 mM-K tends to fall when the membrane potential is raised, as would be expected if the decrease in potassium permeability had more effect than the rise in electrochemical potential difference.

Fig. 6 provides direct evidence for the conclusion that depolarization increases potassium conductance (Hodgkin & Huxley, 1952*c*, 1953). This quantity is defined as

$$g_K = I_K/(E - E_K),$$

where I_K is the membrane current transported by K^+ ions, and $(E - E_K)$ is once more the difference between the membrane potential and the equilibrium potential for potassium. Taking E_K at the intersection of the two curves

(74 mV) and I_K as (influx − efflux) × 96,500 coulombs/mole, it can be calculated from Fig. 6 that g_K is about 0·05 m-mho/cm² at 80 mV membrane potential, about 0·12 m-mho/cm² at 62 mV, and about 1 m-mho/cm² at 35 mV. In the steepest part of the curve the conductance increases *e*-fold for an 8 mV decrease of membrane potential. The absolute conductances are smaller, and the change with membrane potential somewhat less than that expected from Hodgkin & Huxley's (1952*c*) data, but the present results are not at all accurate, and there may be no real discrepancy between the two sets of experiments. If there were a genuine difference, it would not be hard to explain, since one method used *Loligo* axons and currents lasting less than 50 msec, while the other used *Sepia*

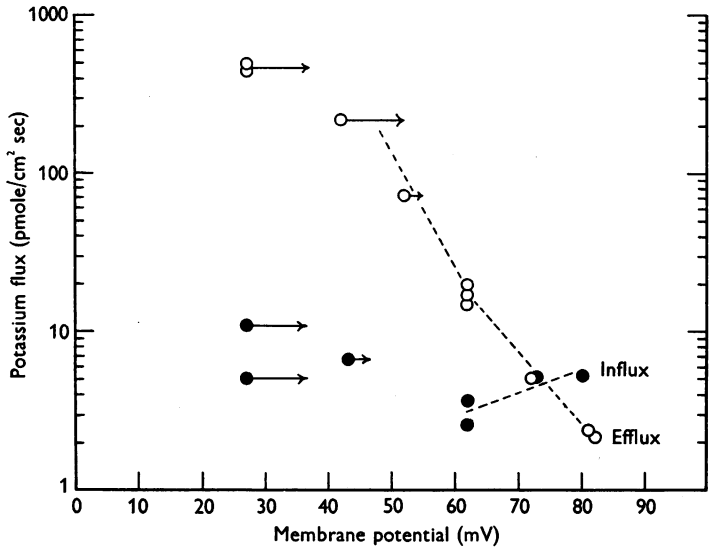

Fig. 6. Effect of membrane potential (external minus internal) on potassium efflux (○) and potassium influx (●) with an external potassium concentration of 10·4 mM. The values are taken from Tables 6 and 7, and are plotted as the nominal membrane potentials with arrows indicating approximate corrections. All fibres had been treated with 0·2 mM-DNP.

axons and currents lasting for 20 min. The species difference could account for the disagreement in absolute conductance, since *Sepia* fibres have a higher membrane resistance than those from *Loligo* (Cole & Hodgkin, 1939; Weidmann, 1951), while a difference in slope might arise if prolonged depolarization tended to reduce potassium permeability.

The influence of potassium concentration on the membrane potential for equal potassium fluxes

If the movements of potassium are entirely passive in axons poisoned with dinitrophenol one would expect that the influx would be equal to the efflux when the membrane potential was 58 mV × log $[K]_i/[K]_o$. The potentials at

which the fluxes were equal can be estimated from the collected results in Table 8. With $[K]_o = 10{\cdot}4$ mM the fluxes were equal at 74 mV, as shown in Fig. 6. With 20·7 mM-K the ratio of influx to efflux was 0·46 at 58 mV and 2·7 at 72 mV; hence the equilibrium potential may be taken as 65 mV. With 52 mM-K the ratio was close to unity at 43 mV. With 104 or 207 mM-K values of 24 or 6 mV are obtained by using the empirical equation (2.0) to extrapolate from the ratios of 1·30 or 1·90 observed at 27 or 12 mV respectively. These estimates, which are tabulated in the second column of Table 9, are based partly on the electrical data in Table 1, and partly on flux ratios derived from Tables 3, 4, 6 and 7. Since the average internal potassium concentration varied slightly in the different groups of fibres, there are several ways of comparing observed and calculated values of E_K. One method is to assume that the deviation of the membrane potential from E_K at different external

TABLE 9. Comparison of observed and calculated values of E_K at different external potassium concentrations

$[K]_o$ (mM)	Membrane potential at which K influx = K efflux in DNP-poisoned fibres (mV)	−58 mV log $[K]_o/270$ (mV)	−58 mV log $[K]_o/[K]_i$ (mV)
10·4	74	82	78
20·7	65	65	63
52	43	41	41
104	24	24	25
207	6	7	7

potassium concentrations is independent of the internal potassium concentration. In this case $[K]_i$ should be taken as 270 mM, this being the average value for the experiments of Table 1. Alternatively, it can be assumed that the membrane potential is the same in all fibres, and that E_K is given by the average internal potassium concentration in each group of experiments. We regard this method as less fair than the former, but there is not a great difference between them, and except at 10·4 mM-K both give values which agree very closely with the experimental estimates of E_K. The discrepancy at 10·4 mM-K may be explained on the assumption that the channels which allow potassium to move through the membrane are not perfectly selective, and that other ions such as sodium begin to compete effectively when the potassium concentration is under 1/45 of the external sodium concentration. A similar hypothesis is probably required to explain the facts that neither the membrane potential during the positive phase, nor the apparent value of E_K in *Loligo*, obey the Nernst relation at low potassium concentrations (Hodgkin & Huxley, 1952*b*).

Although there are some residual uncertainties connected with Table 9, the general agreement between the calculated and observed values of the equilibrium potential over a wide range of potassium concentrations establishes

the passive nature of the potassium fluxes in poisoned axons in a very satisfactory manner. This is important because it might otherwise be supposed that the interaction described in the next section had something to do with active transport.

Evidence for interaction

It has been shown that in a depolarized axon (E around 30 mV) increasing $[K]_o$ from 10·4 to 104 mM reduces the potassium efflux by 2·6 and increases the influx by about 30. Neither result is consistent with independence, which predicts a constant efflux and an influx proportional to external concentration when the membrane potential is kept constant. This establishes interaction in a depolarized axon, but it does not necessarily follow that the same type of interaction occurs at all concentrations and membrane potentials. The results in Table 8 suggest that the influx increases more rapidly than concentration over the range 10–100 mM-K, and over the range of membrane potentials from 30 to 70 mV. (Outside these limits we have no information, but one might expect that with sufficiently high external potassium concentrations the influx would eventually saturate.) It is difficult to be sure that the converse effect—reduction of efflux with rising concentration—is present over the whole range studied. The efflux at 52 mM-K and a membrane potential of about 70 mV is less than a third of the efflux at 10·4 mM-K and the same potential (see Table 6), but Expt. 8 in Table 6 suggests that at a membrane potential of about 62 mV, a rise in external potassium from 10·4 to 52 mM did not have much effect on the efflux. There was no good reason to question the validity of this particular experiment, and as the majority of the results deviate so markedly from independence, we are inclined to attribute the apparent agreement in this one case to imperfections in the method of controlling the membrane potential. If the potential were to decrease somewhat in 52 mM-K, instead of being perfectly constant, there would be an appreciable tendency for the efflux to rise, both through an increase in membrane permeability and through an increase in the force driving ions outwards, which might just balance out the effect of interaction in decreasing the efflux. Such a factor might be less important at 30 mV than at 60 mV, since the permeability varies less rapidly at low membrane potentials.

The best method of testing the independence of the potassium movements is to consider the relation between the flux ratio and the electrochemical potential difference ($E-E_K$). It is at once clear from Table 8 that the ratio varies over a very wide range. Thus the efflux is 60 times greater than the influx when $E-E_K$ is about -50 mV, and the influx is 40 times greater than the efflux when $E-E_K$ is about $+30$ mV. Hence the flux ratio alters by a factor of 2400 for an 80 mV change in the driving force. The change in flux ratio predicted by equation (1) is only 24 for 80 mV.

In Fig. 7 the flux ratio has been plotted logarithmically against $E-E_K$. Plotted in this fashion the independence relations

$$\frac{\text{Influx}}{\text{Efflux}} = \exp\frac{(E-E_K)F}{RT}, \tag{1.0}$$

or

$$\frac{\text{Influx}}{\text{Efflux}} = \frac{[K]_o}{[K]_i} e^{EF/RT}, \tag{1.1}$$

or

$$\log_{10}\left(\frac{\text{Influx}}{\text{Efflux}}\right) = \frac{(E-E_K)}{58\text{ mV}}, \tag{1.2}$$

give a straight line with a tenfold change in 58 mV. Our data can be reasonably well fitted by a straight line, but a tenfold change occurs in about 23 mV. This means that instead of equations (1.0), (1.1) and (1.2) we have to write

$$\frac{\text{Influx}}{\text{Efflux}} = \exp\frac{(E-E_K)n'F}{RT}, \tag{2.0}$$

or

$$\frac{\text{Influx}}{\text{Efflux}} = \left\{\frac{[K]_o}{[K]_i} e^{EF/RT}\right\}^{n'} \tag{2.1}$$

or

$$\log_{10}\left(\frac{\text{Influx}}{\text{Efflux}}\right) = \frac{(E-E_K)}{23\text{ mV}}, \tag{2.2}$$

where n' is 2·5. In view of the considerable scatter of the results, no great reliance can be placed on the exact numerical value chosen here for n'. However, it is clear that even with generous allowance for experimental error, n' must be substantially greater than 1.

Potassium fluxes and membrane conductance

On the assumption that potassium ions move independently through the membrane it can be shown that at the equilibrium potential for potassium the flux and conductance should be related by

$$g_K = \frac{F^2}{RT} M_K, \tag{3}$$

where g_K is the component of the total membrane conductance contributed by potassium, M_K is the potassium flux, and the other symbols have their usual meaning (Hodgkin, 1951; Hodgkin & Huxley, 1952*a*).

The derivation of this relationship depends directly on the use of equation (1.0) (see Hodgkin, 1951, p. 364). If equation (2.0) is used instead, the flux-conductance relation becomes

$$g_K = \frac{n'F^2}{RT} M_K. \tag{4}$$

Since n' is about 2·5, there is a considerable difference between the conductances worked out from the two formulae. In order to see which best fitted the facts, we compared the fluxes of potassium and the membrane conductance in an axon immersed in a solution containing 104 mM-K. The influx and efflux were roughly equal, and had a mean value of 170 pmole/cm² sec. The membrane conductance, estimated from the spread of electrotonic potential, was about 3 m-mho/cm². As the relation between E and

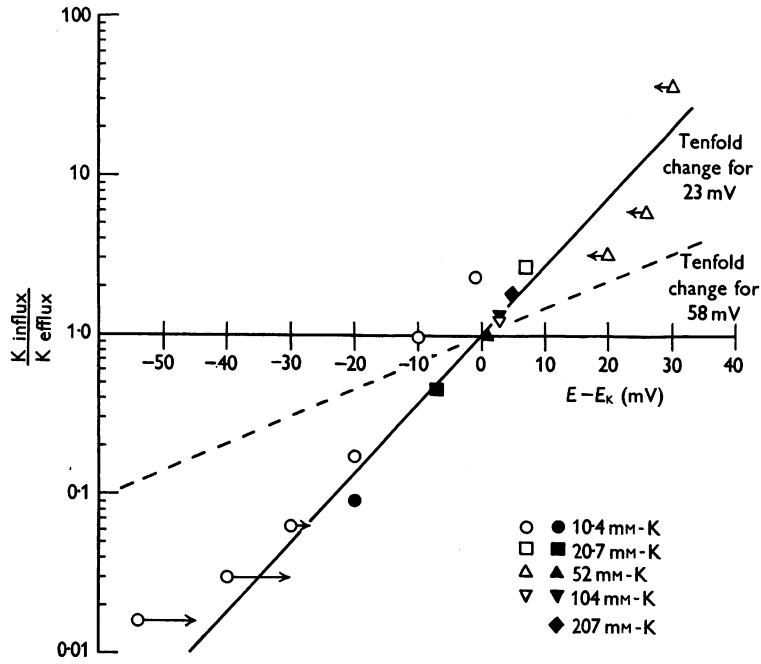

Fig. 7. Effect of driving force, $E - E_K$, on potassium flux ratio in fibres poisoned with 0·2 mM-DNP. Results plotted from Table 8 at nominal potentials; arrows show approximate corrections for cable effects. Filled in symbols are based on flux measurements using 17–29 mm lengths of fibre in the absence of applied current (Tables 3 and 4). The other points were obtained with the apparatus shown in Fig. 1 (Table 8).

log $[K]_o$ has a slope of about 50 mV at this external potassium concentration (see Fig. 3), g_K may be taken as 2·5 m-mho/cm². The value predicted by equation (3) is 0·66 m-mho/cm², while equation (4) predicts 1·65 m-mho/cm². The former quantity may be considered outside experimental error; the latter is probably within it. A similar conclusion was reached in two other experiments with 52 and 207 mM-K, but the condition of the fibres was unsatisfactory, and less confidence can be placed in the results. The apparent values of n' calculated by equation (4) for these three experiments were 3·2, 3·8 and 2·0. This may be regarded as additional evidence that the fluxes are not independent.

A comparison of potassium fluxes and membrane conductance in frog muscle suggests that here also the independence relation does not apply. Thus Keynes (1954) points out that the value of g_K calculated from the potassium flux is only about one-tenth of the overall membrane conductance, although on other grounds one might expect K^+ ions to carry about half the current crossing the membrane. The data for *Carcinus* axons seems to fit rather better with equation (3) (see Keynes & Lewis, 1951*a*; Hodgkin, 1951), but the comparison is of limited value since the membrane conductance varies greatly from one fibre to the next. It is also uncertain whether the membrane potential in these fibres is close enough to E_K for equation (4) to apply.

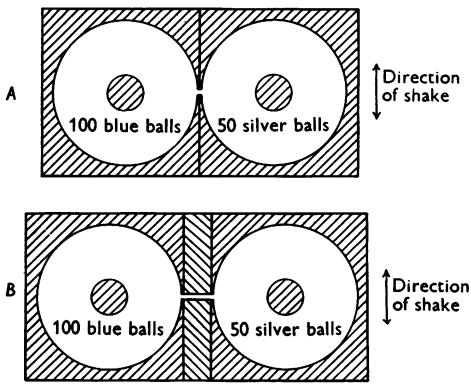

Fig. 8. Diagram of mechanical model. In *A* two flat compartments were separated by a narrow gap; in *B* the gap width was increased by spacers. The sides and bottom of the compartments were made of aluminium; the top was Perspex. The diameter of the circular compartments was 74 mm, and that of the balls 3 mm. The drawing is roughly to scale. The central blocks of aluminium helped to randomize the movement of the balls.

Experiments with a mechanical model

The very large departures from the independence relation described in this paper can be explained by assuming that K^+ ions tend to move through the membrane in narrow channels, or along chains of sites such as might be provided by the negatively charged groups of a cation exchange resin. The essential feature of these systems is that the ions should be constrained to move in single file and that there should, on average, be several ions in a channel at any moment. Since it may not be immediately obvious that this general type of assumption does explain our results, we built a mechanical model to illustrate the hypothesis. This consisted of two flat compartments which could be joined by a short gap as in Fig. 8*A*, or by a long gap as in Fig. 8*B*. 100 steel balls which had been blued by heating were put in the left-hand compartment, and 50 balls, identical except in being silver in colour, were put in the right-hand compartment. The model was then shaken

vigorously by a motor for 15 sec. This caused all the balls to rattle about with a random 'Brownian' movement, and a certain number passed through the gap. The number of blue balls which moved from left to right was counted, and compared with the number of silver balls which had moved in the opposite direction during the same time interval. In order to avoid errors which might have been introduced by asymmetries or any slight tilt of the apparatus, or by small mechanical differences between the blue and silver balls, the experiment was repeated with 100 silver balls in the right and 50 blue balls in the left compartment. Both experiments were performed many times, and the results averaged.

With a short gap separating the two compartments one would expect that the number of balls leaving the side with 100 would be twice as great as the number leaving the side with 50 balls. This prediction was approximately but not exactly fulfilled, the ratio in 19 trials averaging 2·7 (S.E. of mean, 0·2). A very much greater discrepancy appeared when the long gap was employed, for in this case the number leaving the 100-ball compartment was 18 times greater than the number travelling in the reverse direction (34 trials, S.E. of mean, 4). Use of the long gap therefore greatly exaggerated the ratio between the rates of movement upstream and downstream. The reason for this is obvious. A single collision with the short gap is sufficient to transfer a ball across the gap, so that the flux ratio is roughly equal to the concentration ratio. This is not so with the long gap. The number of balls in the gap fluctuated, but on the average there were about three balls in the gap at any time. This means that at least four collisions were needed to transfer a ball right through the gap. Starting with three blue balls in the gap, the first collision of a silver ball moves it into the right-hand position, the second moves it into the middle, the third moves it to the left-hand position, and the fourth finally transfers it into the other compartment. The frequency with which four collisions occur in succession will be 2^4, that is, 16 times greater on the 100-ball side than on the 50-ball side. Hence a very large flux ratio is obtained with the long gap, but not with the short one. The difference between the observed and expected ratios for the short gap probably arose in a similar fashion, for the balls were of finite size, and the gap was not infinitesimal, as was tacitly assumed in the initial argument.

Calculations for a theoretical model

The approximate agreement between equation (2.1) and the experimental data suggests that it would be worth trying to find a theoretical basis for the equation. This can be done by assuming that K^+ ions move in single file along chains of potassium-selective sites which stretch through the membrane, and that all n sites in each chain are occupied by potassium. The solution to the left of the membrane contains potassium at a concentration $[K]_1$ and all ions

in this solution are labelled A. On the right side the concentration is $[K]_2$ and the ions are labelled B. The channels in the membrane will then contain A and B in the following combinations:

Solution 1	Membrane	Solution 2
Species A	$AA...AA$	Species B
	$AA...AB$	
	$AA...BB$	
	.....	
	$AB...BB$	
	$BB...BB$	

The proportion of the total number of channels with one particular combination is denoted by $[A_r B_{n-r}]$. Potassium ions are transferred from left to right by collisions between ions in solution 1 and the left-hand ends of the chains. Each collision makes all the ions in the chain move one place to the right, and one ion is given off to solution 2. The total rate, irrespective of labelling, at which ions are transferred from left to right will be denoted by α, and the rate in the opposite direction by β. Since each such transfer involves the movement of one positive charge through the membrane, it is reasonable to suppose that

$$\frac{\alpha}{\beta} = \frac{[K]_1}{[K]_2} \exp \frac{E_{12} F}{RT}, \tag{5}$$

where E_{12} is the potential difference between solution 1 and solution 2, and F, R and T have their usual significance (see Glasstone, Laidler & Eyring, 1941; Hodgkin & Huxley, 1952*a*). This expression does not give the flux ratio for labelled ions, because a collision of A with a combination such as $A_r B_{n-r}$ merely has the effect of returning B to its original solution, and does not contribute directly to the flux of A passing through the membrane. The only collisions which do transfer A to the right-hand solution are those in which A collides with the combination $A_n B_o$. The frequency with which these occur is $\alpha[A_n B_o]$, since $[A_n B_o]$ is the proportion of $A_n B_o$ to the total number of chains. A similar argument applies to the flux of B from right to left, so that the flux ratio must be

$$\frac{M_{12}}{M_{21}} = \frac{\alpha}{\beta} \frac{[A_n B_o]}{[A_o B_n]}. \tag{6}$$

By the same type of argument it follows that the rate at which any intermediate combination $A_r B_{n-r}$ is converted into $A_{r+1} B_{n-r-1}$ is $\alpha[A_r B_{n-r}]$, while the rate of conversion in the opposite direction is $\beta[A_{r+1} B_{n-r-1}]$. In the steady state, these rates must be equal, so that

$$\frac{[A_{r+1} B_{n-r-1}]}{[A_r B_{n-r}]} = \frac{\alpha}{\beta}. \tag{7}$$

Hence

$$[A_n B_o] = \frac{\alpha}{\beta} [A_{n-1} B_1] = \frac{\alpha^2}{\beta^2} [A_{n-2} B_2] = \ldots = \frac{\alpha^n}{\beta^n} [A_o B_n].$$

Substituting in (6) the flux ratio becomes

$$\frac{M_{12}}{M_{21}} = \left(\frac{\alpha}{\beta}\right)^{n+1},$$

or

$$\frac{M_{12}}{M_{21}} = \left\{\frac{[\mathrm{K}]_1}{[\mathrm{K}]_2} e^{E_{12}F/RT}\right\}^{n+1}. \tag{8}$$

The foregoing argument is unrealistic in at least two respects. In the first place it assumes a 'knock-on' type of transfer, similar to that in the mechanical model, but unlikely to occur in solution. This defect can be remedied by assuming that occasional vacancies arise when potassium ions dissociate from the ends of the chains, and that these vacancies are transferred through the membrane in a random manner by being filled in from adjacent sites. With somewhat more elaborate working, this modification of the hypothesis leads to a result similar to (8) except that the index is n (the number of sites) and not $n+1$. On this basis the experimental finding that $n = 2{\cdot}5$ means that there are 2–3 potassium ions in line across the membrane. The second major weakness is the assumption that all the sites are occupied by ions. We have not worked out the general case of 'unsaturated' chains in full, but preliminary calculations indicate that the index in equation (8) is not constant unless the system is saturated, and that it is roughly, but not exactly, equal to the average number of potassium ions in each chain.

DISCUSSION

The main conclusions from our experiments are (1) that potassium movements in axons poisoned with dinitrophenol are strictly passive, (2) that the potassium permeability (or conductance) increases markedly when the membrane is depolarized, and (3) that the variation of flux ratio with driving force is of the kind expected in a system in which the ions are constrained to move in single file. The first result needs no special discussion since it is exactly what one would expect. The second raises once more the question of the nature of the permeability changes in excitable tissues. One possibility mentioned previously (Hodgkin & Huxley, 1952*c*) is that K^+ ions cross the membrane over a bridge or chain of negatively charged particles. When the membrane potential is high, these particles are strongly attracted to the outside of the membrane, by virtue of their negative charge, and there are relatively few completed chains. When it is low they distribute themselves more evenly, and the number of complete chains is increased. Such a mechanism would also account for the flux ratio results, if it were assumed that two or three of the sites in each completed chain were normally occupied by K^+ ions.

A different way of explaining the anomalous flux ratios is to imagine that K^+ ions cross the membrane through narrow tubes or channels. If these were little bigger than the hydrated potassium ion, there would clearly be a marked

interaction of the kind described for the mechanical model. Even in a relatively large tube, movement of one potassium ion might tend to sweep along a column of water, making it easier for other potassium ions to move in the same direction, and hindering movements in the opposite direction. There are almost certainly other ways of accounting for the interaction between potassium ions, but we have not succeeded in evolving a plausible hypothesis which does not incorporate the concept that movement of one ion tends to assist others to travel in the same direction. It may occur to the reader that K^+ ions might be transported in groups of two or three by a polyvalent carrier. However, such a system would be unsatisfactory because it would require that partly associated carriers should move less easily than fully dissociated or fully associated carriers; and even with this improbable assumption it does not seem to account for the observed reduction in potassium efflux on increasing the external potassium concentration.

The experiments described in this paper help to explain why there is only a small increase in potassium influx during the passage of a nervous impulse. Hodgkin & Huxley (1952*c*) found that while there was reasonable agreement between the observed and calculated leakages of potassium during the spike, the influx observed with tracers was only about one-sixth of that calculated on the basis of independent transport. At the time, the discrepancy was thought to be connected with the fact that the apparent equilibrium potential for potassium did not obey the Nernst relation at low external potassium concentrations. It now seems likely that a more important factor is the interaction between the influx and the efflux of potassium, which makes it difficult for external ions to enter against a predominant stream coming in the opposite direction.

Since the system which permits rapid sodium movements during the early part of the spike has many properties in common with the one which later allows potassium to traverse the membrane more freely, it is natural to wonder whether the single-file behaviour observed for potassium may not also apply to sodium ions. On the whole this does not seem very likely. If equation (2.0) applied to the sodium movements one would expect the passive efflux of sodium during the spike to be small compared to the influx. The observation (Keynes, 1951; Hodgkin & Keynes, 1955) that during the spike the sodium efflux is about two thirds of the influx, suggests that if there is any interaction between sodium ions it is not as powerful as for potassium ions. Hodgkin & Huxley (1952*a*) also obtained evidence supporting the independence of the sodium movements, but their experiments were concerned with net movements rather than with fluxes of labelled ions, and were not well suited to show up the type of interaction considered here. In terms of the theoretical model, the lack of interaction with sodium ions can be explained by assuming that the number of sites occupied in the sodium channels is sufficiently small for

the fluxes to be nearly independent. However, the evidence is not at all direct, and further work is needed to clear up this point. The most useful approach would be to measure sodium flux ratios under a voltage clamp and to compare them with electrical measurements of $E-E_{Na}$, but this might be a somewhat formidable task.

At present there is little to show whether the type of interaction discussed in this paper applies to the passive transport of other ions or molecules through cell membranes. In some cases there is clear evidence that it does not. Thus the movements of chloride through frog skin agree well with the requirements of independent transport (Koefoed-Johnsen, Levi & Ussing, 1952). Movements of glucose molecules through the human red cell are plainly not independent, although apparently passive, but here the interaction is of the kind in which the chance of a molecule entering the cell decreases when the external concentration is raised (Rosenberg & Wilbrandt, 1952; LeFevre, 1948). This is in the opposite direction from the interaction described by us. Another example of interaction, again operating in the reverse direction from ours, is afforded by the surface of *Micrococcus pyogenes*, which allows a large exchange but little net transfer of phosphate under resting conditions (Mitchell, 1953). The only case which seems at all comparable is that of the movement of water through cell membranes, where the permeability coefficient calculated from net rates of water movement is about 8 times larger than that obtained from exchange experiments with heavy water (Ussing, 1953; Jacobs, 1952). It has been concluded that water does not cross the membrane by dissolving as single molecules, but flows in bulk, or at any rate in fairly large aggregates of molecules, through pores in an otherwise impermeable structure. Whatever the exact mechanism, it appears that movement of one molecule of water in the membrane is associated with the movement of others in the same direction. In this sense there is a formal analogy between the passage of water through cell membranes, and the passage of potassium ions through the membranes of giant nerve fibres.

SUMMARY

1. The effects on the potassium fluxes of varying the membrane potential and external potassium concentration were investigated in giant axons from *Sepia* which had first been poisoned with dinitrophenol.

2. The potassium conductance, calculated from the potassium fluxes, increased markedly when the membrane was depolarized with applied current.

3. The passive nature of the potassium movements in fibres poisoned with dinitrophenol was established by the facts that

(*a*) the direction of net movement was always down the electrochemical gradient,

(*b*) the influx and efflux were equal at an apparent equilibrium potential

which agreed with that calculated by the Nernst equation from the internal and external potassium concentrations.

4. Evidence that the potassium movements are not independent, but that these ions interact in crossing the membrane, was provided by the observations that

(*a*) under appropriate conditions an increase of external potassium at constant membrane potential reduced the potassium efflux,

(*b*) the potassium influx at constant membrane potential was not proportional to the external potassium concentration, but increased more steeply,

(*c*) the variation of flux ratio with driving force was much greater than that predicted on the assumption of independent movement. Thus the ratio of influx to efflux changed 2400-fold (1/60 to 40) as the electrochemical potential difference ($E-E_K$) was altered from -50 to $+30$ mV. The change predicted for independence is 24 (1/7 to 3·3).

5. The interaction between potassium ions is of the kind expected in a system in which ions move through the membrane in single file.

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