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MOVEMENT OF RADIOACTIVE POTASSIUM AND MEMBRANE CURRENT IN A GIANT AXON

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One remarkable property of nerve fibres is that they are capable of passing large outwardly-directed currents for considerable periods of time when depolarized by 10–50 mV (Cole & Curtis, 1941; Hodgkin & Huxley, 1952). These currents may be nearly one hundred times greater than those associated with a corresponding increase in membrane potential. They are interesting physiologically because they are of the right sign and magnitude to explain the rapid recharging of the membrane capacity during the falling phase of the action potential. Since there is evidence that potassium ions move outwards during activity it has been assumed that the prolonged outward current associated with depolarization is carried by potassium ions (Hodgkin & Huxley, 1952). The experiments described here were designed to test this point and the affirmative answer which they provide has already been mentioned in earlier papers.

The principle of the method is illustrated by Fig. 1. A single nerve fibre from *Sepia* was isolated and soaked in a solution containing radioactive potassium for a few hours. It was then mounted in oil with the central portion in a drop of sea water about 6 mm in diameter. The contents of this drop were changed periodically by operating a pair of syringes coupled in 'push-pull'. After each change the fluid collected in one syringe was ejected on to a nickel dish, dried and analysed for ^{42}K with a Geiger counter. The outward flux of potassium at rest and in the presence of current was calculated from the quantities of ^{42}K leaving the nerve in unit time and from measurements of

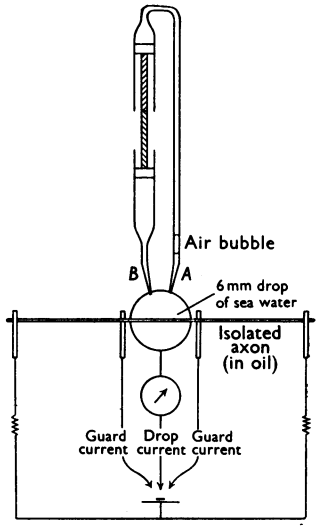

Fig. 1. Diagram illustrating method.

specific activity made at the end of the experiment. The difference between the two fluxes gave the extra leakage of internal potassium associated with any particular current. The electrical quantity with which this must be compared is not the total current through the nerve and external fluid but the component which leaves the axis cylinder in the region occupied by the drop. In the present experiments sufficient current was supplied to the guard electrodes to make their ends equipotential with the drop. Under these conditions no current could flow along the outside of the nerve between the drop and the guards so that the current crossing the membrane in the region of the drop was necessarily equal to that recorded by the galvanometer.

METHOD

Material

Giant axons 170–260 μ in diameter and 40–70 mm in length were isolated from *Sepia officinalis* by the usual methods (Keynes, 1951).

Electrode system and electrical connexions

The method of supplying current to the nerve fibre is illustrated in Fig. 1, and, in greater detail but still diagrammatically, in Fig. 2. The nerve was held at either end by forceps and was mounted about 0·5 mm above the bottom of a Perspex chamber which was filled with oil. A drop of sea water 6 mm in diameter and about 2 mm in height was located and stabilized by a circular groove in the bottom of the Perspex chamber. Electrical contact with the drop was made by two holes (80 μ in diameter) which were plugged with agar sea water and connected through tubes drilled in the Perspex with the sea-water pools P_1 and P_2, which contained Ag-AgCl electrodes. The guard electrodes were placed about 0·6 mm on either side of the drop and consisted of agar wicks about 0·4 mm in thickness. One end of each wick was brought into contact with a small hole filled with agar sea water which communicated through a wider tube with an open pool of sea water (P_3 or P_4). The other end of each wick was connected to the common guard electrode through a glass tube filled with agar sea water and a rubber tube filled with sea water. The resistance of the latter could be varied by compressing the rubber tube with a screw. In practice one tube was compressed to a standard extent giving the fixed resistance R_{g1} while the other was used as the variable resistance R_{g2}.

The mode of action of the whole system is best described by considering the procedure followed in a typical experiment. After the fibre had been mounted, a standard current was applied by closing the switch S with the potentiometer R_1 set to a suitable value. The potentiometer R_2 was increased until removal of the fluid bridge F_1 gave a deflexion of less than 50 μV in the potential difference recorded by the d.c. amplifier. The potential of the drop was then equal to the mean potential of the two guard electrodes. If the nerve had been perfectly uniform and if the resistance of the guards had been equal one would expect that the two guard electrodes would be equipotential. In practice neither condition was easy to realize, and it was therefore necessary to vary R_{g2} until removal of the fluid bridge F_2 as well as F_1 caused no detectable shift in potential. This adjustment ensured that both guards were at the same potential as the drop. The setting of R_{g2} was not at all critical and only had to be made once in each experiment. The position of R_2, on the other hand, had to be altered fairly frequently since the fraction of the total current which flowed into the drop varied with the membrane conductance.

If the circuit for supplying current was disconnected it was often possible to detect a change in p.d. when the fluid bridge F_1 was removed. This effect was due to small differences in resting potential and its sign was usually such as to make the potential of the guards lower than that of the drop. Under these conditions current must have been flowing from drop to guards along the

fluid outside the nerve. This means that the drop region was not in a condition of zero membrane current, as it should have been if the analysis is to be exact. The difficulty was overcome by taking resting measurements with the lead to the drop disconnected and supplying the guards with sufficient current to bring them to the same potential as the drop.

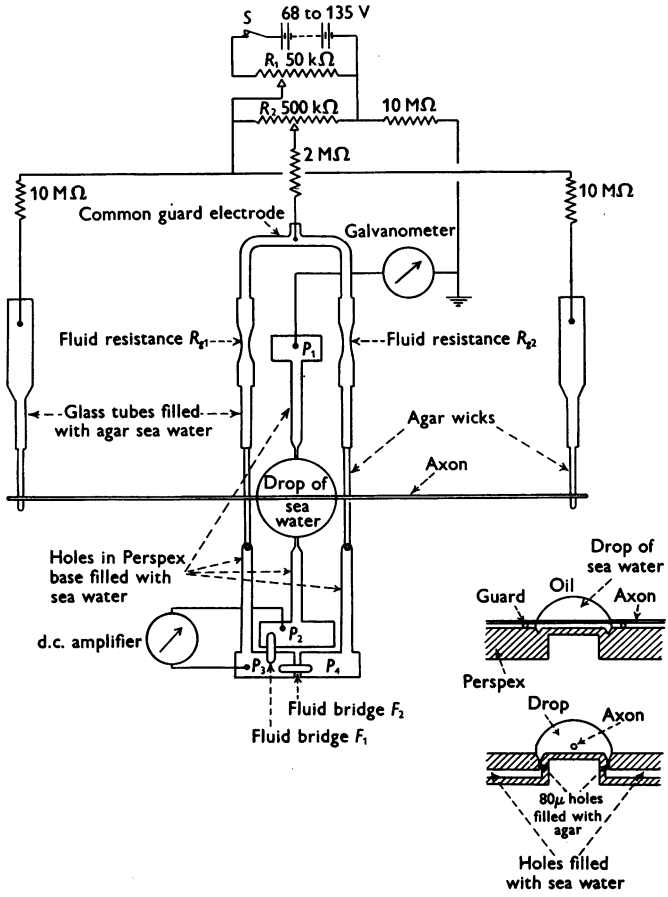

Fig. 2. Diagram of nerve chamber and electrical connexions. Inset: vertical sections through drop, in plane of nerve (above) and at right angles (below). The Perspex was cut away below the drop in order to allow β rays to reach a Geiger counter.

The reason for using fluid bridges (F_1 and F_2) and fluid resistors (R_{g1} and R_{g2}) instead of the metal equivalent of these elements is that it makes the operation of the whole apparatus independent of the potential differences across the Ag-AgCl electrodes used for supplying current or recording potential. It was therefore unnecessary to take any special precautions to obtain uniform electrodes or to prevent them polarizing when currents were applied.

Mode of action of guard system

In considering the action of the guard electrodes it is important to know whether their operation will be upset by the use of a finite distance (0·6 mm) between guard and drop instead of an infinitesimal one as assumed in the simplified discussion on p. 404. This is best done by treating

the nerve as a linear cable and calculating the distribution of potential and membrane current by standard methods. The results of such an analysis are given in Fig. 3. A fairly low value for the membrane resistance has been chosen in order to represent the condition of a depolarized axon. It will be seen that the external voltage gradient is zero about half-way between the edge of the drop and the guards. (The exact distance is 0·303 mm from the edge of the drop.) These points of zero voltage gradient define the length of nerve over which current crossing the membrane goes to the central electrode. Outside these limits any current in the external fluid necessarily goes to the guards. This means that the collecting length of the central electrode is not the drop itself but the drop plus about 0·3 mm of axon on either side. A correction here would be uncertain since it would depend on the variation of membrane conductance with membrane potential. Fortunately this source of error was offset by a very similar one arising from longitudinal diffusion of potassium ions in the external fluid. The cross-sectional area of the guard electrodes was large compared to that of the external fluid so that the concentration of ^{42}K should have been nearly as low at the guarded points as in the drop. This means that there should have been a point of zero diffusion gradient about half-way between guards and drop so that the collecting length for ^{42}K should have been nearly the same as that for membrane current.

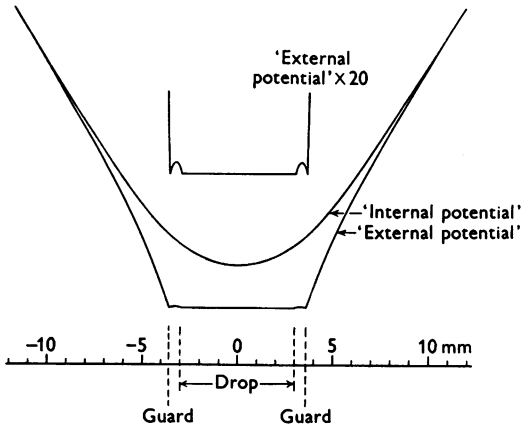

Fig. 3. Theoretical changes of potential in a linear cable produced by current flowing into a large central electrode (the drop) and into two adjacent electrodes (the guards). The curves show the change in potential from its resting value in the axis cylinder and external fluid and were calculated on the following assumptions: width of drop, 6 mm; standard equations of cable theory with the external resistance zero in the drop and twice the internal resistance elsewhere; axon diameter 200 μ; specific resistance of axis cylinder, 50 Ω cm; membrane resistance 1000 Ω cm^2. The guard electrodes were taken as infinitesimal and the cable as infinite. The ratio of guard to drop current is determined by the condition that guard and drop are equipotential.

Modified method for large cathodal currents

The guard system worked well for currents in which the component into the drop was less than 2 μA but was unsatisfactory for higher currents. With increasing cathodal currents a progressively larger fraction of the total current went to the guards and eventually damaged the nerve. The change in the relative proportions of drop current to guard current is a necessary consequence of the rectifying properties of the membrane which make the membrane resistance fall when the axon is depolarized. In theory it should be possible to improve matters by reducing the gap between guards and drop. But this was not practicable because surface tension effects tended to constrict the fibre if the gap was too short. In any case short gaps were unstable mechanically and therefore unsatisfactory.

In order to avoid these difficulties the guard electrodes were removed and current outside the drop was reduced to a relatively low value by wiping the fibre periodically with a solution of high resistivity. The composition of this solution was: 750 mM-dextrose, 10 mM-K, 11 mM-Ca, 37 mM-Mg, 103 mM-Cl and 3 mM-HCO_3; its specific resistance was 6·85 times that of sea water. The drop itself contained sea water, as in the previous method. In order to treat the parts of the fibre immediately adjacent to the sea-water drop, it was necessary to draw the small drop of sugar solution along the nerve until it finally coalesced with the sea-water drop. The sea water in the drop was changed after each wipe so as to avoid contamination from any ^{42}K collected by the drop of sugar solution.

This method was intended for use with large cathodal currents which were mainly concentrated at the edges of the drop. If the effective space constant is small compared with the width of the drop it can be shown that the fraction of the total current which crosses the membrane in the drop is $r_1/\{r_1+r_2+\sqrt{[r_2(r_1+r_2)]}\}$, where r_1 and r_2 are the resistances per unit length of the external fluid and axis cylinder. This statement can be proved by a method similar to that used by Cole & Curtis (1941) without assuming that the membrane conductance is constant. According to Weidmann (1951) $r_1/r_2=1·9$ for an oil-immersed axon which has previously been in sea water. In the present case the wiping solution was 6·85 times more resistant than sea water so that r_1/r_2 is taken as 13 and the drop current is then 0·73 times the total current. This ratio was adopted in calculating the currents enclosed in parentheses in Table 2.

Drop-changer

This was built from two matched syringes of bore 0·7 cm and capacity 1·5 ml. The plungers were operated by a rack and pinion in order to give a smooth motion. When collecting from the drop, both nozzles were placed below its surface and the plungers were moved downwards until the air bubble had travelled from the tip of tube *A* (Fig. 1) to a position about half-way up. This operation transferred the contents of the drop to tube *A* and replaced it with an equal amount of fresh sea water from *B*. The drop-changer was then raised and swung into a position suitable for ejection and refilling. Nozzle *A* was placed above a nickel dish while *B* was dipped into fresh sea water. The plungers were then moved upwards so that all the fluid below the air bubble was ejected on to the nickel dish and *B* was refilled with fresh sea water. The volume of fluid used to wash out the drop was 0·6 ml. which was about 10 times the volume of the drop. The apparatus was tested in the absence of a nerve by starting with a solution of known radioactivity in the drop and measuring the amount left after operating the drop-changer. This showed that 90–95% of the radioactivity was removed in a single change. The collecting efficiency sometimes appeared to be less good when the nerve was in position and two changes were normally made after a period of current flow. Errors due to inefficient collection would have had little effect on the estimates of resting leakage because these were usually based on a number of measurements made with the nerve in an approximately steady state. Under these conditions a 'carry-over' from one drop to the next would not influence the result.

Radioactive tracer methods

Samples of K_2CO_3 or $KHCO_3$ were irradiated at A.E.R.E., Harwell, turning some of the potassium into ^{42}K, and were subsequently converted into ^{42}KCl by the methods mentioned by Hodgkin & Keynes (1953). Artificial sea water containing ^{42}K was made up with a K concentration of 20 mM and the concentration of other ions approximately as stated by Keynes (1951, table 1). Axons were left in the radioactive sea water for 1–4 hr and were then transferred to ordinary sea water (10 mM-K) for about 10 min in order to wash off extracellular ^{42}K. The next operations were to mount the fibre in the measuring chamber and to determine the amount of labelled potassium in the central part of the axon with a screened Geiger counter placed below the measuring cell. This determination influenced the choice of a suitable time for collecting ^{42}K in the drop of sea water but was not used in the final calculation.

The ^{42}K content of each drop was determined by drying the drop on a nickel dish and counting the β particles emitted with an end-window Geiger counter of conventional design. The results

were standardized by comparing them with the counting rates produced by a weighed quantity of a ^{42}K solution of known concentration.

Since potassium ions were collected from only 6 mm of nerve the counting rates were low, and it was sometimes necessary to count each sample for 30–60 min. The labour and loss of efficiency resulting from prolonged manual counting led us to design a simple form of automatic counter which handled twelve samples without attention. A description of this device would be out of place since it was not finished until the experiments were nearly complete.

At the end of each experiment about 15 mm of the central part of the axon was cut out and dried on a quartz thread. The total quantity of ^{42}K was obtained by counting the fragment of nerve in the same way as the dried drop of sea water.

Potassium analyses

After the ^{42}K content of the dried nerve had been measured it was stored in a quartz tube and subsequently analysed for total potassium. These determinations were carried out with the help of Dr Keynes by the method of activation analysis (Keynes & Lewis, 1951).The method gave both sodium and potassium but about half the sodium was extracellular since we did not soak the fibres in a choline solution. Potassium concentrations were calculated from the total potassium and the axon diameter using a correction for a layer of extracellular fluid 20 μ in thickness. This figure, rather than 13 μ (Keynes & Lewis, 1951), was chosen because it gave a reasonable value for the internal sodium concentration. However, the total correction for extracellular potassium was only 2% so that the difference is unimportant.

EXPERIMENTS AND RESULTS

A typical experiment and method of calculation

Although the sequence of operations altered slightly during the course of the work, it is easiest to describe the procedure by considering a single experiment in detail. The essential results are given in Table 1; qualitatively this shows that the outward flux of potassium is reduced by an anodal current and increased by a cathodal one.

The method of calculation is illustrated by considering the extra leakage due to a current of 1 μA lasting 9 min (sample 8). Samples 7 and 10 gave a mean resting leakage of 1·065 counts/min per min which is in good agreement with values obtained in the rest of the experiment. Most of the extra leakage due to current occurred in sample 8, but about 10% was not removed in a single change and appeared in sample 9. The extra leakage due to current was therefore taken as the total quantity in samples 8 and 9 minus the amount due to resting leakage, i.e. $52{\cdot}0 + 9{\cdot}8 - 14{\cdot}8 \times 1{\cdot}065 = 46{\cdot}0$ counts/min. At the end of the experiment the central 15 mm of axon was found to give 783 counts/min (all counting rates have been corrected for decay). Two months later activation analysis showed that this piece of axon contained 115,000 p.mole of potassium (1 p.mole $\equiv$ 1 $\mu\mu$mole $\equiv 10^{-12}$ mole). The last two measurements give the counting rate of potassium in the nerve, and this ratio is assumed to apply to potassium ions which crossed the membrane during the experiment as well as to those left in the axon (see p. 411 for discussion of possible errors). On this basis the extra leakage of potassium associated with outward transport of charge is $46 \times 115{,}000/783 = 6760$ p.mole. The total charge crossing the

membrane is $1{\cdot}07 \times 9 \times 60 = 578$ μcoulomb or 6000 p.mole of monovalent cation. The average current density in the drop can be obtained by dividing figures such as these by the duration of current and the area of membrane in the drop. Resting fluxes are obtained in a similar manner. Since the counter was standardized with a weighed sample of the solution used to make the nerve radioactive it was also possible to calculate the fraction of labelled potassium present. The complete results of this experiment, together with others, are summarized in Table 2.

TABLE 1. Results of a typical experiment

Axon 3: Diameter 177 μ

Time (min)	Time interval (min)	Condition of nerve	Sample	Counting rate of sample (counts/min)	Resting leakage (counts/min per min)
5	Excitability test: threshold 27				
22	9·6	Resting	1	10·2	1·06
32	10·4	Resting	2	11·5	1·10
42	10·0	0·107 μA (*a*) for 9·25 min	3	6·9	—
52	10·0	Resting	4	11·6	1·16
62	10·0	0·545 μA (*c*) for 9·25 min	5	26·9	—
69	3·5	Resting	6	6·0	—
76	10·0	Resting	7	11·8	1·18
86	10·0	1·07 μA (*c*) for 9·0 min	8	52·0	—
93	4·8	Resting	9	9·8	—
101	10·0	Resting	10	9·5	0·95
111	10·0	0·545 μA (*c*) for 9·25 min	11	33·2	—
118	3·8	Resting	12	4·0	—
125	10·2	Resting	13	6·5	0·64
135	Excitability test: threshold 26				
141	15 mm cut out and dried				
—	Counting rate in excised 15 mm determined later as 783 counts/min				
—	Total potassium in excised 15 mm determined later as 115,000 p.mole				

Notes. (*a*) indicates an anodal current; (*c*) a cathodal current. All counting rates have been corrected for background and decay. The times in the first column refer to the mid-time of each operation.

Results

Six complete experiments were carried out by the guard method and three by the sugar method. The results are given in Table 2 and are seen to be similar to those described in the previous paragraph. An instructive way of examining the data is to plot the increment in the outward flux of potassium against the current density as in Fig. 4. The straight line in this graph was drawn through the origin with a slope given by the Faraday, and the fit to the cathodal points is evidence that steady cathodal currents are carried mainly by potassium ions moving outward through the membrane. A similar conclusion may be drawn from the fact that the ratios in column (8) of Table 2 are near unity.

TABLE 2. Collected results

(1) Axon	(2) Resting leakage (p.mole cm^{-2} sec^{-1})	(3) Current (μA)	(4) Mean current density ($\mu A/cm^2$)	(5) Duration of current (sec)	(6) Chemical equivalent of total current (p.equiv)	(7) Extra K leakage (p.mole)	(8) Ratio (7)/(6)	(9) Mean increase in outward K flux (p.mole cm^{-2} sec^{-1})	(10) S.E. of column 7 (as %)
1	58	0·545	16·8	570	3220	3820	1·19	207	4
		−0·107	−3·3	550	−610	−428	(0·70)	−24	17
2	45	0·545	11·6	530	2990	4125	1·38	165	16
		−0·107	−2·3	545	−604	−746	(1·24)	−29	56
		1·07	22·7	540	6000	5470	0·91	215	14
3	83	−0·107	−3·2	565	−626	−639	(1·02)	−34	34
		0·545	16·3	565	3190	2600	0·81	138	15
		1·07	32·0	540	6000	6760	1·13	375	8
		0·545	16·3	565	3190	3850	1·21	204	12
4	54	1·07	23·9	550	6080	6540	1·08	267	7
		2·58	58·0	225	6020	6330	1·05	630	7
		1·07	23·9	560	6200	5340	0·86	214	11
5	40	−0·107	−2·18	565	−626	−469	(0·75)	−17	23
		2·08	42·4	260	5600	6730	1·20	528	7
6	40	(0·78)	(17·9)	560	(4510)	3780	0·84	155	9
		(4·45)	(102)	120	(5540)	5330	0·96	1020	6
7	52	(0·78)	(17·5)	573	(4620)	5410	1·17	212	5
		(3·72)	(83·6)	120	(4630)	4590	0·99	860	5
8	85	(8·31)	(230)	60	(5160)	4300	0·83	1980	17
9	35	−0·26	−6·6	1170	−3150	−714	(0·23)	−16	59
Mean	55						1·04		

Additional measurements

Axon number	1	2	3	4	5	6	7	8	9	Mean
Diameter (μ)	172	250	177	237	260	232	236	192	208	218
Potassium concentration (m.mole/l.)	304	244	306	254	306	251	276	306	288	282
Fraction of potassium exchanged ...	0·168	0·079	0·065	0·214	0·051	0·15	0·065	0·041	0·076	

Notes. All axons except 4 were excitable at the end of the experiment. Axons 6, 7, 8 were studied by the 'sugar' method: the bracketed values in cols. (3) and (4) were calculated by the method described in the text. A minus sign in cols. (3) and (4) indicates an anodal current. Col. (6) is col. (3) × col. (5)/96500. Current densities and fluxes are average values obtained by dividing drop current by the area of membrane in the drop. Anodal ratios have been omitted in calculating the mean of col. (8). Temperature 16–20° C. Col. (10) gives the standard error due to the counting rate of the samples.

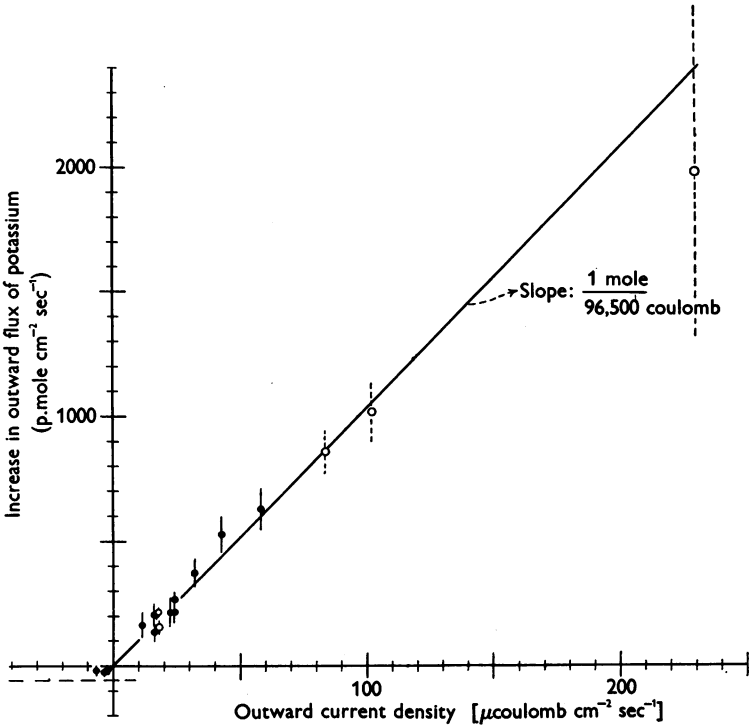

Fig. 4. Abscissa: mean outward membrane current density in drop (=total membrane current in drop divided by area of membrane in drop). Ordinate: mean increment in potassium outflux associated with flow of current (=outflow÷by area of membrane in drop). The vertical lines show ± twice the S.E. estimated from the observed counting rates. Full circles and continuous lines were obtained by the guard method; hollow circles and dotted lines by the modified method for large currents. The horizontal line at −55 p.mole cm^{-2} sec^{-1} is drawn at a level corresponding to complete suppression of the average resting outflux.

Sources of error

Counting errors. These were estimated in each experiment and the results are given in Table 2, column (10). The large standard error of the extreme point in Fig. 4 arose because only freshly isolated axons will remain excitable after very large currents. This meant that the duration of treatment in ^{42}K was shorter than we should otherwise have employed.

Errors in collecting potassium. A trace of ^{42}K must have been lost by diffusion into the small holes used to make electrical connexion with the drop: the error here was calculated as about 0·3%. The effect of diffusion from the parts in oil was probably offset by a similar error in measuring current (see p. 406).

Errors in potassium analysis. Keynes & Lewis (1951) estimated the standard error of their method as ±2%. Our measurements may have been somewhat less accurate since we used smaller quantities of axoplasm.

Errors in measuring specific activity. In working out the results it was assumed that the specific activity of the potassium which left the axon during the experiment could be obtained from the ratio of ^{42}K to total potassium at the end of the experiment. The time between the application of

current and the end of the experiment varied between 30 and 110 min and averaged 50 min. It is therefore necessary to consider how much the specific activity might alter in this time. Most of the axon was in oil so that its specific activity could not alter, apart from radioactive decay which was always allowed for. Nor would a net leakage of potassium into the drop have any effect unless the membrane could distinguish between labelled and unlabelled potassium. The process which could have altered the specific activity is an entry of unlabelled potassium from the drop into the axon. Taking the drop width as 6 mm, the axon diameter as 220 μ and the inward flux as 17 p.mole cm^{-2} sec^{-1} (Keynes, 1951) it is found that the total entry of potassium in 50 min is 2000 p.mole. This is only 1·3% of the total potassium in 15 mm so that the error here is likely to be small.

The method of calculation would be upset if a substantial part of the potassium were bound so firmly that it could not exchange with ^{42}K. In this case the relevant factor for converting counting rate into quantity of potassium should be taken from the quantity of exchangeable potassium and not from the total potassium. However, the experiments of Hodgkin & Keynes (1953) indicate that the fraction of bound potassium in *Sepia* axons is less than 10% so that the error here is unlikely to be important.

Errors in method for large currents. This method assumes a ratio of external to internal resistance and is clearly liable to larger errors than the first method. The use of an equation based on an infinitely large drop would make the drop current too large.

DISCUSSION

The results described in this paper are subject to two qualifications. In the first place the measurements refer not to current densities or potassium fluxes in a uniform fibre but to quantities averaged over a length of 6 mm in which there was considerable variation of membrane potential. This should not upset the correlation between potassium movement and current, but it does mean that there is doubt about the range of current density and flux to which the measurements apply. In order to form some idea of the way in which membrane current varied over the drop we assumed that the steady state relation between membrane potential and current density was similar to that in *Loligo* (Hodgkin, Huxley & Katz, 1952, fig. 13). On this basis it can be shown, by a method similar to that of Cole & Curtis (1941), that an average current density of 100 $\mu A/cm^2$ over 6 mm would correspond to a current density of about 1000 $\mu A/cm^2$ at the edge and a maximum depolarization of roughly 50 mV. This implies that the outward flux of potassium at the edge would be about 10,000 p.mole cm^{-2} sec^{-1}, which is 200 times greater than the resting outflux. These estimates are obviously rather uncertain but they indicate that the experiments described here apply to the range of current density and membrane potential which we used with *Loligo*.

The second reservation which must be made is that the approximate equivalence of outward potassium flux and membrane current does not necessarily mean that potassium is the only ion concerned in carrying current. In order to establish this point in a rigorous manner it would be necessary not only to make more accurate measurements but also to study potassium influx at the same time as the outflux. If there were no change in permeability one would expect that depolarization would increase the outward potassium flux

and decrease the influx. In this case the contribution of potassium ions to the current would be larger than that found by our method. On the other hand, since the permeability to potassium almost certainly rises when the fibre is depolarized there may be an increase in influx, so that our method may overestimate the contribution of potassium ions. Although we cannot eliminate this possibility it is not thought to be particularly important. With the larger cathodal currents the membrane potential would be near zero and the ratio of inward to outward flux ought not to exceed the concentration ratio across the membrane. In this case the influx would be less than 4% of the outflux so that it may reasonably be neglected. There is more doubt about the weaker cathodal currents, but it seems unlikely that the influx could have exceeded one-fifth of the outflux. On general grounds one would expect the transport number of potassium to approach unity over the range of membrane potentials in which the membrane behaves like a potassium electrode but to fall off markedly as the potential approaches its resting value.

Little need be said about the subsidiary results in Table 2. The resting fluxes are similar to Keynes's (1951) average value but somewhat greater than the figures which he gives for fresh fibres. This is not surprising since we found it necessary to soak axons in ^{42}K sea water for long periods in order to obtain reasonable counting rates. The average figure for the internal potassium concentration also agrees reasonably with those given by Keynes & Lewis (1951) for fibres which had been isolated for several hours. Any comment on the contribution of potassium movement to anodal currents would be premature since our method gave no information about variations in potassium influx, which are likely to be of considerable importance under these conditions.

SUMMARY

1. A method for comparing membrane current and potassium outflux was applied to isolated axons from *Sepia officinalis*.

2. The outward flux of potassium was decreased under an anode and increased under a cathode.

3. Over a wide range of cathodal currents the quantity of potassium leaving 6 mm of axon was equivalent to the total electric charge passing through the same area of membrane in the same time.

4. It is concluded that the steady outward current associated with depolarization is mainly carried by potassium ions.

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