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THE COUPLING OF SODIUM EFFLUX AND POTASSIUM INFLUX IN FROG MUSCLE

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Many types of living cell contain more potassium and less sodium than the surrounding medium, and these concentration differences appear to be built up and maintained by ion pumping mechanisms in the cell membrane. Except when an appreciable transfer of anions also takes place, the active movements of sodium and potassium are roughly equal and opposite, and the question arises as to exactly how the sodium efflux and potassium influx are linked together. At one extreme a form of 'chemical' coupling can be envisaged which would link the outward transfer of each Na^+ ion so tightly to the inward transfer of a K^+ ion that there would be no separation of electrical charge across the membrane, however large the fluxes were; such a pump would be electrically neutral in its operation. At the other extreme, if the sodium pump extruded a stream of Na^+ ions with their positive charges, in such a way as to create a potential difference across the membrane—the inside becoming negative—which would attract K^+ or other cations inwards, the coupling would be purely electrical. Such a pump, or one which operated in an intermediate fashion with partial chemical coupling, could be described as 'electrogenic'. One way of distinguishing experimentally between these possibilities is to consider the absolute size of the membrane potential (E_m) at a time when a net uphill transfer of sodium and potassium is known to be taking place. If E_m was greater than E_K, the Nernst equilibrium potential for K^+ ions, the coupling could be largely electrical, since as far as passive movements of K^+ ions were concerned, there would be a net driving force in the inward direction. On the other hand, if E_m was smaller than E_K, the passive movements of K^+ ions would necessarily be outwards, so that the occurrence notwithstanding of a net uptake of potassium would imply that a chemically coupled process must be at work. In the latter situation the argument applies unequivocally, but this is not the case when E_m is greater than E_K, since the excess membrane potential might arise from a

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source other than active sodium transport, such as the existence of a large concentration gradient for a penetrating anion like Cl^-. Furthermore, with a neutral coupled pump working in the presence of an external diffusion barrier, the potassium concentration immediately outside the membrane might be appreciably lower than in the bathing solution, making the true as opposed to the apparent value of E_K greater and not smaller than E_m.

In order to apply these arguments in the case of frog muscle, the findings of Desmedt (1953) can be exploited. He showed that if sartorius muscles are loaded with sodium and depleted of potassium by exposure for 24 hr or longer at a low temperature to a K-free soaking-in solution, and are then transferred to a recovery solution containing 10 mM-K at room temperature, large movements of sodium and potassium take place against the concentration gradients during the next hour. It is not difficult to estimate both E_m and E_K during the recovery period, and our first results, which were obtained during a visit by Mrs Rybová to England in the summer of 1961, agreed with those of Kernan (1962) in showing that E_m may be appreciably larger than E_K in the early stages of recovery, and that later the two potentials become roughly equal. This suggested that the active uptake of potassium depended mainly on an electrical coupling with the efflux of sodium. However, although subsequent control experiments disposed satisfactorily of the possibility that the membrane potential might have been held up by chloride, they also led to the observation that in some muscles there could be a large net uptake of potassium while E_m remained well below E_K. In a preliminary note (Keynes & Rybová, 1963) it was proposed that both types of coupling might normally be operative and, perhaps more speculatively, that their relative contributions to the total potassium influx might vary with the circumstances. This conclusion has been strengthened by further experiments to be described below, and by the elegant work of Adrian & Slayman (1964 and in preparation) on the uptake of rubidium by potassium-depleted muscles, in which they were able to apportion the rubidium influx into its electrically coupled and chemically coupled components.

METHODS

Pairs of sartorius muscles were dissected from medium-sized specimens of *Rana temporaria*, and mounted for the experiments at roughly their resting length in the body, with the pelvic tendons gripped in small stainless-steel clamps. The muscles were then loaded with sodium and depleted of potassium by various periods of exposure to a K-free solution kept at 2° C in a well stirred water-bath. The volume of soaking-in solution was 500 ml. and the number of pairs of muscles treated together was never more than three. Throughout the soaking-in period the muscles were raised and lowered on their holders once per second by an electric motor.

When the muscles were judged to be adequately loaded with sodium, they were mounted with their inner surfaces uppermost in a small rectangular chamber fixed to a mechanical stage, usually still in the K-free solution but now at room temperature. After a few determinations of E_m had been made in the K-free solution, one member of the pair was next cut out, blotted, weighed, and put in a platinum crucible for subsequent ashing and analysis by flame photometry. Its potassium content was assumed to be identical with that of the companion muscle before recovery, and enabled the starting level of E_K to be calculated. The K-free solution was then sucked out of the chamber, and replaced with several changes of a recovery solution containing 10 mM-K; a slow flow of this solution through the chamber was usually maintained while the membrane potentials of the fibres were being measured, in order to provide some stirring. After about an hour, during which at least four groups of determinations of E_m were made, the test muscle was cut out for analysis in the same way as its companion, to obtain the final value of E_K.

Membrane potentials were measured with glass micro-electrodes filled with 3 M-KCl whose resistance was generally not less than 10 MΩ. The precautions described by Adrian (1956) were taken in order to avoid errors from tip potentials. In the first set of experiments (see Fig. 1) the output of a voltage calibrator connected in series with the muscle chamber was adjusted to back off exactly the membrane potential of each fibre, and the reading was then noted. This procedure did not allow many fibres to be sampled, and a better technique was to connect the d.c. amplifier output to a Honeywell potentiometric recorder, so that the micro-electrode could be inserted into a number of fibres in quick succession, and the potentials measured later, at leisure, on the paper record. A systematic traverse across the muscle was made with the help of the mechanical stage, and the potentials of 20–30 fibres were recorded in about 5 min. In order to make the measurements as objective as possible, the sole criterion for rejecting a record was a failure of the potential to remain at a steady level for several seconds after the micro-electrode had been lowered so as to penetrate a fibre.

Solutions. The solution normally used for depletion of the potassium content of the muscles had the following composition: 89 mM-NaCl, 25 mM-$NaHCO_3$, 3 mM-Na_2HPO_4, 0·9 mM-$CaCl_2$, 1·5 mM-$MgSO_4$, equilibrated with a 5 % CO_2, 95 % O_2 gas mixture. On some occasions 0·9 mM-Na gluconate was also present, and the concentration of calcium was sometimes increased to 1·8 mM; these changes made no obvious difference to the results. On other occasions, more drastic alterations were made to the soaking-in solution; these are described in the text. The recovery solution contained: 73 mM-NaCl, 25 mM-$NaHCO_3$, 3 mM-Na_2HPO_4, 10 mM-KCl, 0·9 mM-$CaCl_2$, 1·5 mM-$MgSO_4$, 26 mM-dextrose, 5 % CO_2, 95 % O_2. Again, any appreciable deviation from this composition is mentioned in the text. Both solutions had a pH of 7·4.

RESULTS

Hyperpolarization during recovery

Figure 1 summarizes the results of measuring the membrane potentials in the fibres of nine muscles while they extruded sodium and regained potassium in a solution containing 10 mM-K at room temperature, after having first spent 24 hr in the K-free soaking-in solution at 2° C. The average values over successive 5 min intervals after the start of recovery are shown, together with their S.E.S. In the K-free solution the average membrane potential was well over 100 mV; on raising the external $[K^+]$ to 10 mM it quickly fell to 71·5 mV, where it remained for the next 45 min. During the second half of the recovery period the potential fell slowly to a final value in the neighbourhood of 63 mV. Analyses of companion

muscles which were identically Na-loaded and K-depleted, but which were not exposed to the recovery solution, gave [Na] = 80·5 ± 3·2 m-mole/kg wet weight and [K] = 47·4 ± 2·0 m-mole/kg; their water content was 799 ± 3 ml./kg. Assuming 130 ml. of the water to have been extracellular, the concentration of potassium in the fibre water before recovery was calculated as 47·4/0·669 = 70·7 mM. Making the further assumption that the activity coefficients were the same inside and outside the fibres, the Nernst equilibrium potential for K^+ ions at the beginning of recovery was

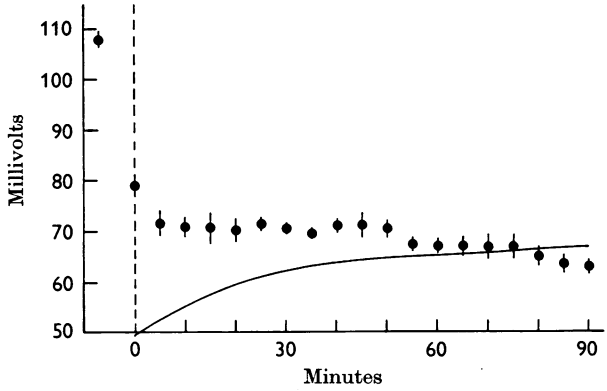

Fig. 1. Determinations of the average resting membrane potential in sodium-loaded frog sartorius muscle fibres before and after transferring the muscles (at $t = 0$) to a recovery solution containing 10 mM-K at about 20° C. The length of the bar through each point is ± 1 × S.E. The solid line shows the way in which E_K varies with time, calculated as described in the text.

given by $E_K = 58 \log_{10} (70{\cdot}7/10) = 49{\cdot}4$ mV. Analyses of the test muscles on completion of the 90 min recovery period gave [Na] = 38·8 ± 3·2 m-mole/kg and [K] = 94·2 ± 2·0 m-mole/kg; the water content was 784 ± 3 ml./kg. Hence on the same basis as before, the final value of $[K]_i$ was calculated as (94·2 − 1·3)/0·654 = 142·0 mM, and E_K as 66·8 mV. In order to estimate the value of E_K at intermediate times it may be taken from Desmedt's (1953) analyses of muscles treated in similar solutions that the recovery process approaches completion with a half-time of the order of 20 min, and the line in Fig. 1 is drawn accordingly.

It may be seen in Fig. 1 that during the first 30 min of recovery, when the uptake of potassium is proceeding most rapidly, E_m exceeds E_K by 15–20 mV. This finding is in line with the very similar work of Kernan (1962). His estimate for $(E_m - E_K)$ in the normal sodium recovery solution was somewhat smaller (11 mV), but in a choline recovery medium he found an initial membrane potential of 84 mV, and $(E_m - E_K)$ was over 30 mV. Before it is concluded that this evidence favours the electric and not the chemical coupling hypothesis, it is necessary to consider the implications

of the two assumptions made in calculating E_K that have already been mentioned, and of certain others to which attention has not yet been drawn. The first assumption was that the extracellular space was no more than 130 ml./kg. This value was that found by Desmedt (1953) with a polysaccharide, and it has been confirmed in a few experiments at Babraham with [^{14}C]labelled sucrose, which were conducted by Dr K. Fugelli. If the extracellular space (e.c.s.) had really been, say, 200 ml./kg, the initial value of $[K]_i$ would have been 79·0 mM, and E_K would have been 52·1 mV. An underestimate in the assumed e.c.s. is therefore unlikely to account for more than a small fraction of the apparent difference between E_m and E_K. The second assumption was concerned with the activity coefficient of intracellular K^+ ions. Lev (1964) inserted potassium-sensitive glass electrodes into frog muscle fibres and found $\gamma_K = 0{\cdot}77$; this is close to the standard value for 0·1 M-KCl or 0·1 M-NaCl at room temperature, and suggests that any inequality in the activity coefficients on the two sides of the membrane is unlikely to have given rise to gross discrepancies between the true and calculated values of E_K. Another assumption made tacitly is that it is justifiable to compare a figure for E_K representing an average for all the fibres throughout the muscle, with a figure for E_m obtained by penetrating only the superficial fibres. If, however, there was a difference between the deep and superficial fibres at the end of the soaking-in treatment, it would probably be in the direction of a greater depletion of the potassium in the fibres at the surface of the muscle, so that the estimate of E_K worked out for these fibres would tend to be too high. The occurrence of a non-uniform distribution of potassium would therefore strengthen the conclusion that E_m was initially greater than E_K rather than weakening it. At the same time, it is quite possible that because of diffusion effects the superficial fibres recover their potassium sooner than the deep ones, so that the line drawn in Fig. 1 should really rise more steeply, making E_K closer to E_m during the later stages of recovery.

In the experiments of Fig. 1 the mean value of E_m in the K-free soaking-in solution at room temperature, immediately before transferring to the recovery solution, was 108 mV. Since Cl^- ions are distributed passively in frog muscle (Adrian, 1961), $[Cl]_i$ was correspondingly small at the start of recovery, and must have tended to hold E_m up after $[K]_o$ was raised to 10 mM. It seemed rather unlikely that this effect could have maintained E_m above E_K for as long as an hour, because the experiments of Hodgkin & Horowicz (1959) had shown that chloride was redistributed quite rapidly after changes in $[Cl]_o$, the process having a time constant of the order of 4 min. However, it was necessary to examine the possible contribution of the chloride concentration ratio to the high values of E_m

observed during the early stages of recovery. The first approach was to use a recovery solution in which propionate was substituted for all the chloride, but this suffered from the disadvantage that any chloride inside the fibres at the beginning of recovery now tended to hold E_m down, and thus to bias the results in the opposite direction. Some of these experiments suggested strongly that under certain conditions the membrane potential could actually remain for several minutes in a reversed state, with the inside of the fibres at about +20 mV, before reverting abruptly to a more normal level in the region of −40 mV; but this interesting phenomenon did not seem to be directly relevant to the present study, and was not followed up. The second approach was to load the muscles with lithium instead of sodium, and then to measure E_m in the usual recovery solution; it was hoped that the contribution of chloride to the membrane potential would be the same as before, while that of active transport would be greatly reduced, since lithium is only slowly extruded from frog muscle fibres (Keynes & Swan, 1959*b*). In four lithium-loaded muscles, E_m was indeed 4–16 mV *smaller* than E_K about 5 min after transferring the muscles to the recovery solution, but this observation was of limited value, because although no more than a normal proportion of the intracellular potassium had been lost, the muscles failed to maintain a high resting potential in the K-free Li solution, and the initial value of $[Cl]_i$ corresponded to a potential of 42–64 mV instead of the expected 100 mV.

A better method of demonstrating that E_m could not be held up by chloride for a prolonged period was to block active transport with ouabain, which at a concentration of 10^{-5} M has been shown to interrupt both the net uphill transfer of sodium and potassium (Johnson, 1956) and the efflux of labelled sodium from frog muscle (Edwards & Harris, 1957; Horowicz & Gerber, 1965; Keynes, 1965). Table 1 summarizes the results of a number of experiments, and shows that as long as the ouabain was applied in such a way as to achieve its full inhibitory effect, the hyperpolarization at the beginning of the recovery period was indeed abolished. In two of the first four experiments (marked with asterisks) there was still some initial hyperpolarization, probably because the glycoside was added to the recovery solution only, and some active transport took place before it had time to act; comparison of these test and control muscles showed that in the recovery solution there was a slight (but not significant) fall in the Na content of $2{\cdot}8 \pm 3{\cdot}3$ m-mole/kg, and a definite rise in the K content of $6{\cdot}0 \pm 2{\cdot}4$ m-mole/kg. In all the later experiments, ouabain was added to the soaking-in solution as well, not less than 40 min before the end of the soaking-in period, and during recovery the change in Na content was reduced to $-0{\cdot}2 \pm 1{\cdot}4$ and that in K to $+1{\cdot}5 \pm 1{\cdot}4$ m-mole/kg. In three

of these experiments, the membrane potential had fallen to under 60 mV in the K-free soaking-in solution, so that they did not provide a good test of the effect of ouabain on the possible contribution of chloride to E_m. In the remaining seven experiments, measurements made 5–10 min after transferring to the recovery solution showed that, with active transport blocked, E_m was smaller than E_K by $3{\cdot}1 \pm 1{\cdot}2$ mV, instead (as in Fig. 1) of being much larger; and after 60 min in the recovery solution, E_m was $11{\cdot}1 \pm 1{\cdot}3$ mV below E_K instead of being roughly the same. The most

TABLE 1. Membrane potentials in muscles treated with recovery solution containing 10 mM-K and 10^{-5} M-ouabain

Soaking-in time (hr)	E_m in soaking-in solution (mV)	Initial		Final		K content	
		E_m (mV)	E_K (mV)	E_m (mV)	E_K (mV)	Initial (m-mole/kg)	Final (m-mole/kg)
24*	31	35	47	36	51	45·2	53·3
24*	75	60	54	52	58	57·2	68·2
24*	30	34	53	28	55	54·9	59·9
24*	68	57	56	43	55	61·0	60·8
21	51	42	55	34	54	61·1	57·1
17	64	53	58	47	59	66·1	70·9
21	42	30	58	24	58	67·0	67·0
6½	46	45	58	42	60	67·3	75·4
4½	120	60	61	56	63	80·7	83·9
8	92	66	63	51	62	77·7	77·6
23	74	54	59	42	60	68·9	72·4
5	113	56	63	51	61	75·3	72·1
24	79	55	(58)	49	58	—	65·9
24	82	56	(60)	49	60	—	72·0

* In these experiments there was ouabain only in the recovery solution; in all the other experiments, ouabain was also added to the soaking-in solution, at least 40 min before the end of the soaking-in period.

Temperature was about 2° C during period of soaking-in, and was 19–24° C during recovery.

direct evidence on the abolition of the hyperpolarization by ouabain was obtained in the last two experiments of Table 1, where the test and control muscles were grouped in a different way, the membrane potentials being measured both in a muscle transferred to normal recovery solution and in its companion treated with ouabain. At a time when E_m was 13·7 and 8·4 mV greater than E_K in the muscles which were reabsorbing potassium, it was 2·8 and 4·2 mV below E_K in the paired muscles with identical ionic concentration gradients unable because of the ouabain to follow suit.

Another way of blocking active transport is to lower the temperature to around 0° C. For the experiment illustrated in Fig. 2 a muscle chamber was built with a double wall through which cooling fluid could be circulated from a refrigerated bath, so that the temperature could be changed while the membrane potentials were being measured. After a pair of muscles had been depleted of potassium by 25 hr exposure at low temperature to

the usual soaking-in solution, one muscle was taken for analysis and the other was mounted in the chamber. Membrane potential determinations were then made at 3° C, first in the K-free soaking-in solution (in which E_m was 66·3 mV) and then in the recovery solution containing 10 mM K. After 20 min E_m had fallen to a value very close to the figure for E_K calculated from the analysis of the companion muscle. The temperature was now raised to 19° C, and quickly the membrane potential also rose by about 10 mV. The change in E_K corresponding to the change in temperature was only an increase of 3·2 mV, so that E_m was now well above E_K. On the assumption that Cl^- ions were in electrochemical equilibrium across the membrane immediately before the temperature was changed,

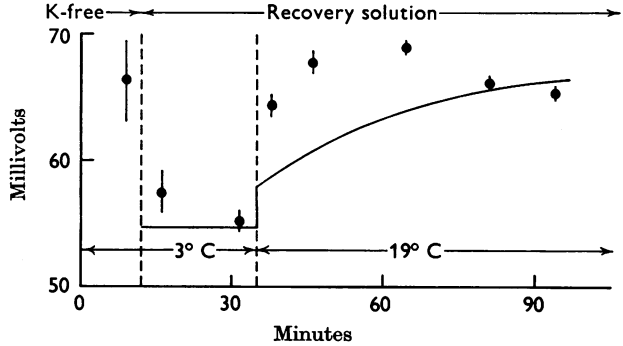

Fig. 2. The change in membrane potential when active transport is initiated by raising the temperature from 3 to 19° C. The solid line shows the calculated value of E_K. Each point is an average for 32–45 fibres, and the bars show $\pm 1 \times$ S.E. In this experiment, as in most of the others, the scatter in the values of E_m in individual fibres was much less after recovery than it was in the K-free solution.

chloride could not have contributed more than 3·2 mV to the rise in membrane potential, and it may be concluded that the greater part of the hyperpolarization must have arisen from the acceleration of active transport accompanying the 16° C rise in temperature. As may be seen from comparison of the line indicating the calculated value of E_K with the points indicating the measured values of E_m, the difference between E_m and E_K was apparently greatest about 15 min after the onset of recovery, and when the uptake of potassium was complete an hour later the two potentials were roughly equal. However, the change in E_K may again have been faster in the superficial fibres than in the deep ones (see p. 869), so that equality between E_m and E_K may really be reached somewhat sooner than Fig. 2 suggests. This experiment was repeated on three other occasions, on each of which a similar rise in E_m to a value above E_K was observed when the temperature was changed. A control experiment with a freshly dissected muscle which was not sodium-loaded gave no comparable

rise in E_m. Similar but much more extensive observations on changes in membrane potential after initiating net transport of ions by a shift in temperature are described by Adrian & Slayman (in preparation) and by Frumento (1965).

Depolarization during recovery

At one period in the course of this work, considerable difficulty was experienced in reproducing the results illustrated in Fig. 1. In most of the muscles examined during February, March and April of 1963, the membrane potential had fallen below 40 mV after 24 hr in the K-free soaking-in solution and, although the ability of the muscles to extrude sodium and regain potassium did not seem to be impaired, they did so at a value of E_m which was well below E_K instead of above it. As far as could be discovered, the solutions used at this time were identical in pH and composition with those that gave high potentials during recovery at other times, the muscles were dissected with equal care and were mounted at the same lengths, and changes in the soaking-in solution such as doubling [Ca] and adding gluconate made no difference. The most plausible explanation for the atypical behaviour of these muscles is that the condition of the frogs had been adversely affected by the spell of exceptionally cold weather in January 1963. However, the correctness of this suggestion, which is now not easy to verify directly, is less important than the observation that a net inward movement of potassium can take place against the electrochemical gradient, i.e. with $E_m < E_K$. When the results of a number of experiments were arranged as in Table 2 in order of the value of E_m after 24 hr in the soaking-in solution, it became clear that whenever this value was above 50 mV there was the normal hyperpolarization during the recovery process, but that if E_m was less than 50 mV just before transferring the muscle to the recovery solution, the opposite behaviour was observed. It must be repeated that after a 24 hr period of potassium depletion in the normal soaking-in solution, most muscles still have a relatively high resting potential, and during their recovery E_m is greater than E_K as in Fig. 1 and as in the experiments of Kernan (1962) and Adrian & Slayman (in preparation). With only two exceptions, all the muscles in Table 2 whose membrane potentials were below 50 mV after 24 hr in the soaking-in solution were either examined during the period already mentioned in the spring of 1963, or had been treated with soaking-in solutions containing little or no calcium. The exceptions were two experiments performed in January, 1964 (those in which E_m in the soaking-in solution is shown as 38* and 30*), when the soaking-in period was appreciably prolonged. After some unsuccessful attempts had been made to reproduce the 1963 behaviour by loading muscles with chloride before transferring them to the recovery solution, it turned out that low mem-

brane potentials could best be achieved by reducing the concentrations of calcium and magnesium in the solutions, or better still by omitting them altogether. As can be seen in Table 2, this technique was used in a number of experiments whose main purpose was to check that the 1963 results were not merely a figment of the experimenters' imagination.

Leaving for the Discussion (see p. 878) the problem of explaining how recovery can take place with E_m either greater or smaller than E_K, it

TABLE 2. A comparison of E_m and E_K at the beginning and end of recovery, with experiments listed in order of the value of E_m in the K-free soaking-in solution

E_m in the soaking-in solution (mV)	Initial		Final		K content	
	E_m (mV)	E_K (mV)	E_m (mV)	E_K (mV)	Initial (m-mole/kg)	Final (m-mole/kg)
113	76	60	62	65	71	87
99*a*	68	61	66	66	76	92
92	69	60	54	68	69	95
90	65	61	66	66	74	89
87	67	49	67	65	47	89
84	68	60	67	68	72	94
79	72	58	65	68	66	93
74	62	56	61	63	60	80
70	70	59	65	67	66	90
68	64	59	66	64	69	87
60	60	59	53	66	73	94
60	63	60	63	67	73	99
60	58	53	65	64	52	81
58*b*	55	40	60	59	33	68
55	43	27	70	60	20	71
51*d*	51	48	70	62	46	79
51	52	48	57	63	44	80
45*c*	44	48	53	64	45	87
43	39	45	57	59	54	74
43*	40	47	60	68	43	100
38*	19	37	40	51	30	53
38*d**	29	50	44	61	47	76
36	43	50	64	65	48	88
31*e*	28	34	60	61	27	78
30*	32	43	49	58	38	70
30	28	43	48	59	37	70
29	26	49	50	68	49	102
29	39	47	50	60	44	72
28*c*	28	43	50	61	39	78
28*c*	24	27	44	55	20	63
23*c*	13	28	19	57	21	68
21	22	47	39	64	42	84
18	22	49	33	60	47	73
18	17	54	42	65	55	86
16	22	50	34	62	48	80
10*d**	−2	8	49	49	10	50

* Soaking-in period increased to 30–48 hr.

a, soaking-in solution had 0·09 mM-Ca and 1·5 mM-Mg; recovery solution was normal.

b, both solutions had 0·09 mM-Ca and no Mg.

c, both solutions had no Ca and no Mg.

d, soaking-in solution had 0·09 mM-Ca and no Mg; recovery solution was normal.

e, soaking-in solution had no Ca and no Mg; recovery solution was normal.

Temperature was about 2° C during period of soaking-in, and was 18–23° C during recovery.

remains to reconsider one or two of the possible sources of error in arriving at the values of E_K, in case these values could have been grossly overestimated. First, there are two reasons why E_K was probably slightly underestimated. The more important of these is that E_K was calculated from the K content of the companion muscle before any recovery had taken place, whereas E_m was measured a few (5–10) min after recovery had begun, by which time the test muscle had already regained a little potassium. In the bottom ten experiments of Table 2, the average K content increased during recovery from 37·5–75·6 m-mole/kg; if after the first $7\frac{1}{2}$ min the K content had already risen by 7 m-mole/kg, the values of E_K given in the table would have been too low by about 4 mV. There were also indications from the [^{14}C]sucrose measurements made by Dr Fugelli that the extracellular space of the 1963 muscles was appreciably greater than the standard value of 0·13 used in the calculations, and this again would have resulted in a slight underestimate of E_K. Against these considerations must be set the possibility of an overestimate of the difference between E_m and E_K caused by a non-uniform distribution of potassium within the muscles; as argued on p. 869, the superficial fibres which yielded most of the values for E_m might have contained less potassium than the deeper ones, and might even have failed to regain their potassium during recovery. The latter possibility seemed rather unlikely, if only because the final K content of the depolarized muscles was little lower than in those which exhibited hyperpolarization (see Table 2), but it was important all the same to see whether there was any obvious difference between the membrane potentials of the fibres close to the surface and of those buried more deeply within the muscle. In one of the experiments on a muscle treated with Ca-free solutions, measurements over a period centred 8 min after the start of recovery gave a mean E_m for fifteen fibres classified as 'superficial' of $25·2 \pm 2·4$ mV, while the same number of fibres classified as 'deep' gave $22·2 \pm 2·0$ mV; in another experiment of the same kind the average for twenty-one superficial fibres was $11·0 \pm 1·2$ mV, and the average for twenty-one deep fibres was $13·9 \pm 1·2$ mV. These figures suggest that any variation in E_m with distance from the surface of the muscle was too small to account for the difference between E_m and E_K, which on ten occasions exceeded 15 mV.

DISCUSSION

If, in the conventional circulating carrier scheme for coupled transport of sodium and potassium shown in Fig. 3, the carriers X^- and Y^- are prohibited from crossing the membrane except in combination with K^+ or Na^+, respectively, then the net movements of sodium and potassium

must be exactly equal and opposite, and there is no separation of charge across the membrane. Under these conditions the mechanism operates in a strictly neutral and tightly coupled fashion. However, a simple modification of the scheme is to envisage that there can be a limited movement of the carrier X^- in its uncombined form, as indicated by the dashed lines in the figure. If such a movement is permitted, operation of the mechanism involves a forced transfer of electric charge through the membrane; the pump becomes electrogenic, and the movements of sodium and potassium

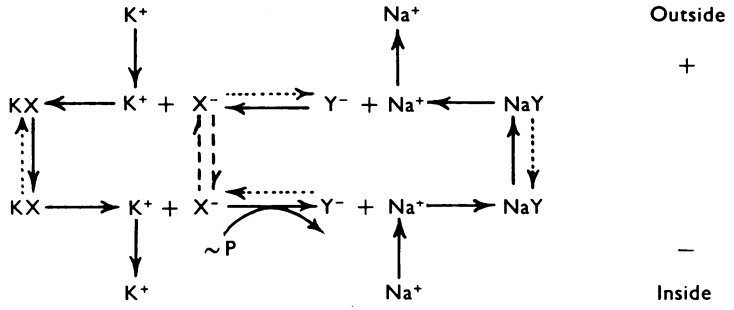

Fig. 3. A circulating carrier scheme for coupled active transport of sodium and potassium. If X^- and Y^- are unable to cross the membrane except with potassium or sodium, the pump will operate in a tightly coupled and electrically neutral fashion. If the carrier X^- can also cross by itself, as shown by the interrupted lines, the pump will be electrogenic and the coupling will be partly electrical. A similar argument would apply if X and Y were uncharged, or if they had more than one negative charge, and transported several ions at a time.

are to a certain extent electrically coupled. The argument is presented in this way not to establish the validity of the carrier hypothesis, which in the absence of any evidence as to the nature of X and Y must remain theoretical, but to emphasize that there is no conceptual difficulty in the proposition that the active efflux of sodium and the active influx of potassium are linked *both* electrically *and* chemically. Moreover, given some adjustment with circumstances of the passive permeability of the membrane, it is not hard to see why sometimes one mode of coupling might appear to predominate, and sometimes the other. At all events, the results on frog muscle described here can readily be explained on a dual hypothesis, but would be difficult to reconcile with exclusively electrical or exclusively chemical coupling.

In their studies of the uptake of rubidium by sodium-loaded muscles, Adrian & Slayman (in preparation) estimated that an electrical coupling of less than one-fifth of the sodium efflux with the rubidium influx could account for the large hyperpolarizations that they observed, and that the other four-fifths of the active fluxes could be neutrally coupled. It is, un-

fortunately, hard to make satisfactory calculations for the coupling between the sodium and potassium fluxes, because there are no data for the absolute size of the potassium conductance (g_K) of frog-muscle membranes under conditions exactly comparable with our experiments. However, Hodgkin & Horowicz (1959, Table 8) found an average g_K of 480 μmho/cm^2 in four fibres with an external [K] of 10 mM and membrane potentials of around 70 mV, and with this conductance a value for $(E_m - E_K)$ of 10 mV would correspond with an inward potassium current of 4·8 μA/cm^2, or an influx of 50 pmole/cm^2.sec. In Desmedt's (1953) experiments the initial rate of uptake of potassium was, from his Fig. 3, about 1 m-mole/kg wet weight.min. At the beginning of recovery in our Fig. 1 it was probably a little larger, say 1·5 m-mole/kg.min, but in the experiments at the top of Table 2 it was smaller, about 0·75 m-mole/kg.min. If the surface/volume ratio for frog muscle is taken as 0·415 cm^2/mg wet weight (see Keynes & Swan, 1959*a*), the net influx of potassium was therefore between 30 and 60 pmole/cm^2.sec. This suggests that a hyperpolarization of the order of 10 mV could just about be explained if the whole of the sodium efflux was electrogenic, and unless g_K was appreciably smaller in the sodium-loaded muscles than in the isolated fibres of Hodgkin & Horowicz (1959), the calculation does not leave much room for a large neutrally coupled component of the fluxes. But the potassium conductance is a rather variable quantity which was appreciably smaller under some other conditions studied by Hodgkin & Horowicz (1959), and these considerations do not rule out the possibility that when during recovery E_m is greater than E_K, part of the sodium efflux is, nevertheless, coupled chemically and in a non-electrogenic fashion with the potassium influx.

It was mentioned in the introduction that if there was a diffusion barrier between the site of the sodium pump and the bulk of the external medium, then during an uptake of potassium the value of $[K]_o$ might fall appreciably below the figure of 10 mM used in calculating E_K, and an apparent hyperpolarization might be produced by a strictly neutral pump. An explanation of this kind has been given for the post-tetanic hyperpolarization in mammalian non-myelinated nerve fibres (Ritchie & Straub, 1957). Adrian & Slayman (in preparation) have calculated that during the early stages of recovery the fibres at the surface of the muscle are likely to take up potassium so fast that the rise in $[K]_o$ to 10 mM at the centre of the muscle will be considerably delayed. However, the values of E_m with which we are concerned are mainly those for the superficial fibres, and a diffusion effect of this kind would give rise to errors in the calculated time courses of the changes in E_K in fibres in different parts of the muscle rather than falsifying the initial difference between E_m and E_K for the surface fibres. In order to explain hyperpolarization of the superficial

fibres it would be necessary to suppose that there is a diffusion barrier at individual fibre level, and it is not obvious where this may be located. In nerve fibres it is well recognized that the Schwann cells may restrict access between the nerve membrane and the bathing medium, but in muscle fibres there are no comparable enveloping structures. It could perhaps be suggested that the sodium pump sites are placed deep in the sarcoplasmic reticulum at some distance from the outer surface of the muscle fibres, but subsidiary hypotheses would then be needed to explain why the recorded membrane potential should be governed by the potassium concentration within the reticulum rather than by the true external concentration. Further difficulties for any explanation of hyperpolarization in terms of a diffusion barrier are raised by the fact that those muscles in which depolarization was observed were probably absorbing potassium at especially high rates (see analyses in Table 2).

Under conditions when E_m remains well below E_K while recovery takes place, any passive movement of K^+ ions down the electrochemical gradient must necessarily be outwards. It follows that since the net movement of potassium is actually inwards, the chemically coupled component of the potassium influx must be big enough to balance the outward leak of potassium and still leave a sufficiently large net influx of potassium to cause a rapid uptake. Two factors may help towards this state of affairs. First, with a maintained depolarization of the membrane, g_K and hence the passive leak of potassium may be small because of anomalous rectification (see Adrian & Freygang, 1962). Secondly, the total size of the sodium efflux may be larger than it usually is during recovery with hyperpolarization, so that more can be spared to offset a simultaneous leak and, incidentally, to balance electrically the outward movement of Cl^- ions which must have been appreciable in some of the experiments where E_m was initially very small. Thus in the first ten experiments of Table 2 the average sodium content of the muscles at the beginning of recovery was 50·6 m-mole/kg wet weight and at the end was 32·9 m-mole/kg, whereas for the last ten experiments the initial sodium level was 82·2 m-mole/kg and the final level was 47·2 m-mole/kg. Despite the tendency of the sodium pump mechanism to reach saturation at high values of $[Na]_i$ (Keynes, 1965), both the total and the net sodium efflux must have been appreciably larger in the latter group of muscles.

It is impossible to say from the evidence at our disposal whether the chemically coupled and electrically coupled fluxes are in a fixed ratio to one another, or whether the ratio varies with internal sodium concentration, as does not seem impossible. This point requires further investigation, and we must be satisfied for the present with demonstration provided by the experiments described here that depending on conditions

either mode of coupling may be operative, or perhaps both at the same time.

SUMMARY

1. The nature of the coupling between the active efflux of sodium and the active influx of potassium was studied in frog muscle by comparing the absolute value of the resting membrane potential, E_m, with that of the Nernst equilibrium potential for K^+ ions, E_K, at a time when a net uptake of potassium was taking place.

2. When muscles were loaded with sodium and depleted of potassium by exposure to a K-free solution at 2° C for 24 hr, and were then transferred to a recovery solution containing 10 mM-K at room temperature, E_m was normally 10–20 mV greater than E_K at the beginning of recovery, and when the uptake of potassium was complete 1–2 hr later the two potentials became roughly equal.

3. When recovery was blocked by treatment with ouabain or by lowering the temperature, the initial hyperpolarization was abolished. This eliminated the chloride concentration gradient as a possible cause of the difference between E_m and E_K.

4. It was concluded that under normal conditions the fluxes were at least partly coupled in an electrical fashion, and that the sodium pump could be regarded as electrogenic.

5. In one group of muscles examined during the early part of 1963, and in muscles treated with solutions containing little or no calcium and magnesium, the membrane potential measured in the K-free solution was usually well below 50 mV after 24 hr, and although in the recovery solution such muscles rapidly reabsorbed potassium and lost sodium, they did so at a value of E_m which was 5–30 mV smaller than E_K.

6. It was concluded that under these conditions the neutrally coupled component of the potassium influx must predominate.

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