THE COMPONENTS OF THE SODIUM EFFLUX IN FROG MUSCLE

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SUMMARY

1. In normal Ringer solution containing 2·5 mM-K only 37 % of the efflux of labelled sodium from a freshly dissected frog muscle is blocked by treatment with ouabain; in sodium-loaded muscles the ouabain-sensitive fraction of the efflux increases to 75 %.

2. Under all conditions, the ouabain-insensitive component of the sodium efflux is markedly reduced if the sodium in the external medium is replaced by lithium; at least in sodium-loaded muscles, the ouabain-sensitive component is increased in lithium Ringer.

3. Only the ouabain-sensitive component of the efflux is affected by the external potassium concentration.

4. In the presence of 2·5 mM-K the sodium influx in a freshly dissected muscle is not significantly altered by ouabain, but in a K-free medium the influx and the efflux are both reduced by nearly 20 %.

5. The sodium efflux can therefore be regarded as consisting of (1) a sodium-potassium coupled component that is blocked by ouabain and involves a sodium-sodium exchange in the absence of external potassium, and (2) a potassium-insensitive component that is unaffected by ouabain and tends to reach saturation at relatively lower internal sodium concentrations.

6. The evidence is considered for attributing component (1) to an efflux of sodium from the sarcoplasm proper, and component (2) to an efflux from the sarcoplasmic reticulum. Although such an interpretation is consistent with many of the observations, a definite identification of the possible sodium compartments in frog muscle cannot yet be made.

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INTRODUCTION

The studies on the sodium efflux in frog muscle to be described here originated in some experiments designed to follow up the work of Horowicz (1965) on the inhibitory effects of cardiac glycosides and of sodium-free solutions on the efflux of ^{22}Na from frog sartorius fibres. It was readily confirmed that in freshly dissected muscles less than half the sodium efflux was blocked by ouabain, and that the residual glycoside-insensitive efflux was further cut down by removal of external sodium. It was suggested (Keynes, 1966) that the sodium efflux from frog muscle may be divided into: (1) an exchange diffusion component, unaffected by glycosides, reduced by lithium, and saturating at relatively low internal sodium concentrations; (2) a net transport component, blocked by glycosides, increased by lithium, and only saturating at much higher values of $[Na]_i$. Another clear distinction between the two components that emerged from later experiments was that only (2) is sensitive to external potassium; the magnitude of the exchange diffusion flux is unaffected by changes in $[K]_o$. The evidence for such a division of the sodium efflux into two apparently independent components forms the bulk of this paper.

If this fractionation of the sodium efflux is valid, it must then be asked whether the intracellular sodium in frog muscle is contained in a single compartment with two different types of transport mechanism operating in parallel in the surrounding membrane, or whether there are two separate intracellular compartments arranged either in series or in parallel. The two forms of the twin-compartment hypothesis were considered and dismissed by Keynes & Swan (1959) in favour of a single-compartment theory involving a power law relationship between efflux and $[Na]_i$ (see also Keynes, 1965), but as has been pointed out by Caldwell (1968) fresh facts have come to light since 1959 which necessitate a re-appraisal of the situation. In the first place, an anatomical basis for a twin-compartment system has been provided by recent studies of the sarcoplasmic reticulum by electron microscopy (see Peachey, 1965; Smith, 1966); and, secondly, Lev's (1964) determination of the apparent activity coefficient of the Na$^+$ ions in frog muscle fibres as about 0·3 instead of the expected 0·7 suggests rather strongly that more than half of the intracellular sodium must be sequestered in a part of the fibre not normally penetrated by micro-electrodes. The experiment that originally led to the rejection of the idea that frog muscle consists of two intracellular compartments whose sodium exchanges in parallel with that in the extracellular space has therefore been repeated with some variations in the conditions. It appears that if labelled sodium can be exchanged directly between the compartments as well as moving into the extracellular space, many of the observations can be explained. However,

as is clear from Caldwell's (1968) account of earlier work on the partition of the ionic content of muscle fibres, this is a difficult field in which to perform really decisive experiments, and much more evidence will be needed for a complete identification of these two hypothetical sodium compartments.

<div align="center">METHODS</div>

The techniques used to study the influx and efflux of labelled sodium were the same as those employed by Keynes & Swan (1959) and Keynes (1965), and need no further description here. Extensive use was made of paired sartorii dissected from the same frog to provide control and test muscles. For experiments in 2·5 mM-K the solutions contained 111·2 mM-NaCl or LiCl, 1·37 mM-K phosphate pH 7·6, and 1·8 mM-CaCl$_2$; to obtain 10 mM-K, 7·5 mM-KCl was added. For K-free solutions, 1·37 mM-Na or Li phosphate pH 7·6 was substituted for the K phosphate.

<div align="center">RESULTS</div>

Effects of ouabain and lithium on freshly dissected muscles. In the first group of experiments, the effects of lithium and ouabain on the sodium efflux from freshly dissected muscles were observed by the procedure illustrated in Figs. 1 and 2. When the solutions contained 10 mM-K, as in Fig. 1, the efflux from a muscle treated with 10^{-4} M ouabain was reduced to about 60 % of that from a paired control. Substitution of lithium for sodium in the external medium further reduced the efflux from both muscles, the absolute extent of the reduction being about the same in each case. When the solutions contained no potassium (Fig. 2), the effect of ouabain was distinctly smaller, and again lithium cut down the effluxes from the control and poisoned muscles by about the same amount. Table 1 summarizes the measurements made in solutions with 0, 2·5 and 10 mM-K, and confirms that the effect of ouabain was significantly less in a K-free solution than in one containing 2·5 or 10 mM-K. In order to be able to express all the effluxes as a fraction of the efflux in normal Na Ringer solution, four determinations were made of the ratios of the unpoisoned efflux in 0 and 10 mM-K to that in 2·5 mM-K, which were respectively 0·87 ± 0·03 and 1·33 ± 0·04. The adjusted mean values in the muscles treated with ouabain were then seen to be approximately independent of [K]$_o$, suggesting that the ouabain-insensitive fraction of the efflux was also potassium-insensitive. The validity of this conclusion was strikingly confirmed in two experiments of the kind shown in Fig. 3, in which the efflux from a ouabain-treated muscle was found to be unchanged when the external [K] was raised from 0 to 10 mM, although in the unpoisoned companion muscle the efflux was more than doubled.

Effects of ouabain and lithium on sodium-loaded muscles. In the second group of experiments, the same procedure was followed, but the sodium
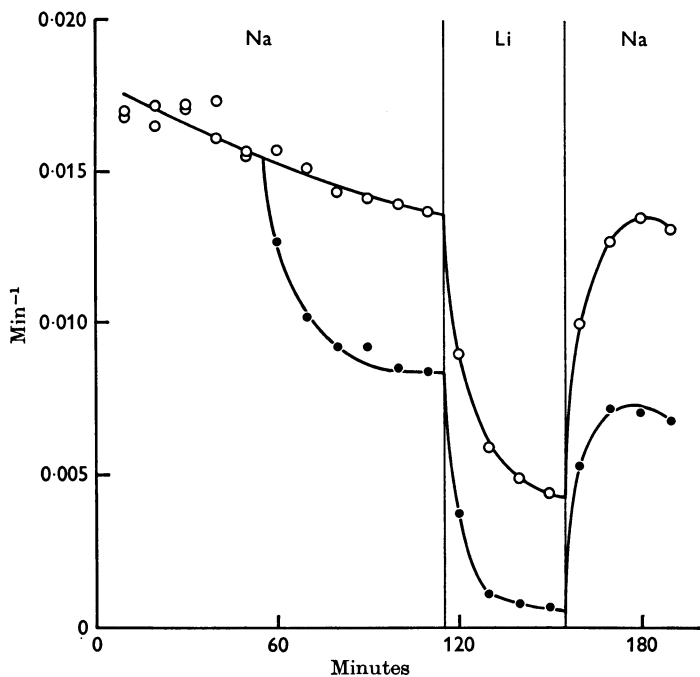
Fig. 1. The effect of lithium on the rate constants for loss of labelled sodium from a pair of freshly dissected sartorius muscles. The muscles were loaded with ^{22}Na for 3 hr, and washed in several changes of inactive Ringer containing 2·5 mM-K for 30 min before starting the measurements. The washing-out solutions all contained 10 mM-K and 111 mM-Na or Li. Open symbols: control muscle. Filled symbols: 10^{-4} M ouabain added. Temperature 21·5° C. Experiment of 13. i. 66.
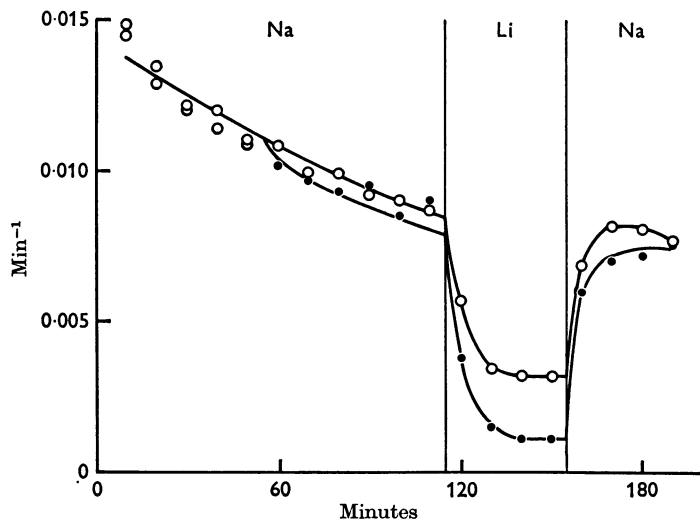

Fig. 2. An experiment similar to Fig. 1, except that all the inactive solutions were K-free. Symbols as before. Temperature 21·7° C. Experiment of 14. i. 66.

TABLE 1. The effects of ouabain and lithium on the sodium efflux in freshly dissected frog sartorius muscles. Each figure is the ratio of the efflux to that in Na Ringer solution without ouabain. The adjusted means in sections B and C show the size of the efflux relative to that in normal Ringer with 2·5 mM-K. In the experiments marked with an asterisk the concentration of ouabain was 10^{-5}M; otherwise it was 10^{-4}M

| Date	Temperature (°C)	In ouabain	In lithium	In ouabain + lithium
		A. Normal Ringer, [K] = 2·5 mM		
4. vi. 65	24	0·58	—	0·11
9. vi. 65*	25	0·63	—	0·17
10. vi. 65*	24	0·57	0·55	0·12
1. x. 65	23	0·72	0·49	0·10
11. x. 65	22	0·63	0·44	0·22
Mean and S.E.	24	0·63 ± 0·03	0·49 ± 0·03	0·14 ± 0·02
		B. K-free Ringer, [K] = 0 mM		
22. xii. 65	23	0·83	0·65	0·22
23. xii. 65	21	0·85	0·59	0·14
14. i. 66	22	0·93	0·39	0·13
Mean and S.E.	22	0·87 ± 0·03	0·54 ± 0·08	0·16 ± 0·03
Mean × 0·87	—	0·76	0·47	0·14
		C. High-K Ringer, [K] = 10 mM		
4. i. 66	20	0·73	0·41	0·11
11. i. 66	19	0·54	0·24	0·15
13. i. 66	21	0·60	0·32	0·05
Mean and S.E.	20	0·62 ± 0·04	0·32 ± 0·05	0·10 ± 0·03
Mean × 1·33	—	0·82	0·43	0·13
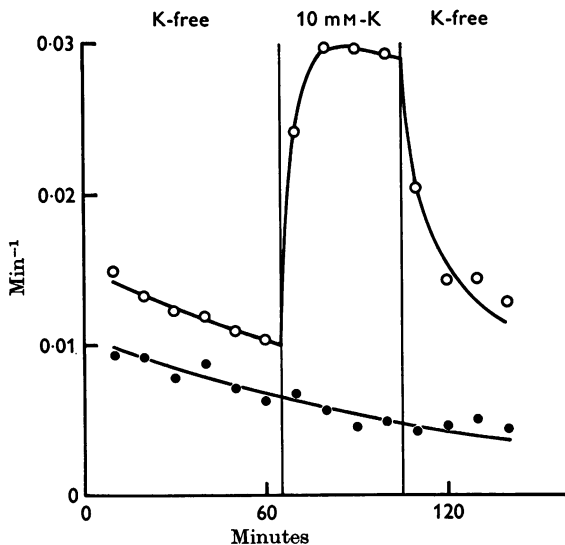
Fig. 3. After spending 160 min in a labelling solution, one muscle was transferred to inactive K-free Ringer containing 10^{-4} M ouabain, while the control was put into a similar solution without ouabain. The efflux measurements were begun 40 min later. Symbols as before. Temperature 22·5° C. Experiment of 12. v. 66.

content of the muscles was raised by soaking them for about 24 hr in inactive K-free Ringer solution at 2° C before putting them in the ^{22}Na solution, which was also K-free, at room temperature. As may be seen in Fig. 4, the sodium efflux in 2·5 mM-K was now reduced much further by ouabain, the glycoside-insensitive component accounting for only 25 % of
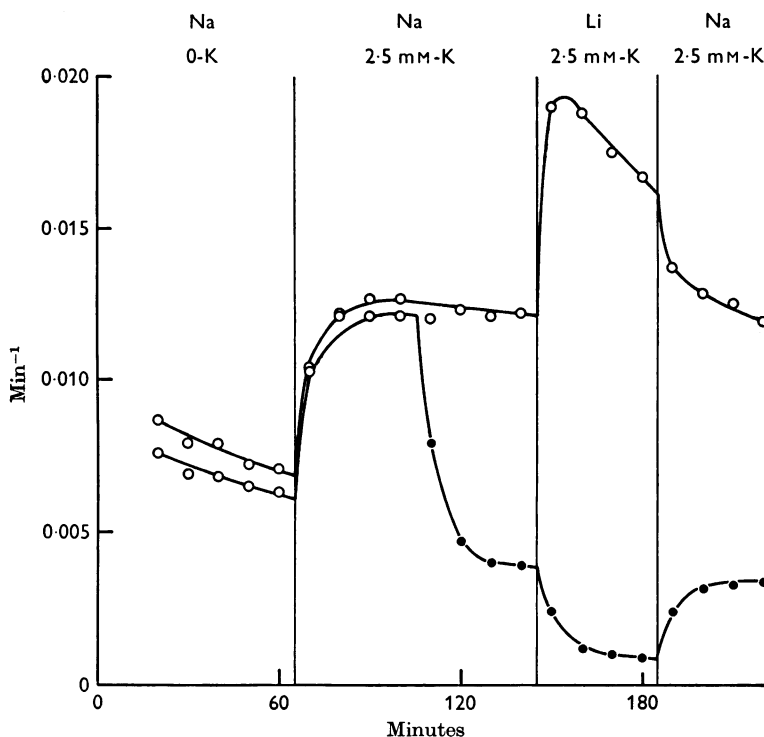

Fig. 4. Before the first collecting period, the muscles spent 22½ hr in inactive K-free Ringer at 2° C, then 4 hr in K-free ^{22}Na Ringer at room temperature, and finally 30 min in several changes of inactive K-free Ringer at room temperature. Solutions were then changed as shown. Symbols as before (open: control; filled-in: in 10^{-4} M ouabain). Temperature 21·3° C. Experiment of 23. vi. 65.

the total efflux (Table 2) instead of 63 % (Table 1). This component was still cut down to about a tenth of the original total efflux by removing the external sodium, whereas in the control muscle the lithium effect was reversed, as reported previously (Keynes, 1965). The effect of ouabain on the efflux into a lithium solution was thus very marked. From the experiment of Fig. 4 and three others like it, the average ratio of the efflux in K-free Ringer to that in 2·5 mM-K was 0·48 ± 0·03. Table 2 shows that the adjusted means of the effluxes from the ouabain-treated muscles in K-free Na and Li Ringer were roughly equal to those in similar solutions with

2·5 mM-K, confirming once more the lack of influence of $[K]_o$ on the glyco-side-insensitive component of the sodium efflux.

Effects of ouabain and lithium at low temperature. Since it seemed possible that the two components of the efflux might be differently affected by changes in temperature, the experiment illustrated in Fig. 5 was performed, following a similar procedure again, but with the muscle kept at 1·5° C

TABLE 2. The effects of ouabain and lithium on the sodium efflux in sodium-loaded frog sartorius muscles. Each figure is the ratio of the efflux to that in Na Ringer solution without ouabain. The adjusted mean in section *B* shows the size of the efflux relative to that in normal Ringer with 2·5 mM-K. In the experiments with 2·5 mM-K the concentration of ouabain was 10^{-5} M; in those with K-free solutions it was 10^{-4} M

Date	Temperature (°C)	In ouabain	In lithium	In ouabain + lithium
	A. Normal Ringer, [K] = 2·5 mM			
22. vi. 65	23	0·18	1·40	0·09
23. vi. 65	21	0·32	1·57	0·07
24. vi. 65	22	0·26	1·75	0·13
25. vi. 65	21	0·25	—	0·11
Mean and s.e.	22	0·25 ± 0·03	1·57 ± 0·10	0·10 ± 0·01
	B. K-free Ringer, [K] = 0 mM			
18. i. 66	21	0·73	1·27	0·17
19. i. 66	20	0·69	1·90	0·29
20. i. 66	18	0·72	1·33	0·16
Mean and s.e.	20	0·71 ± 0·01	1·50 ± 0·20	0·21 ± 0·04
Mean × 0·48	—	0·34	0·72	0·10

throughout the period spent in inactive Ringer. The behaviour of the glycoside-insensitive efflux was little altered, but the control muscle now showed a reversed lithium effect as if it had become sodium-loaded in the initial stages of the experiment. The effect of low temperature was only examined on one other occasion, when a very similar result was obtained; but it may be noted that Keynes & Swan (1959, Fig. 9) had also observed an abolition or slight reversal of the lithium effect after a somewhat shorter period at the low temperature. It is not at present clear whether the glycoside-sensitive component of the efflux does indeed increase much more markedly in response to lithium when the temperature is close to 0° C, or whether an explanation is to be sought in terms of a rise in the sodium content of one of the hypothetical compartments during exposure to low temperatures.

Effect of ouabain on sodium influx. Although a reduction of sodium efflux in a sodium-free external medium is often taken as evidence for the existence of the type of exchange diffusion mechanism postulated by Ussing (1949), such a conclusion rests on assumptions about the nature of the coupling between influx and efflux that have yet to be validated experimentally. Another way in which the occurrence of exchange diffusion could be established would be by a demonstration that treatment

with inhibitors like the glycosides blocks part of the sodium influx as well as part of the efflux. Such a demonstration has been provided for human erythrocytes by Garrahan & Glynn (1967), and an attempt was therefore made to see whether the sodium influx in frog muscle was affected by ouabain. The method used was that employed by Keynes & Swan (1959) to investigate the effect of external [Na] on the sodium influx, in which
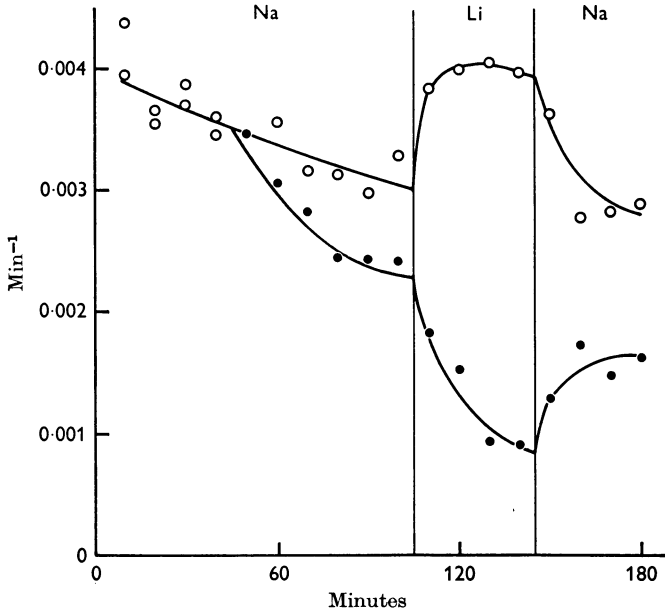

Fig. 5. After spending 160 min in ^{22}Na Ringer at room temperature, the muscles were washed in several changes of inactive Ringer at 1·5° C during the next 50 min. The temperature was maintained at 1·5° C throughout the rest of the experiment. All the solutions contained 2·5 mM-K. Symbols as before. Experiment of 10. v. 66.

the uptake of radioactivity by a test muscle exposed to ^{22}Na for 10 min was compared with that for its companion from the same frog acting as a control, allowance being made for any small differences in muscle weight or in the specific radioactivities of the solutions. As was admitted when it was first described, this technique is open to various objections as a way of determining the absolute size of the sodium influx, but it has again served quite well to measure relative values.

A typical experiment is shown in Fig. 6, and the results of a number of them are summarized in Table 3. In normal Ringer solution containing 2·5 mM-K, the influx was not significantly changed by treatment with 10^{-4} M ouabain, although the efflux, as given by the slope of the straight line (on a semi-logarithmic plot) drawn through the later counts, was once again (see Table 1) reduced by about a third. Since the slow phase of loss

of ^{22}Na did not follow a strictly exponential time course, the lines drawn to find the extrapolated uptake at zero time were made to pass through the counts at (*a*) 30 and 60 min, and (*b*) 40 and 100 min; but both procedures gave the same values for the flux ratios. It may be tentatively concluded that in 2·5 mM-K the ouabain-sensitive sodium efflux does not
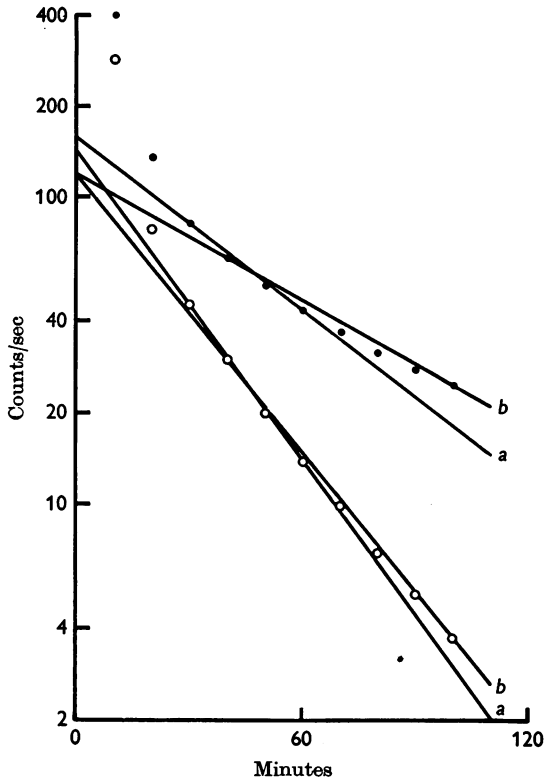

Fig. 6. The effect of ouabain on sodium influx. ●, muscle weighing 48·2 mg was pre-treated with 10^{-4} M ouabain for 40 min, and then spent exactly 10 min in ^{22}Na ouabain Ringer; the washing-out solution also contained 10^{-4} M ouabain. O, paired control weighing 49·1 mg was subjected to similar procedure without ouabain. All solutions contained 2·5 mM-K. Temperature 22·0° C. The straight lines used to determine the uptake of radioactivity were drawn through the counts at (*a*) 30 and 60 min, (*b*) 40 and 100 min. Experiment of 4. iv. 66.

have a substantial exchange diffusion component. This experiment cannot, of course, reveal whether or not the same is true for the ouabain-insensitive efflux.

When the experiment was repeated in K-free Ringer solution, the efflux was reduced by less than 20 %, again in reasonable agreement with the results in Table 1, but the influx now fell by about the same extent. It

would therefore appear that in frog muscle, as in red cells (Garrahan & Glynn, 1967), the ouabain-sensitive sodium efflux does involve a sodium–sodium exchange when external potassium ions are removed.

Changes in sodium efflux during long periods in lithium Ringer. The experiments described thus far establish that the sodium efflux is made up of two rather different components, but throw no light on the possible compartmentation of the sodium in frog muscle, since similar components are observed (see pp. 594–595) in tissues where it is unlikely that there is more than one intracellular compartment. We therefore turn to some

TABLE 3. The effect of 10^{-4} M ouabain on the sodium influx in frog muscle. The results are expressed as the values for the test muscle divided by the corresponding figures for the control muscle from the same frog, which had not been treated with ouabain. *a*: extrapolation made through the counts at 30 and 60 min. *b*: extrapolation made through the counts at 40 and 100 min

Date	Temperature (°C)	Ratio of influxes		Ratio of effluxes	
		a	*b*	*a*	*b*
A. In normal Ringer containing 2·5 mM-K					
5. x. 65	24·1	1·16	1·22	0·74	0·73
7. x. 65	24·8	1·27	1·32	0·68	0·68
8. x. 65	23·3	1·20	1·22	0·64	0·64
1. iv. 66	23·4	1·03	1·03	0·71	0·70
4. iv. 66	22·0	1·08	1·03	0·54	0·45
5. iv. 66	19·8	0·80	0·87	0·57	0·66
6. iv. 66	22·0	0·90	0·98	0·60	0·61
Mean and s.e.	—	1·06 ±0·06	1·10 ±0·06	0·64 ±0·03	0·64 ±0·03
B. In K-free Ringer					
21. iv. 66	22·7	0·83	0·88	0·87	0·92
26. iv. 66	24·2	0·72	0·73	0·71	0·69
27. iv. 66	23·0	0·86	0·89	0·87	0·90
2. v. 66	16·3	0·94	0·96	0·80	0·85
3. v. 66	18·9	0·72	0·81	0·80	0·86
Mean and s.e.	—	0·81 ±0·04	0·85 ±0·04	0·81 ±0·03	0·84 ±0·04

observations that do bear on the question of compartmentation. Keynes & Swan (1959) showed that the behaviour of the sodium efflux during a long exposure to a sodium-free solution, followed by a return to normal Ringer solution, was compatible *at first sight* with the idea that the intracellular sodium was contained in two compartments which exchanged their contents in parallel with the sodium of the extracellular phase, if it was assumed that the rate constants for the effluxes from the two compartments were roughly equal in normal Ringer, and that one rate constant was cut down much more than the other on transfer to lithium Ringer. The control muscle in the experiment illustrated in Fig. 7 behaved in much the same way as those examined by Keynes & Swan (1959) in that towards the end of the first period in lithium Ringer the rate constant fell to a steady low level as if the radioactivity had all been lost from the compart-

ment unaffected by lithium, but immediately jumped back to a value close to its original one when the external sodium was replaced. However, Fig. 7 also shows why the simplest form of the parallel-efflux model was eventually rejected. For had it been valid, the lithium-insensitive compartment would now have been entirely depleted of its ^{22}Na, and could not have influenced the loss of radioactivity any further, so that a final re-exposure to lithium should have brought the rate constant down at
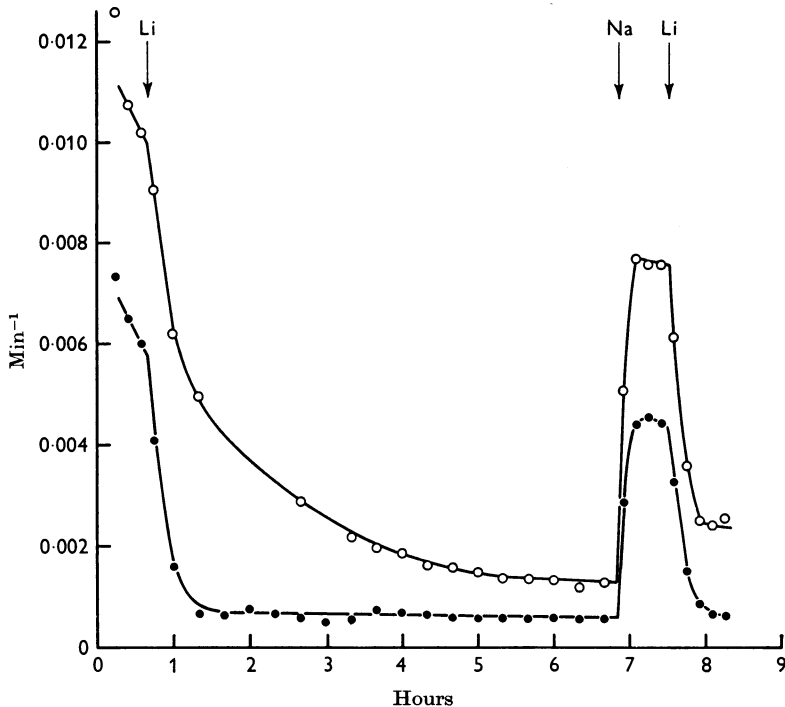

Fig. 7. Changes in the sodium efflux from paired frog sartorii during a long period in lithium Ringer solution. The muscles spent 3 hr in ^{22}Na Ringer at room temperature and were then transferred to inactive Na Ringer with and without 10^{-4} M ouabain. Counts taken during the first 30 min in inactive Ringer are not shown. The solutions were changed at the arrows as indicated. Open circles: control muscle. Filled circles: muscle in 10^{-4} M ouabain. Temperature about 20° C. Experiment of 31. i. 67.

once to the lowest level reached earlier. In fact, the second exposure to lithium did not reduce the sodium efflux much more rapidly than the first one. Another point that should be made here, in confirmation of the conclusion of Keynes & Swan (1959), is that a model consisting of two compartments arranged in series rather than parallel could not behave like the control muscle in Fig. 7. For if the external medium did not have access to the membrane separating the innermost and intermediate compartments,

such a system could not exhibit the observed *gradual* reduction in rate constant on removal of external sodium, but *abrupt* rise on its restoration.

A new finding illustrated in Fig. 7 was that in the muscles treated with 10^{-4} M ouabain the rates of reduction and restoration of the efflux on removing and replacing external sodium were about the same. Moreover, the objection to the parallel-efflux model explained in the preceding paragraph no longer applied. Under these conditions the behaviour of the muscle could be accounted for equally well by two compartments in parallel, or two in series, or for that matter by a single intracellular compartment.

In order to examine the multi-compartment hypothesis in a more quantitative way, the results of nine experiments similar to Fig. 7 were analysed by fitting relationships of the form

$$\text{total } ^{22}\text{Na in muscle} = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} \qquad (1)$$

and
$$\text{rate of loss of } ^{22}\text{Na} = k_1 A_1 e^{-k_1 t} + k_2 A_2 e^{-k_2 t}. \qquad (2)$$

where A_1 and A_2 are in c/s, k_1 and k_2 are rate constants in min^{-1} and t is time in min.

Since in most cases $A_1 e^{-k_1 t}$ was negligibly small at the end of the period in lithium Ringer, k_2 and A_2 could be obtained from the last values for the efflux and total radioactivity remaining in the muscle, the total time that had elapsed also being known. It was then a simple matter to work out k_1 and A_1 from the efflux and total radioactivity at zero time. In two cases where $A_1 e^{-k_1 t}$ had not quite reached zero, the values of k_1, k_2, A_1 and A_2 were obtained by a process of successive approximation. It will be seen from Table 4 that in the control muscles the rate constant fell in lithium Ringer to about a fifth of its initial value in sodium Ringer, but was restored nearly to its original level during the second period in normal Ringer. In the ouabain-treated muscles the efflux fell more markedly in lithium Ringer, and did not recover quite as completely. In the controls, the average value of the ratio of k_2 at the end of the second period in lithium Ringer to k_2 at the end of the first period was 2·0; in the poisoned muscles the ratio averaged 1·2, and in all but the last two cases it was close to unity. The analysis suggests that in the control muscles, while one of the rate constants decreased on transfer to lithium, the other was roughly doubled; in the ouabain-treated muscles the calculated value of k_1 was even higher. However, the slowness of diffusion in the muscle interspaces must have introduced a larger error in the latter case; thus recalculation of the results using the counts for the second 20 min period in Li instead of the first gave values for k_1 that were only about 10 % lower for the controls, but were nearly 50 % lower for the poisoned muscles. Finally, it may be noted that $A_1/(A_1+A_2)$ was only about one third as great in the ouabain-treated muscles as it was in the controls.

The most obvious way of modifying the parallel-efflux model to over-come the objection discussed on p. 591 is to add a series connexion between the two intracellular compartments as indicated in Fig. 9, thus providing a pathway for partially reloading the lithium-insensitive compartment with ^{22}Na during the second period in normal Na Ringer. This idea seems to be supported, at least qualitatively, by the experiment shown in Fig. 8,

TABLE 4. Values for rate constants (in min^{-1}) and activity ratios $A_1/(A_1+A_2)$ obtained by fitting eqns. (1) and (2) to the data for experiments of the kind shown in Fig. 7. The rate constants in Na represent the last value for the initial period in normal Ringer, and the peak value reached during the second period in normal Ringer. Temperature 20·0–20·5° C

| Date	First period in Na	First period in Li k_1	k_2	$A_1/(A_1+A_2)$	Second period in Na	Second period in Li k_1	k_2	$A_1/(A_1+A_2)$
			A. Control muscles, unpoisoned					
24. i. 67	0·0097	0·025	0·0029	0·40	0·0077	0·030	0·0023	0·180
26. i. 67	0·0117	0·024	0·0022	0·42	0·0089	0·104	0·0034	0·042
31. i. 67	0·0102	0·021	0·0013	0·39	0·0077	0·131	0·0026	0·028
6. ii. 67	0·0162	0·028	0·0024	0·49	0·0132	0·100	0·0079	0·057
8. ii. 67	0·0143	0·024	0·0024	0·42	0·0135	0·083	0·0063	0·070
16. ii. 67	0·0135	0·034	0·0030	0·20	0·0108	0·065	0·0057	0·077
28. ii. 67	0·0131	0·022	0·0028	0·40	0·0108	—	—	—
6. iii. 67	0·0143	0·027	0·0049	0·29	0·0119	—	—	—
15. iii. 67	0·0191	0·046	0·0044	0·24	0·0178	—	—	—
Means	0·0136	0·028	0·0029	0·36	0·0114	0·085	0·0047	0·076
			B. Paired muscles treated with 10^{-4} M ouabain					
24. i. 67	0·0039	0·108	0·0012	0·02	0·0029	0·128	0·0013	0·007
26. i. 67	0·0036	0·099	0·0008	0·02	0·0034	0·122	0·0010	0·011
31. i. 67	0·0060	0·066	0·0006	0·06	0·0046	0·110	0·0006	0·024
6. ii. 67	0·0102	0·044	0·0007	0·14	0·0065	0·100	0·0008	0·037
8. ii. 67	0·0126	0·024	0·0012	0·29	0·0077	0·114	0·0013	0·035
16. ii. 67	0·0065	0·053	0·0006	0·05	0·0061	—	—	—
28. ii. 67	0·0037	0·016	0·0002	0·11	0·0014	0·089	0·0001	0·005
6. iii. 67	0·0115	0·020	0·0013	0·31	—	0·100	0·0023	0·011
15. iii. 67	0·0169	0·034	0·0019	0·22	0·0118	0·100	0·0030	0·026
Means	0·0083	0·052	0·0009	0·14	0·0056	0·108	0·0013	0·020
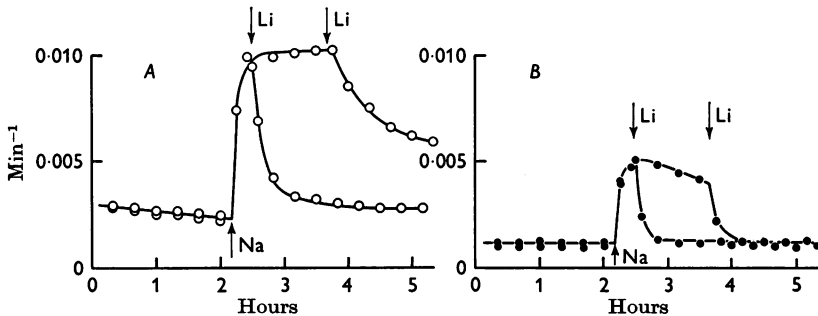
Fig. 8. The effect on the final efflux in Li Ringer of varying the duration of an interpolated period in Na Ringer. Counts taken during a preliminary 3 hr period in Li Ringer are not shown. In each experiment, both muscles were transferred from Li to Na Ringer at the first arrow; one muscle was then left in Na Ringer for only 20 min (until the second arrow), while the other remained for altogether 90 min. A: muscles not treated with ouabain. B: the washing-out solutions contained 10^{-4} M ouabain. Temperature 21° C.

from which it is clear that in the unpoisoned muscle the final decline of the rate constant in Li Ringer was appreciably delayed by allowing a longer period of hypothetical reloading. Once again, in the experiments on ouabain-treated muscles the rate constant fell rapidly to a constant low level in lithium Ringer, and returned to the same value after re-exposure to sodium Ringer for either 20 or 90 min. If the model of Fig. 9 is correct, and as will be seen on pp. 596–598 this has certainly not been proved, the simplest explanation for the action of ouabain would be that it blocks the connexion between compartments 1 and 3, converting the system into two compartments in series with a lithium-sensitive membrane outermost. This seems preferable to supposing that ouabain acts on the internal membrane separating compartments 1 and 2, to which it presumably does not have ready access.

<div align="center">DISCUSSION</div>

It is naturally attractive to suppose that the mechanisms by which different types of cell maintain a low internal sodium concentration are likely to have some features in common. It was therefore satisfactory to find that frog muscle fibres behaved like human erythrocytes (Garrahan & Glynn, 1967) in exhibiting a ouabain-sensitive sodium–sodium exchange on removal of external potassium, but not in 2·5 mM-K. The evidence on the behaviour of the ouabain-sensitive component of the sodium efflux towards substitution of lithium for external sodium is that in squid axons (Baker, 1968) and erythrocytes (Garrahan & Glynn, 1967) there is little change; in freshly dissected frog muscles the same is true, since from Table 1 A the 51 % reduction in efflux in lithium seen in unpoisoned muscles can virtually all be accounted for by the effect on the ouabain-insensitive components $(63 - 14 = 49 \%)$. Sodium-loaded muscles provide an exception and show a marked increase in ouabain-sensitive efflux in lithium Ringer (see Fig. 4 and Table 2), perhaps resembling the increase seen in squid axons with other sodium substitutes such as choline or dextrose (Baker, 1968). The ouabain-sensitive sodium efflux is always dependent on external potassium, though clearly the sodium–potassium coupling ratio may vary both from tissue to tissue and with internal sodium concentration, as suggested by several authors.

Under normal physiological conditions, the ouabain-insensitive sodium efflux accounts for a greater proportion of the total sodium efflux in frog muscle than it does in squid axons or erythrocytes, e.g. for 63 % (Table 1) as against about 30 % (Baker, Blaustein, Manil & Steinhardt, 1967) or 33 % (Garrahan & Glynn, 1967). The difference may not be particularly significant, and since in sodium-loaded muscles the proportion falls to 25 % (Table 2), it could merely indicate that the ouabain-insensitive channel

tends to saturate at lower internal sodium levels than the ouabain-sensitive channel, and so becomes relatively less predominant when $[Na]_i$ increases. Both in frog muscle and in erythrocytes (Garrahan & Glynn, 1967) the ouabain-insensitive component of the sodium efflux is also insensitive to the external concentration of potassium; in squid axons this component of the efflux does depend on external potassium, and is reduced in a K-free solution (Baker, 1968). The greatest differences between the three tissues are seen when external sodium is replaced by lithium. In squid axons there is an appreciable rise in the sodium efflux (see Keynes, 1965, fig. 9), which is now known to be due entirely to an effect on the ouabain-insensitive components (Baker, 1968). In erythrocytes the ouabain-insensitive efflux is very slightly reduced on complete removal of external sodium (Garrahan & Glynn, 1967), while in frog muscle, as we have shown here, it is always markedly reduced. An interesting recent discovery is that in squid axons part of the ouabain-insensitive sodium efflux is dependent on the presence of calcium in the external medium (Baker *et al.* 1967). In contrast, Garrahan & Glynn (1967) reported a slight rise in the sodium efflux from ouabain-treated erythrocytes when calcium was removed. In some preliminary experiments on frog muscle, we did not find that changes in calcium concentration had any marked effects on the ouabain-insensitive sodium efflux, but this point deserves more detailed investigation. In conclusion, it seems clear that the properties of the sodium pump or pumps in these three tissues are quite far from being identical, although they certainly present some common features.

There is no reason for thinking that erythrocytes or squid axons may possess more than one intracellular sodium compartment, and the greater relative size of the ouabain-insensitive sodium efflux in frog muscle fibres may therefore reflect the presence in these alone of a second sodium compartment. As has already been mentioned, there is a good case for exploring the possibility that frog muscle may resemble the series-parallel system shown in Fig. 9, where compartment 1 would represent the sarco-plasm proper, compartment 2 the sarcoplasmic reticulum, and compart-ment 3 the extracellular space. Although one of the strongest reasons for proposing, not for the first time, that striated muscle fibres should be treated as multi-compartment systems is the recent anatomical evidence, there is an immediate difficulty in identifying anatomically the direct connexion between compartments 2 and 3, since the electron micrographs of Peachey (1965) and others suggest that the reticulum is located entirely within the sarcoplasm, and could only communicate with the extracellular phase via compartment 1. However, as has been pointed out on p. 591, the tracer evidence is definitely incompatible with a purely series system, and demands the existence of such a direct pathway. Moreover, the mechanism

for activating muscular contraction by release of calcium from the sarco-plasmic reticulum appears to require some form of preferential connexion between the external surface of the fibre and the lateral vesicles via the T-system, and sodium might also traverse this pathway. It may be pointed out that although few if any of the transverse tubules can be seen in electron micrographs to communicate with the outer surface, there is no
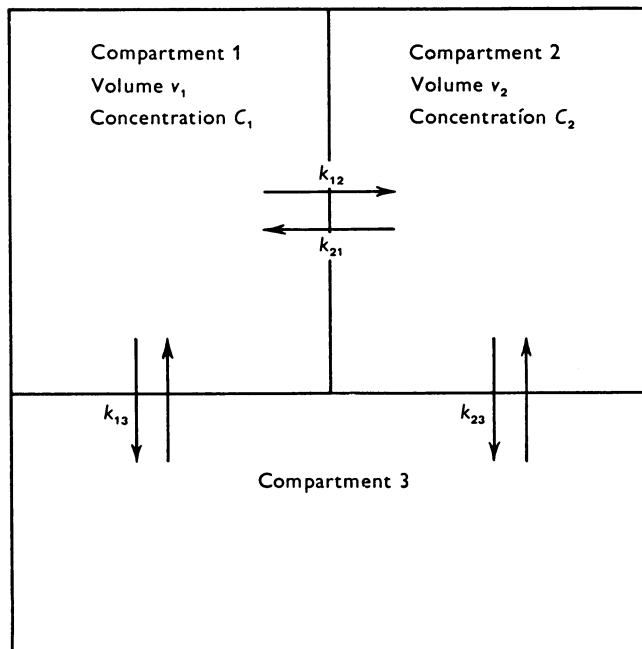

Fig. 9. A series-parallel multi-compartment model, in which compartment 1 is tentatively identified with the sarcoplasm and compartment 2 with the sarco-plasmic reticulum.

longer any doubt that large molecules can penetrate into them (Peachey, 1965), and the lack of a visible channel between the reticulum and the extracellular phase should therefore not be allowed to rule out the possi-bility of its existence.

The next step is to see what the time course of loss of radioactivity from a series-parallel system would be. If it is assumed that the flux from any compartment is directly proportional to the concentration in that com-partment, i.e.

$$\text{flux } 1 \to 2 = \alpha_{12} . C_1, \tag{3}$$

where C_1 is the concentration in compartment 1 and α_{12} is a constant, then the rate constant k_{12} can be written as

$$k_{12} = \frac{\alpha_{12} . A_{12}}{v_1}, \tag{4}$$

where A_{12} is the area of membrane between compartments 1 and 2, and v_1 is the volume of compartment 1. For the loss of Na* into an inactive medium, it can be shown (cf. Sheppard, 1962) that the total activity remaining in compartments 1 and 2 at time t is given by

$$\text{Na}_T^* = \frac{(k_{13}+q)v_1 . C_{10} + (k_{23}+q)v_2 . C_{20}}{(q-p)} . e^{pt}$$

$$+ \frac{(k_{13}+p)v_1 . C_{10} + (k_{23}+p)v_2 . C_{20}}{(p-q)} . e^{qt}, \tag{5}$$

where C_{10} and C_{20} are the concentrations at zero time, and p and q are the roots of

$$(x + k_{12} + k_{13})(x + k_{21} + k_{23}) = k_{12} . k_{21}. \tag{6}$$

For the parallel-efflux model, we put $k_{12} = k_{21} = 0$ and the solution becomes

$$\text{Na}_T^* = v_1 . C_{10} . e^{-k_{13}t} + v_2 . C_{20} . e^{-k_{23}t}. \tag{7}$$

The solution for the series system is obtained by putting $k_{13} = 0$. It is

$$\text{Na}_T^* = \frac{r . v_1 . C_{10} + (k_{23}+r)v_2 . C_{20}}{(r-s)} . e^{st}$$

$$+ \frac{s . v_1 . C_{10} + (k_{23}+s)v_2 . C_{20}}{(s-r)} . e^{rt}, \tag{8}$$

where r and s are the roots of

$$x^2 + x(k_{12} + k_{21} + k_{23}) + k_{12} . k_{23} = 0. \tag{9}$$

The fact that eqns. (1) and (5) resemble one another in being the sum of two exponential terms provides, as is well known (Sheppard, 1962), no support for the validity of the series-parallel model, since eqns. (7) and (8) have the same form and could equally well have been fitted to the results. Another inescapable fact is that the complexity of eqn. (5) makes it impossible to determine unequivocally from results like those in Table 4 the sizes of the individual rate constants or the initial amounts of radioactivity in the separate compartments; extra assumptions or measurements must be made if all the parameters are to be evaluated. Moreover, in at least one respect the results do not seem easily reconcilable with eqn. (5), for in both sets of muscles k_1 was appreciably greater during the second period in Li Ringer than it was during the first, and in the control muscles the same may have been true for k_2; but since the exponents p and q are functions only of the rate constants and not of the initial concentrations, k_1 and k_2 should have been the same on both occasions. As has already been mentioned (see p. 592), the values of k_1 for the ouabain-treated muscles

were grossly distorted by diffusion time effects; but in the case of the controls the discrepancy is hard to explain, unless of course the assumption of first-order kinetics embodied in equation (3) is invalid. In this connexion, it must not be forgotten that Keynes (1965) presented a number of observations that could plausibly be interpreted in terms of a single compartment with higher order kinetics; these observations would still need to be explained if there are really two intracellular compartments rather than one. A further stumbling block is provided by the relatively smaller size of $A_1/(A_1 + A_2)$ in the ouabain-treated muscles, when it might have been predicted that in these muscles Na would have accumulated in compartment 1, thus raising the value of $C_{10}/(C_{10} + C_{20})$.

In view of these difficulties, and since our analysis has in any case been simplified undesirably by the omission of any consideration of the effects of variation in the size and ease of access to the external solution of the individual muscle fibres, we feel that a more detailed attempt to fit the results to eqn. (5) would not be profitable. But although it must be recognized that the two components of the sodium efflux, whose existence does seem to be well established by our experiments, are unlikely to be each confined to a particular compartment, the idea that striated muscle fibres should be regarded as a series-parallel two compartment system certainly deserves further exploration. Quite a number of the facts that we have described would be compatible with such a system in which the efflux from the sarcoplasm (compartment 1) was mainly potassium- and ouabain-sensitive and was raised in lithium Ringer, while the efflux from the reticulum (compartment 2) was mainly insensitive to potassium and ouabain and markedly reduced by lithium. Obviously it would be interesting to examine some of the consequences of this hypothesis in isolated muscle fibres, where solutions can be changed quickly and the effects of variation in fibre diameter avoided; and muscles which lack a sarcoplasmic reticulum should also be examined. A recent observation that may be of considerable assistance in this context is that of Eisenberg & Gage (1967), who have shown that treatment of sartorius muscles with Ringer solution to which 400 mM glycerol has been added appears somehow to disrupt the connexion between the T-system and the fibre surface. If it could be assumed that this procedure reduced k_{23} in the model of Fig. 9 to zero without affecting the other rate constants, it would provide a valuable tool for evaluating the other unknowns. But preliminary tests have suggested that glycerol treatment may affect more than one component of the system, in which case it will not lead to an immediate answer to the difficult problems posed here.

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