J. Physiol. (1959) 147, 626–638

THE PERMEABILITY OF FROG MUSCLE FIBRES TO LITHIUM IONS

By R. D. KEYNES and R. C. SWAN
From the Physiological Laboratory, University of Cambridge

(*Received* 20 *April* 1959)

When Overton (1902) studied the excitability of frog muscle in different media, he found that lithium was the only cation which could be substituted for sodium without causing the fibres to become inexcitable. This suggests that in some ways the behaviour of the muscle membrane towards lithium must be similar to its behaviour towards sodium. In other respects, however, the membrane discriminates between sodium and lithium, as is shown by the experiments described in the preceding paper (Keynes & Swan, 1959) on the effect of lithium on the efflux of labelled sodium from frog muscle. The results of this work on the sodium efflux could not be interpreted without knowing more about the rate of movement of lithium ions through the membrane, and this paper is therefore concerned with some measurements of the lithium influx into freshly dissected muscles, and with the rate at which lithium moves outwards from lithium-loaded muscles. Since lithium has no usable radioactive isotopes (the half-life of ^{8}Li is under 1 sec), we were only able to measure the net rate of transfer of lithium into and out of the muscles, using a flame photometer to determine the lithium contents of muscles treated in various ways. But some useful figures were obtained for the resting influx of lithium from a solution similar in its composition, except for the substitution of Li for Na, to normal frog Ringer's solution, and the extent to which lithium can be actively extruded from the interior of the muscle fibre was also studied. Finally, a few observations were made with intracellular micro-electrodes to see whether there was any obvious difference between the action potentials recorded in Na and in Li Ringer's solution.

METHODS

Material

Pairs of sartorius muscles were dissected from small specimens of *Rana temporaria*, and tied to glass racks at their body length, as described by Keynes & Swan (1959). In order to minimize interfibre diffusion times, muscles weighing between 20 and 40 mg were preferred, but a few larger

muscles were used on occasions when no small frogs were available. The muscles were dissected as cleanly as possible at the pelvic ends, leaving only enough connective tissue for them to be tied to the racks without crushing the ends of the fibres. When there was any suspicion that either muscle of the pair had been damaged in the course of dissection, both were rejected. They were also rejected if at the conclusion of the experiment either muscle contained more than about half a dozen fibres whose myoplasm was visibly clotted. Excitability was checked visually at the beginning and end of each experiment; except in cases where the final solution to which they were exposed contained 10 mM-K, they always gave good twitches, even when the experimental soaking procedure had lasted for nearly 3 days. The experiments were done during the months of March, July and August.

Solutions

For the determinations of lithium influx, and for recording action potentials, the 'normal' Ringer's solution contained 2·5 mM-K and had the composition shown in Table 1. In order to load the muscles with Na or Li, they were sometimes soaked at a low temperature in the low-K 'soaking-in' solution shown in Table 1, and sometimes for a shorter period in a similar solution from which the potassium phosphate was omitted. The 'sodium recovery' solution was similar to the medium devised by Boyle & Conway (1941) to imitate closely the inorganic composition of frog plasma, except that (following Desmedt, 1953), it contained 10 mM-K. On some occasions a K-free modification of it was used, the KCl being omitted, but the other components left unchanged. The 'lithium recovery' solution had Li substituted for all the Na except the 0·9 mM added as gluconate; since $LiHCO_3$ was not available as such, it was made up by taking appropriate quantities of 'Specpure' Li_2CO_3 and HCl A.R. Both recovery solutions were equilibrated with 3 or 5% CO_2 in O_2.

TABLE 1. Composition of solutions (mM)

Normal Ringer's		Soaking-in solution		Sodium recovery medium		Lithium recovery medium	
NaCl or LiCl	111·2	NaCl or LiCl	120	NaCl	73	LiCl	78·5
K_2HPO_4	1·14	K_2HPO_4	0·125	Na_2HPO_4	2·5	K_2HPO_4	2·5
KH_2PO_4	0·23	KH_2PO_4	0·025	NaH_2PO_4	0·5	KH_2PO_4	0·5
$CaCl_2$	1·8	$CaCl_2$	0·9	KCl	10	KCl	4·5
				$CaCl_2$	0·9	$CaCl_2$	0·9
				$NaHCO_3$	25	$LiHCO_3$	25
				$MgSO_4$	1·5	$MgSO_4$	1·5
				Na gluconate	0·9	Na gluconate	0·9
				Glucose	26	Glucose	26

Analyses

After the muscles had been exposed to the various solutions as described under 'Results', they were cut off the glass racks and their excitability was checked. They were next thoroughly blotted, a few millimetres at either end was cut off to minimize errors from connective tissue and remnants of attachments to other muscles, and their central parts were put into platinum crucibles, in which they were weighed. After drying at 110° C, they were reweighed, and were then dry-ashed during the night in an oven at about 600 °C. The ash was dissolved in a drop of strong HCl (the ash of Li-treated muscles did not dissolve completely in pure water, perhaps because of the relative insolubility of Li_2CO_3), and the crucibles were then nearly filled with distilled water; their capacity was slightly over 5 ml. The exact dilution for each muscle was determined by weighing the crucibles, and 1·0 ml. samples were next taken by pipette and made up to 25 ml. in standard flasks for Na and K determinations with an EEL flame photometer. The sensitivity of the flame photometer for Li was appreciably less than for Na, so that to determine Li it was necessary to spray fluid directly from the crucibles, the 4 ml. remaining being quite enough to take readings in triplicate.

RESULTS

The lithium influx into frog muscle

The main problem in measuring the influx of lithium into sartorius muscles was that of discriminating between lithium which had merely exchanged with the sodium in the extracellular space of the tissue, and that which had entered into the interior of the fibres. Two methods of approach were tried, giving results that were in tolerable agreement. The first consisted in analysing pairs of muscles after one had been exposed to Li Ringer's solution for about 0·5 hr, which should have been long enough for all the extracellular Na but not much of the intracellular Na to have been replaced by Li (see Na values in Table 2), and after the other had been in the same solution for a further 2 hr. The difference between their Li contents was taken to represent Li that had penetrated into the fibres during the extra 2 hr. The disadvantage of this

TABLE 2. The rate of gain of lithium in pairs of muscles soaked for various periods in a solution containing 111·2 mM-Li and 2·5 mM-K

Muscle *A*			Muscle *B*			
Time in Li (min)	Li content (m-mole/kg)	Na content (m-mole/kg)	Time in Li (min)	Li content (m-mole/kg)	Na content (m-mole/kg)	Rate of gain of Li (m-mole/kg.min)
24	25·6	—	158	24·6	—	−0·007
24	16·7	2·9	144	22·6	1·3	+0·049
24	19·1	2·7	144	24·5	2·2	+0·045
24	31·0	4·1	144	39·0	3·4	+0·067
30	15·8	9·2	150	17·0	5·3	+0·010
30	17·5	5·7	150	20·3	2·8	+0·023
30	17·9	7·9	150	19·7	3·3	+0·015
Mean	20·5	5·4	—	24·0	3·1	+0·029

technique was that it depended on there being a good correlation between the amounts of extracellular Li in the members of each pair. However, the figures in Table 2 show that despite appreciable variation in total Li content between the pairs, in six cases out of seven the muscle exposed for the longer period contained slightly more Li than its companion. The average rate of gain of Li in the seven experiments was 0·029 m-mole/kg.min. Taking the muscle density as 1·05 g/ml., the extracellular space as 13%, and the mean fibre diameter as 80μ (see Keynes, 1954), 1 kg of frog sartorius contains 830 ml. of fibres whose total surface area is $4{\cdot}15 \times 10^5$ cm^2. The average lithium influx was therefore about 1·2 pmole/cm^2.sec.

The second method involved the exposure of muscles to Li Ringer's solution for about 2 hr, followed by a period of washing in normal Ringer's solution long enough to ensure removal of all the extracellular Li, and finally analysis of the Li remaining in the muscles. The experiments on Li efflux described in the following section showed that once Li had entered the fibres it was not lost very rapidly, so that it could be expected that 30 min of washing in

normal Ringer's solution would not cause a serious depletion of the intracellular Li. As a check on this assumption the muscles were treated in pairs; both were soaked in Li Ringer's solution for the same period, and one was then washed in normal Ringer's solution for 30 min, the other for about 70 min. It may be seen from Table 3 that during the extra 40 min of washing the amount of lithium in the muscles only decreased by about 15%. The Li contents of the muscles in group *A* should therefore represent a fairly reliable estimate of the intracellular Li gained during 2 hr in Li Ringer's solution (if the muscles had still contained traces of extracellular Li, this would offset

TABLE 3. Lithium content of pairs of muscles first soaked about 2 hr in a solution containing 111·2 mM-Li and then washed for various periods in normal Ringer's solution

	Muscle *A*		Muscle *B*		Rate of gain of Li
Time in Li (min)	Washing time (min)	Li content (m-mole/kg)	Washing time (min)	Li content (m-mole/kg)	of muscle *A* (m-mole/kg.min)
151	28	7·6	65	6·5	0·050
119	25	8·6	65	7·2	0·072
120	32	7·1	68	5·8	0·059
120	36	5·5	72	4·1	0·046
120	41	5·9	75	6·1	0·049
Mean					
126	32	6·9	69	5·9	0·055

any slight losses of intracellular Li during the 30 min period of washing). The average rate of gain of Li for 5 muscles was 0·055 m-mole/kg.min. On the same basis as before, this corresponds to an influx of 2·2 pmole/cm^2.sec. Although the second method therefore gives a slightly higher average figure than the first, the individual values overlap one another (see Tables 2 and 3) and there is no essential disagreement between the two techniques.

The efflux of lithium from frog muscle

In order to study the rate at which lithium would move outwards from frog muscle fibres, it was necessary to subject the muscles to a preparatory period of loading with lithium. This was done by soaking the muscles at a low temperature (2° C) either for 44–69 hr in a low-K lithium solution (see Table 1), or for 27–40 hr in a similar solution with potassium omitted altogether. The muscles were mounted on racks at their body length, and oscillated up and down in a large volume of solution once per second throughout the period of soaking-in; the solution was changed every 12 hr. At the end of the soaking-in period almost all the sodium in the muscles (see Table 6), and a large part of their potassium (see Tables 4–6) had been replaced by lithium. It is of interest to note in passing that in spite of virtually complete removal of both external and internal sodium (the 1 m-mole Na/kg remaining in the muscles may have been located in the connective tissue, as the inexchangeable fraction described by Harris & Steinbach (1956)), the muscles

were still capable of twitching in what appeared from visual inspection to be a normal fashion.

The principle on which all the experiments were based was to determine the starting levels of lithium, potassium and sodium by analysing one muscle from each pair on completion of the preparatory period, and then to determine the changes in cation levels after the second muscle had been exposed for a few hours to a recovery solution. The closeness of correlation between the starting levels in the individual muscles of each pair was not examined specifically, but the remarkably small differences in lithium content for the shortest recovery periods (see Tables 4–6), despite a much greater range of variation between different pairs, suggests that the agreement between paired muscles must have been rather good. In the experiments of Tables 4 and 5, in which the test muscles were treated with recovery solutions containing sodium, the preparatory period was concluded by soaking both muscles for 1 hr at 2° C in a low-K 120 mM-NaCl solution, in order to replace extracellular lithium, and so to make the two members of the pair directly comparable as far as intracellular lithium was concerned. In the experiments of Table 6 this was unnecessary, both control and test muscles being taken directly out of lithium solutions.

When Li-loaded muscles were soaked in a sodium recovery solution containing 10 mM-K (for composition see Table 1), the lithium was found to emerge slowly from the fibres. The analytical values for all three alkali metal cations are given in Table 4, and the changes in lithium with time are also plotted in Fig. 1. In order to make some allowance in Fig. 1 for variation in starting levels, the lithium contents of the test muscles are expressed as percentages of the contents of the control muscles. The average lithium content of the nine controls was 50·4 m-mole/kg, and the average initial rate of loss of lithium was of the order of 4 m-mole/kg.hr. The results are not sufficiently precise to reveal the exact time course of lithium loss, but an exponential with a time constant of just over 10 hr fits the data fairly well (a straight line would give an equally good fit, but it seems more likely that the disappearance of lithium pursues a roughly exponential course). The average rate constant for lithium loss was 0·079 hr^{-1}; ignoring the two muscles for which the recovery periods were shortest and the values therefore least reliable, the average was 0·095 hr^{-1}. This may be compared with the much faster loss of sodium from Na-loaded muscles allowed to recover in an identical solution. Desmedt's (1953) results correspond to an initial rate constant for sodium loss of about 1·2 hr^{-1} (see Keynes, 1954). The outward movement of lithium was thus over ten times slower than the outward movement of sodium under similar conditions.

To check that the muscles used in our experiments were capable of a rapid net sodium extrusion, six pairs of muscles were loaded with sodium by

soaking in low-K 120 mM-NaCl, and one muscle of each pair was allowed to recover for 1–3 hr in the high-K recovery medium. The average starting levels were 64·4 m-mole Na/kg and 48·8 m-mole K/kg; after recovery the test muscles all contained substantially less sodium and more potassium, the final levels being 36·1 m-mole Na/kg (a loss of 28·3 m-mole/kg) and 81·3 m-mole K/kg (a gain of 32·5 m-mole/kg). These figures agree satisfactorily with

TABLE 4. Changes in cation content (expressed in m-mole/kg wet weight) of pairs of Li-loaded muscles soaked for various periods at room temperature (17–22° C) in a high-K sodium recovery medium

Recovery time (hr)	Dry weight (%)	Contents of			Change in			Rate constant for loss of Li (hr^{-1})
		Li	K	Na	Li	K	Na	
0	16·8	74·1	11·5	27·4				
0·8	16·1	75·1	17·1	26·9	+ 1·0	+ 5·6	−0·5	—
0	20·0	37·3	35·4	37·5				
1·3	21·2	35·1	48·9	27·6	− 2·2	+13·5	−9·9	0·047
0	18·5	59·0	24·0	32·0				
2·1	19·4	49·2	42·8	27·4	− 9·8	+18·8	−4·6	0·087
0	22·6	40·0	59·1	16·5				
2·5	21·8	29·2	80·2	17·7	−10·8	+21·1	+1·2	0·126
0	20·9	45·9	36·4	31·1				
3·1	19·8	36·4	56·0	31·9	− 9·5	+19·6	+0·8	0·075
0	21·4	43·3	51·0	18·4				
4·0	21·7	28·5	84·5	17·9	−14·8	+33·5	−0·5	0·105
0	19·0	49·3	35·2	33·5				
4·4	19·8	30·5	60·9	28·2	−18·8	+25·7	−5·3	0·092
0	19·1	41·5	52·0	23·8				
5·5	19·4	25·9	84·0	20·4	−15·6	+32·0	−3·4	0·086
0	18·5	63·2	19·3	29·7				
6·3	18·7	35·1	58·1	31·0	−28·1	+38·8	+1·3	0·093

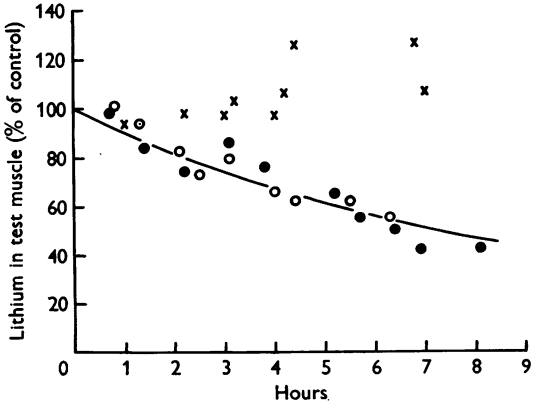

Fig. 1. The decline with time of the Li contents of test muscles. ○, in a high-K sodium recovery medium. ●, in a K-free sodium recovery medium. ×, in a high-K lithium recovery medium. For further details see Tables 4, 5 and 7. Temperature 16–22° C.

Desmedt's observations, but the recovery periods were not short enough to yield further values for the initial rate of sodium loss.

While lithium was lost from the muscles, potassium was regained. Table 4 shows that the movements were not exactly reciprocal, the potassium entry exceeding lithium loss by an average of 11·1 m-mole/kg (S.E. ± 1·3 m-mole/kg). The changes in sodium content, on the other hand, were small and their average (a loss of 2·3 m-mole/kg, S.E. ± 1·2 m-mole/kg) did not differ significantly from zero. The percentage dry weight of the muscles also remained unaltered (average change +0·1%, S.E. ± 0·3%), showing that there were no gross volume changes during recovery. The extra gain of potassium was of the right order to have occurred passively in consequence of the type of Donnan equilibrium discussed by Boyle & Conway (1941), the recovery solution being hypertonic relative to the soaking-in solution by just about the 10 mM-KCl that it contained.

Two explanations might be advanced for the observed interchange of lithium and potassium. One would be that the sodium pump is able to extrude lithium actively in exchange for potassium, albeit at under a tenth of the rate at which it can extrude sodium. However, it may well not be legitimate to classify an outward transfer of lithium into a solution containing no lithium as an active transport process, and an alternative hypothesis would be that lithium is somehow exchanged passively for sodium, and that the sodium which thus enters the cell is then extruded in the normal manner in exchange for potassium. These possibilities were examined by doing two further sets of recovery experiments, to see whether lithium could be extruded (*a*) into a K-free sodium recovery solution, and (*b*) into a high-K recovery solution containing lithium instead of sodium.

For (*a*) the procedure adopted was exactly the same as before, but the 10 mM-KCl was omitted from the recovery solution, the other components being left unaltered. As may be seen from Table 5, the average starting level for lithium was slightly lower (at 38·0 m-mole/kg) than in the experiments of Table 4, but again lithium was slowly lost from the muscle. It is clear from the column of rate constants in Table 5 and from the percentage changes plotted in Fig. 1 that the rate of loss was indistinguishable from that observed in the presence of 10 mM-K; the average rate constant was 0·092 hr^{-1} (or, without the first two figures, 0·096 hr^{-1}). The movement of potassium, on the other hand, was now outwards instead of inwards, and in most cases was slightly greater than the loss of lithium. The losses of lithium and potassium were nearly balanced by a gain of sodium, the over-all average change in total cation content being −6·5 m-mole/kg. It would therefore appear that the process by which the bulk of the lithium leaves the fibres is not dependent on a simultaneous inward movement of potassium. Moreover, since the lithium efflux is not blocked by removal of external potassium, as the sodium pump is,

lithium seems unlikely to be transported outwards through the channel normally responsible for the net sodium efflux.

It was necessary to verify the correctness of the statement just made that net sodium efflux is blocked in a K-free medium, since this point has apparently not been tested previously (as was pointed out by Hodgkin, 1957). Six pairs of muscles were loaded with sodium by soaking in K-free 120 mM-NaCl at 3° C for 40–44 hr, and one muscle from each pair was then transferred to the K-free sodium recovery solution at 20° C. It will be seen from the analyses

TABLE 5. Changes in cation content (in m-mole/kg) in pairs of Li-loaded muscles soaked for various periods in a K-free sodium recovery solution (18–21° C)

Recovery time (hr)	Dry weight (%)	Contents of			Change in			Rate constant for loss of Li (hr^{-1})
		Li	K	Na	Li	K	Na	
0	19·3	29·3	64·5	24·4				
0·7	20·5	28·8	61·0	29·3	− 0·5	− 3·5	+ 4·9	0·026
0	22·6	37·5	73·5	18·2				
1·4	22·1	31·6	65·8	19·3	− 5·9	− 7·7	+ 1·1	0·122
0	20·4	28·2	71·2	25·3				
2·2	19·2	20·8	60·2	35·0	− 7·4	−11·0	+ 9·7	0·139
0	20·5	50·2	46·1	27·0				
3·1	20·6	43·1	34·0	40·5	− 7·1	−12·1	+12·5	0·049
0	21·7	34·9	64·6	26·3				
3·8	21·8	26·6	50·1	34·5	− 8·3	−14·5	+ 8·2	0·072
0	16·8	54·7	34·7	36·6				
5·2	17·4	35·6	18·3	62·0	−19·1	−16·4	+25·4	0·083
0	18·2	33·7	67·0	18·3				
5·7	16·9	18·6	46·7	56·2	−15·1	−20·3	+37·9	0·104
0	19·1	30·7	68·3	21·0				
6·4	18·8	15·3	50·1	51·5	−15·4	−18·2	+30·5	0·092
0	19·1	47·4	31·6	35·2				
6·9	19·0	19·8	13·9	75·1	−27·6	−17·7	+39·9	0·126
0	21·1	33·2	74·0	20·5				
8·1	20·6	14·0	46·4	59·5	−19·2	−27·6	+39·0	0·107

listed in Table 6 that in every case the test muscle gained further sodium instead of losing any, and lost a very nearly equivalent quantity of potassium. This established that a net sodium extrusion cannot take place from frog muscle when there is no potassium in the external medium.

The last set of experiments was designed to see whether lithium-loaded muscles were able to bring about a net extrusion of lithium into a high-K recovery solution in which lithium was substituted for all the sodium except the 0·9 mM added as gluconate (for composition see Table 1). The results set out in Table 7 and plotted in Fig. 1 suggest that in the recovery solution there was either no change in lithium (of the slight loss observed in four of the muscles, about 2·2 m-mole/kg can be attributed to the rather low external lithium concentration in the recovery solution) or a definite rise. There is thus no doubt that frog muscles are unable to extrude lithium at a rate comparable

to that at which they can extrude sodium. However, these experiments do not establish unequivocally that there was no movement of lithium whatever against the concentration gradient, since if lithium had been entering the fibres during the recovery period at the same rate as in the influx measurements (order of 3 m-mole/kg.hr), and if the efflux had been zero, some of the muscles should have shown a larger gain of lithium than they did. In other words some of them seem to have been just able to hold their own for several hours in the recovery solution, and this indicates that they were, in fact, able

TABLE 6. Changes in sodium and potassium content of pairs of Na-loaded muscles soaked for various periods at 20° C in a K-free sodium recovery medium

Recovery time (hr)	Dry weight (%)	Contents of K (m-mole/kg)	Contents of Na (m-mole/kg)	Change in K (m-mole/kg)	Change in Na (m-mole/kg)
0	22·6	58·0	55·5		
1·2	22·7	52·0	60·4	− 6·0	+ 4·9
0	19·3	51·5	62·6		
1·8	20·2	48·6	67·7	− 2·9	+ 5·1
0	20·1	52·0	59·0		
2·6	20·0	46·5	64·1	− 5·5	+ 5·1
0	20·9	49·6	61·9		
3·7	20·0	40·3	71·7	− 9·3	+ 9·8
0	21·2	50·0	68·4		
4·8	18·8	27·7	82·4	−22·3	+14·0
0	20·9	68·0	49·8		
6·8	21·6	52·9	63·8	−15·1	+14·0

TABLE 7. Changes in cation content (m-mole/kg) of pairs of Li-loaded muscles soaked for various periods at 16–19° C in a high-K lithium recovery medium. The pairs marked with asterisks had spent 26 hr in a K-free Li solution at 2° C; the others spent 44–51 hr in the low-K Li solution (see Table 1) at the same temperature

Recovery time (hr)	Dry weight (%)	Contents of Li	Contents of K	Contents of Na	Change in Li	Change in K	Change in Na
0	21·0	64·1	55·6	1·0			
1·0	21·3	60·3	51·5	1·2	− 3·8	− 4·1	+0·2
*0	17·8	70·3	60·2	1·5			
2·2	18·5	69·0	60·3	1·7	− 1·3	+ 0·1	+0·2
0	17·9	89·7	27·0	0·9			
3·0	17·9	86·8	33·9	1·1	− 2·9	+ 6·9	+0·2
*0	19·6	57·5	68·0	2·9			
3·2	19·9	59·2	61·0	1·9	+ 1·7	− 7·0	−1·0
0	14·8	107·2	13·7	0·8			
4·0	17·6	104·0	21·6	1·1	− 3·2	+ 7·9	+0·3
0	17·5	83·9	32·6	1·2			
4·2	—	88·7	34·9	1·4	+ 4·8	+ 2·3	+0·2
*0	22·2	49·0	70·0	1·8			
4·4	22·7	61·5	62·2	1·5	+12·5	− 7·8	−0·3
0	22·2	60·5	66·1	0·8			
6·8	21·3	76·1	49·4	1·0	+15·6	−16·7	+0·2
0	21·4	67·0	56·7	0·8			
7·0	20·9	71·2	47·7	1·0	+4·2	− 9·0	+0·2

to extrude lithium very slowly. But many further experiments would be needed in order to arrive at an accurate quantitative estimate of the extent to which active transport of lithium can occur.

The effect of lithium on the electrical activity of frog muscle

Resting and action potentials were recorded with micro-electrodes by using methods and precautions similar to those described by Adrian (1956). The first point to be established was that there was no significant change in the resting potential on exposure to Li for periods of 15–30 min. Thus in one muscle the average resting potential was initially 92·6 mV (S.E. $\pm$ 1·4 mV for 13 fibres) in normal Ringer's solution; after 15 min in Li it was 91·5 $\pm$ 2·0 mV (8 fibres); and on restoring normal Ringer's solution it was 92·5 $\pm$ 1·4 mV (11 fibres).

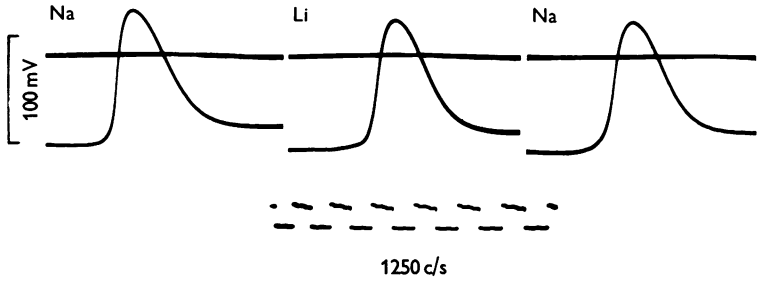

Fig. 2. Resting and action potentials recorded (*a*) in normal Ringer's solution, (*b*) after 20 min in Li Ringer's solution, (*c*) after restoring normal external [Na]. Each record is from a different fibre in the same muscle. Temperature 21° C.

Another muscle gave very similar results. The action potential was equally unaffected, the average spike heights (for 3 fibres in each case) being 122 $\pm$ 3 (in Na), 119 $\pm$ 1 (in Li), and 122 $\pm$ 2 mV (in Na). The recordings reproduced in Fig. 2 show that there was also no obvious change in the rate of rise of the spike, whose shape was virtually identical in the three cases.

In view of the small number of muscles examined, and the appreciable variability between individual fibres, these observations do not exclude the possibility that there are immediate *slight* changes in the resting and action potentials. But they suggest that any such changes are not likely to exceed 1–2 mV. It also remains possible that larger changes may occur after the internal [Na] has fallen lower than it was given time to do in these experiments, which did not extend beyond a period of 30 min in Li Ringer's solution.

DISCUSSION

The main conclusion to be drawn from the work described here is that there is a clear difference in selectivity between what may be termed the 'spike' and 'recovery' channels for transport of ions through the cell membrane. In agreement with observations on squid axons (Hodgkin & Katz, 1949), isolated

frog nerve fibres (Huxley & Stämpfli, 1951) and other excitable tissues (further references are given by Schou, 1957), there is very little immediate change in the electrical activity of frog muscle fibres when Li is substituted for Na in the external medium. This implies that the 'spike' channel, that is to say the passive permeability mechanism which in the resting state allows only a slow trickle of Na^+ ions to flow down the concentration gradient into the interior of the cell, but which permits a greatly accelerated entry during the rising phase of the spike (Hodgkin & Horowicz, 1959), hardly discriminates at all between Na and Li. In the preceding paper (Keynes & Swan, 1959) it has been seen that roughly half the total Na influx of 4·2 pmole/cm^2.sec probably arises from an exchange diffusion process, leaving an influx of some 2 pmole/cm^2.sec reasonably attributable to a passive entry of Na via the spike channel. If the spike channel is unable to differentiate between Na and Li, this figure fits fairly well with the estimate of 1–2 pmole/cm^2.sec for the Li influx.

In contrast with the lack of discrimination displayed by the spike channel, the recovery mechanism certainly does seem to distinguish between Na and Li. Whereas under suitable conditions Na can rapidly be extruded from Na-loaded fibres, muscles similarly loaded with Li lose their intracellular Li relatively slowly when soaked in a high [K] recovery solution. Comparison of the rate constants for loss of Na and for loss of Li into Na recovery solutions suggests that Na can be extruded rather more than ten times faster than Li, but this estimate probably errs in favour of Li, since at least part of the Li which emerges into these solutions seems to do so through the operation of a Li/Na exchange process rather than being transported by a Li pump coupled with an uptake of K. Another method of deriving a figure for the rate at which Li can be extruded is to suppose that in the first five pairs of muscles in Table 7 the Li efflux was exactly keeping pace with the Li influx, and that the influx was the same (say 2 pmole/cm^2.sec) as that measured in fresh muscles. On this basis the rate of extrusion of Li would have been about 3 m-mole/kg.hr from muscles whose average Li content was 77 m-mole/kg, corresponding to a rate constant of 0·04 hr^{-1}. As has already been mentioned (see p. 630) the rate constant for extrusion of Na under equivalent circumstances is slightly over 1 hr^{-1}, so that the relative efficacy with which the recovery channel handles Na and Li would come out in the region of 25/1. There are indications (see, for example, Hodgkin & Horowicz, 1959) that the Na influx tends to increase with time after dissection; if the Li influx had behaved similarly, the muscles of Fig. 7 would have been both gaining and extruding lithium somewhat faster than has just been assumed. Although the argument is complicated by the possibility that under the conditions of Table 7 the passive efflux of lithium via the spike channel was not negligible, it seems fair to regard 25/1 as an upper limit and 10/1 as a lower limit for the relative efficacy of extruding Na and Li.

The behaviour of the muscle membrane towards Li seems similar to that of the erythrocyte membrane, since Maizels (1954) showed that in human red cells the inward passive movements of Na and Li had roughly equal rate constants, but that there was little or no active outward movement of Li. Another tissue where active transport of Li has recently been examined is the isolated frog skin. Zerahn (1955) found that when the skin was bathed with Ringer's solution in which 80% of the Na was replaced by Li the short-circuit current soon fell to about a tenth of its value in normal Ringer's solution, at which level it could apparently be maintained for some while; at the same time, appreciable amounts of Li accumulated in the skin. He established that some active transport of Li was taking place from one side of the skin to the other, and although precise values for the relative affinity of the mechanism for Na and for Li cannot easily be derived, his results do not seem inconsistent with our evidence that the ratio lies between 10 and 25 times. If in general Li enters cells not much less readily than Na, but is extruded much more slowly, then on prolonged exposure to Li one would expect much of the intracellular K to be displaced by Li and in excitable tissues the membrane potential would gradually fall. A slow depolarization in Li of various types of muscle and nerve fibre has often been recorded (Schou, 1957), and a tendency for Li to accumulate inside cells at the expense of K would account satisfactorily for at least part of the well-known toxicity of the ion.

If mammalian C fibres are stimulated in Li solutions, they give normal action potentials (at any rate for short periods), but the post-tetanic hyperpolarization and positive after-potential are immediately abolished (Ritchie & Straub, 1957; Greengard & Straub, 1958). On the assumptions that, as in muscle, the Li that enters the fibres during the impulse is not quickly extruded afterwards, and that consequently there is in Li no enhanced uptake of K after passage of an impulse, these observations have been used by their authors to support interesting hypotheses as to the origins of the hyperpolarization and after-potential. Thus the differences in the behaviour of excitable membranes towards Na and Li have already proved to be of analytical value.

SUMMARY

1. Two methods of determining the size of the lithium influx into frog muscle fibres gave values of 1–2 pmole/cm^2.sec.

2. When Li-loaded muscles were soaked in a sodium recovery medium containing 10 mM-K they lost their Li with a rate constant just under 0·1 hr^{-1}, this figure being about one tenth of that observed by Desmedt (1953) for the extrusion of Na into the same solution.

3. When K was omitted from the recovery medium loss of Li still took place at the same rate, suggesting that efflux of Li into a sodium solution is

not obligatorily linked to an influx of K. It was verified that extrusion of Na from Na-loaded muscles was effectively blocked in a K-free medium.

4. When Li-loaded muscles were soaked in a lithium recovery medium containing 10 mM-K, their Li content remained stationary for about 4 hr, and then began to increase.

5. Micro-electrode recordings showed that there was very little immediate change in either the resting or the action potential when Li was substituted for Na in the external medium.

6. It is concluded that the passive permeability mechanism responsible for generating the action potential does not discriminate between Na and Li, but that the active transport mechanism is only capable of extruding Li from the interior of muscle fibres 1/10–1/25 as fast as it extrudes Na.

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