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THE SODIUM PUMP IN SKELETAL MUSCLE IN RELATION TO ENERGY BARRIERS

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In a previous paper (Carey, Conway & Kernan, 1959) it was shown that, when frog sartorii were immersed during the night in K-free Ringer–Conway fluid containing 120 mM-Na, the average content of Na in the muscle was $59{\cdot}0 \pm 0{\cdot}7$ m-equiv/kg (S.E. of mean for 166 expts). Three muscles were used for each experiment.

On reimmersion for 2 hr at room temperature in similar fluid containing 104 mM-Na and 10 mM-K (referred to here as the 120,0/104,10 procedure) there was considerable active secretion of Na, which is in agreement with the results of Desmedt (1953). The secretion averaged $17{\cdot}6 \pm 0{\cdot}8$ m-equiv Na/kg in a large number of experiments, allowing 2 m-equiv Na/kg for passive diffusion from the interspaces in the total Na loss. (In the oxygen-uptake experiments described here the conditions were somewhat different, and the average secretion was found to be 22 m-equiv Na/kg.)

If the reimmersion fluid contained 120 mM-Na (the 120,0/120,10 procedure) there was, on the average, little or no excretion of sodium though a very occasional experiment might show it markedly. Also, if the K-free soaking fluid contained 104 mM-Na, and the reimmersion fluid contained also 104 mM-Na, there was likewise no significant Na secretion (Carey, *et al.* 1959).

A partial explanation for this difference was given by the consideration that a secretion of Na in the second immersion, with 10 mM-K present, could be regarded as a restoration of the conditions following the effects of the K-free immersion. One would expect then that the limit of the secretion would be set by the Na levels in the muscles if 10 mM-K were present in *both* the soaking and the reimmersion fluids. A number of experiments carried out with 10 mM-K present throughout suggested that the maximum average *loss* of Na to be expected in the 120,0/104,10; 120,0/120,10 and the 104,0/104,10 procedures was $20{\cdot}0 \pm 1{\cdot}0$; $3{\cdot}6 \pm 3{\cdot}5$ and $7{\cdot}8 \pm 1{\cdot}1$ m-equiv/kg respectively. The average experimental *losses* observed were $19{\cdot}6 \pm 0{\cdot}8$, $-5{\cdot}2 \pm 9{\cdot}6$ and $1{\cdot}0 \pm 0{\cdot}9$. The elucidation of the

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very large average difference between the 120,0/104,10 and the 104,0/104,10 procedures appeared to be carried a step further.

Yet this did not explain the fact that in a large number of experiments with companion sets of muscles with the 120,0/104,10 and the 104,0/104,10 procedures, a certain number of results appeared in both procedures with similar muscle Na levels after the first immersions, but there was more secretion of Na in the one than in the other procedure. A plausible explanation of this seemed then to lie in the hypothesis that soaking in the 120 mM-Na K-free Ringer's fluid caused a stimulation of the sodium pump as compared with the soaking in the 104 mM-Na K-free Ringer's fluid.

If this is so, then in the reimmersion period with the 120,0/120,10 procedure it could be expected that the large secretion of Na would also occur but be masked by an Na entrance of about the same magnitude. It was decided to study this matter, as described in the present paper, by inhibiting the secretion of Na in the 120,0/120,10 and in the 104,0/104,10 procedures by iodoacetate (2 mM) and by ouabain (10^{-5} M). If the active secretion were occurring before inhibition the muscle Na should increase markedly because of its unbalanced entrance. The fact emerged, as will appear, that there was very little increase in the muscle Na content.

We came then to examine the energy barrier presented to the secretion of Na. The energy barrier to secretion could be lessened by decreasing the Na content of the external fluid, or by raising the K content (and so lessening the membrane potential) or by both procedures. The results show quite clearly that under the conditions of our experiments there exists a critical energy barrier above which there is no significant average secretion of Na, and below which Na can be excreted in surprising amounts in a short time. Studies of oxygen uptake as described here agree with this view.

METHODS

The companion sartorius muscles of *Rana temporaria* were used in all the experiments described here. With a few slight variations described below, the muscles were treated in the manner already described (Carey *et al.* 1959) in the studies of Na extrusion. The Ringer's fluids used were variants of the Ringer–Conway fluid (Boyle & Conway, 1941). Where the Na level of the reimmersion fluid was raised or lowered, the glucose level was altered so as to restore osmotic equilibrium. In the oxygen experiments the bicarbonate of the Ringer's fluid was replaced by an equivalent amount of phosphate buffer.

Chemical methods

Before analysis for Na and K the muscles were blotted on moist filter paper and carefully weighed. They were then digested in hot HNO_3, and after oxidation was complete, the acid was evaporated, and the residue taken up in 15 ml. of de-ionized water. This was analysed by means of a Beckmann flame photometer.

Inhibitors. The inhibitors used included 10^{-5} and 10^{-6} M ouabain, 2 mM sodium iodoacetate and 2 mM potassium cyanide. The sodium and potassium salts of the inhibitors were

added in substitution for NaCl and KCl so as not to alter the total cation concentrations. In studying inhibitor effects companion muscles were used, one group acting as control.

Membrane potentials

These were measured by the micro-electrode technique of Graham & Gerard (1946). The electrical arrangement employed has already been described (Carey *et al.* 1959) but the following modification was used. The microcapillaries were drawn mechanically to about 0·5 μ tip diameter and were filled with 3 M-KCl in the cold by the method of Caldwell & Downing (1955). Electrodes were selected having impedances in the range 7–15 MΩ. Tip potentials were kept at a minimum by this method.

Oxygen-uptake measurements

The Warburg manometric technique was used for this purpose. The muscles were allowed at first to cling to the walls of the manometer flasks which were filled with 3 ml. of the phosphate Ringer's fluid. The conventional procedure was then followed, the Warburg bath being equilibrated at 20° C. In most cases the sensitivity of the manometers was increased by a factor of approximately 7 by the use of the hydraulic leverage principle of Burk & Hobby (1954). This was necessary where small muscles were used. In a number of experiments, after the initial loading of the muscles in K-free Ringer's solution containing 120 mM-Na, one muscle of each companion set was analysed immediately to determine the Na level, and the other was placed in the manometer flask in Ringer's fluid containing 104 mM-Na and 10 mM-K, and its oxygen consumption was measured after 2 hr. It was then analysed for its Na content. Similar experiments to these were conducted with the 120,0/120,10 and the 104,0/104,10 procedures.

The fluids used

The K-free soaking fluids contained 120 mM-Na or 104 mM-Na. The two fluids containing 120 mM-Na had the compositions shown in Table 1. Fluid *B* was used for soaking when the reimmersion was conducted in manometers, phosphate being substituted for bicarbonate. Fluids *C* and *D* which were used corresponded in general composition with *A* and *B*, the difference lying in the Na and Cl contents, which were 104 mM and 72·5 mM, respectively, and also in their containing 26 mM of glucose. The series 120,0/120,10; 120,0/115,10;

TABLE 1. Composition of fluids used

	(m-mole/l.)	
	A	*B*
Sodium	120	120
Calcium	0·9	0·9
Magnesium	1·2	1·2
Chloride	88·5	88·5
Phosphate	3·0	16·7
Bicarbonate	25·0	—
Sulphate	1·8	1·8
Gluconate	0·9	0·9
pH	7·4	7·4

120,0/110,10; 120,0/104,10 and 120,0/80,10 had all the same soaking fluid (*A*). The reimmersion fluids all contained 10 mM-KCl in addition, the Na contents being 120, 115, 110, 104 and 80 mM, respectively. The total osmolarity was adjusted by glucose to be approximately the same in each group. It was assumed that 56 mM-NaCl was the approximate osmotic equivalent of 98 mM glucose (Hodgkin & Keynes, 1955), whence glucose was incorporated to the extent of 0, 7·0, 16·0, 26·0 and 68·0 mM, respectively, making a total osmolarity

(arrived at by adding up the ion concentrations and the NaCl equivalent to the glucose) of 260 mM within about 1 %. This is no doubt approximate, but is considered sufficiently accurate for the purpose of the investigations.

RESULTS

Examination of a possible osmotic effect on the difference between the extrusion of Na in the 120,0/104 *and the* 120,0/120,10 *procedures*

At an early stage in the investigations the possibility of some osmotic effect affecting the excretion was examined. When, for instance, after immersion during the night in K-free Ringer's fluid containing 120 mM the muscles were reimmersed in fluid containing 104 mM-Na and 10 mM-K, or alternatively in fluid containing 120 mM-Na and 10 mM-K, there was a difference in molarity amounting to 16 mM-NaCl. This difference was made up with sucrose in a series of thirteen sets of experiments. This equalization of the osmotic pressures of the recovery solution had little or no effect on the excretion of sodium.

TABLE 2. Effect on the secretion of Na of changing the Na content of the reimmersion fluid

Procedure	Mean Na content of muscle after 2nd immersion (m-equiv Na/kg)	No. of observations	Mean excretion of Na, allowing for interspace (m-equiv Na/kg)
120,0/120,10	57·6 ± 2·3	22	1·4 ± 2·4
120,0/115,10	54·7 ± 2·2	14	3·7 ± 2·3
120,0/110,10	39·5 ± 1·6	21	18·2 ± 1·7
120,0/104,10	37·3 ± 1·4	30	19·6 ± 1·5
120,0/80,10	34·0 ± 1·7	13	19·8 ± 1·8
104,0/104,10	45·9 ± 0·6	122	1·0 ± 0·9
104,0/80,10	32·5 ± 2·0	16	12·6 ± 2·2

Recently we carried out a series of experiments in which the osmolarity of the external fluids was maintained constant by the addition of glucose, as described in Methods, and here also no appreciable effect was produced. The results are summarized in Table 2, from which it will appear that from 22 experiments with the 120,0/120,10 procedure the average Na secretion was 1·4 ± 2·4 m-equiv/kg, whereas with the 120,0/104,10 procedure the average excretion from 30 experiments was 19·6 ± 1·5. In both sets of experiments the total osmolarity of 260 mM was the same, owing to suitable glucose additions.

Investigation of the possibility of an active excretion of Na in the 120,0/120,10 *and the* 104,0/104,10 *procedures being balanced by a similar degree of passive entrance*

This question was investigated by inhibiting the outward secretion of Na by means of iodoacetate (2 mM) and by ouabain (10^{-5} M), also by

cyanide (2 mM). The effects on the large Na secretion in the 120,0/104,10 procedure of such inhibitors has been previously described (Carey *et al.* 1959). Iodoacetate (2 mM) and ouabain (10^{-5} M) cause complete or practically complete inhibition of the excretion under our conditions, and cyanide (2 mM) produced a 60 % inhibition in the average. Such inhibitions produced very little or no increase in the Na content of the muscles with the 120,0/120,10 or the 104,0/104,10 procedure. The results are summarized in Table 3. If the total of 35 experiments with the 120,0/120,10

TABLE 3. Effects of ouabain, iodoacetate and cyanide in the 120,0/120,10 and the 104,0/104,10 procedures

Procedure	Inhibitor used	No. of expts.	Mean Na content (± S.E.) of muscles after the 2 hr reimmersion period (m-equiv/kg, referred to wt. after 1st soaking)		
			Without inhibitor	With inhibitor	Difference due to inhibitor
120,0/120,10	Ouabain	14	54·8 ± 1·8	54·0 ± 1·9	−0·8
	Iodoacetate	14	53·0 ± 3·3	55·1 ± 3·3	+2·1
	Cyanide	7	53·0 ± 1·5	50·9 ± 1·9	−2·1
104,0/104,10	Ouabain	32	44·5 ± 1·6	46·1 ± 1·4	+1·6
	Iodoacetate	12	44·4 ± 2·3	42·7 ± 2·7	−1·7
	Cyanide	12	42·9 ± 2·0	42·0 ± 2·7	−0·9

are considered together, the mean increase in Na content produced by the inhibitor is 0·1 ± 2·1 and with the 104,0/104,10 procedure it is 0·4 ± 1·3 m-equiv/kg. Thus while such inhibitors may possibly cause an increase of 4·2 or 3·0 m-equiv Na/kg taking twice the S.E. of the mean, the effect is none the less quite small compared with, say, the average secretion of about 18 m-equiv Na/kg that would occur in the 120,0/104,10 procedure. The answer to the above question is, then, that on the average active secretion does not occur to any appreciable extent during the reimmersion periods in the 120,0/120,10 and the 104,0/104,10 experiments.

Effects of lessening the energy barrier to Na excretion

With the failure of an adequate explanation of the secretion differences described above from osmotic effects or those of stimulation of the sodium pump during the night, we came to examine the results of changing the energy barrier to Na excretion. The energy barrier to the Na excretion can be decreased either by decreasing the external Na concentration or increasing the K content. This is again considered in the Discussion.

Decrease in the external Na concentration of the re-immersion fluid. It was already apparent as the result of numerous experiments that dropping the external Na concentration in the 120,0/120,10 procedure from 120 to 104 (the average concentration of the frog plasma) produced a large Na excretion. The effects on the energy barrier of changing by steps from

120 mM in the second immersion to 80 mM-Na are summarized in Table 4.

In this series it will be seen that a big increase in the Na secretion occurs at the 110 mM-level, after which but little further increase occurs. A point of special importance here is that lowering the external Na from 120 to 104 mM causes an average fall of membrane potential which in itself considerably affects the energy barrier. Also in changing from the 104,0/104,10 to the 104,0/80,10 procedure there is a fall of potential (see Table 4).

TABLE 4. Energy barrier to Na excretion by Na-rich sartorii immediately after reimmersion

Procedure	Membrane potential (E_m) (mV)	$E_m F$ (cal/m-equiv Na)	$RT.\ln[Na_o]/[Na_i]$ (cal/m-equiv Na)	Total cal/m-equiv	Average Na excretion (m-equiv/kg)
120,0/120,10	67·3 ± 1·5	1·55	0·44	1·99	1·4 ± 2·4
120,0/115,10	(66·0)	1·52	0·41	1·93	3·7 ± 2·3
120,0/110,10	(64·7)	1·49	0·38	1·87	18·2 ± 1·7
120,0/104,10	63·4 ± 0·7	1·46	0·35	1·81	19·6 ± 1·5
120,0/80,10	56·8 ± 0·8	1·32	0·22	1·54	19·8 ± 1·8
120,0/120,30	48·9 ± 0·7	1·12	0·44	1·56	14·6 ± 1·8
120,0/120,60	41·6 ± 1·0	0·96	0·44	1·40	15·6 ± 2·9
104,0/104,10	71·7 ± 0·9	1·65	0·51	2·16	1·0 ± 0·9
104,0/80,10	54·8 ± 0·7	1·27	0·39	1·66	12·6 ± 2·2
104,0/104,30	46·6 ± 2·2	1·07	0·51	1·58	9·0 ± 1·7

The brackets used with some membrane potentials indicate interpolated values. The membrane potential (E_m) is taken for convenience as positive (outside minus inside).

Figure 1 shows a frequency distribution of potentials measured immediately after reimmersion in the 104,0/104,10 and the 120,0/104,10 procedures. The distributions are for 25 sartorii in one procedure and the 25 companion muscles in the other. For each muscle the membrane potentials of six fibres were investigated. The means for the two series were 71·7 ± 0·9 and 63·4 ± 0·7, giving a difference of 8·3 ± 1·1 mV.

It is also important to note that similar differences remained to the end of the 2 hr reimmersion as shown in Table 5. Thus the mean potential after 2 hr with the 104,0/104,10 procedure was −65·0 ± 0·8 mV and that for the 120,0/104,10 procedure was −56·0 ± 1·1. Although a much smaller number of muscles were here examined, from the similarity of the potentials obtained it appeared unnecessary to accumulate more results. (Here it may be noted that in a previous communication (Carey *et al.* 1959) frequency distributions of potentials with the 104,0/104,10 and 120,0/104,10 procedures were given, but in these the observations were made on a large number of fibres *from a single muscle*, and owing to the variability with regard to the single muscle cannot be regarded as in any way contradictory to the present results).

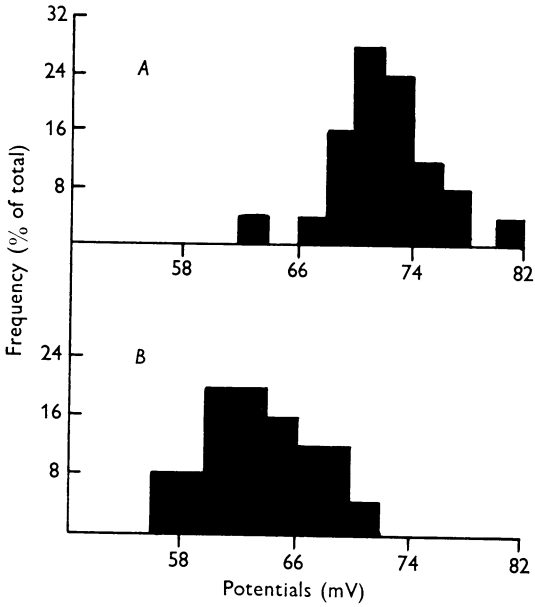

Fig. 1. Frequency distribution of membrane potentials measured immediately on reimmersion under the following conditions: *A*. Muscle left during night at 2° C in fluid *C* and reimmersed in same fluid with 10 mM-K/l. *B*. Muscles left during night at 2° C in fluid *A* and reimmersed in fluid *C* with 10 mM-K/l. 25 sets of companion muscles used.

TABLE 5. Energy barriers to Na excretion at the beginning and end of reimmersion with different procedures

Procedure	[Na] in the fibre water (m-equiv/l.)	RT. ln $[Na_o]/[Na_i]$ (cal/m-equiv Na)	Membrane potential (mV)	$E_m F$	Total energy barrier (cal/m-equiv Na)
120,0/120,10					
Beginning of reimmersion	56·4	0·44	67·3 ± 1·5 (5)	1·55	1·99
End of 2 hr reimmersion	54·3	0·46	70·1 ± 0·8 (5)	1·62	2·08
120,0/104,10					
Beginning of reimmersion	56·4	0·35	63·4 ± 0·7 (25)	1·46	1·81
End of 2 hr reimmersion	27·2	0·78	56·0 ± 1·1 (5)	1·27	2·05
104,0/104,10					
Beginning of reimmersion	41·4	0·51	71·7 ± 0·9 (25)	1·65	2·16
End of 2 hr reimmersion	40·0	0·56	65·0 ± 0·8 (5)	1·50	2·06

The numbers in brackets represent the number of sartorii used. The figures for the Na content of the fibre water are calculated from the average data previously given (Carey *et al.* 1959). The 25 muscles used for 120,0/104,10 were companions of those used with 104,0/104,10.

Increase of the KCl content of the fluid in the second immersion. Such increase results in a fall of the membrane potential and hence a lessening of the energy barrier against which the sodium pump works. The effects of changing from 10 to 30 mM-K and to 60 mM-K are also summarized in Table 4. It will be seen that lowering the energy barrier in the 120,0/120,10 or the 104,0/104,10 procedures by increasing the KCl content again produced a marked secretion of Na. It would appear, then, that the lack of significant Na secretion in the reimmersion periods of the 120,0/120,10 and 104,0/104,10 procedures under our conditions relates to the energy barrier. When this reaches or passes a certain critical level no marked secretion of sodium occurs, and when the barrier falls below this critical level a large active secretion of Na is set going.

Oxygen uptake in relation to sodium excretion

Concerning the relation of oxygen uptake to Na excretion, Levi & Ussing (1948), using separate sets of data for oxygen consumption and for ^{24}Na efflux, determined the relation between them. They concluded that part of the isotopic efflux was due to 'exchange diffusion'.

Keynes & Maisel (1954) aimed at a greater precision of treatment by correlating the measurements of oxygen uptake and isotopic effluxes on the same muscles, as well as by obtaining wide variations in these quantities by raising the external K concentration (Hegnauer, Fenn & Cobb, 1934). In this way and as given in Table 1 of their communication, for five experiments the range of the ratio of Na ions excreted (as measured by the isotopic efflux) per molecule of oxygen taken up was from 2·0 to 5·7 with an average of 4·1. With such a range and with so few results the mean can have no exact significance, and it is purely coincidental that the figure is the same as the mean we obtained with a large number of results and quite different treatment. In fact, if four of their five experiments comprising two sets of companion muscles are taken and the ratio computed from the *extra* efflux and the *extra* oxygen uptake the mean ratio is 5·1.

In the treatment of Keynes & Maisel (1954) it is assumed that the rate of active extrusion of Na is measured by the isotopic efflux. It is now known that owing to exchange diffusion such measurements of ^{24}Na effluxes may give results very different from the real Na extrusion. This not only appears from the results of the present communication but one may also cite the following references: Levi & Ussing (1948); Keynes & Swan (1959); Carey *et al.* (1959).

Here the oxygen uptake associated with the large net extrusion of Na in the 120,0/104,10 procedure has been measured and compared with the oxygen uptake in the 120,0/120,10 and the 104,0/104,10 procedures, in which no significant average extrusion of Na takes place (Conway, 1957;

Carey *et al.* 1959). From such experiments the true mean relation between amount of Na extrusion and oxygen uptake appears from two different ways of treating the results.

Relation between the net Na extrusion and O_2 uptake in the 120,0/104,10 *procedure.* Thirty-five sets of experiments were carried out as described in Methods, all the measurements referring to a recovery period of exactly 2 hr. A correlation of $0{\cdot}68 \pm 0{\cdot}09$ was found between the two quantities and the regression line relating the Na excretion to the O_2 uptake was found to be

$$O_2 \text{ uptake (m-mole/kg)} = 0{\cdot}26 \times (\text{Na excreted (m-equiv/kg)}) + 6{\cdot}04. \quad (1)$$

From this regression equation it is seen that 3·85 m-equiv of sodium excreted is associated with 1 m-mole of oxygen uptake. The regression equation need only be used for the purpose of calculating the mean oxygen uptake with no Na excretion, the result being 6·04 m-mole/kg. As the mean oxygen uptake in the 35 experiments was 11·7 m-mole/kg, 5·6 m-mole/kg was therefore associated with the 22·1 m-equiv Na/kg excreted, giving a mean ratio of 3·90 m-equiv Na/m-mole O_2. Such values for the ratio are not significantly different statistically from 4·0.

Instead of taking the companion muscles to ascertain the Na levels after the first immersions, they were used to ascertain the O_2 uptake in the 120,0/120,10 procedure, with a similar series for the 104,0/104,10 procedure, since these latter procedures could be regarded as giving the basic O_2 uptake when there was no significant excretion of sodium. The results for the 120,0/120,10 and the 104,0/104,10 experiments were practically identical, and are here taken together as 22 experiments in all, with a mean oxygen uptake in the 2 hr reimmersion period of $10{\cdot}5 \pm 0{\cdot}8$ m-mole/kg. The 22 experiments with companion muscles for the 120,0/104,10 procedure gave a mean O_2 uptake of $16{\cdot}3 \pm 1{\cdot}0$ m-mole/kg, the difference between this and the previous figure being $5{\cdot}8 \pm 1{\cdot}3$, corresponding to a mean Na excretion of 22·1 m-equiv/kg. This results therefore in a mean ratio of Na excreted to O_2 uptake of 3·81, which again does not significantly differ statistically from 4·0. It will be noticed that the excretion of Na in the 120,0/104,10 procedure was accompanied by an oxygen uptake 56% greater than that found when there was no Na excretion.

The relation between the O_2 uptake and the Na level in the muscle fibre water

With thirty-five sets of experiments the correlation between O_2 uptake and the Na content after the first immersion in K-free fluid was only $0{\cdot}19 \pm 0{\cdot}16$, which is not significant. The important fact here is that the O_2 uptake is highly correlated with the excretion of Na but is not significantly correlated with the Na concentration in the muscle fibres. It may

at first appear surprising that two variables (here O_2 and muscle Na concentration) may be highly correlated with a third (the Na excretion) but not significantly with each other. This, however, is a frequent statistical occurrence, but to analyse it fully in the present instance would require more data than are at present available.

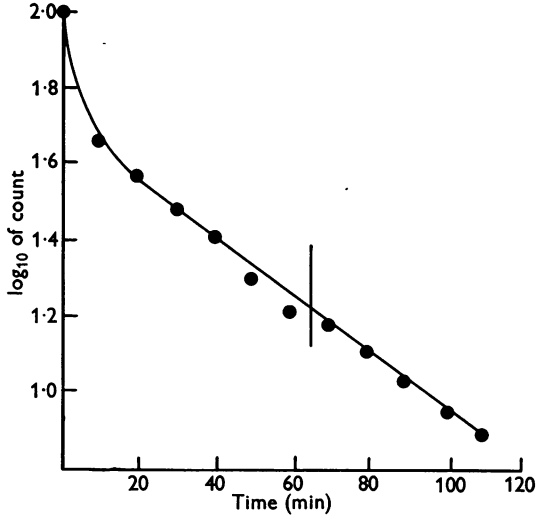

Fig. 2. Effect of iodoacetate on loss of ^{24}Na from Na-rich muscle in the 120,0/120,10 procedure. Inhibitor added at time indicated by vertical bar.

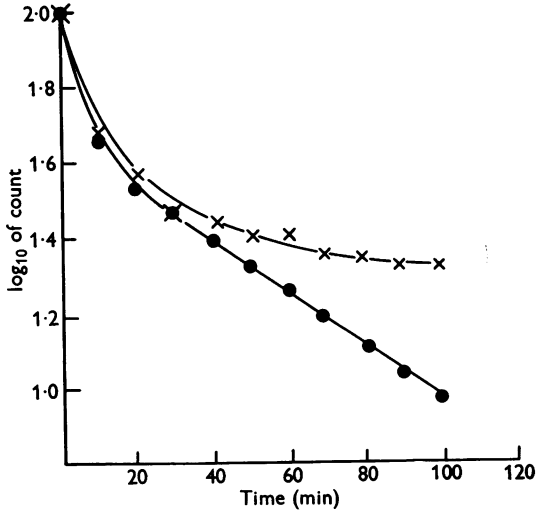

Fig. 3. Effect of ouabain on loss of ^{24}Na from Na-rich muscle in 120,0/120,10 procedure. Inhibitor present from beginning of reimmersion. ●, control; ×, inhibited.

Sodium effluxes from Na-rich muscles, when the excretion is inhibited by iodoacetate (2 mM) *or ouabain* (10^{-5} M)

With the 120,0/120,10 or the 104,0/104,10 procedures no significant average secretion of Na over the 2 hr of the reimmersion period was found, and, as considered above, the use of inhibitors of excretion such as iodoacetate (2 mM) and ouabain (10^{-5} M) showed that no significant mean excretion of Na balanced by an equal uptake of Na occurred. It was of special interest, therefore, to observe the effect of such inhibitors on the effluxes of ^{24}Na. Figure 2 shows the results of a typical experiment, yet iodoacetate practically completely inhibited the large average excretion of Na found with the 120,0/104,10 procedure (Carey *et al.* 1959). The virtually unchanged rate constant appears under these conditions, therefore, to be associated to a large extent with 'exchange diffusion' (Ussing, 1949).

With ouabain (10^{-5} M), on the other hand, which also causes practically complete inhibition of excretion in the 120,0/104,10 procedure, there was a marked inhibition of the ^{24}Na efflux in experiments of the 120,0/120,10 types. This is illustrated in Fig. 3. In this context it may be noted that Edwards & Harris (1957) examined the effect of strophanthin on ^{24}Na effluxes from Na-rich muscles and also found a marked inhibition (1·6–2·2 times).

DISCUSSION

The results show that there exists a critical energy barrier of about 2·00 cal/m-equiv for the active secretion of sodium under the conditions of the experiments described. The energy barrier to the excretion of sodium may be written

$$dG/dn = RT \ln [Na_o]/[Na_i] + E_m F. \quad (2)$$

This expression gives the energy per equivalent of sodium excreted, at a time when $[Na_o]$ and $[Na_i]$ are concentrations in the external fluid and in the fibre water respectively, while E_m is the membrane potential (outside minus inside).

The expression could be abbreviated to

$$dG/dn = F(E_{Na} + E_K), \quad (3)$$

where

$$E_K = \frac{RT}{F} \ln \frac{[K_i]}{[K_o]}$$

and

$$E_{Na} = \frac{RT}{F} \ln \frac{[Na_o]}{[Na_i]}$$

but equation (2) is preferred in this context as it expresses directly an osmotic and an electrical component of the energy barrier. That an upper

limit of the electrochemical potential for sodium must exist against which Na ions can no longer be secreted would seem sufficiently obvious, and for the frog's skin was determined by Ussing (1949), who investigated the maximum potential against which the active Na transport could occur. Harris (1954) has also referred to the experiments of Harris & Maizels (1951) on red corpuscles as suggesting that the lower limit of the Na levels encountered could be set by the possible electrochemical potential derivable from the extruding mechanism. This, however, would simplify overmuch, since such levels of a 'balanced state' will be set in part by the rate of sodium entrance, and it is likely that a higher electrochemical level could be reached by the active transport system. The significance of the present study relates to the following points:

1. The critical-energy barrier is rather sharp for the experimental conditions described, and on one side of it a high secretion rate of sodium occurs continuing over an hour or so, while on the other side of the barrier no sodium is excreted.

2. Under the conditions where the muscles are loaded with Na after soaking during the night at about 0° C in K-free Ringer's fluid, the critical energy barrier is much lower than would be expected from a consideration of the normal conditions *in vitro* or conditions *in vivo*. It is this fact that has previously obscured its occurrence.

3. It so happens also that under the conditions mentioned, with high Na levels in the muscles, a real entrance of sodium into the muscle (as distinct from the apparent isotopic flux) does not occur significantly over the 2 hr period of the immersion. This latter was shown by the absence of any significant increase in the sodium content during the 2 hr reimmersion period, when the excretion was inhibited by iodoacetate (2 mM) or by ouabain (10^{-5} M).

These points make this preparation fruitful to study with respect to the relation between Na excretion and O_2 uptake and to the similar relation under anaerobic conditions between the Na secretion and the lactic acid formed (E. J. Conway & M. Mullaney, unpublished observations). Light is also thrown on the fact that whereas cyanide (2 mM) was found in our laboratory to inhibit, to the extent of 60%, the excretion of Na in the 120,0/104,10 procedure, such inhibition was not observed by Frazier & Keynes (1959). For under our conditions, decreasing the energy barrier by raising the external [K] from 10 to 20 mM entirely removed any cyanide inhibition, and it would appear therefore that the difference in cyanide action observed could be due to different metabolic states of the muscles used.

In the existence of this critical energy barrier there now appears to be an explanation of the fact that, while there is no significant excretion over the 2 hr reimmersion with the 104,0/104,10 procedure, there is a large amount of sodium secretion in the 120,0/104,10 procedure.

It is to be noted that the difference in energy barrier when the external sodium concentration is decreased below that of the soaking fluid is due to a change in both terms of equation (2), electrical as well as osmotic. This is because the measured membrane potential is less in the reimmersion period of the 120,0/104,10 procedure than in that of the 120,0/120,10 experiments. In this way, although the value of $RT \ln [Na_o]/[Na_i]$ becomes greater at the end of the 2 hr excretion period in the 120,0/104,10 than its initial value in the 120,0/120,10 experiments, the total energy barrier becomes approximately the same as in the latter procedure.

The oxygen uptake experiments fit in well with the above considerations. The secretion of sodium is significantly correlated with the oxygen consumption, after subtracting a basic oxygen consumption when there is no Na secretion, as with the 104,0/104,10 procedure, or practically so as in the 120,0/120,10 procedure. As has been shown, the mean ratio of the number of Na^+ ions secreted to molecules of O_2 taken up is approximately 4·0.

An apparent difficulty in relation to the critical-energy-barrier concept for isolated Na-loaded muscles arises when one considers the question of the normal secretion of Na from the muscle fibre. Here the energy barrier must be about 4·00 cal/m-equiv, or twice the critical level as dealt with above. But the secretion of Na would appear to be extremely small compared with the rate of about 8 m-equiv Na/kg over 30 min in the 120/104 experiments on frog muscle.

In this context it may be pointed out that for the *in vivo* secretion of sodium from mammalian muscle (Conway & Hingerty, 1948) a definite net secretion has been shown from rat muscle when this is loaded with Na after 4 weeks on a K-free diet, during which period the plasma [K] falls markedly. When the rats are restored to a K-rich diet, and the plasma [K] goes above the normal level in 1 day, an excretion of Na occurs, but only at a mean rate over 3 days of about 0·05 m-equiv/kg/30 min. When so much KCl is ingested that the plasma [K] increases to 9·8 mM after about 1 day, the excretion rate of the sodium increases, but still only to about 0·1 m-equiv/kg/30 min. These results were confirmed by Muntwyler & Griffin (1951) with some difference in detail. The normal energy barrier to secretion in rats, from the available data (Conway & Hingerty, 1948), is about 4·0 cal/m-equiv, and the secretion from the Na-rich muscle fibres occurred at about 3·0–3·5 cal/m-equiv. The precise levels cannot be readily calculated, owing to uncertainty as to the location of Na in normal mammalian

muscles. As the plasma [K] falls there is an entrance of Na ions, and as it rises there is an excretion, indicating a critical barrier somewhere in the region of 3–4 cal/m-equiv.

The question then arises, do these two levels, one of about 2·0 cal/m-equiv in isolated Na-rich muscles, and the much higher level for normal frog or mammalian muscle, represent two modes of Na secretion? While there may be two modes, none the less they can be interpreted as one mode in accordance with the redox pump theory.

The critical energy barrier and the redox pump theory

The nature of the Na secretion may be briefly stated in accordance with the redox pump theory, as follows: The reductant of a redox system in the cell forms an ionic complex with the Na ion, and the following reaction takes place

$$\text{Red.Na}^+ = \text{Ox} + \text{Na}^+ + e. \tag{4}$$

It may be taken that there are enough free Na^+ ions to saturate the reductant.

The potential of this system may be written as E_1, and the potential of a second system which receives electrons from it may be written as E_2. As an equivalent of electrons passes from the first system to the oxidant of the second, there is a corresponding release of Na^+ ions into the cell. The energy change may be written

$$dG/dn = F(E_2 - E_1) + RT \ln [\text{Na}_i]. \tag{5}$$

If the first system can operate in the membrane and release its Na externally after transmitting electrons to the second system, then

$$dG/dn = F(E_2 - E_1') + RT \ln [\text{Na}_o] + E_m F. \tag{6}$$

The potential of the first system has increased to E_1' in this occurrence. This is a necessary consequence, since the value of dG/dn in equations (5) and (6) is the same, and $(RT \ln [\text{Na}_0] + E_m F)$ is much greater in equation (6) than $RT \ln [\text{Na}_i]$ in equation 5.

From equations (5) and (6) one may write:

$$F(E_2 - E_1) = F(E_2 - E_1') + RT \ln [\text{Na}_0]/[\text{Na}_i] + E_m F. \tag{7}$$

The expression on the left represents the source from which the energy for the secretion of Na can be derived.

In a series of hypothetical instances where $[\text{Na}_0]$ and E_m were to rise gradually, then E_1' would rise correspondingly until a point would be reached when the quantity $F(E_2 - E_1')$ were reduced to zero. It would be necessary that the reduction should be not quite to zero, in order that electrons should pass. But if it became zero the critical energy barrier

would be reached, and also if ($RT \ln [Na_0]/[Na_i] + E_m F$) actually exceeded $F(E_2 - E_1)$ no electrons could pass.

In this we have a clear explanation of the critical energy barrier. If this is of the first kind—around 2·0 cal/m-equiv—then the existence also of the second level, as *in vivo*, could be explained as follows.

The expression $F(E_2 - E_1)$ can be expanded to

$$F(E_2 - E_1) = F(E_{20} - E_{10}) - RT \ln \left[\frac{[Red]_2}{[Ox]_2} \frac{[Ox]_1}{[Red]_1} \right]; \quad (8)$$

and while E_{20} and E_{10} are characteristic redox constants, it is to be expected that the expression under the ln sign could change very appreciably under different metabolic conditions. Thus a change of $[Red]_2/[Ox]_2$ from 3·0 to 0·3 and a corresponding change of $[Ox_1]/[Red]_1$ over similar ranges could result in a total change of potential of 112 mV at room temperature, with a corresponding change in the energy level of about 2·58 cal/m-equiv.

It is of interest now to list briefly the various results which fit in with this view of critical energy barriers.

1. It seems to provide an explanation as to why with the 120,0/104,10 procedure a large average secretion of Na was obtained, whereas with the 104,0/104,10 or 120,0/120,10 procedures there was no significant average secretion.

2. It explains also why, with the 120,0/120,10 or the 104,0/104,10 procedures, on lessening the membrane potential by increasing the external [K] in the reimmersion period, full secretion can occur as in the 120,0/104,10 experiment.

3. It explains the results of the oxygen-uptake experiment in a very satisfactory manner. There appears to be a by-pass of electrons (which are ultimately received by oxygen in aerobic conditions), which is blocked when the energy barrier to secretion of Na reaches the critical level. When allowed to pass fully, by a lessening of the energy barrier, the ratio of Na ions secreted to the extra molecules of oxygen taken up is near an average of 4·0. The extra oxygen uptake is significantly correlated with the sodium secreted, and there is a large difference between the oxygen uptake in the 104,0/104,10 or the 120,0/120,10 procedures and the 120,0/104,10 procedure, corresponding to the sodium excreted in the latter experiments.

Besides these results, which are well explained, one may also draw attention to the following.

4. It can explain the fact that, when the K content of the Ringer's fluid in which fresh sartorii muscles are immersed is increased, one gets a marked increase in oxygen uptake (Solandt, 1936).

5. It can also provide an explanation why, when the external K content of Ringer's fluid is reduced or eliminated, the secretion of Na is greatly inhibited.

SUMMARY

1. The fact that an average net excretion of 18 m-equiv Na/kg occurs in the 120,0/104,10 procedure, while little or none is excreted on the average in the 104,0/104,10 and 120,0/120,10 procedures, can now be well explained by the existence of a critical energy barrier to the excretion of Na from the Na-rich muscle. ('The 120,0/104,10 procedure' implies soaking during the night in K-free Ringer's fluid containing 120 mM-Na, and reimmersion for 2 hr at room temperature in Ringer–Conway fluid containing 104 mM-Na, and 10 mM-K. The other numbers are to be interpreted similarly.)

2. At the beginning of the reimmersion period with the 120,0/104,10 procedure the average membrane potential was $63{\cdot}4 \pm 0{\cdot}7$ mV, whereas for the 104,0/104,10 procedure it was $71{\cdot}7 \pm 0{\cdot}9$ mV; 25 sartorii and their companion muscles being used for these determinations. The initial energy barriers for the two procedures were 1·81 and 2·16 cal/m-equiv Na.

3. At the end of the period of reimmersion the membrane potentials were $56{\cdot}0 \pm 1{\cdot}1$ and $65{\cdot}0 \pm 0{\cdot}8$ mV respectively, and the energy barriers were 2·05 and 2·06 cal/m-equiv.

4. When the excretion of sodium is prevented in the Na-rich muscles by the energy barrier reaching the critical level or passing beyond it, full net excretion can be established by decreasing the external sodium or by raising the external potassium concentration. Both procedures decrease the energy barrier. When the excretion is set going by decreasing the external sodium concentration only, the membrane potential drops to a somewhat lower level.

5. The correlation coefficient of the net excretion of Na with O_2 uptake was found to be $0{\cdot}68 \pm 0{\cdot}09$, and the regression equation for the relation between Na excretion and the oxygen uptake indicates that on the average 3·8 m-equiv Na were excreted per m-mole of extra O_2 taken up.

6. The extra O_2 uptake in the excretion of Na showed no significant correlation with the Na level in muscle.

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