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THE DEPENDENCE OF EFFLUX OF SODIUM FROM FROG MUSCLE ON INTERNAL SODIUM AND EXTERNAL POTASSIUM

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The use of isotopic sodium to follow the efflux of sodium from cells, in particular muscle, has led to some confusion because there is the possibility of a considerable self exchange not requiring energy to which may sometimes be added the effects of net changes. The net movement of sodium from muscle can be made to fall by reducing the external sodium concentration (Keynes & Swan, 1959*a*). On the other hand, net output can be stimulated temporarily by reduction of the external sodium (Shaw & Simon, 1955; Carey, Conway & Kernan, 1959). The apparent contradiction can be resolved in terms of the relation between efflux and internal concentration (Keynes & Swan, 1959*a*; Mullins & Frumento, 1963) on the one hand, and the external sodium concentration as one factor determining the possibility of active efflux on the other. In the present paper, use has been made of a number of older results obtained in sodium media and results more recently obtained in sodium-free media to compare the efflux-content relations and in particular to show the importance of the external potassium level.

The suggestion is made that there is a saturatable exchange flux between sodium and either sodium, choline or lithium and in addition a potassium-contingent active component of efflux. The definition of 'active' in this context presents difficulty because potassium can stimulate an extra efflux to a sodium-free solution (as shown by Keynes & Swan) where there is no obvious energy requirement.

The results obtained have necessarily included some bearing on the extracellular space. The space is kinetically inhomogeneous and totals about 0·25 ml./100 g tissue. Its presence explains a number of anomalies met in the study of potassium movement as discussed by Sjodin & Henderson (1964).

METHODS

The methods were similar to those used by Edwards & Harris (1957) with modifications in later experiments in the counting procedure and solutions, which will be described. The movement of isotopic sodium from the sartorius muscle was followed after a preliminary period of loading which was for 2 hr or longer at 20° C or in some cases overnight at 4° C. Attention was paid to the load time in order to permit correction for incomplete equilibration.

In a number of runs in which sodium-free solution was used, the portions were subsequently analysed for sodium by flame photometry with an estimated accuracy of ±2%. A convenient method for running the combined tracer and analytical experiment was to pass the tissue on a holder along a series of small tubes containing the solution. The tubes fitted into the well of a scintillation counter and after assay of the radioactivity the fluid could be held directly under the intake of the flame photometer. The muscles were mounted on stainless-steel hooks and tied on a fine tube used to introduce CO_2–O_2 mixture. Following the remarks of Mullins & Frumento (1963) on the deleterious action of bubbling, use was made of a vertical flat Teflon sheet to separate the fluid in the tube with the tissue suspended in one side and the stream of bubbles passing up the other. In this way a circulation of the fluid was induced.

A number of early experimental results have been assembled to obtain the collected flux results plotted in Fig. 8; those obtained with *Leptodactylus* muscles using sodium solution *A* are distinguished in Fig. 8. Other experiments in sodium media were run in sodium solution *B* or, in a few cases in solution *C*. It was thought that the presence of insulin and lactate might help maintain a steady sodium level in low Na tissues (Smillie & Manery, 1960; Kernan, 1962 *b*) but no clear evidence was obtained. The choline solution used was as mixture *C* but with choline replacing sodium, and in the lithium mixture lithium similarly replaced sodium.

The sodium mixture *A* contained (mM): NaCl, 90; NaH_2PO_4, 1; $NaHCO_3$, 30; KCl, 2·5; $CaCl_2$, 1; $MgCl_2$, 1 (for *Leptodactylus* Fig. 8). The sodium mixture *B* contained (mM): NaCl, 90; $NaHCO_3$, 20; KCl, 3 (or as specified); $CaCl_2$, 1; $MgSO_4$, 1. The sodium mixture *C* contained (mM): NaCl, 97; $NaHCO_3$, 20; KCl, 3 or zero; Na lactate, 3; Ca acetate, 1; Mg acetate, 1; insulin, 0·05 u./ml. The sucrose mixture contained: sucrose, 6%; (mM) K lactate, 5; Ca acetate, 3; Mg acetate, 1. The potassium mixture contained (mM): K methyl sulphate, 90; K lactate, 3; $KHCO_3$, 20; Ca acetate, 1; Mg acetate, 1. The magnesium acetate mixture contained (mM): Mg acetate, 110; K lactate, 3; Ca acetate, 1.

At the end of the experiments the residual radioactivity was assayed either after ashing (earlier experiments) or directly in the well of the scintillation counter. Cation analyses were made with a flame photometer and are estimated to be accurate to 2%.

RESULTS

Sodium collected in 1 min. The sodium and the chloride collected in a 1 min wash, either in 20 mM potassium bicarbonate in 6% sucrose or in 110 mM magnesium acetate, from a number of sartorius and semitendinous muscles (not used for sodium efflux study) were used to calculate an equivalent space. In sucrose mixture the sodium space was 10·0 ± 0·29 ml./100 g (S.E. 67 analyses) and in the same series the chloride space was 11·9 ± 0·30 ml./100 g. The difference between sodium and chloride values is significant to the 0·1% level. In twenty-four pairs of analyses after use of magnesium-acetate wash the chloride space was 11·7 ± 0·50 and the chloride space was 13·8 ± 0·73 ml./100 g; the difference is significant to the 5% level. In ten pairs of freshly dissected muscles the sodium space came to 10·5 ± 0·82 and the chloride space to 15·4 ± 1·5 ml./100 g; the difference is significant to the 2% level. An explanation of the greater amount of chloride collected may well be that there is a significant loss of cellular chloride in the 1 min during which, of course, by no means all the extracellular material has been removed. Comparisons

etween the 1 min spaces of fresh and soaked tissues in sixteen experiments showed a mean increase of 1·7 ml./100 g which is of doubtful significance. Clearly the material collected in 1 min represents the most variable superficial part of the extracellular space.

The second extracellular space (denoted by compartment 1 in Harris (1963) and corresponding to Carey & Conway's (1954) 'special region' and Mullins & Frumento's (1963) 'extracellular space'). The further quantity of extracellular sodium and its rate of emergence has to be found by difference because under all conditions of loading there is a comparable amount of sodium in the cells. An assumption has to be made about the kinetic law followed by the cellular sodium in order to make an extrapolation of its time curve. Under steady-state conditions the simple

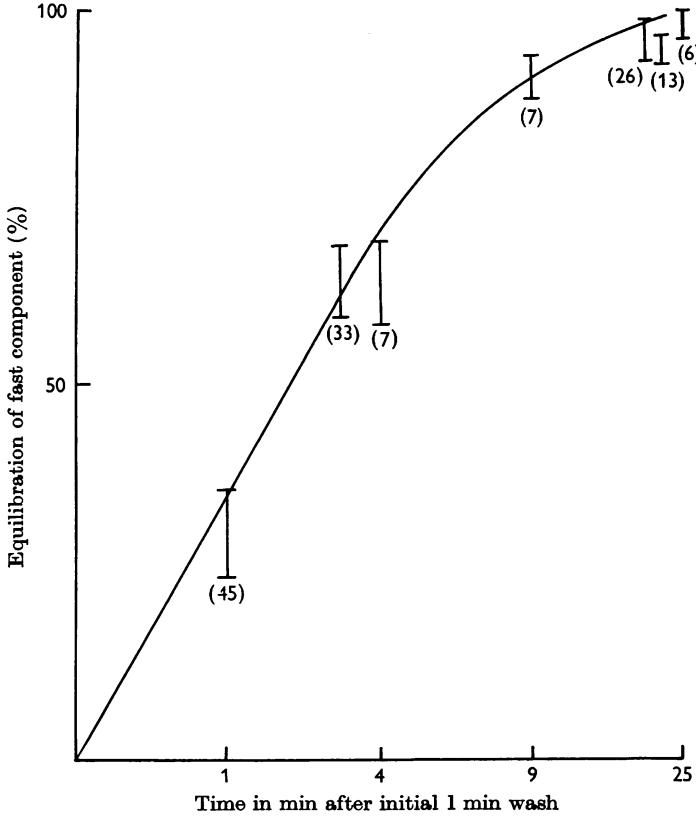

Fig. 1. The time course of the equilibration of the extracellular space after an initial 1 min wash. The values are obtained by difference between total sodium and extrapolated internal sodium at 20° C. The line is drawn according to the diffusion equation for a flat sheet and fitted by eye to the observations. The bracketed figures are the numbers of results and the vertical bars are two standard deviations in length.

exponential law seems to hold. Making the extrapolation and subtracting from the total sodium remaining after the initial 1 min wash gives the amount of more slowly lost extracellular material, and from readings during the wash out a time curve can be constructed. For muscles equilibrated for a few hours in saline mixtures a mean additional space equal to $12{\cdot}2 \pm 0{\cdot}42$ ml./100 g was found (S.E. 47 values) and after use of low sodium media the figure was insignificantly different $14{\cdot}2 \pm 1{\cdot}3$ (10 values).

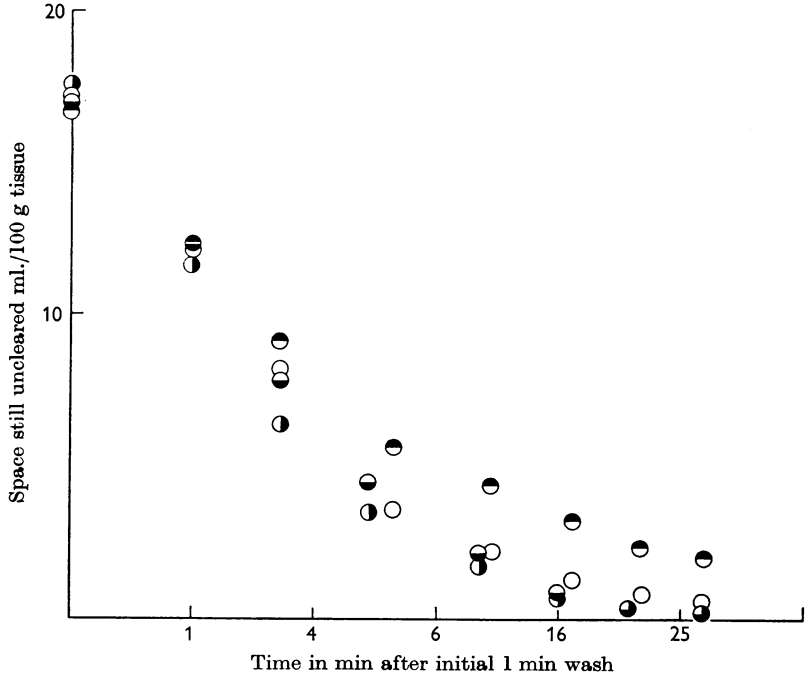

Fig. 2. The time course of clearance of the extracellular space after an initial 1 min wash. Values were obtained using ^{24}Na and $^{35}SO_4$ together in each of two runs. The sodium space was taken as the difference between the total Na and the extrapolated value of the slow fraction at the time in question. Wash out at 0° C (◓ SO_4, ○ Na); wash out at 20° C (◒ SO_4, ◑ Na).

The time course of loss of this presumed extracellular material did not appear to depend on the solution being used for the washing so, to put the points on Fig. 1, all values have been averaged, and the standard deviations shown. The line drawn through the most significant point was calculated for diffusion from a sheet; at 20° C the second space is half cleared in 1·74 min and reaches $1/e$ (the course plotted is not exponential) in 3 min. This 'time constant' agrees well with Mullins & Frumento's figure for sodium moving into choline solution. Movement of chloride or sulphate from the space proceeds with a similar course and the values fall within the scatter of the values found with sodium. Using $^{24}Na^+$ and $^{35}SO_4^{2-}$ together, the wash out of the sulphate and the fast component of the sodium were compared at 20 and 0° C (Fig. 2). At 20° C the agreement is excellent but appreciable sulphate is held up at 0° C. The result obtained with sodium at 0° C is more complicated

than Fig. 2 indicates because when an efflux run is made with a temperature change from 0 to 20° C, after 20 or 30 min a greater release of sodium is found in the next 10 min than that expected from other runs started at 20° C. Careful examination of the specific activity of the sodium in eluates to Na-free solution showed that soon after the temperature change the sodium had a higher degree of exchange than that in later portions so it is likely that some 'extracellular sodium is, like sulphate, retained at 0° C. The amount, estimated from the specific activity data, was between 2 and 3 m-equiv/kg. This phenomenon, whatever its explanation, removes the advantage of starting wash-out experiments at 0° C with the object of first clearing the space and then obtaining flux measurements at 20° C on a maximal quantity of cellular sodium.

The internal sodium: efflux when internal sodium diminishes compared with the steady state. Comparisons between the time course of loss of labelled sodium from pairs of muscles in sodium-salt solutions and in sodium-free solutions having lithium, choline or potassium as major cation showed that although in the first 30–90 min the efflux to the Na-free solution can be higher than to the sodium solution it eventually becomes lower. This transition can be seen in Fig. 3.

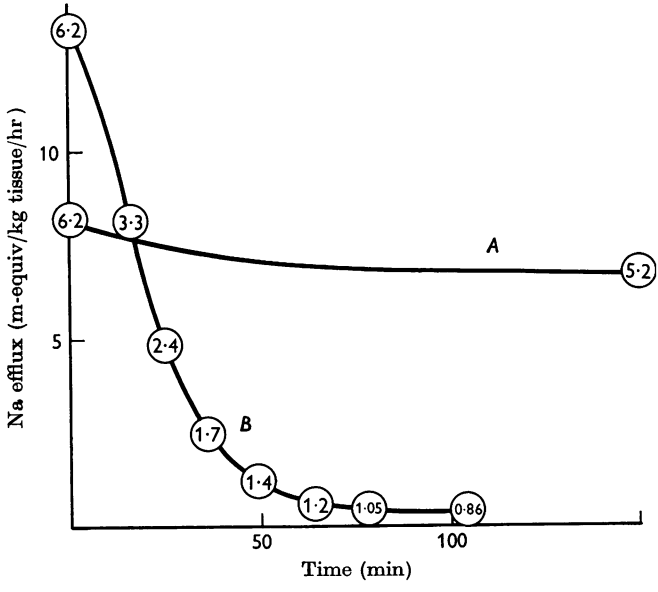

Fig. 3. Efflux–time curves obtained with paired muscles loaded for 2 hr with sodium and then washed out either into inactive sodium mixture (curve *A*) or into potassium mixture (curve *B*) at 20° C. Contents at various times are shown in the circles. The initial content of intracellular sodium (6·2 m-equiv/kg) was obtained from an extrapolation of the logarithmic plot of the content–time curve for the sodium mixture. The final content is calculated from the analysis by subtraction of unexchanged (1 m-equiv/kg) and extracellular sodium. The latter was taken from the difference between the initial labelled sodium and the extrapolated content of intracellular sodium. At the end of exposure to the potassium mixture the tissue contained 1·2 m-equiv unexchanged Na/kg.

The interpretation of such experiments can usefully be considered next. It is necessary to relate sodium flux to movement of labelled sodium. The latter has to be considered along with knowledge of the internal sodium level as a function of time. The simplest case is when Na-free solution has been used to wash out the labelled sodium as in curve *B*, Fig. 3.

After the tissue has been loaded in the radioactive mixture for a certain time its content of sodium is not fully exchanged for two reasons. First, the limited time is insufficient to complete the presumed first order process which operates in sodium-containing media with rate constant usually between 1 and 1·5 hr^{-1} for fresh tissue, and second, the tissue appears to include 1–2 m-equiv/kg of inexchangeable sodium (Conway & Carey, 1955; Harris & Steinbach, 1956; Keynes & Swan, 1959*a*). The former factor can be allowed for directly either when a control efflux into a sodium solution is carried out or when measurements of specific activity in the later eluates are made. Provided the residual sodium is quite inert its presence could be ignored. However, it was noticed that if muscles were stored overnight in K-free solution before attempting to label their Na, they contained more unexchanged Na (allowing for the incomplete loading as measured from the specific activity in the eluates) than did fresh muscles. Mullins & Frumento (1963) report an inexchangeable Na content of muscles so treated amounting to 5 m-equiv/kg, which is more than twice the value found starting with fresh tissue. Hence it is probably preferable to load the muscles by overnight exposure to the K-free labelled sodium solution when a high Na tissue is desired, though this was not always done in the present experiments.

Before leaving this aspect of the procedure the pair of experiments illustrated in Fig. 4 may be considered because the solutions used favoured accurate sodium analyses in the portions of eluate. The points plotted are based on the sodium analyses and the labelled sodium was only used to find the amount of labile sodium remaining in the tissue after 3 hr on the assumption that the specific activity of the residual sodium would be equal to that of all the later eluates. This left 1·5 and 1·9 m-equiv/kg as the respective contents of inert sodium after use of sucrose and magnesium-acetate mixtures. Had one based the curves exclusively on analyses they would have been shifted to the right by the amounts mentioned. The curves also show that output to magnesium acetate (+3 mM-K salt) from a given sodium content is slower than to the sucrose (+5 mM-K salt) mixture though the rates are nearly the same at the lower and higher limits examined.

Subject to the preceding considerations the Na-free media provide efflux–content relations in which the observed fall of efflux with time is a consequence of the falling Na content (e.g. as Fig. 3, curve *B*).

The case is quite otherwise when sodium exchanges for sodium. For the first order process the rate constant determining the fall in content of labelled ions is the same as that applying to the time differential; that is, to the flux (see for example in Keynes & Swan, 1959*a*, Table 2). The fall in output of labelled ions is attributable to the diminishing specific activity of the internal pool and not to a reduction of the total flux.

In the following the 'flux' is usually given as amount per kg tissue per hr; this comes directly by multiplication of the rate constant and the internal sodium. It can be converted to the more fundamental flux per unit area assuming, say, 400 cm^2 per g tissue and the contents can be converted to internal concentrations assuming 0·55–0·60 g water per g tissue. The relations between flux rate constant and internal Na can usefully be set down here:

$$d[Na^x]_i/dt = -k[Na^x)_i \equiv -KA/V[Na^x]_i \quad \text{(loss of labelled Na)}, \quad (1)$$

$$d[Na]_i/dt = \text{influx} - \frac{KA}{V}[Na]_i \quad \text{(net change of total sodium)}, \quad (2)$$

$$\text{influx} = \frac{KA}{V}[Na]_i \quad (t = \infty) \text{ (steady state after net change complete)}, \quad (3)$$

$$\text{so,} \quad \ln \frac{Na_i - Na_i\,(t = \infty)}{Na_i\,(t = 0) \; - Na_i\,(t = \infty)} = -KtA/V \quad (4)$$

(adjustment of Na_i during net change).

The extreme examples of net efflux and efflux during exchanges lead to the more complex case when sodium is gained or lost during the washing out of labelled sodium in a sodium-containing medium. To obtain the time course of the flux one needs time curves of both labelled and total sodium contents. Since the sodium level should follow a course having the same rate constant as that of the loss of labelled ions (equations (4) and (1) above) it is possible to calculate total sodium contents given an initial value and one measured after a certain time interval so long as KA/V is reasonably steady.

When there is a net gain of sodium during washing out the turnover rate observed applies to an increasing pool of material and a combination of falling rate constant and increasing content can be the consequence of a constant saturated efflux.

Na efflux to Na-free media. The curves in Fig. 4 are particular examples of experiments run in Na-free solutions. Most of the results to be described next are based on the use of labelled sodium with correction for the incomplete exchange of the labile fraction; where possible a note is made of the amount of inert sodium. To obtain a range of initial sodium contents experiments were made both on comparatively fresh tissue, as used by Keynes & Swan (1959*a*) and for Fig. 3, and on tissues which had been

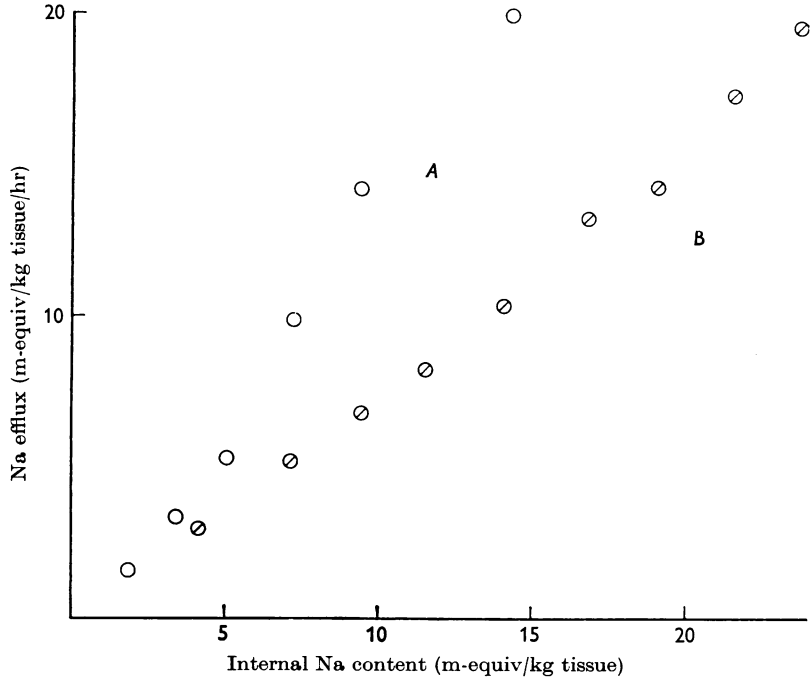

Fig. 4. The relation between sodium efflux and content found in two runs in which all but the final tissue-sodium figures plotted were measured by analysis. At the end of the runs the tissue in the sucrose mixture contained 1·5 m-equiv unexchanged sodium and the other 1·9 m-equiv/kg. These quantities are additional to the residual contents of labelled sodium used for plotting and if taken into account would move the curves over to the right by the respective amounts. ○, in sucrose mixture; ⊘, in magnesium acetate mixture.

enriched with Na overnight, as used by Mullins & Frumento (1963) and for Figs. 5 and 6.

The results can be grouped according to the solution used. The highest efflux from a given sodium content was to the potassium methyl sulphate mixture (Fig. 5, curve *A*). When the muscle was put in this solution there was a contracture lasting about 1 min. The efflux values are so high that it was not possible to obtain values for tissues having a particularly high Na content. In any case with high fluxes a diffusion limitation would be approached. As a contrast to the potassium solution the magnesium-acetate mixture (with 3 mM-K salt) provided low effluxes (Fig. 5, curve *B*). In its lower range curve *B* will be seen to correspond to some results in K-free solutions given later.

The relations obtained in the choline or lithium mixtures were sensitive to the presence of potassium. This corresponds to Keynes & Swan's (1959*a*)

observation that omission of K caused a reduced efflux in Li solution. Figure 6, curve *A*, shows points from three runs in choline and four in lithium mixtures *without* potassium. It was confirmed that use of potassium in the mixture accelerated output. The effect obtained depended upon the internal sodium content at the time of making the test and on some other

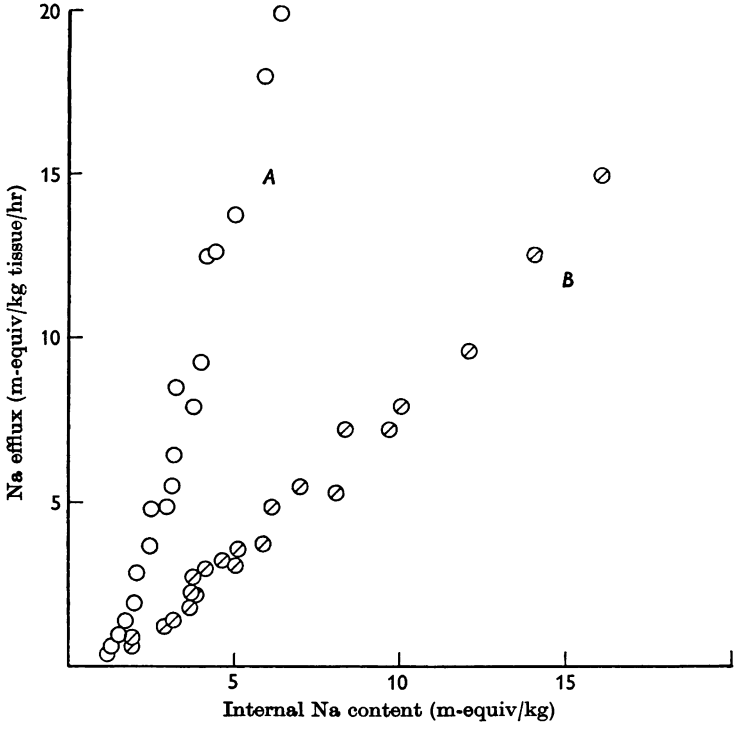

Fig. 5. Sodium efflux–content relations found in potassium mixture (○) and magnesium-acetate mixture (⌀) at 20° C. Contents plotted do not include the in-exchangeable Na. In one of the potassium runs this was measured (1·2 m-equiv/kg) and in the three others the values were 0·4, 1·3 and 1·9 m-equiv/kg.

unknown factor. The points on the branch *B* in Fig. 6 were obtained from three experiments with 3 mM-K salt in the lithium mixture plus one with 0·5 mM-K salt in the lithium mixture plus two experiments in choline mixture with 3 mM-K salt. There is a clear trend towards saturation as described by Mullins & Frumento (1963) in relation to results obtained under similar conditions. In two other runs using choline mixture with 3 mM-K salt the saturation did not set in at such a low efflux; the points are on branch *C* in Fig. 6. To produce a response to potassium an internal level of exchangeable sodium exceeding about 3 m-equiv/kg is necessary. The response is greater in the extreme condition of use of isotonic potassium methyl sulphate (Fig. 5*A*). The run in sucrose mixture

(Fig. 4*A*) provides an efflux–content relation resembling though not identical with that found in the runs of Fig. 6*B*. In the sucrose mixture the tissue loses phosphate (Harris & Steinbach, 1956) and shrinks despite the use of less than the isosmotic concentration.

Portions of the efflux–content relation obtained in presence of potassium if plotted on a log–log scale followed a law with efflux proportional to

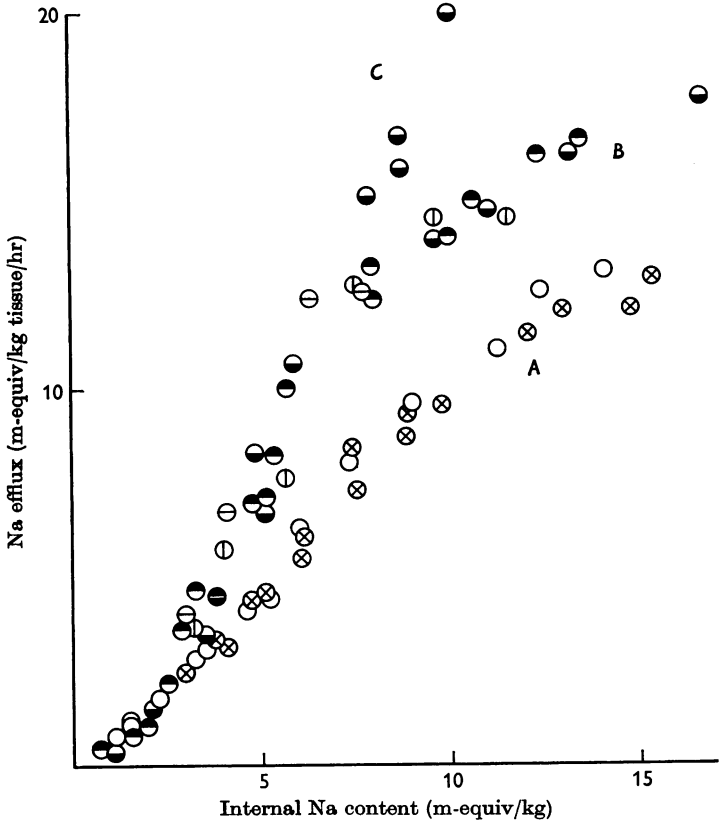

Fig. 6. The efflux–content relations found (*A*) in K-free choline or lithium mixtures (*B*) with 0·5 mM-K plus lithium or choline or 3 mM-K plus lithium and also 3 mM-K plus choline in one experiment (*C*) with 3 mM-K plus choline in two further experiments.

Symbol	Solution	Final inexchangeable sodium contents not included in total sodium content (m-equiv/kg tissue)
○	K-free lithium	5·2, 1·1, 3·8, 2·6
⊗	K-free choline	3·2, 1·3, 1·7
◐	0·5 mM-K plus lithium	2·4
⊖	0·5 mM-K plus choline	2·0
◓	3 mM-K plus lithium	0·7, 1·7, 1·9, 1·4
◒	3 mM-K plus choline	0·4, 0·7, 1·1

Na content raised to a power between 2·2 and 3·1 over a limited range. Keynes (1963) has remarked that the use of media having a high bicarbonate concentration leads to an efflux less dependent on internal sodium than in absence of bicarbonate. In the isotonic potassium medium the steepest relation was seen (Fig. 7); in the other potassium-containing mixtures the power 2·2 is a typical example but the portion around 2 m-equiv/kg tended to be steeper. Mullins & Frumento (1963) in the

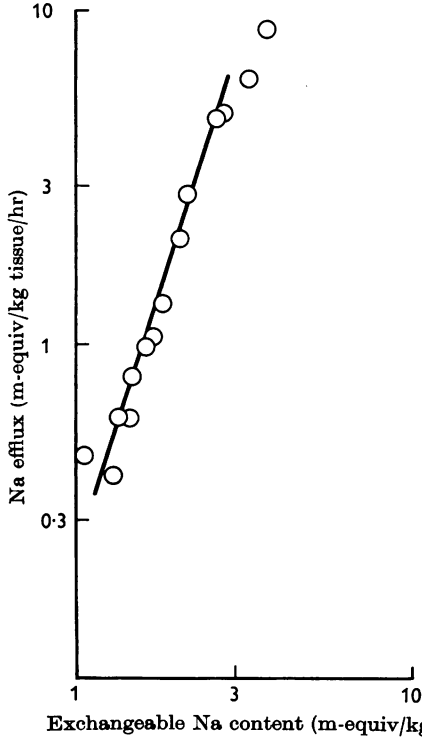

Fig. 7. Points from portions of two efflux–content relations obtained in the potassium solution showing the cubic region.

same context report a range of powers between 2 and 3·5. The suggestion may be made here that the greatest dependence on internal sodium level is found under conditions which most favour the onset of an 'active' exchange between internal Na and external K, although the curvature at the foot is present even in the absence of active movement. The active process would not be defined as one necessarily requiring an energy supply but rather as a chemically mediated interchange of the two ions which *normally* requires energy to work against a gradient. There is analytical evidence that the tissue takes up potassium from the choline plus potassium

mixture (Mullins & Frumento, 1963) and a similar uptake of K was found to take place from the lithium mixture. Net transfers of K from bath to tissue between 5 and 6 m-equiv/kg tissue were measured in 1 hr exposures to mixtures having 3·5 mM-K salt. It is of course likely that there was a concurrent loss of some K in exchange for choline or lithium which would offset some of the Na-for-K exchange.

Efflux to sodium solutions. The output of labelled sodium from a loaded muscle can occur with little or no net change in content. In this case the logarithmic plot of the time curve yields a rate constant for turnover and, by extrapolation, an internal level. A rough check on the absence of net change is afforded by comparison of the final content of sodium with the initial content of labelled sodium. A number of these experiments, some of which have been described (e.g. Edwards & Harris, 1957), provided the flux–content relation plotted in Fig. 8. The open circles were obtained from

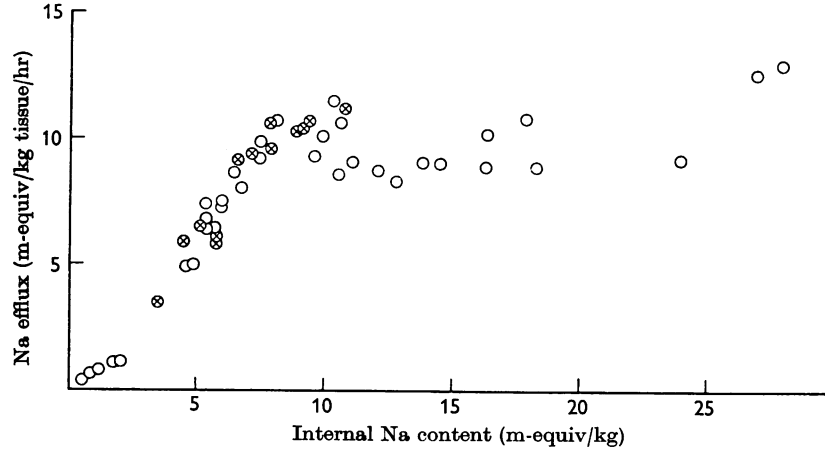

Fig. 8. The sodium efflux–content relation found in sodium solutions under conditions when net output was probably minimal. Either the final sodium contents were compared with initial labelled contents or, in other experiments, the rate constant was found in K-free or strophanthin-containing media.

Rana and those marked with crosses from *Leptodactylus* muscles. Points for contents exceeding 3·5 m-equiv/kg were obtained using normal sodium media (*A* or *B* in Methods) while the few points for low sodium contents were obtained using media which were mixtures of either sucrose mixture or choline mixture with the sodium solution to give 10–16 mM sodium salt. The tissues were loaded in the normal sodium concentration for 1 hr, then their sodium content was reduced by adding sodium-free diluent to the load solution and keeping them in the mixture for a further 2 hr. Finally, the efflux of labelled sodium was followed in the usual way but the wash-out solution was made up with an (inactive) sodium concentration equal

to that which had been present in the diluted load solution. Without the preliminary load in high sodium solution the tissue sodium did not become so completely exchanged during the loading treatment.

A part of the flux value plotted in Fig. 8 may have been associated with active movement. This is because the comparison of initial (labelled) sodium content with final analytical content includes several sources of error so some net change cannot be excluded. However, there does appear to be a saturation of the 'exchange' flux at between 9 and 10 m-equiv/kg/hr when the content exceeds about 10 m-equiv/kg. The form of the flux–content relation resembles that found in K-free, Na-free media (Fig.6*A*).

TABLE 1. Observations which show that an increased efflux of labelled sodium is associated with the net extrusion of sodium from muscles having initially a high sodium content

Conditions	Net efflux	Effect on labelled sodium efflux
10 mM-K salt added to sodium salt solution	About 30 m-equiv Na lost per kg tissue per hr with rate constant 1·2 hr^{-1} (Frazier & Keynes, 1959)	Flux increased by between 1·7 and 4·6 times, mean 2·7. (Keynes, 1954)
4 mM-K salt added to sodium salt solution	Initial rate of Na loss up to 18 m-equiv per kg per hr (new measurement)	Flux increases by between 1·6 and 2·2 times (Edwards & Harris, 1957)
Stretch applied in medium having 3 mM K salt	Na content falls to 70 % of initial value in 10 min	Flux increased by between 2 and 3 times (Harris, 1954)

When conditions are altered so that net output is stimulated the labelled sodium efflux increases; it appears that the net efflux is added to the exchange efflux. To show the parallel between the two processes some observations, mostly from published sources, are collected in Table 1. The purpose of this is to emphasize that the limitation to efflux seen in Fig. 8 must be removed or moved up by a factor of between 2 and 4 when active output is known to be taking place. Similarly when conditions are changed so that active movements are suppressed, e.g. by omission of potassium, or addition of cardiac glycosides such as strophanthin, the tracer efflux is reduced (Edwards & Harris, 1957).

This kind of comparison has led to the conclusion that the observed tracer movement is made up of an ion-exchange component plus a variable active component. However, the similar behaviour of the sodium in K-free, Na-free media and in near-absence of active movement in Na solution (Fig. 8) suggests that the ion-exchange component is not particularly specific. The further feature shown by comparing the points in Fig. 6 curves *B* and *C* with Fig. 8 is that in the presence of potassium the absence of external sodium can lead to a greater efflux from a given Na content than corresponds to normal exchange; this is attributed to the presence

of an active component of efflux which reduction of the external sodium level will have made less energy demanding.

Other cation movements. Analyses were routinely made for alkali cations; these enable some deductions to be made about the compensatory movements which occur when the tissue sodium is lost. The figures in Table 2 are for tissues after a 1 min wash in magnesium acetate (which collected the sodium equivalent to about 12% space). The figures are in two groups, one for tissues which had been loaded for 2–3 hr before the wash out and the other for tissues which had first been enriched with sodium.

TABLE 2. Cation contents of muscles before and after treatment with Na-free lithium or choline solutions. Tissues washed 1 min in magnesium acetate solution before analysis. The variability is indicated by the S.E. of the mean; the number of experiments is shown in parentheses

Details	K (m-equiv/kg tissue)	Na (m-equiv/kg tissue)	Weight ratio: treated/fresh
Tissue loaded 2–3 hr in sodium mixture *B* (3 mM-K)	83·5 ± 1·2	19·8 ± 0·7 (15)	1·03 ± 0·008 (15)
After a further 3–3·5 hr in lithium or choline mixture (with 3 mM-K)	54·5 ± 2·0 (6)	2·7 ± 0·4 (6) (Li = 32, 37 m-equiv/kg)	0·89 ± 0·017 (8)
Tissue loaded with sodium in K-free solution overnight	52·5 ± 1·70	58·0 ± 1·15 (15)	Not measured
After a further 3–3·5 hr in lithium or choline mixture (part of this time with K present)	59 ± 1·42 (15)	Between 1·5 and 5 (Li gain = 35 ± 6·5 (5)	0·97 ± 0·013 (15)

The changes in the first group amount to a loss of about 29 m-equiv K and 17 m-equiv Na per kg. In the two analyses made the gain of lithium was 32 and 37 m-equiv/kg; the loss in weight between the loaded condition and the end of the wash out would correspond to a net loss of about 16 m-equiv cation from the tissue so a balance is obtained by a combination of cation exchange and net loss. There is no reason to doubt that the argument applies equally to choline solution though choline analyses were not made. In the second group there is perhaps a gain of 6·5 m-equiv K/kg, provided K ions are supplied at some stage of the output. The gain agrees with Mullins & Frumento's estimate and with a separate result given in the text. There is a loss of 56 m-equiv Na per kg and a gain of 35 m-equiv Li per kg from the lithium media; the shrinkage corresponds to a net cation loss of about 3 m-equiv/kg, so taking into consideration the wide individual variations an approximate balance is again obtained. An obvious gap in our knowledge about the system is the effect of the Na-free media on the phosphate esters in the tissue and on the rate of the liberation of inorganic phosphate, which is known to be promoted in sucrose solution (Harris & Steinbach, 1956; Mullins & Noda, 1963).

Sodium output and oxygen consumption. Conway, Kernan & Zadunaisky (1961) have shown that the net output of sodium is related to the extra oxygen consumed above a standing rate of between 3 and 5·7 m-mole/kg/hr. The question of whether an appreciable part of the standing rate is associated with the maintenance of a constant sodium content can be approached by comparing oxygen consumption in sodium and non-sodium media. This has been done by the Warburg method (Table 3) for a number of periods

TABLE 3. Oxygen consumption (μl./hr/g tissue) by muscles in different media at 20° C

(1) Using fresh muscles and 5 mM lactate in all media. 3 mM-K salt added to the sodium, lithium and choline media

Time interval (hr)	Media			
	Sodium	Choline	Lithium	Potassium
0–1	135	284	130	158
1–2	122	484	111	115
		Sodium	Sodium	Sodium
2–3	121	237	116	100
3–4	89	122	81	89

(2) Conditions as before, no lactate with Mg solution

Time interval (hr)	Choline	Magnesium acetate	Potassium
0–1	109	75	87
1–2	154	69	63
	Sodium	Sodium	Sodium
2–3	66	63	93

(3) Using muscles which had been stored overnight in K-free solution. No lactate added to media

Time interval (hr)	Sodium	Lithium	Potassium
0–1	48	60	59
1–3	76	65	74

after an initial equilibration of 30 min. It can be seen that in lithium and potassium media the oxygen consumption is about the same as in sodium solution. The only anomaly is provided by choline which causes a high oxygen consumption. Possibly this can be related to oxidation of the choline.

The comparatively high oxygen consumption observed in groups 1 and 2, Table 3, must be attributed in part to the presence of lactate (Smillie & Manery, 1960), which is said to be converted aerobically to glycogen, and in part to the use of fresh tissue.

DISCUSSION

Extracellular space. Use of the 1 min wash procedure has a practical convenience because the adherent film of fluid is removed; the quantity of solutes held superficially depends on blotting and is a variable factor. At the start of an experiment blotting is to be avoided in any case. The total

extracellular space measured as the sum of the space holding the material removed in 1 min plus the remaining fast component is about 25%; this compares well with inulin spaces measured in the muscles of a number of frogs kept in similar conditions. This value of the space necessitates revision of the treatment of a number of results previously obtained with potassium because it accounts for the fast component and leaves a purely exponential component (compare Sjodin & Henderson, 1964). The space also enters the calculation of internal concentrations and the correction required for the extracellular components of the changes in recovery experiments of the Steinbach–Conway type.

The sodium flux. The most important feature of the collected results is the finding that the steady-state sodium exchange and the Na-choline and Na-Li exchanges share a curved flux-content relation. The nature of the dependence of flux on content means that the diffusing carrier requires a certain minimum load of sodium ions which seems to be between 2 and 3 according to the conditions. The eventual saturation implies that the supply of carriers and their rate of passage is limited and that such limitation might arise from interference between inward and outward moving carriers. Given an incomplete selectivity for sodium the carrier on its outward journey can give rise to the leakage flux of potassium and in a similar way can allow the loss of lithium from Li-loaded cells to sodium solution (Keynes & Swan, 1959*b*).

When active sodium movement is stimulated by the presence of external potassium (Fig. 6) the efflux remains raised so long as the cells contain sufficient sodium. The increased efflux may be consequent upon removal of interference between inward and outward moving passive carriers if active movement involves a change diverting them to a different inward path. At the same time the process of loading the carriers inside the cell is kept common to both active and passive exchanges.

The inhibiting effect of magnesium in high concentration may be connected with its depressing influence upon the enzymes involved in active movement. It is known to depress reversed electron transfer (Chance & Hagihara, 1963).

Work on other, more active, cells has provided evidence for a relation between sodium pumping and metabolism; both are promoted by external potassium and inhibited by the cardiac glycosides. Whittam & Willis (1963) and Whittam (1962*a*) have shown that the oxygen consumption of kidney and brain tissue is depressed by omission of sodium or addition of ouabain; that is, when active sodium movement is stopped. The ATP-ase activity of human erythrocyte ghosts varies with their internal sodium content (Whittam, 1962*b*). Judah & Ahmed (1964) have made the attractive proposal that the effects of low sodium concentration and of

ouabain may be consequent upon the accumulation of calcium and inhibition of some enzymes by this ion rather than there being a direct relation between sodium and metabolism. This idea is susceptible of experimental test.

Besides exerting a direct or indirect control of biochemical processes the internal sodium and external potassium levels determine the energy requirement for excretion of sodium against the gradient of electrochemical potential. If the energy requirement exceeds a certain value, the 'critical energy' of Conway, then active output cannot take place. The effluxes in Fig. 8 are considered to be wholly or nearly of an exchange nature. When either the energy requirement is diminished (for example, by increasing external potassium) or the biochemical system is stimulated—as happens apparently with insulin and lactate (Kernan, 1962*a*, *b*, and compare Manery, Gourley & Fisher, 1956 and Smillie & Manery, 1960) the active process can set in to drive the internal sodium down to a lower level. The value approached is set by the combination of increased energy requirement and lower supply of internal sodium to the carrier system. It is questionable how important the latter influence is in a normal environment, but its existence when internal sodium is sufficiently low is indicated by the running together of the flux–content curves in Fig. 6. The observation (Fig. 6) that efflux to sodium-free media is stimulated by potassium can be regarded as indicative of a coupled Na-K active process. However potassium cannot stimulate efflux from a muscle which already has a low sodium content; as mentioned above, this may reflect a change in calcium level.

The saturation seen in the exchange flux was mentioned as likely by Frazier & Keynes (1959) and the constancy of flux from tissues having a range of contents was noted by Harris (1950). It is interesting to note that the immediate consequence of transfer from a sodium solution of a tissue having a high sodium content (say > 10 m-equiv/kg) which is not giving active efflux to a reduced sodium solution containing some potassium is an increased efflux on account of the onset of the active process. The flux shifts to a value, for example, on Fig. 6*B* or *C* from one on Fig. 8. Later, as the content falls the flux passes through its former value and finally becomes less. Evidently if one starts with a tissue having a sodium content of only 6 m-equiv/kg, as in many fresh tissues, there will be little opportunity to observe the brief acceleration of output before the falling internal level has its own influence in reducing output. The time curves (Figs. 1–3) of Keynes & Swan (1959*a*) show that after change to lithium from sodium solution the flux falls in 15–20 min to a lower figure. The tracer efflux can be restored more quickly on return to sodium solution which of course provides inactive sodium both to dilute the labelled sodium in the cells and to

exchange with it. There is little evidence for a parallel active Li output (Keynes & Swan, 1959*b*). Similar results on Na efflux were obtained in the present experiments using potassium salt to reduce the internal sodium. After 90 min the content of labelled sodium was about 1 m-equiv/kg (there was also present about the same amount of unexchanged sodium). If, at this time the tissue was put into sodium mixture (solution *C* in Methods was used), there was a rise in the rate of loss of the tracer which must have entailed a much greater rise in the actual sodium efflux because

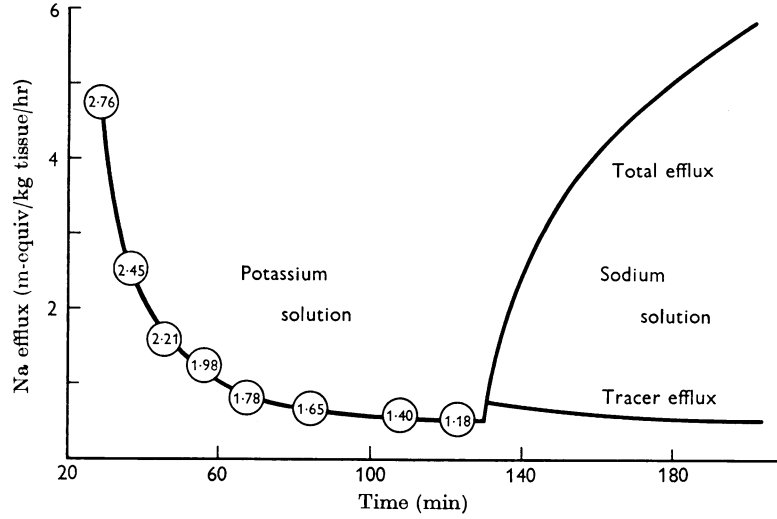

Fig. 9. The time course of fall of sodium efflux and sodium content in potassium solution and the restoration of efflux when the tissue is transferred to sodium solution (Methods, *C*). Contents are given in the circles. The lower line after 130 min gives values proportional to the movement of radioactivity; as in fact inactive sodium is now entering the cell the true efflux (proportional to radioactivity moving/specific activity) is a rising function of time. The estimated true efflux is given by the upper line. This is based on an assumed influx of 8·8 m-equiv/kg per hr taken from the measured exchange flux of the paired muscle in sodium solution together with the efflux–content relation plotted in Fig. 8.

of the falling specific activity. In Fig. 9 the observed time curve was used together with an estimate of the final internal sodium content to construct an estimated time curve of the efflux. The main object is to emphasize the large increase of the total Na efflux brought about by admitting more sodium. Evidently, in the condition that efflux depends on a power of the internal sodium level, then dilution by inactive material will lead to the observed increase in rate of tracer output. The alternative explanation requiring the presence of a specific fraction of sodium capable only of exchange against sodium is not called for. In this connexion Dick & Lea (1964) have been led to postulate a specific sodium self-exchange in

amphibian oocytes because the efflux does not respond as much to osmotic shrinkage as to the sodium content at constant volume. To apply the same argument to the muscle cell, part of which actually swells in hypertonic media (Dydynska & Wilkie, 1963), is perhaps unjustified at present.

Some variations in the procedure of changing from a potassium wash-out solution to a sodium solution have been tested. After adding 2 mM iodoacetate (I.A.A.) to the sodium solution during wash out after a prolonged period in potassium solution the rate constants governing loss of the labelled sodium increased (I.A.A. 1·1 hr^{-1}, control 1·0 hr^{-1}; I.A.A. 1·0 hr^{-1}, control 0·7 hr^{-1}). As after use of the poison the final tissue sodium contents were found to be higher than in the controls (by 3 and 7 m-equiv/kg, respectively) it must be inferred that the I.A.A. caused both the efflux (given by the product of rate constant and sodium concentration at the time) and the influx to rise, with the greater effect on influx. If strophanthin (4×10^{-6} g/ml.) was added to the sodium solution used after a prolonged immersion in potassium wash-out solution, the tracer efflux was still accelerated relative to the rate in potassium but the rate constant (0·67 hr^{-1}) was less than in a parallel control transferred to sodium without strophanthin (1·14 hr^{-1}). The poisoned muscle took up more sodium than the control (3 m-equiv/kg after 3 hr in sodium solution of which only 1 hr was in presence of the poison). It is probable that the strophanthin abolishes a small active component of efflux but it may well have also reduced the influx because the observed change in sodium content is rather small.

It may perhaps be useful to elaborate the idea of a combined active and passive movement mechanism (see Fig. 10). Suppose that sodium emerges in combination with the sodium-preferring form of a carrier which also takes out a small leakage component of the internal potassium. In sodium salt solutions the sodium can be exchanged and the potassium lost against equivalent sodium gain. In sodium-free media much of the sodium can exchange for the other ion provided. By shuttling the unaltered form of the carrier a largely non-specific passive ion exchange could proceed. However, a competition between calcium and sodium for the occupation of sites (cf. Judah & Ahmed, 1964) could lead to inhibition of sodium movement on account of excessive calcium loading in certain low-sodium conditions. Considerations based on the mass law would lead then to the ratio between the sodium and calcium forms depending on the ratio of the square of the sodium concentration to that of the calcium.

To bring about active ion movement it is further supposed that the carrier can be converted near the outer surface to a potassium-preferring form, provided that sufficient energy is available. This is the stage at which the potential energy demand of the active ion movement is

equated to the energy available from a chemical change. If the transformation can occur the carrier sheds sodium and takes up potassium. The K-loaded form may lie in the entrance to a pore along which there is an electric field. In this way for a given loading of the exchanger the K flux through the composite system could depend on the electric field; on the other hand the exchanger provides a site for interference with potassium movement such as that caused by rubidium. The process of net ion movement might continue by a reconversion of the K-preferring form

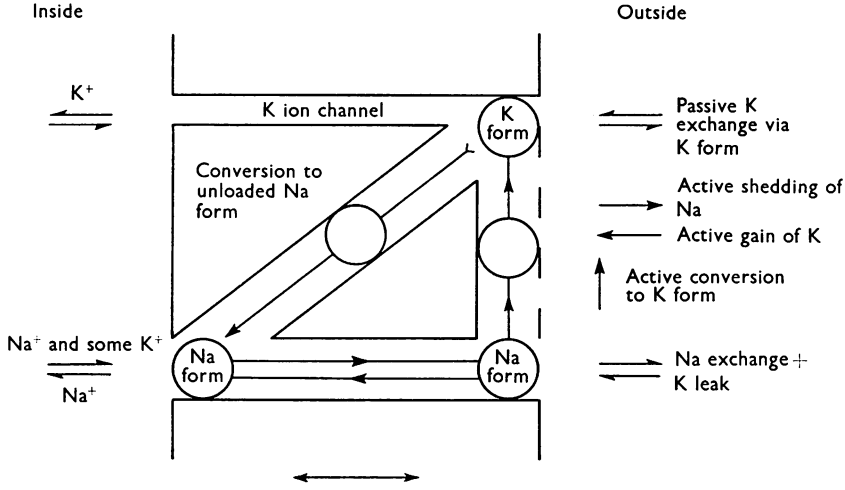

Fig. 10. Scheme discussed for alternative active cycling giving sodium–potassium exchange and passive shuttling giving sodium–sodium exchange with some potassium leak (see text).

of the exchanger to the Na-preferring form so that K ions are shed into the pore and the charged carrier returns to pick up Na ions from the interior of the cell. The active movement requires two successive conversions. The alternative diversion of the carrier system either to active cycling or passive shuttling provides Frazier & Keynes' (1959) requirement that 'it means that there must be little or no simple passive inward diffusion of sodium during recovery' in relation to net output from high-Na muscles.

The higher efflux of sodium to potassium solution than to the other Na-free media can be explained by the low energy demand for conversion to the K-preferring form of the carrier so that instead of moving K ions on the passive Na-preferring form the more fully K-loaded K-form is used.

It may be noted that the cycle proposed involves separate movements of the charged carrier and K ions inwards so that given unequal mobilities a diffusion potential would appear. This could be the origin of the electrogenic effect of active sodium movement (Kernan, 1962*a*, *b*).

SUMMARY

1. The efflux of sodium from frog sartorious muscles has been measured using various media.

2. The efflux to media which are both Na- and K-free and the efflux under exchange conditions in Na media are similar in amount and dependence on internal sodium level. Between sodium contents of 1 and 3 m-equiv/kg tissue the efflux depends on a power, between 2 and 3, of the sodium content; there follows a roughly linear dependence tending to saturation when internal sodium is about 10 m-equiv/kg.

3. The efflux to sodium, lithium or choline media is increased in presence of external potassium and the saturation efflux is moved up. In Na-free potassium salt solution the saturation effect is not seen. As in (2) there is always a range of internal sodium contents over which the efflux depends on a power between 2 and 3 of the sodium content.

4. The similarity noted in (2), together with the stimulating effect of added potassium is interpreted in terms of a mechanism providing alternative passive ion exchange or active Na-K exchange.

5. Rates of oxygen consumption in sodium and sodium-free media were compared. The similarity between rates in sodium, potassium and lithium media indicates that only a small part of the total respiration is contingent upon active sodium movement. In choline media the oxygen rate was reversibly increased.

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