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THE BEHAVIOUR OF THE SODIUM PUMP IN RED CELLS IN THE ABSENCE OF EXTERNAL POTASSIUM

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SUMMARY

1. When red cells loaded with ^{24}Na are incubated in balanced glucose salt solutions, the removal of external potassium reduces sodium efflux by about one third. Ouabain reduces the residual sodium efflux by about one half.

2. The ouabain-sensitive efflux of sodium into potassium-free solutions is accompanied by an equal ouabain-sensitive influx of sodium.

3. If sodium in the external potassium-free salt solution is progressively replaced with choline, both the ouabain-sensitive sodium efflux and the ouabain-sensitive sodium influx are reduced until, with only 5 mM-Na externally, both become very small.

4. At sodium concentrations intermediate between 140 mM and 5 mM, ouabain-sensitive sodium influx and ouabain-sensitive sodium efflux remain equal within the limits of experimental error. The relation between the magnitude of the ouabain-sensitive sodium exchange and the external sodium concentration is roughly linear.

5. As the external sodium concentration is decreased beyond 5 mM towards zero, ouabain-sensitive sodium efflux increases again.

6. In the presence of 5 mM-K, the ouabain-sensitive sodium efflux is scarcely affected by replacing most of the external sodium with choline.

7. In the presence of ouabain, sodium efflux is unaffected by external potassium and is little affected by replacing external sodium with choline.

8. The results suggest that in the absence of external potassium the ouabain-sensitive transport mechanism catalyses a one-for-one exchange of sodium ions across the cell membrane. The relation between this exchange and Ussing's classical 'exchange diffusion' is discussed.

9. The exchange does not occur in the presence of external potassium at physiological concentrations.

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10. Calculations of sodium efflux were based on the specific activities of total cell sodium: separate experiments confirmed that these were the same as the specific activities of sodium lost to sodium-free solutions. No evidence of a slowly exchanging sodium fraction was found.

INTRODUCTION

It is now widely accepted that the high concentration of potassium and the low concentration of sodium in living cells is maintained by active sodium extrusion and active potassium uptake, and that the energy for these movements of ions comes from the hydrolysis of adenosine triphosphate (ATP) at the inner surface of the cell membrane. To get much further in understanding the processes involved, there seem to be two approaches. One is to look for substances acting as intermediates in the 'transport ATPase' system, and experiments along these lines are being carried out in a number of laboratories. The other approach is to look more closely at the over-all behaviour of the transport system, particularly under abnormal conditions, in the hope that knowledge of the kinds of behaviour of which the system is capable will indicate to some extent the kind of mechanism it must have, and will provide a framework for considering the role of any intermediates that may be discovered. The experiments described in this and the following four papers represent an approach of this kind.

Under normal conditions the pump in the red cell membrane expels sodium ions and takes up potassium ions. This paper shows that in the absence of potassium the system may behave in an alternative mode, in which it catalyses a one-for-one exchange of sodium ions across the membrane.

Experiments in which cells were incubated in solutions lacking both sodium and potassium led to the discovery that the transport system was surprisingly sensitive to sodium ions at the outside surface of the membrane, and this sensitivity is the subject of the second paper (Garrahan & Glynn, 1967*b*).

The third paper (Garrahan & Glynn, 1967*c*) confirms that a single transport system is responsible for the active exchange of sodium for potassium and for the one-for-one exchange of sodium ions. The factors that determine the relative magnitudes of the two exchanges are discussed.

The fourth paper (Garrahan & Glynn, 1967*d*) describes experiments on the stoicheiometry of the system—the ratio between the number of ions carried and the number of molecules of ATP hydrolysed—both when the system is catalysing an exchange of sodium for potassium and when it is simply exchanging sodium.

The fifth paper (Garrahan & Glynn, 1967*e*) describes experiments suggesting that the transport ATPase system is to some extent reversible, so that by driving sodium and potassium ions the 'wrong way' it is possible to incorporate inorganic phosphate into ATP.

The behaviour of the sodium pump in the absence of external potassium

When red cells are put in solutions lacking potassium, the efflux of sodium is reduced by about one third and the cells cannot achieve a net loss of sodium against even a small concentration gradient (Glynn, 1956). This inability, and the insensitivity of the sodium efflux under these conditions to the absence of glucose, might suggest that the transport system was inactive in the absence of external potassium. Yet addition of ouabain causes a further 50% fall in the sodium efflux (Glynn, 1957). The paradox can be resolved by suggesting that, in the absence of external potassium, the transport system catalyses a one-for-one exchange of sodium ions across the membrane. This would explain the inability of the cells to achieve a net loss of sodium and, if the exchange consumed little or no energy, it would be compatible with the insensitivity to glucose. The hypothesis leads to two predictions. In the absence of external potassium, the ouabain-sensitive efflux of sodium should require the presence of external sodium, and it should be accompanied by an equal ouabain-sensitive sodium influx. When this work was started there was some, rather fragmentary, evidence that a decrease in external sodium reduced sodium efflux into potassium-free solutions, and there was good evidence that the efflux was accompanied by a ouabain-sensitive sodium influx (Glynn, 1957), but a stringent test of the two predictions had not been made. It therefore seemed worth while to carry out a thorough investigation of the need for external sodium, and a comparison of ouabain-sensitive efflux and influx of sodium in red cells immersed in potassium-free solutions. The results, which confirm the predictions, are described in this paper. Preliminary accounts of most of these experiments have already been published (Garrahan & Glynn, 1965, 1966*a*).

METHODS

Flux measurements

Sodium efflux. Red cells from fresh heparinized blood were loaded with ^{24}Na by incubating them for 4–6 hr in a labelled glucose Ringer solution (see below) containing 5–10 mM-K. The loaded cells were washed 5 times with 2–3 volumes of ice-cold, non-radioactive, K-free Ringer solution. (This was sodium Ringer solution in the experiments of Figs. 1 and 2, choline Ringer solution in the experiments of Figs. 3, 4 and 5 and of Table 1, and Tris Ringer solution in the experiments of Table 2. The solution contained glucose (11 mM) in

the experiments of Figs. 1 and 3.) Between washes the cells were centrifuged for 3 min at 1500 *g*, and after the last wash they were centrifuged at this speed for 7 min. The packed cells were suspended in more of the same wash solution at a haematocrit 10 times that required for the final incubation (0·5–2·0 %), and portions of this suspension were added to suitable volumes of the incubation media in a series of stoppered glass tubes immersed in an ice-bath. A further portion of cell suspension was put aside so that the concentration of ^{24}Na in the cells could be determined later. The tubes were transferred to a bath at 37° C, and individual tubes were removed at intervals, returned to the ice-bath for 5 min and then centrifuged for 3 min at 1500 *g*. The supernatants were put aside for the later determination of radioactivity. In most experiments tubes were removed at 15 min intervals for an hour or more, but in a few experiments efflux was estimated solely from the loss of radioactivity in 30 min. During the incubation the tubes were inverted 2–3 times every 15 min, and the contents were mixed again just before centrifuging.

For each set of conditions, a graph of ^{24}Na loss against time was extrapolated to zero time to give an estimate of the very small amount of ^{24}Na that was extracellular at the beginning of the incubation. This initial loss was subtracted from the measured losses, and the fraction of the initial intracellular radioactivity that remained inside the cells at each time was calculated, and plotted on a logarithmic scale against time. Straight lines were obtained with slopes equal to the rate constants for efflux. In the experiments in which only 30 min time points were available the rate constants for efflux were calculated from the equation

$$\text{rate constant} = 2 \ln \frac{\text{counts in cells at beginning of incubation}}{\text{counts in cells after 30 min}} \text{ hr}^{-1}.$$

Multiplication of the rate constants by the mean sodium contents gave the effluxes. In some experiments, the degree of haemolysis was estimated from the absorption at 541 mμ of the supernatants, and the loss of sodium resulting from haemolysis was deducted from the measured losses. The correction was never more than a few percent.

Sodium influx. The procedure was similar to that for the efflux experiments except that ^{24}Na was not present during the pre-incubation but was included in the incubation media. After 30 min of incubation the tubes were transferred to the ice-bath, cooled for 6 min, and centrifuged for 3 min at 1500 *g*. The cells were washed 4 times with at least 50 volumes of ice-cold, unlabelled Na-Ringer solution and lysed in convenient volumes of water. The radioactivity and 541 mμ-absorption were determined later, and the uptake of ^{24}Na was related to cell volume assuming that the 541 mμ-absorbance of packed cells was 284. Influx was calculated from the uptake of radioactivity in 30 min using the formula.

$$m_i = \frac{kx}{1 - e^{-kt}},$$

where m_i is the influx, x is the amount of labelled sodium taken up in time t and k is the rate constant for efflux. (This formula assumes that Na efflux is directly proportional to $[Na]_i$, over the small range of variation of $[Na]_i$ that occurs during the experiment.) The value of k was determined in a simultaneous efflux experiment. (It is perhaps worth pointing out that, when both influx and efflux are determined from the movements of ^{24}Na after 30 min, the corrections to both ^{24}Na uptake and ^{24}Na loss are similar in magnitude—about 10 %—and are in the same direction, so that the ratio of uptake to loss is not very different from the ratio of influx to efflux.)

Measurement of radioactivity. ^{24}Na was measured using either a Panax well-type crystal scintillation counter, or a Nuclear Chicago liquid scintillation counter without added scintillator (see Garrahan & Glynn, 1966*b*). When the liquid scintillation counter was used for influx experiments the samples were first deproteinized with trichloracetic acid. At a concentration of 5 g/100 ml., trichloracetic acid did not affect the counting efficiency.

Counting was generally continued for a time long enough to include 10,000 counts, so the standard error of counting was about 1 %.

Sodium and potassium estimations were made with an 'Eel' flame photometer. The samples were diluted with distilled water to give concentrations within 20 % of the standards. Measurements were generally made in quintuplicate, alternating standard and unknown, and results obtained in this way gave a mean with a standard error of between 1 and 2 %. When small quantities of sodium were estimated in the presence of much potassium, care was taken to use sodium standards and blanks with similar potassium concentrations. Cells were lysed, but not ashed, before analysis.

Haemoglobin was estimated as oxyhaemoglobin by measuring the absorption at 541 mμ using a Hilger 'Uvispek' spectrophotometer.

Suspending media containing different concentrations of sodium were prepared by mixing 'sodium Ringer solution' and 'choline Ringer solution' in suitable proportions. Glucose was added as a solid to give a final concentration, usually, of 11 mM.

'Sodium Ringer solution' generally contained (mM): Na 155; Mg 1; Ca 1; Cl 155; phosphate (pH 7·4) 2·5. 'Choline Ringer solution' contained (mM): choline 151; Mg 1; Ca 1; Cl 155; orthophosphoric acid titrated with Tris base to pH 7·4 (37° C) 2·5. (10-K) Na Ringer solution and (10-K) choline Ringer solution were similar to the above but with 10 mM of sodium or choline replaced by potassium.

The 'Tris Ringer solution', used for washing the loaded cells in the experiments of Table 2, contained (mM): Tris (pH 8·3 at 5° C, 7·4 at 37° C) 172; Mg 1; Ca 1; phosphate (pH 7·4) 2·5; Cl 117.

Sources of materials. ^{24}Na was obtained as a sterile isotonic solution of NaCl (ref. SGS1P) from the Radiochemical Centre, Amersham. Sodium and potassium chlorides were obtained 'specpure' from Johnson Matthey Ltd., London. Tris was '121' grade from Sigma, London, Ltd. Choline chloride was obtained from British Drug Houses Ltd. and recrystallized as follows: 200 g of the salt were dissolved in 200 ml. of hot absolute ethanol (A.R. grade) and filtered through a hot Buchner funnel. The solution was cooled and 4 volumes of petroleum ether (40–60° fraction, A.R. grade) were added to it. The mixture was kept overnight at −20° C and filtered. The precipitated choline chloride was washed with acetone, dried in a stream of air and kept in a desiccator over silica gel. Other salts, orthosphoric acid, and glucose were of A.R. grade. Ouabain was obtained from British Drug Houses Ltd., or Sigma, London, Ltd. and dissolved in the appropriate salt solution on the day of use.

RESULTS

Measurements of sodium efflux

Specific activity of effluent sodium. In measuring sodium efflux, what is actually observed is the loss of radioactivity from cells loaded with ^{24}Na. To convert loss of radioactivity into loss of sodium it is generally assumed that the specific activity of the sodium leaving the cells is equal to the average specific activity of the sodium in the cells. The first two experiments were designed to see whether this assumption was justified. Fresh cells, in one experiment, and cold stored cells, in the other, were loaded with ^{24}Na and allowed to lose sodium into sodium-free salt solutions. Radioactivity and sodium concentrations were estimated in the cells and in the supernatants and the specific activities compared. The results summarized in Table 1 show that the specific activity of sodium leaving the cells was not significantly different from the average specific activity

of the total cell sodium. Incidentally, this means that these red cells did not contain a significant amount of slowly exchanging sodium (cf. Solomon, 1952).

Sodium efflux in the presence and absence of potassium. Figs. 1 and 2 show the loss of sodium from fresh red cells loaded with ^{24}Na and incubated in balanced glucose salt solutions with and without potassium, and with and

TABLE 1. Experiments to compare the specific activities of intracellular and effluent Na

	Specific activity (counts/min/mμmole)
Expt. 1. Fresh cells containing 5·7 m-mole Na/l. cells	
Total cell Na	1204
Na lost from cells	1180
Expt. 2. Cold-stored cells containing 50 m-mole Na/l. cells	
Total cell Na	58·8
Na lost from cells	57·3

Cells were loaded with ^{24}Na for about 5 hr, washed 5 times with choline–Ringer solution, and incubated for 2 hr at 37° C in a glucose–choline–Ringer solution (Expt. 1) or a (10-K) glucose–choline–Ringer solution (Expt. 2).

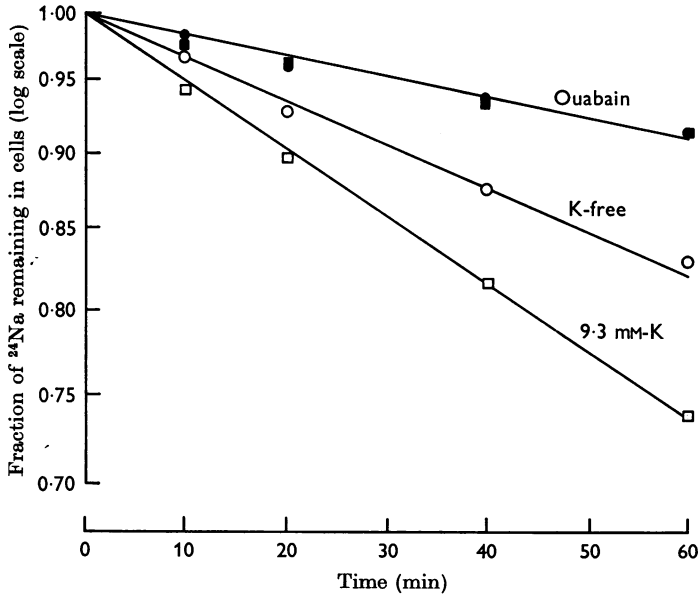

Fig. 1. The effects of external K (9·3 mM) and of ouabain (5×10^{-5} g/ml.) on the efflux of Na from fresh red cells. The cells were incubated for up to 1 hr at 37° C. The K-free incubation medium contained (mM); Na 147; Mg 1; Ca 2·2; Cl 149; phosphate 2·5; glucose 11. The pH was 7·4. In the 9·3 mM-K medium, K replaced an equivalent quantity of Na. □, 9·3 mM-K medium; ○, K-free medium; ■, 9·3 mM-K medium with ouabain; ●, K-free medium with ouabain.

without ouabain (5×10^{-5} g/ml.). The slopes of the curves give the rate constants of efflux. The results show that:

(i) Sodium efflux follows first order kinetics for at least 1 hr.

(ii) Sodium efflux is reduced by about one third in a potassium-free solution.

(iii) Sodium efflux is the same whether the potassium concentration is 10 or 100 mM.

(iv) Ouabain reduces sodium efflux into potassium-containing solutions by about two thirds.

(v) Potassium has no effect on sodium efflux in the presence of ouabain.

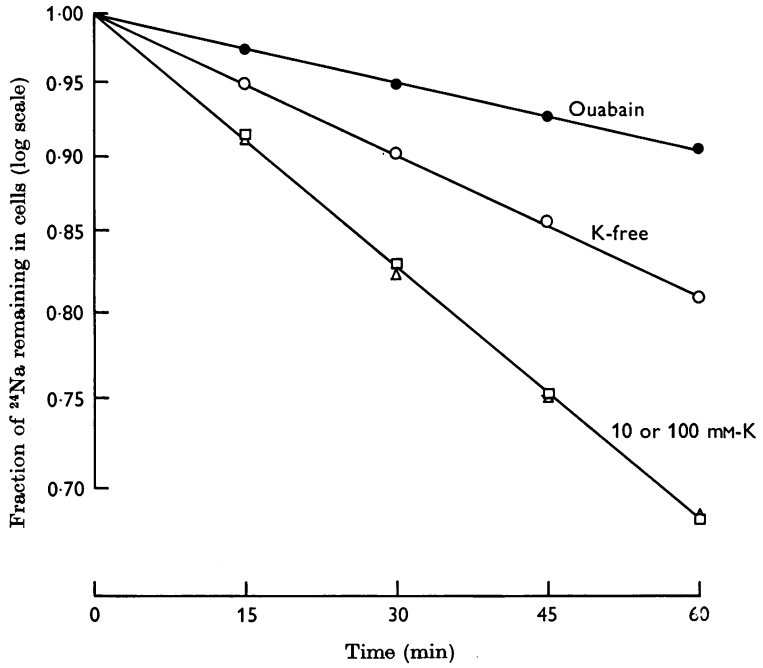

Fig. 2. The effects of external K (10 and 100 mM) and of ouabain (5×10^{-5} g/ml.) on the efflux of Na from fresh red cells. The cells were incubated for up to 1 hr at 37° C. The composition of the K-free medium was as described in the legend of Fig. 1. K replaced an equivalent quantity of Na in the 10 mM-K and 100 mM-K media. □, 10 mM-K medium; △, 100 mM-K medium; ○, K-free medium; ●, K-free medium with ouabain.

The effect of external sodium on sodium efflux. Fresh cells loaded with ^{24}Na were incubated in balanced glucose salt solutions containing different levels of sodium. Isotonicity was maintained with choline. Figure 3 shows the efflux of sodium as a function of external sodium concentration, in the presence and absence of potassium and in the presence and absence of

ouabain. In the presence of ouabain sodium efflux is relatively low and was little affected by the level of sodium externally. With 10 mM-K outside, sodium efflux was high and was little affected by reduction of the external sodium from 140 to 25 mM. Further reduction increased sodium efflux slightly. The most interesting result is given by the middle curve. With 140 mM-Na outside, sodium efflux in the absence of ouabain was well above the ouabain level. There was a much smaller efflux when external sodium was 25 mM, and with only 5 mM-Na outside the effluxes in the presence and absence of ouabain were not significantly different. Further reduction of the level of external sodium caused sodium efflux to rise again, gradually between 5 and 1 mM-Na, more steeply between 1 mM and zero.

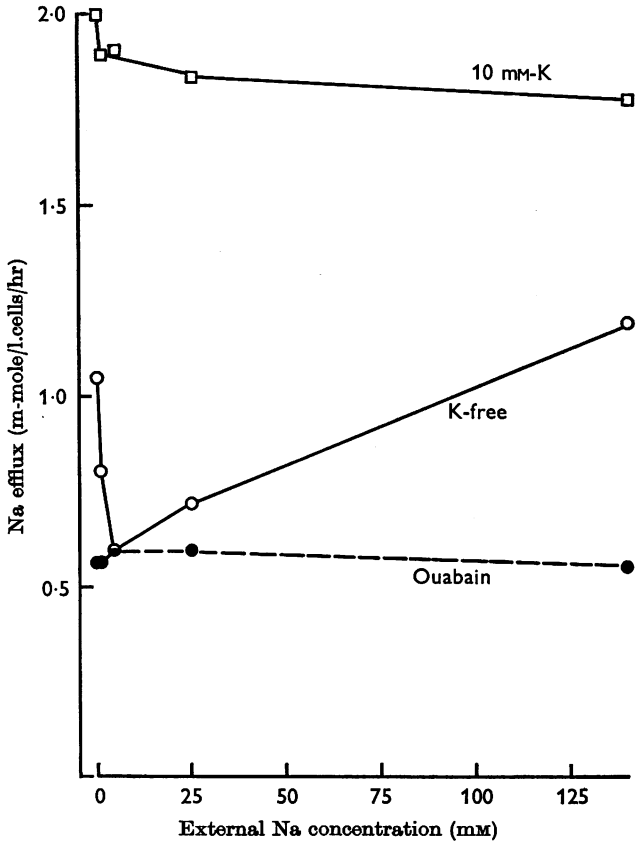

Fig. 3. The effects of external Na on the efflux of Na from fresh red cells into 10 mM-K media and K-free media in the presence and absence of ouabain (5×10^{-5} g/ml.). The cells were incubated for up to 1 hr at 37° C. The incubation media were prepared as described in the Methods section. Each point in the figure was calculated from an efflux curve similar to those in Figs. 1 and 2. □, 10 mM-K medium; ○, K-free medium; ●, K-free medium with ouabain.

Further consideration of the behaviour between 5 mM-Na and zero is given in the next paper (Garrahan & Glynn, 1967*b*). An experiment in which the behaviour between 126 and 4 mM was examined in more detail is illustrated in Fig. 4.

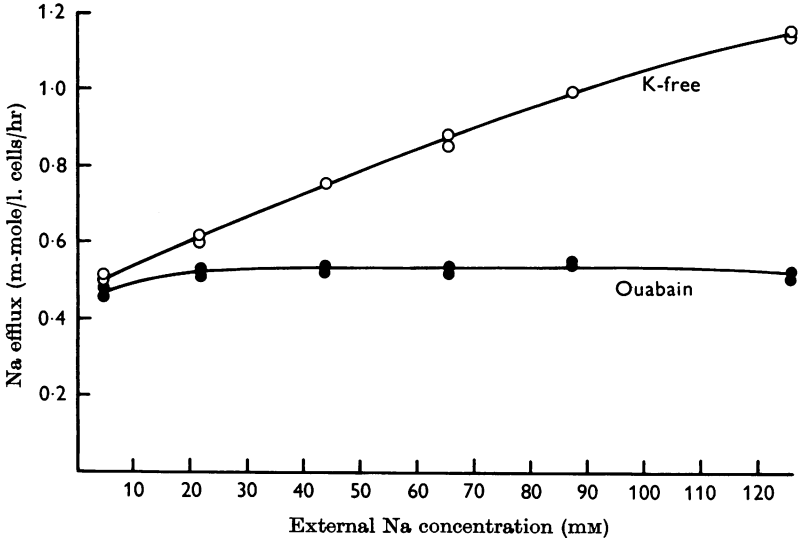

Fig. 4. A more detailed investigation of the effects of external Na, at concentrations between 4·4 and 126 mM, on the efflux of Na into K-free media in the presence and absence of ouabain (5×10^{-5} g/ml.). Incubation was at 37° C. The incubation media were prepared as described in the Methods section. Each point in the figure was calculated from the loss of ^{24}Na in 30 min. This experiment was done under the same conditions, at the same time, and on cells from the same batch as the experiment of Fig. 5. ○, K-free medium; ●, K-free medium with ouabain.

From these experiments, and others like them, it is clear that the ouabain-sensitive sodium efflux in the absence of external potassium is abolished or greatly reduced by removing all but 5 mM of the external sodium. This behaviour fulfils the first prediction made in the introduction.

Comparison of sodium efflux and sodium influx

The second prediction was that the ouabain-sensitive efflux of sodium into potassium-free solutions should be accompanied by a ouabain-sensitive sodium influx of equal magnitude. A stringent test of this prediction can be made by measuring both fluxes in identical batches of cells at a number of different levels of external sodium. The results of an experiment of this kind are shown in Figs. 4, 5 and 6. Figure 4, which has already been referred to, shows sodium efflux in the presence and absence of

ouabain as a function of external sodium at concentrations between 4 and 126 mM. Figure 5 shows sodium influx in separate but identical batches of cells measured at the same time and under the same conditions. From the curves in these two figures, the ouabain-sensitive efflux and influx at each level of external sodium have been calculated, and the results are plotted in Fig. 6. The agreement between the ouabain-sensitive efflux and

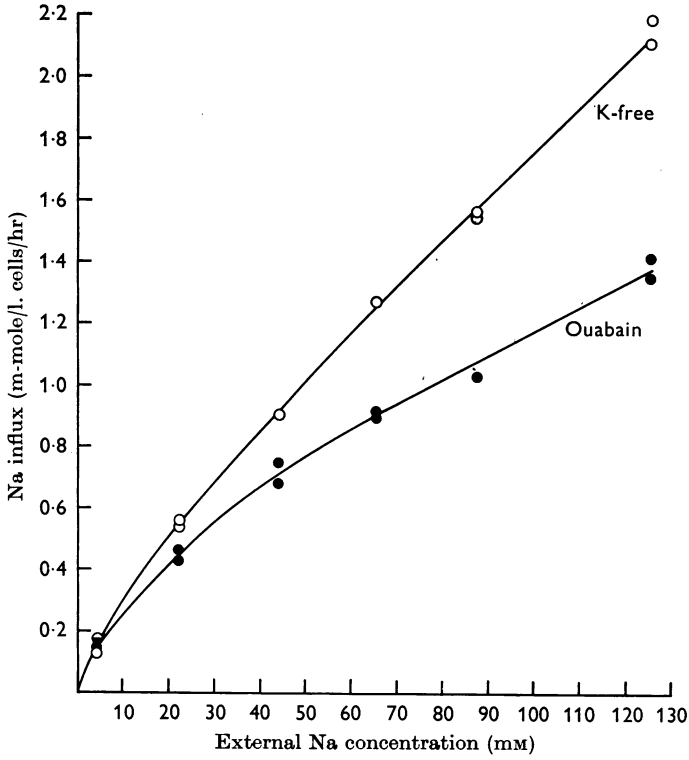

Fig. 5. An experiment to determine the effects of external Na concentration on the flux of Na into fresh cells suspended in K-free media in the presence and absence of ouabain (5×10^{-5} g/ml.). Incubation was at 37° C. The incubation media were prepared as described in the Methods section. Each point in the figure was calculated from the uptake of ^{24}Na in 30 min. This experiment was done under the same conditions, at the same time, and on cells from the same batch as the experiment of Fig. 4. ○, K-free medium; ●, K-free medium with ouabain.

influx is good, except at high sodium levels where the scatter in the results is greater. Two further experiments were therefore carried out, in which influx and efflux were measured with 135 mM-Na outside. Each of these experiments was done in quintuplicate. The results are given in Table 2 and show good agreement between the fluxes.

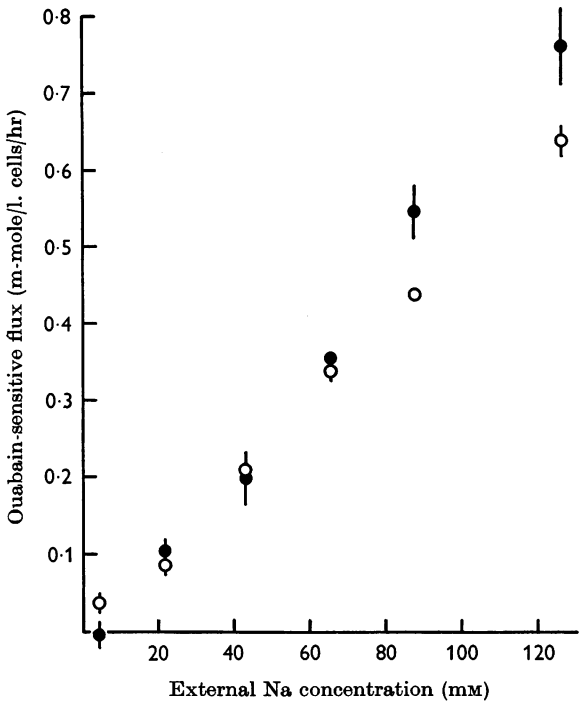

Fig. 6. A comparison of ouabain-sensitive Na efflux and ouabain-sensitive Na influx at six different levels of external Na. This figure is based on the combined results of the experiments of Figs. 4 and 5. The ouabain-sensitive fluxes at each level of Na were calculated by multiplying the differences between the rate constants with and without ouabain by the estimated Na concentrations in the cells in the ouabain-free media halfway through the incubation. ○, efflux; ●, influx. The vertical lines show ±1 S.E.

TABLE 2. Experiments to compare ouabain-sensitive Na efflux and ouabain-sensitive Na influx in a high-Na, K-free medium

	Control flux (m-mole/l. cells/hr)	Flux in the presence of ouabain (5×10^{-5} g/ml.) (m-mole/l. cells/hr)	Ouabain-sensitive flux (m-mole/l. cells/hr)
		Expt. 1	
Na efflux	1·667 ± 0·006	0·631 ± 0·004	1·036 ± 0·008
Na influx	2·802 ± 0·014	1·767 ± 0·009	1·035 ± 0·018
		Expt. 2	
Na efflux	1·598 ± 0·008	0·607 ± 0·003	0·991 ± 0·010
Na influx	2·871 ± 0·018	1·865 ± 0·029	1·006 ± 0·037

The cells were incubated for 30 min at 37° C in a medium containing (mM): Na 139; Mg 1; Ca 1; Tris 17; phosphate 2·5; Cl 150; glucose 9·9. The pH was 7·4.

Insensitivity to external calcium and magnesium

Influx. Cells incubated in solutions lacking both calcium and magnesium showed a slightly greater ouabain-resistant influx of sodium, but the ouabain-sensitive flux was not significantly affected at any level of external sodium.

Efflux. Removal of calcium caused a slight increase in the ouabain-resistant efflux of sodium but the ouabain-sensitive efflux was not significantly affected at any level of external sodium. The effect of removing magnesium on sodium efflux was not investigated.

DISCUSSION

The experiments reported in this paper show that in the absence of external potassium the red cell membrane catalyses a one-for-one exchange of sodium ions. This kind of exchange is what would be expected from the hypothetical 'exchange diffusion' mechanism, first put forward by Ussing (1949) to account for the rapid loss of ^{24}Na from frog muscle, the essence of which is that the ions cross the membrane by carriers that can move

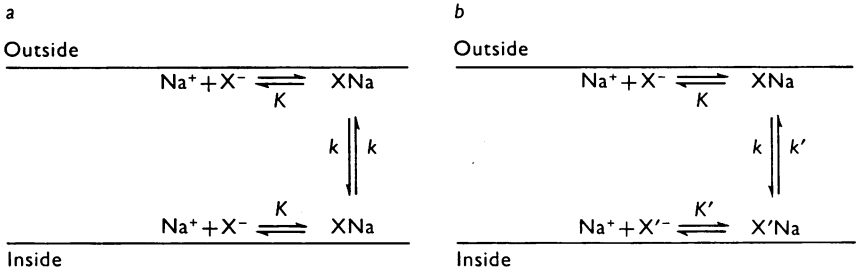

Fig. 7. *a*, Ussing's (1949) classical 'exchange diffusion'. *b*, A modified scheme in which (i) the carrier is allowed to change affinity as it passes across the membrane, and (ii) the velocity constants for the inward and outward movements of the complex may differ. In this scheme each vertical arrow denotes both translocation and chemical transformation. These two processes might occur simultaneously or in sequence, but in either case it is assumed that only XNa gains access to the outside surface and only X'Na gains access to the inner surface.

only when carrying ions. There is, however, a peculiar feature of the sodium:sodium exchange in red cells that requires some modification of the original Ussing model. In that model a carrier X^- is supposed to be in equilibrium with Na^+ ions at each surface of the membrane, and the complex XNa, but not the free carrier, is supposed to be able to pass backwards and forwards across the membrane (see Fig. 7*a*). Let K be the dissociation constant of the sodium carrier complex, k be the velocity constant for the passage of the complex each way across the membrane,

and $[Na]_o$ and $[Na]_i$ be the sodium concentrations in the solutions in contact with the outer and inner surfaces of the membrane. If the chemical reactions at each surface are in equilibrium, and the rate of exchange is limited by the rate of transfer of complex, it is easily shown that

$$\text{influx} = \text{efflux} = \frac{kX_t}{\frac{K}{[Na]_i}+\frac{K}{[Na]_o}+2}, \tag{1}$$

where X_t is the total amount of carrier per unit area of membrane.

From this equation it follows that when $[Na]_o$ is much greater than $[Na]_i$ the fluxes should be independent of $[Na]_o$. In the experiment shown in Fig. 6, the internal sodium concentration was about 9 m-moles/l. cell water yet the fluxes, though remaining equal, increased roughly linearly with external sodium concentration up to 126 mM.

There seem to be two alternative ways of modifying the Ussing scheme so that it is compatible with the observations. The most obvious way is to suggest that the carrier is altered during its passage across the membrane so that its affinity for sodium ions is much less at the outer surface—i.e. K is different at the inner and outer surfaces (see Appendix). The alternative is to suppose that the loaded carrier leaves the outer surface less easily—i.e. the velocity constants for the inward and outward journeys are different. As the two suggestions are not incompatible—indeed, in general, any process affecting the affinity of the carrier may also be expected to affect its distribution—it is simplest to consider the revised scheme shown in Fig. 7*b* where K and K' represent the dissociation constants of the sodium carrier complex at the outer and inner surfaces, and k and k' represent the velocity constants for the inward and outward journeys. Let X_t be the total amount of carrier in X form, X_t' be the total amount of carrier in X′ form, and C_t be the total amount of carrier in all forms.

Using the usual Michaelis Menten derivation we may easily show that

$$\text{influx} = \frac{kX_t}{1+\frac{K}{[Na]_o}}, \tag{2}$$

$$\text{efflux} = \frac{k'X_t'}{1+\frac{K'}{[Na]_i}}. \tag{3}$$

As, by hypothesis, the carriers can cross the membrane only in the loaded state, influx = efflux and we may write

$$C_t = X_t+X_t' = \frac{\text{flux}\left(1+\frac{K}{[Na]_o}\right)}{k}+\frac{\text{flux}\left(1+\frac{K'}{[Na]_i}\right)}{k'}. \tag{4}$$

Rearranged this becomes

$$\text{flux} = \frac{kC_t}{\frac{k}{k'}\cdot\frac{K'}{[Na]_i}+\frac{K}{[Na]_o}+\frac{k}{k'}+1} \tag{5}$$

which reduces to

$$\text{flux} = \frac{kC_t}{\frac{K'}{[Na]_i}+\frac{K}{[Na]_o}+2}, \quad \text{where } k = k', \tag{6}$$

or

$$\text{flux} = \frac{kC_t}{\frac{k}{k'}\frac{K}{[Na]_i}+\frac{K}{[Na]_o}+\frac{k}{k'}+1}, \quad \text{where } K = K', \tag{7}$$

and to equation (1) where $k = k'$ and $K = K'$. From equations (6) and (7) it is obvious that provided $K > K'$, i.e. the carrier has a lower affinity at the outside surface, or $k' > k$, i.e. the carrier complex tends to the outer surface, the flux will be sensitive to $[Na]_o$ even when $[Na]_o$ is much greater than $[Na]_i$.

The effects of differences between K and K' and between k and k' are similar even if the carrier carries a number of sodium ions. For a carrier with three identical sites, for example,

$$\text{flux} = \frac{kC_t}{\frac{k}{k'}\left(\frac{K'}{[Na]_i}\right)^3+\frac{3k}{k'}\left(\frac{K'}{[Na]_i}\right)^2+\frac{3k}{k'}\cdot\frac{K'}{[Na]_i}+\left(\frac{K}{[Na]_o}\right)^3+3\left(\frac{K}{[Na]_o}\right)^2+3\frac{K}{[Na]_o}+\frac{k}{k'}+1}.$$

The derivation of the above equations assumes that the average rate at which the sodium carrier complex moves across the membrane is proportional to the concentration of the complex at the surface from which it is leaving. If the movement across the membrane involves an enzymic reaction, this proportionality will hold only if the enzyme is far from saturation: otherwise the kinetics will be more complicated.

The idea that a change of affinity occurs during the transfer of sodium ions across the membrane is attractive because it seems likely that a change of affinity from Na-selective to K-selective must occur somewhere in the system when it is functioning normally and exchanging sodium for potassium. (Evidence that the same system is responsible for sodium:sodium exchange and for sodium:potassium exchange is presented later (Garrahan & Glynn, 1967*c*).) A model for the transport system based on the idea that the change of affinity from Na-selective to K-selective is also responsible for the properties of the sodium:sodium exchange is discussed in the last of these papers (Garrahan & Glynn, 1967*e*)—see also Baker & Connelly (1966). It is, however, possible that this change of affinity takes place at a step in the reaction sequence beyond those involved in the sodium:sodium exchange.

A difference between the velocity constants for the outward and inward movements of the sodium carrier complex could arise in a number of ways.

For example, (i) a charged complex could be driven towards the outer surface by an electrical gradient produced by fixed charges (cf. Chandler, Hodgkin & Meves, 1965); (ii) a complex that was part of a larger molecule could be pulled towards the outer surface by molecular rigidity; (iii) a difference in composition between different parts of the membrane might ensure that the solubility of the complex was greater near the outer surface. An effect formally similar to a difference between the velocity constants would be obtained if the area available for the exchange of sodium ions between the complex and the bathing solution were greater at the outer surface of the membrane.

It is perhaps worth emphasizing that the sodium:sodium exchange described in this paper does not occur in the presence of ouabain and scarcely occurs under normal physiological conditions with 5 mM-K outside the cells. In both these respects it is different from the exchange diffusion postulated by Tosteson & Hoffman (1960) in sheep red cells, and from the hypothetical 'Pump II' postulated by Hoffman & Kregenow (1966) in human red cells.

The relation between the sodium:sodium exchange in red cells and the sodium efflux observed when nerve or muscle are incubated in K-free media will be discussed in the third paper of this series (Garrahan & Glynn, 1967*c*).

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APPENDIX

Change in affinity of a carrier

At first sight the notion that, in a system not supplied with energy, a carrier might alter its affinity while crossing a membrane, looks thermodynamically suspicious. If in the system represented by Fig. 7*b*, the free carrier as well as the complex is permitted to cross the membrane, it seems that with suitable sodium concentrations an uphill movement of sodium could occur without any expenditure of energy. Yet none of the assumptions made in specifying the system seems unreasonable. The solution to the paradox is as follows:

The four reactions occurring in the system form a cycle, and since, by hypothesis, no energy is supplied

$$\Sigma \, \Delta G = 0.$$

For each reaction $$\Delta G_j = RT \ln K_j$$

so that the product of the four equilibrium constants must equal unity. The tendency of the reactions at the surfaces of the membrane is to go

anticlockwise, so that, if the complex tends, say, to be equally distributed between the inner and outer surfaces, the free carrier must tend strongly towards the outer surface. Although with equal sodium concentrations inside and outside, a much smaller fraction of the carrier at the outer surface would be carrying sodium, the total amount of carrier at the outer surface would be greater, and the movements of carrier–sodium complex in the two directions would be equal. With unequal sodium concentrations, the difference in the degree of loading of the carriers would be more than offset by the difference in the total amount of carrier present at each surface, and net movement would always be downhill.

Professor A. L. Hodgkin (personal communication) has pointed out that behaviour such as we have observed could be accounted for without modification of the carrier if a cation in the outside solution competed with sodium ions for the carrier and formed a complex which could not easily pass through the membrane. Calcium and magnesium ions, the obvious candidates for such a role, seem to be excluded by the observations mentioned briefly on p. 170, but it is conceivable that choline ions, or some component of the membrane, could act in this way.

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