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EFFECT OF SODIUM CONTENT ON SODIUM EFFLUX FROM HUMAN RED CELLS SUSPENDED IN SODIUM-FREE MEDIA CONTAINING POTASSIUM, RUBIDIUM, CAESIUM OR LITHIUM CHLORIDE

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SUMMARY

1. Human red cells treated with lactose solution and loaded with NaCl and BCl subsequently exchange cation with a nutrient BCl medium. B is the same in cells and medium, and is either K, Rb, Cs or Li. In these circumstances Na always moves outwards with the concentration gradient, but the efflux is largely active.

2. With suspensions in media containing Ca^{2+}, the total Na efflux depends on the amount of Na in the cells and on the nature of cation B. Thus for any given value of mean cell Na (Na_m) in excess of 30 m-equiv/l. cells, the effect of B on the amount of Na efflux is K > Cs > Rb > Li, while with Na_m between 0·7 and 5 m-equiv/l. cells, the sequence is Cs > Li > K, Rb. With Na_m between 5 and 30 m-equiv/l. cells, intermediate sequences may be demonstrated for the effects of B on Na efflux. This applies both to the efflux itself and to the flux:concentration ratio, FCR.

3. FCR for passive Na efflux in these circumstances is determined by adding strophanthin G to the medium. It varies inversely with the duration of exposure to Ca^{2+} in the exchange and nutrient media, but not with the nature of cation B. FCR for passive efflux is probably little affected by the value of Na_m.

4. By deducting passive from total Na efflux, active Na efflux is obtained, and variations in the latter with cell Na content, and with B, result in FCR curves similar to those obtained with total Na efflux.

5. Total and passive Na efflux have also been measured in Ca-free media. Here the passive efflux is considerable, and with Na + K cells in KCl media FCR increases with Na_m, but in other systems this change is not signi-

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ficant. However, the rate of passive efflux into LiCl media is less than that for KCl or CsCl media. Owing to the magnitude of passive flux in Ca-free systems, the total Na efflux is also increased, but FCR for active Na efflux is quantitatively and qualitatively similar to that occurring in systems containing Ca^{2+}.

6. The effects of B and Na_m on Na efflux give a series of sequences for B which recall some of those obtainable when chemically modified glass membranes separate solutions of salts, and which are attributable to the charge on the membrane and the hydration of the cations involved. However, certain sequences obtained with red cells do not occur with glass membranes. This difficulty is resolved if it be assumed that throughout the range of Na_m (0–80 m-equiv/l. cells) active B influx at the external cell face modifies linked Na efflux according to the series K > Rb, Cs > Li, while with Na_m between 0 and 30 m-equiv/l. cells (and high complementary B), cations escaping passively compete with active efflux of Na inhibiting the latter according to the series K, Rb > Li > Cs. Both these series could theoretically be explained in terms of surface charges and hydration of cations.

7. Li-loaded cells in nutrient KCl or other media failed to show active Li efflux.

INTRODUCTION

It has been shown that if lactose-treated human red cells are loaded with Na and K in varying but complementary amounts, the total efflux of Na into a nutrient medium containing 125–145 m-equiv Na and 25–5 m-equiv K/l. is not proportional to the concentration of cell Na, but is relatively much less at high concentrations (McConaghey & Maizels, 1962). So, too, Whittam & Ager (1965) have found, in similar cell suspensions, that as the amount of Na loaded into the cell is reduced from 150 to 44 m-equiv/l. cell water, active Na efflux declines by only 30 %; thereafter, for lower Na levels, the relation of efflux to cell Na is more nearly linear. These results were attributed by both authors to increased competition for carriers at high cell Na concentrations. In view of these findings, it was thought of interest to examine Na efflux from cells containing very low concentrations of Na. However, with cells in the Na + K medium mentioned above, it is not possible to maintain low Na concentrations, even when the cells are initially virtually Na-free, for in these circumstances cell Na rises to an equilibrium value of 10–15 m-equiv/l. cells (external Na being about 125 m-equiv/l. medium). If, however, the cells are loaded with Na and K and suspended in a nutrient KCl medium, active Na efflux and the rate coefficient for efflux (FCR) may be studied for cell Na concentrations between 125 and 1 m-equiv/l. cell water (McConaghey & Maizels, 1962).

Here too, it was found that Na efflux remained fairly constant over a wide range of cell concentrations, the rate coefficient rising with fall of cell Na, reaching a maximum with cell Na at about 10 m-equiv/l. cell water, and then falling again at lower levels of cell Na. Reverting here to Na efflux in media containing Na + B (where B is K, Rb, Cs or Li between 5 and 25 m-equiv/l. medium), McConaghey & Maizels (1962) found that total uptake of B against the concentration gradient always accompanied output of Na also against the gradient, and that the efficacy of B in promoting Na efflux was K > Rb > Cs > Li. In view of these findings, it was thought of interest to investigate Na efflux and FCR in relation to a wide range of mean cell Na contents (Na_m) of cells loaded with Na + B and suspended in a nutrient medium containing only BCl, where B is the same in cells and medium and may be K, Rb, Cs or Li. The findings are discussed below.

METHODS

These are essentially the same as those described elsewhere (McConaghey & Maizels (1962). Washed human red cells are rocked in lactose solution (6–10 g/100 ml.) for 3 hr at 37° C, and concentrated from 2 to 20% by centrifuging. This completes the 'depletion stage' during which the red cells become highly permeable and lose about 90% of the cation originally present. To about 36 ml. of the concentrated suspension are added 0·5 ml. glucose (50 g/100 ml.), 0·1 ml. M-Na_2HPO_4 and 100 mg adenosine. The whole is then divided into 6 ml. samples, whose cells are loaded with varying, but complementary amounts of NaCl and BCl added to an over-all concentration of 300 mM. The suspension is then rocked for 3 hr at 37° C after which low permeability to cation is restored by adding $CaCl_2$ to 2·5 mM, keeping at 37° C for a further ½ hr and then at 4° C over-night. As a result of adding Ca^{2+}, the rate coefficient for passive cation efflux which had risen to about 0·8 hr^{-1} after lactose treatment falls to about 0·05 hr^{-1}. Alternatively, the calcium may be omitted until the next ('exchange') stage. In either case, the loaded cells are twice washed with ice-cold solution containing 10 g lactose and 0·5 g human serum albumin/100 ml. (as buffer) and adjusted to pH 7·4. After washing, the cells are suspended in 25 vols. of the 'exchange medium'. This usually contains BCl 150 mM, $CaCl_2$ 2·5 mM, phosphoric acid 0·8 mM and, also, 15 mg each of adenosine and inosine, and 1 g albumin/100 ml. The pH of the medium is adjusted with BOH to 7·4 at 20° C. Adenosine alone raises pH by deamination, inosine lowers it by promoting glycolysis, while jointly they cause little change of pH, constancy of pH being also maintained by the albumin buffer in the medium. After suspension in this medium observations may be commenced by incubation at 37° C, samples being withdrawn at appropriate times. If, however, Ca^{2+} was not added in the 'loading stage' it is added now, and the suspension must be kept for ½ hr before incubation: this lowers the rate coefficient for passive cation efflux from the cells from about 0·8 to 0·1 hr^{-1}. Observations begin with the centrifuging of an aliquot at 0 hr and again at 1–3 hr. Special centrifuge tubes are used, drawn out to sealed graduated and calibrated capillaries. After ½ hr at 2000 *g*, the tubes are washed down to the upper face of the column of packed red cells, the cells haemolysed in 64 vol. of water, and their haemoglobin and Na contents estimated. For details, see McConaghey & Maizels (1962). Experimental errors are discussed below.

Adenosine and the energizing of lactose-treated red cells. It was known that lactose-treated cells would not transport cations actively except after treatment with adenosine, presumably because of loss of ATP during incubation. McConaghey & Maizels (1962) found that active transport could be revived and maintained for many hours if adenosine, glucose and

inorganic phosphate were present in cell suspensions during the loading and exchange stages. Whittam & Ager (1965) showed that glucose could be omitted during the loading stage, and adenosine during the stage of actual cation exchange, and that transport would remain fully effective for at least 3 hr. It has now been found that if adequate adenosine (300 mg/100 ml.) is present during the loading stage, it is unnecessary to add glucose or phosphate to the loading medium, while the exchange medium may consist merely of an appropriate isotonic salt solution buffered with albumin or Tris. It must be presumed that here adenosine is phosphorylated with inorganic phosphate derived from the break-down of intracellular phosphoric esters, and that the process is energized by the metabolism of ribose derived from excess adenosine. In such a simplified system, with red cells containing 15 m-equiv Na/l. active Na efflux was 6·7 m-equiv in 2 hr, compared with 7·4 m-equiv with suspensions containing glucose, adenosine and inorganic phosphate during the loading and exchange stages: there was no significant net active Na efflux in the substrate-free control (actually −0·2 m-equiv/(l. cells).hr). However, if incubation is to be prolonged, the more complex medium originally described is to be preferred.

RESULTS

The Na efflux is calculated from the equation: efflux $= V_0.\mathrm{Na}_0 - V_t.\mathrm{Na}_t$ where V_0 and V_t are the cell volumes at times 0 and t relative to the normal cell volume, and Na_0 and Na_t are the cell contents (m-equiv/l. cells) at 0 and t hr. The flux:concentration ratio, or FCR, used as a rate coefficient may be calculated from the equation

$$\mathrm{FCR} = \frac{\text{Na efflux}}{\text{mean cell Na content}} = \frac{V_0.\mathrm{Na}_0 - V_t.\mathrm{Na}_t}{t\{\sqrt{\mathrm{Na}_0.\mathrm{Na}_t} - w.f.\mathrm{Na}_e(\text{mean})\}}$$

$w.f.\mathrm{Na}_e$ (mean) is a small correction for the effect of external Na contamination on the Na gradient from cell interior to exterior; it amounts to about 5%. w is the amount of water per unit volume of cells, f is the asymmetry factor, while Na_e(mean) is the mean external Na content prevailing during the period of observation (Carolin & Maizels, 1965).

The flux:concentration ratio is derived more correctly from Na concentrations (m-equiv/l. cell water) than from Na contents (m-equiv/l. cells), but the relevant equation is complicated (see Maizels, 1956), and the figures for FCR are very similar whether it is calculated from this equation with Na expressed as a concentration, or from the equation given above, with Na expressed as a content. This is because cell water changes but little during incubation: thus even if cell volume altered by ±5%, cell water per unit volume would change by only ±1·5%.

Experimental errors

Errors in Na estimations arise from contamination of the edges of the centrifuge tube during washing or when in use, and can only be avoided by care in handling; this is tedious but unavoidable, particularly when contents below 3 m-equiv/l. cells are being measured. The other source of error is 'wander' in the upper setting of the flame-photometer; this can be minimized by repeated readings: also, if one is reading duplicate values at 0 and again at t hr, it is better to read the first of each paired sample from the 0 and t hr pairs respectively, and then the second of each pair at 0 and t hr. In this way errors which mainly affect the

difference value in the numerator of the equation for FCR are minimized; the effect on the denominator is in any case negligible. With increased care, it has been possible to achieve rather greater accuracy than formerly.

The standard error for duplicates of cell volume is $\pm 1{\cdot}2\%$, and for Na estimations the S.E. is between $\pm 0{\cdot}9$ and $\pm 1{\cdot}25$ according to circumstances. However, these errors are not necessarily additive, and the S.E. for V.Na, the value which is used to calculate efflux may be no greater than the individual errors of V and Na. This arises because the 65-fold dilution for estimating haemoglobin and Na is prepared in accordance with the volume of cells as measured in the graduated tube, a direct reading having an error of $\pm 1{\cdot}2\%$. But V (cell volume relative to normal cell volume) and also V.Na are both referred to the haemoglobin content of the cell dilution, which can be determined with very little error by means of a photo-electric colorimeter; in other words, though Na as actually read is referred to the measured volume of packed cells, V.Na is referred solely to unit weight of cell haemoglobulin, thus tending to correct for errors in reading the volume of the cells.

TABLE 1. Standard error of the means of duplicate values of V.Na expressed as a percentage. V is the cell volume relative to the original cell volume

Number of duplicates	Range of Na values (m-equiv/l. cells)	Average Na content of group (m-equiv/l. cells)	S.E. of V.Na %
25	1–2	1·52	±1·25
36	2–5	3·1	±1·02
32	5–12	7·7	±0·90
35	12–40	23·1	±0·85
20	40–60	51·2	±0·95
24	60–80	70·0	±1·14

The standard error of duplicate values of V.Na expressed as a percentage of the mean of duplicates is given in Table 1. It is somewhat higher for lower values of cell Na because these are more affected by contamination, and also for high values of Na because of decreased sensitivity of the flame photometer. The latter factor might be minimized by further dilution, but only at the risk of additional contamination.

It follows that the S.E. of efflux will vary not only with the amount of efflux, but also with the levels of cell Na at which efflux begins and ends. It may be calculated from: S.E. (efflux) $= \sqrt{[(\text{S.E.})^2_{(\text{Na}_0)} + (\text{S.E.})^2_{(\text{Na}_t)}]}$ where $\text{S.E.}_{(\text{Na}_0)}$ and $\text{S.E.}_{(\text{Na}_t)}$ are the standard errors at times 0 and t, expressed in absolute units (m-equiv/l. cells) and not as percentages. Thus in the case of Na + K curves for cells in a KCl medium (Fig. 2) with Na_m (mean cell Na content) at 3 m-equiv/l., efflux + S.E. is $0{\cdot}66 \pm 0{\cdot}044$ m-equiv/(l. cells).hr (or $\pm 6{\cdot}6\%$); with 10 m-equiv, efflux ± S.E. is $3{\cdot}6 \pm 0{\cdot}12$ m-equiv ($\pm 3{\cdot}3\%$); with Na_m at 70 m-equiv, efflux ± S.E. is $7{\cdot}9 \pm 1{\cdot}2$ m-equiv/l. cells ($\pm 15{\cdot}3\%$) at 1 hr or $15{\cdot}8 \pm 1{\cdot}28$ m-equiv ($\pm 8{\cdot}1\%$) after 2 hr incubation. Accuracy may be further increased by prolonging the period of cation exchange, but beyond 2 hr haemolysis is likely to be significant. It follows that findings need to be confirmed by numerous replicates.

Amount and rate coefficient (FCR) for total Na efflux from cells loaded with Na + B and exchanging with a nutrient BCl medium

Figure 1 represents the average of eight experiments and shows Na efflux (m-equiv/(l. cells).hr) plotted against the mean cell Na content or Na_m (m-equiv/l. cells). Here B is either K, Rb, Cs or Li, Na and B being present

in the cells in complementary amounts totalling 110 m-equiv/l. cells (about 160 m-equiv/l. cell water), and the concentration of B in the exchange medium is about 150 m-equiv, Na being virtually absent. B in the cells and medium is always the same: thus if the cells contain Na + Li, the only salt in the external medium will be LiCl. Individual curves have been standardized on the assumption that in Na + K systems with Na_m at 10 m-equiv, efflux is 3·5 m-equiv/(l. cells).hr, which is in fact an average value. Thus if in an individual experiment, efflux is only 3·3, all points in the four constituent curves (Na + K, Na + Rb, Na + Cs, Na + Li) of each experiment are multiplied by 3·5/3·3, and then the average values of all the adjusted curves are calculated for various values of Na_m.

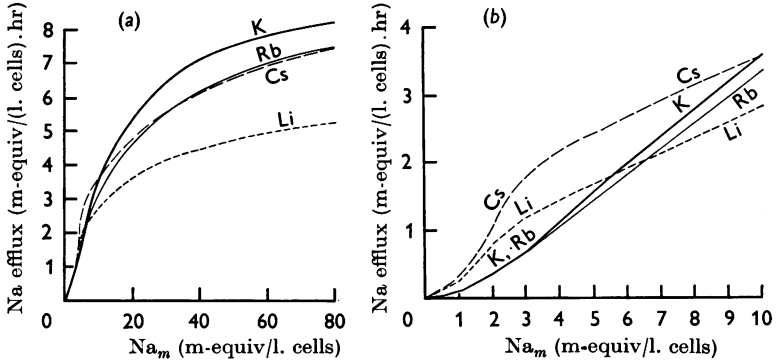

Fig. 1*a*. Total Na efflux from red cells loaded with Na + B and suspended in a nutrient BCl medium containing Ca^{2+}, plotted against mean cell Na content (Na_m); average of 8 experiments. *b*: as *a* but showing detail for Na_m between 0 and 10 m-equiv/l. cells. Complementary cation B indicated as follows: K ——, Rb ——, Cs — — —, Li - - - - - - -.

Figure 1 shows that the total Na efflux declines rather slowly with Na_m until the latter has fallen to about 25 m-equiv/l. cells, below which level efflux falls more sharply and is more directly related to Na_m. This applies especially to Na + K systems, but is also evident with Na + Li cells in LiCl media, though here total efflux is less than in any other system at the same Na_m, until the latter falls below 5-m-equiv/l. cells. It is difficult to discern details for Na efflux with Na_m below 5 m-equiv, because the efflux itself is so small, but clearly at this low level Na efflux from Na + Cs systems and also from Na + Li systems definitely exceeds that of Na + K and Na + Rb systems. Detail is more evident in Fig. 2 where the rate coefficient, FCR, is plotted against log (Na_m). It may be wondered if the slighter differences in the curves of Fig. 2 are significant, and it must be emphasized that in addition to the eight general curves on which Figs. 1 and 2 are based, *detail* was studied by means of special 5-point curves for

Na+Cs and Na+Li in the region of log (Na_m) between −0·4 and 0 (Na_m = 0·4–1 m-equiv/l. cells), for the peaks of Na+Cs and Na+Li systems, and the curves of Na+K and Na+Rb systems between log (Na_m) of 0·2 and 0·8 (Na_m = 1·6–6·3 m-equiv/l. cells), for the peaks of Na+K and Na+Rb curves between 0·6 and 1·2 (Na_m = 4–16 m-equiv/l. cells), and for all these curves in the range of log (Na_m) 1·4 and 2 (Na_m = 25–100 m-equiv/l. cells). Thus if one considers the curve of Na+Rb cells in a

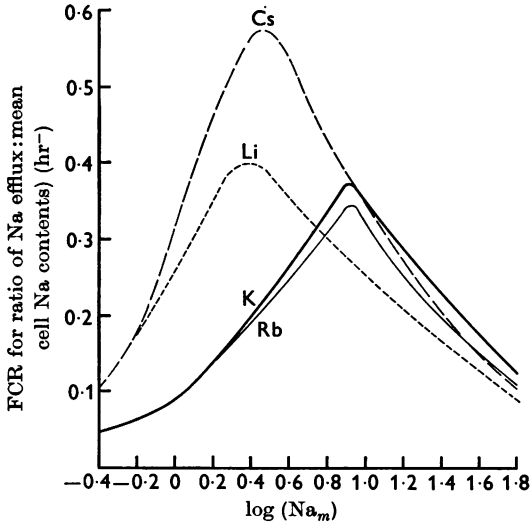

Fig. 2. Total Na efflux from red cells loaded with Na+B and suspended in a nutrient BCl medium containing Ca^{2+}, plotted against log (mean cell Na content) or log (Na_m); average of 8 experiments. Complementary cation B indicated as follows: K ——, Rb ——, Cs — — —, Li ·····.

RbCl medium, this is only a little lower than the curve for Na+K cells in a KCl medium, Na efflux for the former after 2 hr exchange being 6·3 m-equiv/l. cells when log (Na_m) is 1·0 (Na_m = 10 m-equiv/l. cells) compared with 7·0 m-equiv from Na+K cells; however, the Na+Rb curve is not only lower at this point, but also over the whole range of log (Na_m) from 1·8 down to 0·6 (Na_m = 63–4 m-equiv/l. cells), and the finding is also consistent in the individual experiments from which Fig. 2 is derived. Similarly, in the case of Na+K and Na+Li curves, when log (Na_m) is 1·8 (Na_m = 63 m-equiv Na/l. cells), Na efflux from the former is 15·7 ± 1·0 m-equiv in 2 hr, while for the latter it is 10·2 ± 1·0 m-equiv. This difference is probably significant, and is present in all the constituent curves from which the average curve has been constructed. Moreover, Na efflux from Na+Li systems was less than Na efflux in Na+K systems for a range of log (Na_m) between 0·7 and 1·8 (Na_m = 5·0–63 m-equiv/l. cells). Indeed,

for log (Na_m) = 1, the difference in the respective Na effluxes of Na + K and Na + Li systems, together with the total S.E. corresponds to 2·0 ± 0·19 m-equiv/l. cells during 2 hr of efflux. If then these various differences are accepted as significant, it may be said that the total Na fluxes (Figs. 1*a* and *b*) and also FCR (Fig. 2) give a series of sequences for facilitation of total Na efflux by cation B beginning with log (Na_m) at 1·8–1·5 with the sequence K > Rb, Cs > Li, and ending with log (Na_m) at −0·2 to −0·4 with the sequence ?Cs, ?Li > K, Rb. With regard to the latter, at these very low values of cell Na, the precise relation of FCR for Na + Cs to FCR for Na + Li could not be determined, but the average of eight experiments

TABLE 2. Sequences of the complementary cation B in relation to its facilitation of total Na efflux. The figures shown indicate the distribution of sequences for all the individual experiments of this series corresponding to a given range of mean cell Na content, shown also for convenience as the logarithm, log (Na_m). (E.S. = Eisenman sequences with Na omitted)

		Number of observations corresponding to the respective ranges of log (Na_m) and Na_m						
	log (Na_m)	1·8 to 1·5	1·5 to 1·0	1·0 to 0·8	0·8 to 0·72	0·72 to 0·4	0·4 to −0·2	−0·2 to −0·4
Sequences of complementary cation B	or Na_m	63 to 32	32 to 10	10 to 6·3	6·3 to 5·2	5·2 to 2·5	2·5 to 0·63	0·63 to 0·4
	E.S.							
K > Cs, Rb > Li	III*a*, IV or V	8	1	0	0	0	0	0
K > Cs > Rb > Li	III*a*	0	7	2	0	0	0	0
Cs > K > Rb > Li	II*a*	0	3	6	4	0	0	0
Cs > K, Rb > Li	Not represented	0	0	0	0	2	0	0
Cs > K > Li > Rb	Not represented	0	0	3	7	2	0	0
Cs > Li > K > Rb	Not represented	0	0	0	3	5	2	1
Cs > Li > K, Rb	Not represented	0	0	0	0	3	9	4
Li > Cs > K > Rb	Not represented	0	0	0	0	0	0	1
Li > Cs > K, Rb	Not represented	0	0	0	0	0	0	3

Na_m in m-equiv/l. cells.

with Na_m between 1 and 0·3 m-equiv/l. cells suggested that the respective values for FCR were not dissimilar. Complete details of the FCR curves in relation to log (Na_m) and Na_m are set out in Table 2 and are also discussed later, and their relation to the cation sequences of Eisenman's systems (1963) is considered. However, it may be noted now that when the curves for FCR are examined, it will be seen that each presents a peak, whose position with regard to log (Na_m) is fairly consistent in the individual curves on which Fig. 2 is based. The peaks of FCR for Na + K and Na + Rb systems correspond to log (Na_m) of about 0·93 (Na_m = 8·5 m-equiv/l. cells), with the former somewhat higher; the peaks for Na + Cs and Na + Li correspond to log (Na_m) of about 0·45 (Na_m = 2·8 m-equiv/l. cells), with the latter peak somewhat lower and flatter, and possibly shifted slightly to the left.

Active Na efflux: amount and rate coefficient

The preceding comments apply to total Na efflux to which passive efflux (also with the concentration gradient) contributes. The next series of experiments corresponds with the preceding except that parallel observations without and with strophanthin G (20 mg/100 ml. medium) were made, and the difference between the respective observations was taken to give the data for active Na efflux. It may be noted that according to Hoffman (1966) part of the strophanthin G-resistant moiety of Na efflux is insensitive to ethocrinic acid and is passive, while part is sensitive and constitutes an active fraction (Hoffman's pump No. 2). However, the latter, while independent of external K, has an obligatory requirement for external Na. In the present experiments the external medium is Na-free; hence it may be accepted that here the strophanthin G-resistant Na efflux is entirely passive. It will be noted that in the preceding series of experiments, and also in this, $CaCl_2$ was added to restore permeability to normal after it had been raised by depletion in lactose solution. But a distinction must be drawn, because in the first series illustrated in Figs. 1 and 2, $CaCl_2$ was absent during the first 3 hr of the loading stage, but was present during the last $\frac{1}{2}$ hr at 37° C and also over-night at 4° C; $CaCl_2$ was also added to the exchange medium of Stage 3. In these circumstances, FCR for passive Na efflux was 0·03–0·06 hr^{-1}. In this second series of experiments, all suspensions were Ca-free until the cells were transferred to the nutrient exchange medium of Stage 3 which contained 2·5 mM-$CaCl_2$. After $\frac{1}{2}$ hr at room temperature, all samples were divided into two, strophanthin G being added to one set only. Samples were incubated for 80 min at 37° C and the amount of efflux estimated. With this modification FCR for passive Na efflux was 0·08–0·13 hr^{-1}, these high values (compared with 0·05 by the first method) increasing the accuracy of the data for passive efflux, but also increasing S.E. for active Na efflux.

At this point it is necessary to note that some simplification of the experiments was inevitable. For the 5-point curves of Na + K, Na + Rb, Na + Cs and Na + Li systems shown in Figs. 1 and 2 for total efflux only, the effects of K, Rb, Cs and Li were examined in parallel in each experiment involving 40 duplicates or 80 estimations of haemoglobin and Na contents. Doubling this work by investigating in parallel active and passive systems was not found to be possible. Hence 5-point curves were done for single systems like Na + K and Na + Li quite independently, and these sufficed to define the peaks of FCR for active Na efflux, which corresponded closely with the peaks of total Na efflux in Fig. 2. Next, in order to define curves for active Na efflux relatively to one another, the different systems were examined in pairs. Thus FCR curves for active Na efflux in Na + K and Na + Li systems obtained in the same experiment were compared, and so too were curves for Na efflux in Na + K and Na + Rb systems, and Na + K and Na + Cs systems, the Na + K values providing in each case the curve of reference. In these circumstances 3-point curves supported by a

few 4-point curves sufficed for the investigation of total Na efflux, while 3-points sufficed to define the curves of FCR for passive Na efflux, which were roughly linear. Passive efflux requires further consideration.

FCR for passive Na efflux. As passive efflux is small, S.E. is considerable: thus with log (Na_m) at 0·5 (Na_m = 3·2 m-equiv/l. cells), efflux ± S.E. at 80 min is 0·33 ± 0·046 m-equiv/l. cells. Table 3 shows FCR for passive Na efflux corresponding to three different levels of log (Na_m)—1·5, 1·0 and 0·5 (Na_m = 32, 10 and 3·2 m-equiv/l. cells). In each group, Na + K systems are compared with Na + B systems: it will be recalled that in each system cells are loaded with Na and complementary B, and that they subsequently exchange with a pure BCl medium. Table 3 shows three sets of

TABLE 3. Rate coefficients (flux:concentration ratio or FCR) for passive Na efflux from red cells in a BCl medium at 37° C

Series	No. observations	FCR (hr^{-1}) when log (Na_m) is 0·5	1·0	1·5
Na + K	5	0·110	0·112	0·106
Na + Li	5	0·119	0·113	0·109
Na + K	4	0·096	0·100	0·096
Na + Cs	4	0·107	0·092	0·099
Na + K	2	0·101	0·092	0·088
Na + Rb	2	0·107	0·098	0·100

experiments. In the first FCR for passive Na efflux in Na + K systems is compared with data from Na + Li systems; in the second the data are from Na + K and Na + Cs systems, and in the third from Na + K and Na + Rb systems. Each series comprises only a few experiments and hence FCR values vary somewhat in the several series.

The figures in Table 3 suggest that the nature of the cation in the medium (which is also complementary to Na within the cells), whether it be K, Rb, Cs or Li, has little effect on passive Na efflux. And the ratio does not appear to be significantly affected by variations in the Na contents of the cells. Thus at low Na contents, FCR is about 3% more than at moderate contents (10 m-equiv/l. cells) and at high contents it is slightly less. These observations are derived from the twenty-two experiments of Table 3, but even so the differences are not significant, and it would be necessary to carry out a very large number of experiments to decide if FCR were indeed definitely higher at the lower Na content; all that can be said here is that variations in FCR for passive Na efflux, with cell Na content and also with the nature of the complementary cation are slight, and that therefore the shape of the curves of FCR for active Na efflux can differ but little from the curves of FCR for total Na efflux shown in Fig. 2.

FCR for active Na efflux. It will be recalled that total and passive Na effluxes are obtained by examining parallel suspensions in the absence and

in the presence of strophanthin G, and that in order to keep the number of observations within manageable limits (48–56 individual samples), observations were restricted to three or four duplicate values for total Na efflux and three duplicates for passive Na fluxes. Figure 3*a* shows FCR for total, passive and active Na efflux from red cells loaded with Na + K and exchanging cation with a nutrient KCl medium, and Fig. 3*b* shows the findings with parallel observations on Na + Li systems. It will be seen that FCR for active efflux from Na + K cells is similar to FCR for total Na

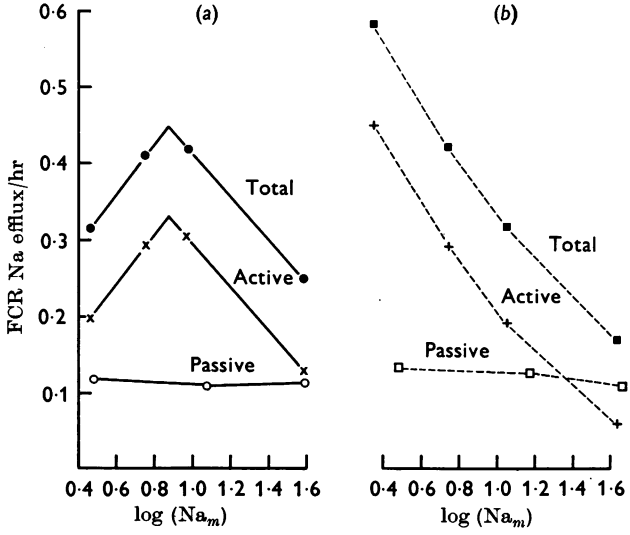

Fig. 3. FCR for total, passive and active Na efflux from lactose-treated red cells (*a*) loaded with Na + K and exchanging with a KCl medium; (*b*) loaded with Na + Li and exchanging with a LiCl medium. Media contain Ca^{2+}. Period of exchange 80 min at 37° C. (*a*) For Na + K cells in a KCl medium, FCR total Na efflux = ●—●, FCR passive Na efflux = ○—○, FCR active Na efflux = ×—×. (*b*) For Na + Li in a LiCl medium, FCR total efflux = ■---■, FCR passive Na efflux = □---□. FCR active Na efflux = +----+.

efflux (Fig. 2) in that it shows a peak corresponding to log (Na_m) of about 0·9 (Na_m = 8 m-equiv/l. cells), the curve falling sharply on either side of the peak. The curve of Na + Li cells is less well defined, but shows that the peak is higher than is the peak for Na + K cells and corresponds to a lower value of log (Na_m). Also it is clear that for higher values of cell Na (40 m-equiv/l.) corresponding to log = 1·6, FCR for active Na efflux from Na + Li cells is less than FCR for Na + K cells. Similarly, Fig. 4 shows FCR for total, passive and active Na effluxes in Na + Cs and Na + Li systems, and it will be seen that the peaks of both curves correspond to a value of log (Na_m) of about 0·4 (3 m-equiv/l. cells), and that at this point FCR for

active Na efflux is greater in Na + Cs systems. Figures 3 and 4 are derived from single experiments. Figure 5 shows details for mean FCR values from pooled experiments: Na + K 9 experiments; Na + Li 4 experiments, Na + Cs 3 experiments, and Na + Rb 2 experiments. In addition, two extra experiments were done to define more precisely the peaks of FCR curves for cells in Na + Cs and Na + Li systems. As in Fig. 2, identical systems in individual experiments at the same Na_m show small variations, which here

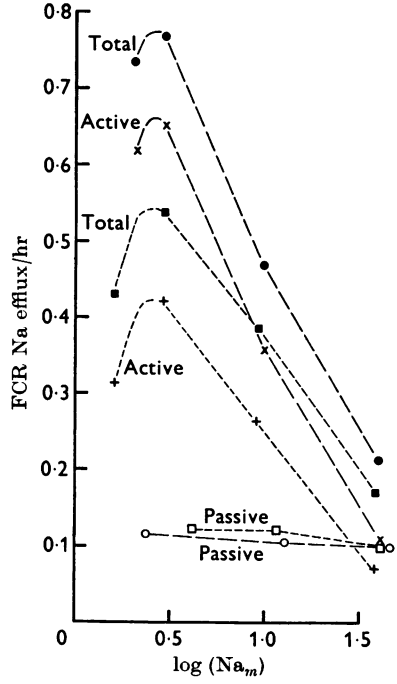

Fig. 4. FCR for total, passive and active Na efflux in Na+Li and Na+Cs systems. Ca^{2+} present. For Na+Li cells in LiCl media: FCR total Na efflux = ■----■, FCR passive Na efflux = □---□, FCR active Na efflux = +---+. For Na+Cs cells in CsCl media: FCR total Na efflux = ●--●, FCR passive Na efflux = ○--○, FCR active Na efflux = ×---×.

too presumably arise from natural differences in the red cells or from minor differences in experimental technique. Hence, as before, all values in a set of FCR data derived from a single experiment are referred to a standard value: FCR (active Na efflux) for Na + K systems when log (Na_m) = 1 (Na_m = 10 m-equiv/l. cells), equals 0·29 hr^{-1}. Thus if in an experiment this value were found to be 0·32, then the values of all points in the associated curves would be multiplied by 0·29/0·32. Individual curves so corrected are all similar in shape and show little quantitative difference.

The mean values from several curves for log (Na_m) = 0·4, 0·6...1·6 have been plotted in Fig. 5*a*, and it will be seen that the FCR values correspond closely with those of Fig. 2, allowing for the fact that the latter shows total values of FCR for Na efflux, from which the value of FCR for passive efflux has not been deducted; this value is not precisely known, but is probably about 0·05 hr^{-1}. (Note Fig. 5*b* shows for comparison corresponding curves in calcium-free systems.)

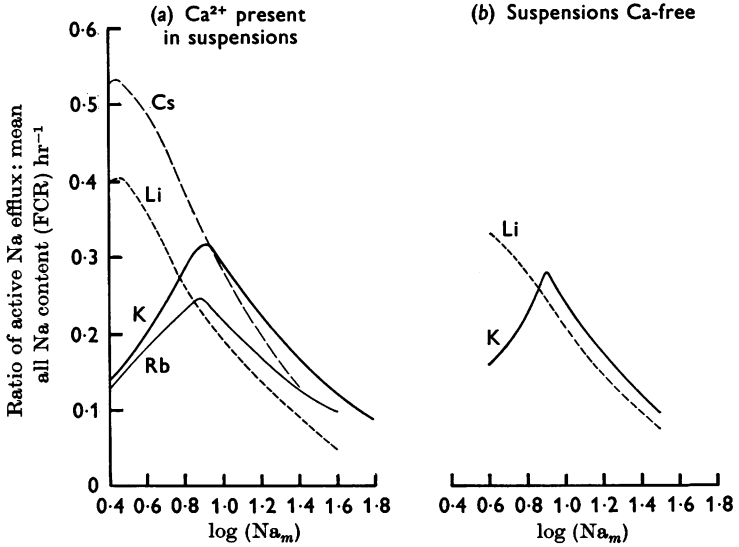

Fig. 5*a*. Flux : concentration ratios (FCR) for active Na efflux plotted against log (Na_m). Suspensions contain Ca^{2+}. Na+K cells in KCl media (9 expts.) ——, Na + Rb cells in RbCl media (2 expts.) ——, Na + Cs cells in CsCl media (3 expts.) – – –, Na+Li cells in LiCl media (4 expts.) ------.

Fig. 5*b*. As *a* but Ca^{2+} absent from suspensions. Na+K systems, 5 expts. Na+Li systems, 3 expts.

Before leaving this section, it is desirable to indicate the sort of S.E. to be expected in the calculation of active Na efflux. Thus for Na+K cells in a KCl medium (Fig. 3*a*), when log (Na_m) = 0·5, 0·9 and 1·6 (Na_m = 3·2, 8·0 and 40 m-equiv/l. cells), the corresponding active Na effluxes are 0·66 ± 0·063, 2·6 ± 0·15 and 5·3 ± 0·72 m-equiv/(l. cells) . hr, and the respective FCRs are 0·20 ± 0·02, 0·32 ± 0·019 and 0·13 ± 0·018 hr^{-1}. It follows that the differences in the data for the respective fluxes and also for FCRs are significant. In the case of Na+Li systems with log (Na_m) at 0·5, 0·9 and 1·6, the respective active effluxes are 1·24 ± 0·066, 1·9 ± 0·14 and 2·6 ± 0·74 m-equiv/(l. cells) . hr. These values, except possibly the last, are significantly different from the corresponding Na+K data. FCRs for active Na efflux at the relevant Na contents in Na+Li systems are 0·38 ± 0·02,

$0{\cdot}235 \pm 0{\cdot}017$ and $0{\cdot}065 \pm 0{\cdot}019$ hr^{-1}. The significance of the data is increased when it is recalled that although S.E. is calculated from the curves on the basis of 60 min efflux, the actual experimental period was 80 min; also the differences shown in Fig. 3 are represented in all the individual curves on which Fig. 5*a* is based.

Active Na efflux in the absence of calcium ions

These experiments are similar to the preceding ones in that active Na efflux is derived from the data for total and passive Na efflux, but they differ in that calcium salts were absent throughout. This introduces a major difficulty because FCR for total Na efflux is now about 0·9 hr^{-1}, to which passive Na efflux contributes 70–90 %. Hence the experimental error is very large. This is considered later.

Passive Na efflux. FCR for passive Na efflux in calcium-free Na + K systems averaged 0·73 in five experiments. However, it was not constant irrespective of the Na content, but increased with rise of Na_m: thus with log (Na_m) at 0·6, 1·0 and 1·6 (Na_m = 4, 10 and 40 m-equiv/l. cells), FCR was respectively 0·68, 0·73 and 0·78 hr^{-1}, the first being 7 % less (range 89–97 %) and the last 7 % more (range 103–114 %) than the middle value (cf. Fig. 6). This rise in Na_m was seen in each of the five experiments, and had been noted previously by Carolin & Maizels (1965).

FCR for passive Na efflux in calcium-free Na + Li systems was consistently less than in the Na + K systems, averaging 0·54 hr^{-1} (see also Fig. 6). The possibility was considered that this was due to calcium contamination of the 'Specpure' lithium chloride, but addition of ethyleneglycol *bis* (aminoethylether) tetra-acetate (EGTA) to the exchange medium showed that FCR for passive Na efflux was still 25 % less in Na + Li systems than in Na + K systems. Thus it seems that lithium in very high concentration (150 m-equiv/l. medium) has a slight calcium-like action. FCR for passive Na efflux in Na + Li systems varies slightly with Na_m; i.e. the value with log (Na_m) = 0·6 was 97 % (range 93–104 %) of the value with log (Na_m) = 1·0, while with log (Na_m) = 1·6, the value was 103 % (range 100–104·5 %). The averages differ by only ± 3 %, and, as there are only three sets of observations, the differences are not regarded as significant. Only two experiments were done with Na + Cs systems, and here FCR for passive systems was similar to that in Na + K systems and was thus greater than in Na + Li systems (see Fig. 6*b*). It is uncertain if FCR increases slightly with log (Na_m).

Active Na efflux in calcium-free systems. Figure 6*a* shows FCR for total, passive and active Na efflux for an experiment on Na + K and Na + Li suspensions. It shows the relative proportions of the passive and active Na effluxes. Figure 6*b* shows corresponding data for Na + Li and Na + Cs

systems. There were in fact five Na + K experiments, three of which were in parallel with Na + Li experiments. The average findings of these are shown in Fig. 5*b*, and the data are similar to those illustrated by the Na + K and Na + Li curves of Figs. 2 and 5*a*. Further, two experiments with Na + Li and Na + Cs systems showed that in these Ca-free suspensions, as in

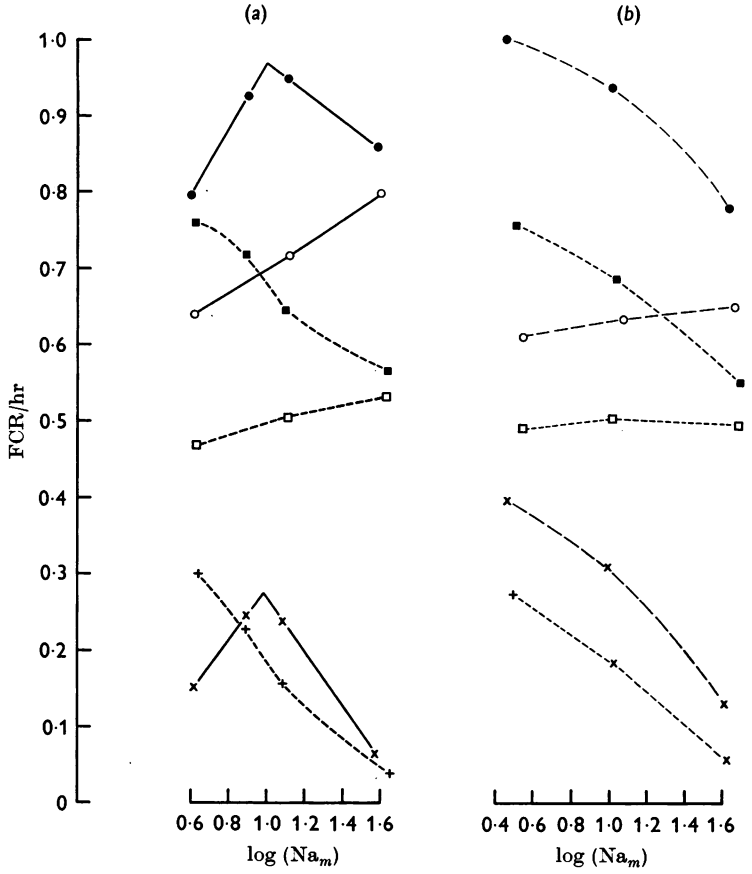

Fig. 6. FCR in calcium-free media for Na efflux from lactose-treated red cells. (*a*) cells loaded with Na + K and exchanging with a KCl medium, *or* cells loaded with Na + Li and exchanging with a LiCl medium; (*b*) cells loaded with Na + Cs and exchanging with a CsCl medium, *or* cells loaded with Na + Li and exchanging with a LiCl medium. Note experiments (*a*) and (*b*) (unlike those of Figs. 3 and 4), were not done at the same time and are not directly comparable. (*a*) For Na + K cells in KCl media: FCR total Na efflux = ●—●. FCR passive Na efflux = ○—○. FCR active Na efflux = ×—×. Also for Na + Li cells in LiCl media: FCR total Na efflux = ■----■. FCR passive Na efflux = □----□. FCR active Na efflux = +----+. (*b*) Na + Cs cells in CsCl media, FCR total Na efflux = ●– – –●. FCR passive, Na efflux = ○— —○. FCR active Na efflux = × – – – ×. Also for Na + Li cells in a LiCl medium, see under (*a*).

those containing Ca^{2+}, FCR for Na efflux is greater in Na + Cs systems than in the Na + Li systems (see Fig. 6*b*). No experiments were done with Na + Rb systems, because, even when passive efflux is small, the curves for active Na efflux in Na + K and Na + Rb systems are fairly similar (Figs. 2 and 5*a*) and in the calcium-free suspensions, where passive permeability is high, it is unlikely that significant differences would be demonstrable. However, the data permit the statement that the effects of K, Cs and Li, and also the effects of variations in Na_m on FCR for active Na efflux are the same whether the cell suspensions contain Ca^{2+} or not, though comparison of Fig. 5*a* and *b* might suggest that active Na efflux is perhaps slightly less in the absence of calcium ions; this is considered further in the next section. The present section concludes with a review of the significance of the data. Thus consider FCR for active Na efflux in Na + K curves (Fig. 6*a*): with log (Na_m) at 0·62 and 0·97 (Na_m = 4·2 and 9·3 m-equiv/l. cells), the respective FCRs are $0{\cdot}15 \pm 0{\cdot}02$ and $0{\cdot}26 \pm 0{\cdot}019$ hr^{-1} and the difference is significant. Consider also FCR for the active Na effluxes of Na + Li and Na + Cs curves of Fig. 6*b* with log (Na_m) at 0·6 (Na_m = 4 m-equiv/l. cells): here the value for the former is $0{\cdot}255 \pm 0{\cdot}018$ and for the latter $0{\cdot}39 \pm 0{\cdot}026$, and the difference in FCR is significant. Reverting now to Fig. 6*a*: FCR for active Na efflux in Na + Li systems with log (Na_m) at 1·56 (Na_m = 36 m-equiv/l. cells) is $0{\cdot}05 \pm 0{\cdot}020$ and for Na + K systems $0{\cdot}07 \pm 0{\cdot}023$ and the difference here is not significant; nor indeed is it significant in Fig. 5*b*, for the Na + Li data here are derived from only three curves. However, in Fig. 5*b*, when log (Na_m) = 0·6, FCR for active Na efflux with Na + K systems equals $0{\cdot}16 \pm 0{\cdot}02$ hr^{-1} and for Na + Li systems $0{\cdot}33 \pm 0{\cdot}02$, hence the difference here is significant.

Calcium and active Na efflux. It was noted above that FCR for active Na efflux might perhaps be slightly less when cell suspensions were Ca-free. Thus comparison of Fig. 5*a* and *b* where curves are derived from average values shows that with log (Na_m) at 0·9 (Na_m = 8 m-equiv/l. cells), FCR for Na + K systems is 0·31 hr^{-1} in the presence of Ca^{2+} and 0·28 in its absence; however, the latter figure was derived from only five experiments, and these were not carried out with the same batches of cells contributing to Fig. 5*a*. Hence it seemed desirable to make a more direct comparison. For this reason, cells loaded with Na and K were transferred to a nutrient KCl medium. This was divided, $CaCl_2$ being added to one half only. Each half was then subdivided into two samples, strophanthin G being added to one of each pair. Figure 7 shows the FCR for total, passive and active Na efflux in the presence and absence of calcium ions, and it will be seen that the FCR for active Na efflux shows no significant difference.

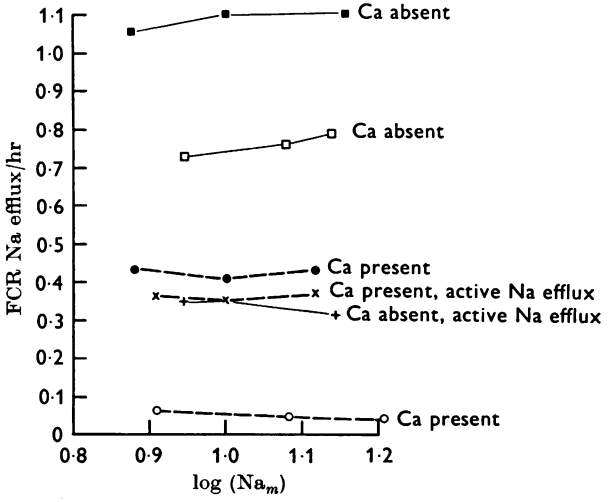

Fig. 7. Effects of the presence and absence of calcium ions on FCR for active Na efflux from Na+K cells in nutrient KCl media. Ca^{2+} present: FCR total Na efflux = ●– – –●, FCR passive Na efflux = ○----○, FCR active Na efflux = ×----×. Ca^{2+} absent: FCR total Na efflux = ■—■, FCR passive Na efflux = □—□, FCR active Na efflux = +—+.

Sodium and lithium transfer

It has been seen that the rate coefficient for active Na efflux from red cells varies considerably according to circumstances, but a reverse trend leading to active influx of Na has never been observed; nor has active efflux of K, Rb, Cs or Li been encountered. However, the proximity of Li to Na in the group table, and the observation that active influx of Li is usually less effective in promoting linked active efflux of Na, has led to attempts to elicit active Li efflux. These have all failed, but are perhaps worthy of record. In the first series, cells and external nutrient medium were arranged to contain 20 m-equiv K and 130 m-equiv Li/l. cell and medium water. Suspensions were incubated at 37° C in the presence and absence of strophanthin G. It was found that after 6 hr there had been no efflux of Li, but a small uptake of Li and K, doubtless due to the Donnan effect. In a similar experiment, but with Na replacing Li, net active efflux was 15·3 m-equiv/l. cells.

In the next experiment, lactose-treated cells loaded with Li and K were incubated in a nutrient KCl medium, without and with strophanthin G. Results are shown in Table 4. It will be seen that FCR for Li efflux is about the same whether strophanthin G be present or not, and the slight observed difference is not significant. On the other hand there is an active efflux of Na, even though the initial cell content is very low (actually a residue of

that originally present in the cells), so that FCR for active Na efflux is 2·7 times FCR for passive efflux. In another experiment FCR for Li efflux into a nutrient KCL medium was 0·031 hr^{-1} for systems devoid of strophantin G and 0·030 in its presence.

TABLE 4. Cation exchanges between lactose-treated red cells loaded with Li and K and exchanging with a nutrient KCl medium for 3 hr at 37° C. Initial cell composition Li 34 m-equiv, K 70·5 m-equiv and residual natural Na 2·3 m-equiv/l. cells. Composition of external medium: K 148, Li 0·51, Na 0 m-equiv/l. $CaCl_2$ 2·5 mM

Strophanthin G	Cell cation (m-equiv/l.) mean content between 0 and 3 hr			Change in cation (m-equiv/l. cells)			FCR(hr^{-1})	
	Li	K	Na	Li	K	Na	Li	Na
Absent	31·8	72·7	1·99	−3·9	+5·4	−0·72	0·041	0·12
Present	31·5	72·4	2·11	−3·5	+5·8	−0·30	0·037	0·047

DISCUSSION

If red cells depleted of cation with lactose solution are loaded with Na + B and then allowed to exchange cation with a nutrient BCl medium, the flux: concentration ratio (or FCR) used as a rate coefficient for total Na efflux alters with the mean Na content of the cells (Na_m). If the curves of FCR for the several Na + B systems are plotted against log (Na_m), each curve shows a peak, but the slopes of the curve to and from the peaks, and the heights of the peaks themselves and their position with regard to log (Na_m) vary according to whether B is K, Rb, Cs or Li (Fig. 2). As the contribution of passive Na efflux to the total efflux is relatively small and also varies much less with change in Na_m (Table 3), it may be presumed that the curves of FCR for active Na efflux will correspond in shape and in the position and heights of the peaks to the curves of FCR of total Na efflux, and this has been shown to be the case (cf. Figs. 2 and 5*a*). Considering in the first instance Na + K systems only, the curves for FCR show that the value is low for high Na_m, rising to a peak which corresponds approximately to log (Na_m) = 0·9 (Na_m = 8 m-equiv/l. cells), falling again below this point. It was suggested by McConaghey & Maizels (1962) that the relatively low rate coefficients for efflux at high Na_m might be due to competition for Na carriers, but if this were the only factor, FCR would be the same whatever the nature of the complementary cation B. In fact it varies and, at high Na_m, Li is associated with the lowest Na efflux and K with the highest: clearly at this level the cations exert an additional effect which is specific. Similarly, in Na + K systems, McConaghey & Maizels (1962) suggested that fall in FCR, as log (Na_m) falls below 0·9, might be due to the presence of a small inexchangeable fraction

of Na within the cells. Again it must be doubted if this can be a significant factor because of the wide variations in the amount of total Na efflux when systems other than Na + K are used. This difference is seen more clearly in Fig. 2 where FCR is plotted against log (Na_m); thus as the latter falls from 0·95 to 0·45 (Na_m = 9–2·8 m-equiv/l. cells), FCR for Na + K and Na + Rb falls by about 40 %, while for Na + Li it rises by 30 % and for Na + Cs by 50 %. That all these findings apply also to active Na efflux in the presence of K, Rb, Cs and Li is apparent when Figs. 2, 3, 4 and 5*a* are compared, so that here too, the complementary cation B has a specific effect. According to the height of the FCR for any given value of log (Na_m), one obtains the sequences detailed in Table 2 and summarized below:

log (Na_m)	Cation sequences	Eisenman's sequences, no.	
1·8– 1·5	K > Rb, Cs > Li	III*a*, IV or V	with Na excluded
1·5– 1·0	K > Cs > Rb > Li	III*a*	with Na excluded
1·0– 0·8	Cs > K > Rb > Li	II*a*	with Na excluded
0·8– 0·72	Cs > K > Li > Rb		
0·72– 0·40	Cs > Li > K > Rb	Not represented	
0·40– –0·20	Cs > Li > K, Rb		
–0·20– –0·40	?Cs or ? Li > K, Rb		

Eisenman's sequences were obtained in purely physical systems, where the selectivity of chemically modified glass membranes to the passage of cations was examined. Eleven cationic sequences were obtained depending on the charge of the membrane and the degree of hydration of the cation. This matter and also the occurrence of similar sequences in biological systems has been investigated and reviewed by Eisenman (1963) and briefly summarized by Carolin & Maizels (1965). In the case of red cells loaded with Na and suspended in a nutrient medium containing 140 m-equiv Na/l. and 10 m-equiv B/l., the rate of total Na efflux (and of B influx) depended on B, which followed the sequence K > Rb > Cs > Li (Eisenman's sequence IV or V with Na excluded) (McConaghey & Maizels, 1962). Carolin & Maizels (1965) also showed that, in certain circumstances, non-metabolizing red cells in NaCl media had the faculty of passively retaining cations against concentration gradients, the avidity of the cells giving the sequence K > Rb > Cs, Na > Li (Eisenman's No. IV). It may be noted that in this passive series Cs and Na are together yet, with active cation movements, Na can never be associated with any other cation, because it is always transported outwards, while the others when transported actively always move inwards. The reason for this selectivity remains unexplained. Returning now to Eisenman's sequences in biological systems: many such systems give typical sequences, but that for the permeability of frog skin is K > Rb > Cs > Li > Na, which is

sequence IV with Li and Na transposed. So, too, the effect of cations on the ATP-ase of crab nerve in the magnesium-activated state is

$$Na > Li > K > Cs > Rb,$$

which is order X but with Cs and Rb transposed. Thus it is possible that Eisenman's parameters may be partly responsible for the cationic series shown above, which apply to facilitation of active Na efflux for Na+B cells in a nutrient BCl medium, and which vary with B and the level of cell Na. Nevertheless, the last four sequences of our series are not represented in Eisenman's orders, and might seem to involve some other factor besides surface charge and the degree of hydration of intracellular Na and of B.

I. M. Glynn has suggested to me (personal communication) that difficulties might perhaps be resolved if active Na efflux were considered not in isolation, but in relation to (i) the active influx of B, and (ii) the possible competition between B and Na at the inner surface of the cell membrane. Garrahan & Glynn (1967) have shown that in the case of the human red cell, when external K (K_e) is low, K influx is much affected by the external Na concentration (Na_e). Thus when the latter is only 0·035 m-mole/l. water (external tonicity being maintained by choline chloride), influx when K_e is 1 mM is 1·35 m-mole/(l. cells).hr, while when Na_e is raised to 130 mM, K influx falls to 0·6 m-mole/(l. cells).hr. This suggests that, when K_e is low and Na_e high, Na ions compete with K ions for a carrier at the outer surface of the cell membrane, though when K_e exceeds 3 mM, variations in the concentration of Na_e have little effect on K influx. The observations may be applied to the present discussion, and to avoid confusion it should be noted that Na_m (as previously) denotes the mean Na content, m-equiv/l. *cells* prevailing during the period of cation exchange; strictly, this should be written Na_{mi} (*mi* being 'mean internal'), but for simplicity Na_m has sufficed. So too, B_m is the mean *cell* content of B (K, Rb, Cs or Li), while B_e is the content of B in the suspending medium; owing to the large volume of medium relative to that of the cells, B_e (unlike cell B) does not alter significantly during an experiment. In the present experiments it may be assumed that when Na_m is high and B_m low, active Na efflux suffers no competition from B_m, though it is affected by the amount of Na_m and the nature of B. It has previously been noted that as Na_m rises from 30 to 80 m-equiv/l. cells, Na efflux increases by only 30% (Fig. 1*a*), and this has been attributed to saturation of carriers. But differences in active Na efflux for any given value of Na_m (e.g. 80 m-equiv/l. cells) may be attributed to linkage between active Na efflux and active influx of B. Assuming that active influx of B is proportional to active Na efflux (and experimentally this may be shown to be the case),

then facilitation of Na efflux by B when Na_m is 80 m-equiv/l. cells would appear to be K:Rb:Cs:Li = 8·2:7·2:7·2:5·2 (Fig. 1*a*). This would explain the finding for active efflux of Na and FCR for Na_m between 30 and 80 (or more) m-equiv/l. cells. On the other hand, with low Na_m and high complementary B, it may well be that competition of B for carriers interferes with the active efflux of Na; if so, the interference will increase progressively as Na_m falls and B_m rises, and this would explain the fall in FCR already noted at low Na_m (Figs. 2 and 5*a*). Therefore the observed FCR peaks result from the adjacent depressions due to saturation of carriers at high Na_m and to a disproportionate depression of Na efflux by B_m at low Na_m. It is, however, clear from Fig. 1*b*, that decrease of active Na efflux with fall of Na_m varies according to the nature of cell B. When Na_m is 3 m-equiv/l. cells, inhibition of active Na efflux presumed to be due to cell B gives the series K, Rb > Li > Cs, and this, if one follows the views of Garrahan & Glynn (1967) regarding active K influx in the presence of external Na, suggests that in the present systems the relative affinities of the different cations at the inner surface of the cells for the carrier responsible for Na efflux is Na > K, Rb > Li > Cs, and this is Eisenman's sequence VIII.

It would obviously be informative if it were possible to define quantitatively the effects of B_m and Na_m on Na efflux independently of the effects of external B. Theoretically this might be achieved by loading red cells with Na, together with K, Rb, Cs or Li, and allowing each of the four batches of cells to exchange cations with a nutrient solution of a salt of a single cation species; thus Na+K, Na+Rb, Na+Cs and Na+Li cells might be allowed to exchange with KCl media. However, it is clear that, even during a short period of incubation, B_m would exchange with K_e and the specific effects of B_m (K, Rb, Cs or Li) would be utterly obscured.

Calcium ions and active and passive Na efflux

The effects of Ca^{2+} are paradoxical: in certain systems (cells treated with iodoacetate or else depleted of substrate during storage) its presence increases cation permeability, especially to K, and this effect is inhibited by such chelating agents as ethylenediaminetetra-acetate (EDTA) and EGTA (Gardós, 1959; Hoffman, 1966). But in the present experiments, treatment with lactose solution increases permeability, and this effect can be prevented by the presence of low concentrations of monovalent cations, and by even lower concentrations of the divalent cations Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+}. But if no cations are added to the lactose solution and high permeability has been allowed to develop, normal permeability can only be *restored* by adding Ca^{2+} to the suspending medium; the other divalent cations of this group are now ineffective (Bolingbroke & Maizels, 1959). How the lactose effect and the inhibitory action of calcium are exerted on

the red cell is at present unknown, and the situation is further obscured by the observation that human red cells contain less than 0·04 mM-Ca (J. D. Judah & M. Maizels, unpublished).

Calcium and passive cation exchanges. Lactose-treated red cells loaded with cation and exposed to an electrolyte medium containing 2·5 mM-$CaCl_2$ for several hours have a rate coefficient for passive Na efflux of 0·05 hr^{-1} compared with a normal value of 0·03. If exposure to $CaCl_2$ is limited to $\frac{1}{2}$ hr before cation exchanges are measured, FCR will be about 0·1 hr^{-1} (Figs. 3 and 4), while if all media are Ca-free throughout, FCR will have increased 25-fold to 0·6–0·8 hr^{-1} (Fig. 6). It has been shown that the latter finding applies to all the various passive exchanges of monovalent cations with the exception of K loaded slowly into red cells and then allowed to exchange with an external NaCl medium, when FCR tends to be low (Carolin & Maizels, 1965). However, present observations show that if FCR for passive Na efflux into KCl and LiCl media are studied in Ca-free systems, loss of cell Na to external LiCl is about 30 % slower than to KCl. Na efflux into CsCl media is about the same as into KCl media, or possibly slightly less. (Note: Fig. 6*a* and *b* were obtained from different batches of cells and the various fluxes are not directly comparable.) It may be shown that passive Na efflux is directly related to passive influx of B (Carolin & Maizels, 1965), and the latter may well determine the former, in which case the greater size of the hydrated Li ion may determine its slower rate of influx, and this in turn might limit the efflux of Na; however, if this were so, then it would be expected that the smaller hydrated Cs ion would enter the red cell more rapidly than K, and that the rate of the complementary Na efflux would be correspondingly great; but in fact it is either the same or less. An alternative suggestion has been made that Li ions in high concentration (150 m-equiv/l. external medium) have a slight Ca-like action.

Calcium ions and active cation exchanges. It has been seen that treatment of red cells with lactose solution increases permeability to passive transfer of cations upwards of twentyfold, but in the case of active cation transfer, FCR for active Na efflux is little if at all affected (Fig. 7 and compare Fig. 5*a* and *b*). This is to be expected, for it is known that red cell ATP-ase is Mg- but not Ca-sensitive (Clarkson & Maizels, 1952), and indeed Dunham & Glynn (1961) have shown that quite small amounts of calcium salts actually inhibit strophanthin G-sensitive ATP-ase, which is closely associated with active cation transport.

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