SODIUM AND POTASSIUM MOVEMENTS IN SHEEP ERYTHROCYTES OF DIFFERENT CATION COMPOSITION

BY C. R. B. JOYCE AND M. WEATHERALL

From the Department of Pharmacology, London Hospital Medical College, London, E. 1

It has become increasingly evident that the exchange of sodium and potassium by the erythrocytes of various mammalian species is less simple than was at first envisaged. The concept of a uniform population of cells, exchanging at a rate depending mainly on temperature (Raker, Taylor, Weller & Hastings, 1950; Sheppard & Martin, 1950; Harris & Maizels, 1951; Solomon, 1952) has not been fully substantiated, and it has been shown that the sodium (Gold & Solomon, 1955; Clarkson & Maizels, 1955) and potassium (Solomon & Gold, 1955; Joyce, Rayner & Weatherall, 1956) of human red cells are better regarded as consisting of at least two fractions exchanging at different rates. The mechanism of transport has also been divided into separate processes, differently affected by changes in environmental conditions, both in horse cells (Shaw, 1955) and human cells (Glynn, 1956). Most of these interpretations assume that the individual cells of a sample of blood behave uniformly, although there is evidence that red cells even in a single sample of blood are not identical in composition (Kruszyński, 1950; Keitel, Berman, Jones & Maclachlan, 1955; Joyce, 1958).

In sheep there is considerable variation in the mean concentrations of sodium and potassium in the red cells of different individuals (Kerr, 1937; Evans, 1954), although the concentrations remain fairly constant from time to time in any particular animal (Evans, 1957). Whether the differences between animals depend on the presence of different proportions of two kinds of cell, one rich in sodium and one rich in potassium, or whether all the cells of a given animal have about the same ratio of sodium to potassium, characteristic of that animal, remains to be established. But it is interesting to compare the rates of exchange of sodium and potassium in sheep with different types of blood, and the results of these observations are described here. A brief pre-

liminary account of this work has been published elsewhere (Joyce & Weatherall, 1957). An earlier study of the exchanges of sheep cells (Sheppard, Martin & Beyl, 1951) involved only one or two animals and did not give information about different types of blood.

METHODS

Blood (30–60 ml.) was drawn from Scottish Blackface sheep at the Rowett Research Institute, Aberdeenshire, and clotting was prevented with heparin (Pularin, Evans Medical Supplies). Some of the sheep have already been referred to, under the same numbers as those used here, by Evans (1957). The blood was brought by rail or air to this laboratory, and used 15–30 hr after bleeding. Experiments were performed in conical flasks with a partition in the floor, so that blood (5 ml.) and radioactive saline medium (5 or 8 ml.) could be kept separate until they were mixed by tilting the flasks. The saline medium contained (mM): Na 143, K 5·5, Ca 1·7, Mg 1·2, Cl 128, HCO_3 25, SO_4 1·2, H_2PO_4 1·2, dextrose 11. Part of the NaCl or KCl used in preparing this medium was derived from spectroscopically pure K_2CO_3 or Na_2CO_3 which had been bombarded with neutrons (at the Atomic Research Establishment, Harwell) and dissolved in appropriate amounts of HCl to give neutral approximately 0·15 M solutions of the chlorides. The exact composition of the medium during incubation was modified by the composition of the added plasma: the potassium concentration was particularly affected, and actual values are reported with the experimental results. The proportion of cells was usually 14–18 % (v/v). The medium was gassed before use with 95 % O_2 and 5 % CO_2 and had a pH of 7·4. The flasks were kept in a water-bath at $37 \pm 0·1°$ C, in a rack on which they could be tilted to mix their contents and rocked by their necks through an angle of 40° at a rate of 3·3 times a minute. This rate was sufficient to prevent visible sedimentation of the cells. From time to time samples (1 ml.) were taken into calibrated tubes, made with a wide upper and narrow lower section. The tubes were kept at 37° C during sampling and then centrifuged at, or slightly above, room temperature for 25 min at 3000 rev/min and 15 cm radius (to the bottom of the tube). The cells packed into the long narrow section and their volume could be determined from measurement against a millimetre scale. The time of beginning centrifugation was taken as the time of separation of cells from the medium. The supernatant fluid was removed and the upper part of the tube and the interface were washed twice with 0·28 M dextrose, which was discarded. The cells were transferred with further washings to volumetric flasks and made up to 50 ml. with 0·1 % (v/v) 0·880 NH_4OH for estimation of haemoglobin, sodium, potassium and radioactivity. Samples (1 ml.) of the whole suspension were diluted for similar estimations.

In some experiments 2 ml. samples, or the entire contents of flasks in which inward movement of radioactive material had proceeded for a chosen period, were centrifuged at 2000 rev/min and 14 cm radius for 3 min. A convenient volume of supernatant fluid was removed and replaced by an equal or larger volume of warm inactive mixture of saline and plasma in the same proportions as in the original medium. The resuspended cells were transferred to fresh flasks, rocked during incubation and sampled as before.

The haemoglobin content of the cell samples was estimated in a Hilger and Watts 'Spekker' absorptiometer with Chance OG1 filters. Small variations in the haemoglobin content of successive samples were attributed to variation in the proportion of cells to supernatant in the samples, and estimates of the sodium and potassium content and radioactivity of the cells were adjusted to a standard quantity of cells accordingly. Radioactivity was estimated by duplicate counts in M6 liquid counters and conventional equipment (type 1014A probe unit and 1009B scaling unit, Dynatron Radio Ltd.). Corrections were made for background and resolving times and for radioactive decay. Potassium was estimated on an EEL flame photometer (Evans Electroselenium Ltd.): standard solutions were made up to contain approximately the concentrations of sodium present in the solutions to be estimated, though this precaution appeared to be unnecessary in the conditions in which the apparatus was used. Sodium was similarly estimated against standard solutions which contained the approximate concentration of potassium. All estimates on the flame photometer were made in triplicate.

The amount of supernatant trapped by the packed cells was estimated with inulin in separate experiments done in the standard conditions. Cells were suspended as usual, except that the medium contained additionally 1–2 % (w/v) inulin. 1 ml. samples were spun in the usual way. 0·5 ml. of supernatant was removed and appropriately diluted to provide standards of comparison. The remaining supernatant was removed, the cell surface and the upper part of the tube gently washed twice, as usual, except that saline medium was used instead of isotonic dextrose, and the cells were resuspended in 1 ml. of inulin-free medium. This suspension was centrifuged and 0·5 ml. of the supernatant was compared with the standards, by means of the colorimetric method for fructose of Bacon & Bell (1948), on the 'Spekker' absorptiometer, using Ilford 602 filters. Hydrolysis of inulin to fructose was brought about by the reagents used in this method without modification.

Several alternative procedures for estimating the trapped volume or eliminating it appeared unsatisfactory. Like Clarkson & Maizels (1955), we found that T1824 in saline solution gave unduly high values for the trapped volume, presumably because a little dye was reversibly bound by the cells in these conditions. The use of ^{24}Na to label the medium (Solomon, 1952) may be subject to a larger error than was then suggested in view of the evidence that there is a rapidly exchanging fraction of the cell sodium (Clarkson & Maizels, 1955; and see below). Resuspension of the cells in sodium- or potassium-free media and recentrifuging has not been adopted because of the likelihood of losses from the cell into the medium used for resuspension.

RESULTS

Volume of fluid trapped during centrifuging

In the experiments described below, estimations have been made of the sodium, potassium and radioactive content of cells centrifuged from media containing these same materials. The estimates have been adjusted for the amount of material contained in that portion of the medium which is carried down with and trapped in the packed cell column. The results of twelve determinations of this volume with inulin are shown in Fig. 1. It will be seen that points showing the volume trapped (y) in cell columns of the size studied (x; 30–60 mm long, containing 0·1–0·2 ml. cells) did not lie on a straight line passing through the origin; but within the range relevant to the present studies departures from rectilinearity appeared to be small enough to be neglected and the adjustment made is based on the straight line which best fits the observations, $y = 0·128x - 0·074$. For a few observations where the packed cell volume was less than 0·12 ml. the adjustment was based on a straight line from the origin to the mean of the lower group of points in Fig. 1.

Sodium movements

Net changes. All experiments were carried out with blood which had been drawn at least 15 hr previously and transported from Aberdeenshire. In most experiments the blood and radioactive saline were warmed to 37° C for only 5–15 min before mixing, and in some, but not all, experiments an appreciable fall in the cell sodium content occurred during the first hour of incubation. This fall was accompanied by a decrease in the cell volume, and the concentration of sodium therefore changed less than the total content; for instance,

in one experiment with blood from sheep 113, the sodium concentration decreased from 98·3 to 95·0 m-equiv/l. cells, a fall of 3·4 %, while the content fell by 11·8 %. The mean level reached after the first hour was in reasonable agreement with values obtained for fresh samples of blood from these animals (J. V. Evans, personal communication). Most experiments finished after 5-6 hr. When they continued through the night, up to 20 % of the cells became haemolysed, but the concentration of sodium in those which remained was not significantly altered.
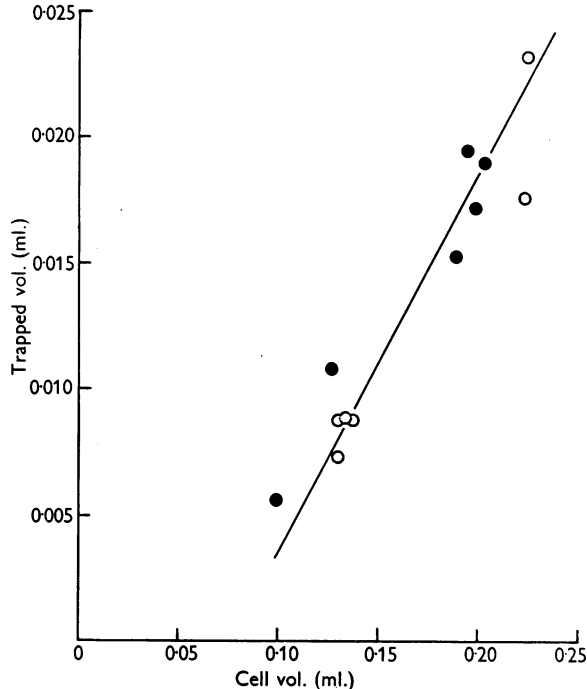
Fig. 1. Trapped supernatant volume (y) for different sizes of cell column (x) in low Na, high K(O) and high Na, low K(●) sheep. The differences between sheep are insignificant. The line ($y = 0·128x - 0·074$) was fitted by the method of least squares.

TABLE 1. Behaviour of cell sodium fractions (sheep 113, Expt. 4)

| Duration of incubation (hr) | Total cell ^{24}Na (counts/min/l.) | Total cell Na (m-equiv/l.) | Slow fraction | | Fast fraction | | |
			^{24}Na (counts/min/l.)	Na (m-equiv/l.)	^{24}Na (counts/min/l.)	Na (m-equiv/l.)	Specific activity (counts/m-equiv)
0·125	29,940	107·7	460	86·1	29,480	21·6	1360
0·5	15,930	97·3	1940	86·1	13,990	11·2	1250
1·0	10,450	94·5	3750	86·1	6,700	8·4	800
1·5	19,000	95·0	5690	86·1	13,310	8·9	1490
						Mean	1225

Specific activity of whole suspension, 1200 counts/m-equiv. All concentrations are based on the volume of the cells at 1 hr: the observed concentrations at 0·125 and 0·5 hr were 99·2 and 93·7 m-equiv/l., because the cells initially occupied a larger volume. The estimated quantities in the slow fraction are derived from the figures in Table 2, and those in the fast fraction by difference ('total' minus 'slow').

Inward sodium movement. The uptake of ^{24}Na was observed, both in cells which were extruding sodium and in cells in a steady state of total sodium. In the former, the initial uptake was large and rapid, so that 30–45 % of the sodium was labelled in samples taken within 8 min of mixing the blood with active medium (Table 1, column 2). Surprisingly, subsequent samples of cells contained less radioactivity and, in spite of the extrusion of sodium, the specific activity of the cell sodium actually declined as long as sodium was being extruded, after which the specific activity rose again slowly. In cells in which the cell sodium was steady, the initial uptake of ^{24}Na was smaller, though still rapid compared with the subsequent rate of gain, and the radioactivity of the cells subsequently increased at a slow steady rate. Such changes occurred in high-sodium cells and in low-sodium cells. The initial uptake of ^{24}Na was up to four to six times greater than the amount which could be due to trapping of the medium among the centrifuged cells and the subsequent decline showed that it was not due to errors in this adjustment. This decrease was sometimes irregular, perhaps because of errors in cell separation and in sodium and ^{24}Na estimation, but the over-all decrease was too large and too consistent to be accounted for in this way. The cell sodium was therefore not uniformly labelled, and a part of it exchanged with the medium much faster than the rest. This part appeared to include most or all of the initially extruded sodium. As some ^{24}Na was taken up rapidly, even by cells in a steady state, the fast fraction did not consist only of the extrudable fraction. Further evidence on this point was obtained by observing the outward movement of ^{24}Na from cells loaded with ^{24}Na.

Outward sodium movement. Outward movement of ^{24}Na was followed by taking samples from the flasks in which cells had been taking up tracer for 0·5 hr or 4 hr, centrifuging for about 3 min, removing as much active supernatant as convenient, replacing it by inactive but otherwise identical medium at 37° C and incubating again. Since a small amount of the original medium was carried over with the cells, the medium was not quite inactive, but its specific activity was always considerably less than that of the cells. Both at 0·5 and 4 hr the cells lost much of their total tracer within 25 min of immersion in the new medium, and thereafter only the cells which had been immersed for 4 hr continued to lose appreciable further amounts of ^{24}Na (Fig. 2). The loss of ^{24}Na from the cells departed considerably from a simple exponential relationship, and confirmed the existence of a rapidly exchanging fraction of the cell sodium.

Measurement of rates of sodium movement. The estimation of rates of movement when the ions are not homogeneous in the tissue has been discussed by Sheppard & Householder (1951), Rescigno (1954), Robertson (1957) and others, and, when there is a net change in the amount of material in the tissue, by Harris & Maizels (1951), Keynes & Lewis (1951), Joyce, Moore & Weatherall (1954) and others. Here there are both lack of homogeneity and net movement,

and interpretation presents some difficulties. It is simplest to assume (and it will be shown that the assumption leads to a reasonable description of the facts) that the total cell sodium is in two fractions, and that only one of these varies in size. It also simplifies the interpretation to assume that there is no movement of material between the fractions, though nothing in the evidence makes this assumption essential, and alternative possibilities are considered later.
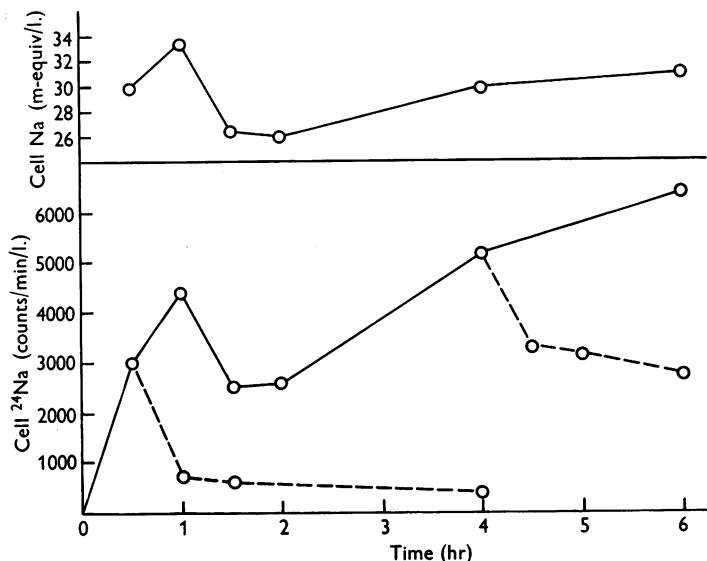

Fig. 2. Inward and outward movement of ^{24}Na. Sheep 137, Expt. 3. Mean cell Na concentration 33·6 m-equiv/l. cells. Solid line, cells in active medium throughout: expected count at complete exchange, 15,880. Interrupted lines, cells transferred to nearly inactive medium at 30–36 min (expected count at complete exchange, 260), or at 240–246 min (expected count at complete exchange, 320).

When the cell sodium is divided into two fractions and there is no net change, the uptake of tracer follows a course described by the sum of two exponentials (Gellhorn, Merrell & Rankin, 1944; Cohn & Brues, 1945; Robertson, 1957). When the two cell fractions do not communicate directly with each other and the quantity of sodium in the medium is so large that uptake of ^{24}Na by the cells does not appreciably alter the specific activity of the medium, the relative sizes of the fractions are determined by plotting $\ln (1 - y/y_{\infty})$ against time, y being the radioactivity of the cells at time t and y_{∞} their radioactivity at the specific activity of the whole suspension. Once exchange of the fast fraction is complete, the points lie on a straight line, of which the slope depends on the rate of movement into and out of the slow fraction, and the value at the intercept measures the proportion of the total cell sodium in the slow fraction. This procedure can be applied also when there is sodium extrusion during the

early part of incubation, if, as seems likely, the changes in total sodium content affect only the fast fraction and if observations are used only after extrusion is complete. Estimates obtained in this way are summarized in Table 2. No estimate is given for the rates of exchange of the fast fractions as they are largely complete in the smallest periods studied and also are seriously affected by uncertainties about the exact time of separation of the cells from the medium. A minimal estimate can be made of the rate of uptake of sodium from experiments in which the suspensions were sampled and centrifuged within a few minutes of adding tracer, and these give figures of > 240 m-equiv/l.

TABLE 2. The exchange of the red cell sodium of sheep

Sheep no.	Expt. no.	Total cell Na		Slow fraction		
		At start* (m-equiv/l.)	Steady state (m-equiv/l.)	(m-equiv/l. cells)	Exchange rate (per hr)	Movement (m-equiv/l. cells/hr)
296	5	100·7	100·7	100·5	0·046	4·62
113	4	107·7	95·0	86·1	0·037	3·18
130	3	105·5	90·2	88·4	0·039	3·45
152	3	—	81·2	75·8	0·050	3·79
W144	5	88·5	83·8	78·5	0·055	4·32
145	3	—	34·2	34·2	0·127	4·34
137	3	—	28·8	26·4	0·085	2·24
137	4	43·0	31·8	27·3	0·112	3·06

* Within 7·5 min of beginning incubation.

cells/hr. Since the cells were probably losing sodium at this time, they must have been extruding sodium at no less a rate; even the maximum rate of net loss observed (about 20 m-equiv/l. cells/hr.) is small in comparison and it is evident that the net extrusion of sodium is effected by a comparatively small difference between the two rates. The slow fraction exchanges at a rate which differs little between sheep and averages $3·76 \pm 0·71$ (S.D.) m-equiv/l. cells/hr. It does not depend significantly on the amount of sodium in the cells or in the slow fraction ($r = +0·51$, $P > 0·1$) and the rate constants have a significant negative correlation with the sodium contents ($r = -0·90$, $P < 0·01$).

Two checks can be made on the validity of this model. Since the fast fraction is assumed to exchange with the medium with great rapidity, its specific activity at any time after the first few minutes of incubation should be approximately equal to that of the medium. The data of Table 2 give the amount of sodium in the slow fraction and allow its ^{24}Na content at any given time to be calculated. The amounts in the fast fraction can be obtained by difference. The values in Table 2 are based on observations made after incubation for two or more hours. They are independent of earlier estimations of sodium and ^{24}Na, which can therefore be used to calculate the specific activity of the fast fraction while its size is varying. Table 1 gives the results with sheep 113 which showed the largest changes in total cell sodium. It will be seen that

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the average specific activity of the fast fraction, so estimated, is 102% of that of the medium. The rather large scatter of the separate estimates is hardly surprising, in view of the errors which may arise from imperfect separation of the cells and from variation in the amount of trapped medium, and it is somewhat fortuitous that the average value is so near to that predicted by the model. Other experiments have shown the same sort of scatter of individual values and no significant difference from 100% exchange of this fraction. The second check is provided by the experiments on efflux, as it is possible to predict the expected counts of cells after transfer to a new medium on the assumption that the fast fraction again reaches the activity of the new medium within a few minutes. An approximate check of this sort can be applied by inspection of Fig. 2. When cells are transferred to an almost inactive medium (interrupted lines), the specific activity of the fast fraction rapidly becomes negligible. Estimates of the fast fraction made as before suggest that an initial loss of approximately 2500 counts/min (corresponding to the unloading of tracer from 3·6 m-equiv sodium/l. cells) should occur in cells transferred at 0·5 hr and approximately 2200 counts/min (corresponding to 3·2 m-equiv sodium/l. cells) at later times. When these deductions are made, the initial steep fall in the count of cells transferred to a new medium is reasonably well accounted for, and the remaining activity is consistent with the expected behaviour of a single slowly exchanging fraction containing 92% of the steady cell sodium.

Potassium movements

Net changes. The cell potassium of individual samples of blood did not vary outside the limits of experimental error during incubation, i.e. within a range of $\pm 3\%$. There was no systematic trend in such variation as did occur, but the possible occurrence of regular changes of 1–2% in the cell K was not excluded by these observations.

Inward movement of potassium. The uptake of ^{42}K, like the uptake of ^{24}Na, followed a steady course after the first hour of incubation. Before this, the uptake was greater than expected from the later observations: and sometimes there was a decline in the cell radioactivity between the first sample, taken within a few minutes of beginning incubation, and samples taken after $\frac{1}{2}$ or 1 hr (Fig. 3). Semilogarithmic plots of $(1-y/y_\infty)$ against time showed no significant departures from linearity between 1 and 6 hr incubation, and always crossed the intercept at a point below unity. It therefore appeared that, as with sodium, there was a small, rapidly exchanging fraction, some of which might be lost from the cells during incubation but some of which persisted. On the assumption that the fast fraction was completely exchanged with the medium within a few minutes, the quantity of potassium so lost never exceeded 2% of the total cell potassium, and was therefore too small to be detected by flame photometry. The persistence of some at least of the fast fraction was confirmed

by experiments in which cells were transferred to an inactive medium so that outward movement of tracer could be followed.

Outward movement of potassium. The outward movement of ^{42}K was followed in the same way as that of ^{24}Na. Because the concentration of potassium in the medium was much lower than that of sodium, the ^{42}K carried over with the cells had much more effect than ^{24}Na on the specific activity of the new medium, and this activity was on different occasions between about one tenth

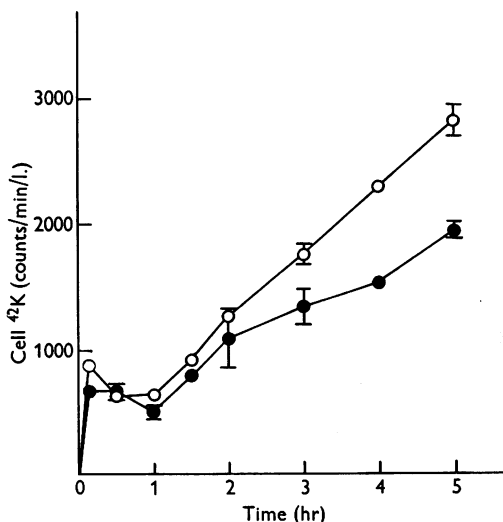Fig. 3. ^{42}K uptake by sheep erythrocytes. (●) Sheep 113, Expt. 4, mean cell K 20·6 m-equiv/l. cells. (○) Sheep 137, Expt. 4, mean cell K 81·2 m-equiv/l. cells. Range smaller than symbol where range marks absent. Expected counts when cells reach specific activity of medium, 135,000 (Sheep 113) and 252,000 (Sheep 137).

of and twice the activity of the cells at the time of removal from the soak-in medium. Cells transferred at 0·5 or at 4 hr lost much tracer within 25 min of immersion in the new medium (Fig. 4).

The loss showed a paradoxical relation to the relative specific activities of the cells and the medium. When transferred at 0·5 hr the cells lost tracer, even if their specific activity was initially less than that of the medium; and if their specific activity was initially higher, the loss of tracer continued to well below the level of the medium. This surprising observation has also been made on human red cells (Solomon & Gold, 1955; C. R. B. Joyce & M. Weatherall, unpublished) but it is more marked and more easily demonstrated in sheep cells. When the cells are transferred at 4 hr they rapidly lose about half their tracer content: the absolute loss is of a similar magnitude to that which occurs when they are transferred at 0·5 hr. This relationship between the specific activity of the medium and that of the cells is explicable only if the latter is an average derived from some cells, or some part of the cells, whose specific

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activity is higher, and some whose specific activity is lower, than that of the medium. The fall would then be due to a greater loss of tracer from the highly labelled fraction than uptake by the remainder.

Measurement of rates of potassium movement. The procedure which has been adopted in interpreting sodium movements can also be applied to the results with potassium. The estimation of the movement rates is slightly complicated because the volume and concentration of potassium in the medium were sufficiently small for its specific activity to decline considerably during the
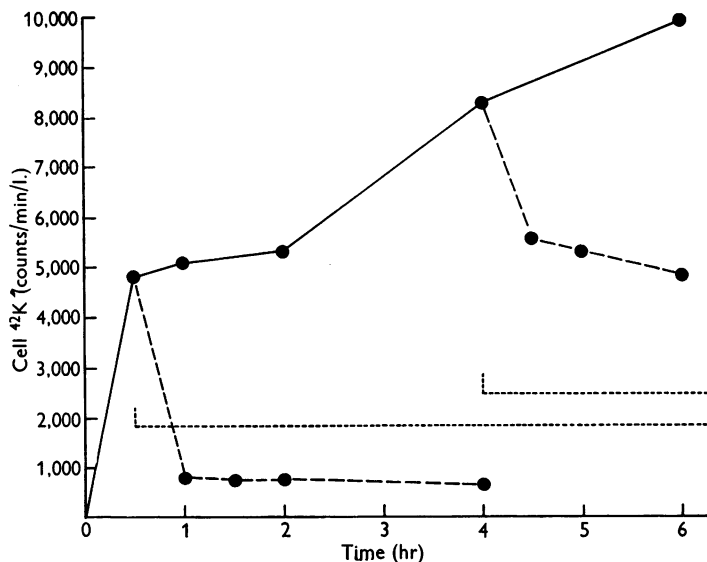

Fig. 4. Uptake and loss of ^{42}K by sheep erythrocytes. Sheep 152, Expt. 2, mean cell K concentration 27·7 m-equiv/l. cells. Solid line, cells in active medium throughout: expected count at complete exchange, 57,460. Interrupted lines, cells transferred to less active medium at 30–36 min, or at 240–246 min. Dotted lines show expected final count of cells so transferred.

experiments. The observed rate constants and relative sizes of the two fractions therefore require some adjustment (Robertson, 1957). The relative sizes of the fractions and the rates of movement into and from the slow fraction for various samples of blood are shown in Table 3. As with sodium, the rate of movement between the medium and the fast fraction is too rapid to estimate, and there is possibly some initial fluctuation in the size of this fraction, as shown by a larger initial uptake of ^{42}K (Fig. 5) than that predicted by the values tabulated. Apart from these initial changes, the fast fraction contained 1·0 ± 0·53 (s.d) m-equiv/l. cells, with no relation to the total concentration of potassium in the cells or to such variation as was observed when blood from the same sheep was examined on different occasions. Consequently, the slow fraction is mainly responsible for the variation in cell potassium between animals. The rate of potassium movement to and from this fraction showed comparatively

little variation in spite of the considerable differences in the quantities of potassium in the fraction. The lowest movement rate was 0·23 and the highest was 0·85 m-equiv/l. cells/hr (the latter value being the mean of two experiments on blood from the same sheep, 130). The blood from sheep 130 was exceptional in that it had the lowest concentration of potassium in its cells, barely higher than that of its plasma: also the total sodium plus potassium content of its blood in a steady state was only 98 m-equiv/l. cells, about 12 m-equiv/l. below the average or 7 m-equiv/l. below the minimum observed in any other animal,

TABLE 3. The exchange of red cell potassium of sheep

Sheep no.	Expt. no.	Medium K (m-equiv/l.)	Cell K (m-equiv/l.)	Slow fraction	
				(m-equiv/l. cells)	Movement (m-equiv/l. cells/hr)
130	1	5·0	7·4	6·3	0·80
130	2	4·7	7·7	6·5	0·90
296	8	7·5	20·0	19·4	0·23
113	4	6·4	20·6	20·0	0·30
152	2	5·9	27·7	26·9	0·36
3	8	8·0	43·7	42·7	0·24
68	8	8·0	79·5	77·1	0·39
145	1	9·6	70·6	69·3	0·35
145	2	7·8	80·7	79·6	0·31
137	2	5·4	85·0	84·6	0·51
137	4	6·3	80·3	80·1	0·60

and the blood showed appreciable haemolysis on arrival on each occasion, unlike that of any other animal. This sheep has already been described by Evans (1957): though grouped with other low-potassium sheep as Keα, its cells behave differently enough to suggest separate classification. The single sheep studied by Sheppard *et al.* (1951) appears to be similar in its very low potassium content, lack of homogeneity and liability to haemolysis. Apart from the blood from sheep 130, Table 3 shows slight positive correlation between the potassium content of the cells or of the slow fraction and the movement rate from it; the correlation (even omitting sheep 130) is not statistically significant $(r = +0·479, P \simeq 0·2)$ and the results give little support to any hypothesis suggesting that the efflux is proportional to the internal potassium concentration.

Observations on cells incubated for longer periods

In two experiments (5 and 8) incubation was continued through the night and samples were taken after 18 or 22 hr. In these samples 15–20 % of the cells had become haemolysed. The concentration of sodium and potassium in the remaining cells did not differ appreciably from the mean concentration between 2 and 4 hr incubation. The ^{24}Na content of the cells was slightly greater, and that of ^{42}K was slightly less, than the amount estimated by extrapolation from the early observations, but none of the observed values lay outside the limits of confidence $(P = 0·05)$ of the expected values.

DISCUSSION

These results confirm the observations of Sheppard *et al.* (1951) upon non-homogeneity in the sodium of sheep blood cells, and extend them to potassium as well. It is desirable to consider possible causes. The departures from a simple exponential uptake of tracer are much too large and consistent to be due to experimental errors, as are the observations of movement of tracer out of the cells into a medium of higher specific activity than the average for the cells. The fast fractions are not due to errors in the estimate of trapped volume: a fast fraction of 1 m-equiv potassium/l. cells would require that the cells trapped about a sixth of their volume of medium, which is improbably large, apart from the evidence of the inulin determinations. Also, if the fast fraction were due to trapped medium, the fast fraction of sodium would be related in size to the fast fraction of potassium in the same proportion as their concentrations in the medium; whereas the sodium fast fraction is of the same order of size or sometimes even smaller than that of potassium. It is most improbable that white cells are responsible for the fast fraction, because experiments were done under conditions in which few leucocytes are likely to have survived, and no buffy coat appeared in the cell columns when they were centrifuged. Also, the amount of cation, particularly sodium, which exchanged rapidly would have to be present in an improbably high concentration to be contained in white cells, even if their number approached that of fresh blood. The same arguments apply even more strongly to the platelets. It follows that the rapidly exchanging sodium and potassium is associated with the red cells, though no evidence has been obtained about how it is associated. It may not be uniformly distributed, as it is known that the ionic composition of the erythrocytes of a given individual is not uniform (Kruszyński, 1950; Keitel *et al.* 1955; Joyce, 1958).

On the other hand, the rapidity of the initial exchange suggests that the ions participating in it may be at the cell surface rather than intracellular. The variation in size of the fast fraction is also in better accord with the concept that it lies near the surface of all the cells than that it is located in a small number of special cells, which would have to change their sodium content twofold or more to account for the observed variations. Harris & Prankerd (1957) have pointed out that there is good agreement between numerous observations on sodium and potassium movements, particularly in human red cells, and those to be expected if superficial ions are more accessible than those situated deeply within cells in which diffusion is slow. Such a model leads to equations in which the uptake of tracer is linearly related to the square root of time, and the output after transferring the cells to a new medium has a similar but slightly more complex relation to the times of immersion in each medium (Harris & Prankerd, 1957). The present observations on uptake are

quite well described by such a function, but the observed output after transfer to an inactive medium failed to follow the theoretical course, being consistently too rapid at first and too slow later. The disagreement is large enough to make estimation of parameters describing the properties of the cells on this model rather unsatisfactory, but it is much less than the disparity with the expected behaviour of a population of uniform cells, and not much greater than the deviations observed from the two-fraction model described here.

If the rapidly exchangeable ions are at the cell surface and lie on the pathway between the medium and the slowly exchangeable ions, slight revision of the tabulated estimates of the size of the fractions and their movement rates is desirable, as the estimates are based on the assumption that both fractions communicate only with the medium. However, with differences in rate as large as those observed here, estimation of the sizes of the fractions and the slow movement rates are little affected by assuming the alternative spatial arrangement, and the possible error is not of much immediate importance.

A corollary of these observations is that a fast fraction with the properties described here is likely to be overlooked in any experiments performed on cells which are washed between removal from the experimental medium and estimation of their tracer content. Such a fraction seems not to be peculiar to sheep, for a rapidly exchanging component has been observed in human erythrocytes by Sheppard *et al.* (1951), Clarkson & Maizels (1955) and ourselves (Joyce *et al.* 1956). Estimations of the rates of sodium movement are of course subject to considerable error if unwarranted assumptions of homogeneity are made and the tracer content of the fast fraction is eliminated by washing.

The properties of the less readily exchangeable sodium and potassium are not easy to examine because the more rapid movements tend to obscure the situation. As changes in the amount of rapidly exchanging sodium are associated with changes in cell volume, it is unwise to make assumptions about the relationship of the cell water to either fraction. Likewise, it is inappropriate to relate the movement rates to unit area of cell membrane while the physical basis of each fraction, or of the gradation from easily to less easily exchangeable ions, is not established. The present results are consistent with dissolved ions separated from the medium by a membrane of low permeability or with adsorbed ions diffusing to and from special sites in the cells. In the first case the differences between high-sodium and high-potassium cells would be due to differences in the cell walls; in the second case, to differences in the cell receptors which adsorb the ions. Further study of these cells will perhaps distinguish between such possibilities.

SUMMARY

1. Inward and outward movement of ^{24}Na and ^{42}K have been followed in the blood cells of sheep with either high or low intracellular sodium concentration.

2. All measurements were made on cells as centrifuged from the incubation medium, with corrections based on inulin determinations for medium trapped in the packed cell column.

3. Changes of cell potassium concentration during incubation were small and unsystematic. Cell sodium frequently declined during the first hour.

4. The uptake of ^{24}Na and ^{42}K was initially very rapid and usually followed by a period in which the cells lost tracer. After 1–2 hr the cell tracer content rose again and continued to do so for at least 24 hr.

5. The results are interpreted as indicating fast and slow fractions of the cell sodium and potassium. The potassium fast fraction contains 0·5–2·4 m-equiv/l. cells. The sodium fraction contains 0–9 m-equiv/l. cells. These values are not correlated with the total sodium or potassium content of the cells or with each other.

6. Movements to and from the fast fraction were too rapid for estimation. Movements to and from the slow fraction were not significantly correlated with cell sodium or potassium concentrations, and were between 2·2 and 4·6 m-equiv/l. cells/hr for sodium and between 0·2 and 0·9 m-equiv/l. cells/hr for potassium.

7. Cell tracer content at 24 hr did not differ significantly from that to be expected if the contents of the slow fraction had continued to exchange at the rate calculated from the early observations.

8. The significance of these results is discussed.

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