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INTERACTIONS BETWEEN TEMPERATURE AND TONICITY ON CATION TRANSPORT IN DOG RED CELLS

By B. C. ELFORD
From the Division of Cryobiology, Clinical Research Centre, Watford Road, Harrow HAI 3 UJ

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SUMMARY

1. The temperature-dependence of the uptake of ^{24}Na and ^{42}K into dog red cells between 38 and 4° C has been investigated. The effects on the cation fluxes of partial dehydration of the cells in hyperosmolar sucrose (50–125 mM) have also been studied.

2. A Hamilton gas-tight syringe was used to pipette accurately reproducible volumes of packed cells which contained in addition to ^{24}Na or ^{42}K either [^{131}I]albumin or [^{51}Cr]EDTA as extracellular markers.

3. At 38° C Na flux (m-equiv/l. isosmolar cell volume.hr) increased from $2{\cdot}8 \pm 0{\cdot}1$ ($n = 8$) in cells of normal volume to 226 ± 8 ($n = 8$) when the cells were shrunken by $27{\cdot}4 \pm 0{\cdot}6$ % ($n = 8$) in media containing sucrose (100 mM). K influx remained relatively constant under these conditions.

4. The exchange of ^{24}Na in shrunken cells followed a single exponential time course but about 9 % of the intracellular Na apparently did not exchange with ^{24}Na in the bathing medium.

5. The steady-state influx of Na in cells of normal volume was maximal at about 22 °C. The temperature dependence of the Na fluxes in shrunken cells was described by an Arrhenius relationship with a change in slope at about 22° C.

6. The K influx in cells of normal volume decreased as the temperature was lowered from 38° C, to about 12° C, at which temperature the flux was at a well defined minimum. Above 12° C, cell shrinkage had hardly any effect on K influx, but below 12° C the influx in shrunken cells was significantly less than in cells of normal volume.

7. The selective increase in Na flux induced by cell shrinkage results from a Na:Na exchange process which cannot be explained in terms of Ussing's (1947) model of carrier-mediated exchange diffusion.

8. The lack of coupling between the effects of temperature and cell volume on the fluxes of Na and K indicates that localized structural changes of lipid-protein complexes specific for Na or K are responsible for

the cation transport characteristics of dog red cells, and that phase transitions in the lipids of the cell membrane are unlikely to account for the temperature dependence of the fluxes.

INTRODUCTION

Red blood cells of the dog and cat differ in many ways from those of man and other mammalian species. The intracellular electrolyte composition approaches that of plasma (Bernstein, 1954) and the membrane ATPase activity is neither enhanced by K in the presence of Na nor is it affected by ouabain (Chan, Calabrese & Theil, 1964). It is not surprising therefore that ouabain has little if any effect on Na or K transport in these cells (Sorensen, Kirschner & Barker, 1962; Sha'afi & Lieb, 1967; Miles & Lee, 1972). Nevertheless, in spite of the apparent lack of an ion-pumping mechanism similar to that in human red cells, erythrocytes of the dog and cat are able to maintain a constant cell volume both *in vivo* and *in vitro*, and do not undergo colloid osmotic haemolysis. However, the cation fluxes are markedly dependent on cell volume, and this dependence, which is reversible, is influenced by the metabolic state of the cells (Davson, 1942; Parker & Hoffman, 1965; Hoffman, 1966; Sha'afi & Hajjar, 1971; Romualdez, Sha'afi, Lange & Solomon, 1972; Elford & Solomon, 1973; Elford & Solomon, 1974*b*).

Dog red cells provide an ideal model system for studying factors that influence the passive transport of Na and K since the measurement of membrane permeability characteristics is not complicated, as it is in human cells, either by the presence of large concentration gradients of ions across the cell membranes, which can give rise to net fluxes of cations, or by the superimposed effects of ion pumps involved with active transport.

This paper is concerned with the influence of temperature on the fluxes of Na and K in dog red cells at normal and reduced volume. A brief report of the temperature dependence of the cation fluxes in cells under isotonic conditions has already been published (Elford & Solomon, 1974*a*).

METHODS

Previous investigations of the relationship between cation fluxes and cell volume in cat and dog erythrocytes have been based on indirect estimations of the relative volume of the cells in different media. These estimations were obtained from an empirical equation relating cell volume to the osmolality of the bathing medium and the amount of 'osmotically active' water in the cells (Sha'afi & Hajjar, 1971; Romualdez *et al.* 1972; Sha'afi & Pascoe, 1972, 1973). Because of the recognized shortcomings of this approach (Sha'afi & Pascoe, 1973), a method was devised for the direct comparison of relative cell volume under the various experimental conditions. This was achieved by means of a Hamilton gas-tight syringe with a Chaney adaptor for pipetting accurately reproducible volumes of packed cells which con-

tained in addition to ^{24}Na (or ^{42}K) either [^{131}]albumin or [^{51}Cr]EDTA as an extracellular marker. This method of measuring tracer uptake in red cells avoids the obvious problems associated with the more traditional techniques which involve the repeated washing of cells in cold non-radioactive solutions supposedly to remove extracellular tracer only (Tosteson & Hoffman, 1960; Garrahan & Glynn, 1967; Sha'afi & Pascoe, 1973).

Materials

Blood was withdrawn from the jugular veins of unanaesthetized Beagles into syringes wetted with heparin; usually 100–200 ml. blood was taken for a single experiment so that cation fluxes could be measured simultaneously under a variety of conditions using red cells from the same animal. Within a few minutes of collection the blood was cooled to 4° C and centrifuged at about 1900 g (MSE Minor). Some of the plasma was removed for subsequent estimation of Na and K content; the remaining plasma and buffy coat were removed, and the cells were then washed three times at 4° C by alternate centrifugation and resuspension in Ringer solution. Except when mentioned specifically in the text there was virtually no lysis of the cells during any of the incubation procedures.

Composition of solutions

In all experiments the cells were suspended in a medium of the following composition (mM): NaCl 142·2, KCl 5·0, Na_2HPO_4 5·2, NaH_2PO_4 0·8, $CaCl_2$ 1·0, $MgCl_2$ 0·25, glucose 5·0 and 1% (w/v) bovine serum albumin (Sigma or Armour fraction V). The osmolality of this solution and its Na content were always close to the respective values of plasma, but the K concentration in plasma (3·8–4·7 mM) was generally slightly lower than that of the bathing medium. The pH of the bathing medium was reduced from 7·4 to 6·8 by the albumin but the buffering capacity of haemoglobin in the red cell suspensions (10–14% haematocrit) was sufficient to raise their pH to about 7·0. Hyperosmolar media contained sucrose (50, 75, 100 or 125 mM) in addition to the usual constituents of the bathing solution. It was very difficult to pipette packed cells after they had been shrunken in media containing 125 mM sucrose.

Isotopes

All radioactive tracers were obtained from the Radiochemical Centre, Amersham. The solution of [^{51}Cr]EDTA (0·1 mc/ml.) was used as supplied. ^{131}I-labelled human albumin solution (1 mc/ml.) was passed through an anion exchange resin (Deacidite FF-1P, Permutit) just before each experiment and was made up as a stock solution (0·1 mc/ml.) in isotonic buffer. ^{24}NaCl was supplied in sterile isotonic solution at an activity of about 0·6 mc/ml.; this was diluted with isotonic bathing medium to give a stock solution with an activity of about 0·3 mc/ml. ^{42}K stock solutions (about 0·3 mc/ml.) were prepared from unprocessed irradiated salts as the specific activity of the sterile isotonic solution supplied by the Radiochemical Centre was inconveniently low.

Uptake and exchange of tracers

Samples (5 ml.) of the washed and packed cells were washed again 2 to 4 times in the appropriate isosmolar or hyperosmolar solution and finally resuspended at a concentration of 5 ml. packed cells (at their initial volume) to 25 ml. bathing medium in stoppered Erlenmeyer flasks which had previously been coated with a water repellent (Repelcote, Hopkin and Williams Ltd). 0·2–0·5 ml. volumes of the stock [^{131}I]albumin (or [^{51}Cr]EDTA) solutions were added to each suspension using a Hamilton syringe. Cell suspensions were usually agitated in a water-bath at 38° C for 30 min, then transferred to shaking water-baths at the temperature of the

uptake of ^{24}Na (or ^{42}K) and shaken for a further 30–60 min at 38° C or for 1–2 hr at lower temperatures. At the end of these pre-incubation periods, small volumes (usually 0·2 ml.) of the stock ^{42}K or ^{24}Na solutions were added to each suspension at 15 sec intervals using a Hamilton syringe. Hereafter, the treatment of the cell suspension was identical to that described previously (Elford & Solomon, 1974*a*).

In some experiments the time resolution of the influx was improved by taking 1·5 ml samples from each suspension and spinning down the cells within about 15 sec. of collection in small plastic vials at 8,000 *g* in an Eppendorf 3200 centrifuge.

In a few experiments in which [^{51}Cr]EDTA was used as the extracellular marker, samples of the lysates and supernatants were left for a week after counting for ^{24}Na, and then the activity of ^{51}Cr in the samples was measured after ^{24}Na had decayed to negligible levels.

Analytical procedures

The concentration of haemoglobin in each lysate was estimated in duplicate using a Gilford spectrophotometer (Model 300-N) to measure absorbance at 540 nm after haemoglobin had been converted to cyanmethaemoglobin by adding 0·2 ml. of lysate to 10 ml. Drabkin's reagent made up from pellets (Acculute, Orthodiagnostics). Absorbance was converted to concentration by means of a haemoglobin standard (Acuglobin, Orthodiagnostics).

A flame photometer (Instrumentation Laboratories, Model 243) was used for duplicate estimations of the concentrations of Na and K in each lysate, in one or two samples of the supernatants and in a sample of plasma. Na and K determinations were made from single dilutions of the lysates using an internal lithium standard; this inevitably resulted in somewhat inaccurate estimations of the K content of the cells as the signal-to-noise ratio was low in the K channel.

Estimation of the fluxes

The uptake of ^{24}Na and ^{42}K into cells of normal volume, and of ^{42}K into shrunken cells as well, usually followed a well-defined linear time course except for some initial deviation from linearity (Elford & Solomon, 1974*a*). It was possible to neglect back diffusion of the isotopes, and the steady-state influx of Na or K in m-equiv/l. cells.hr was calculated from the ratio of the slope of the regression line fitted to the uptake of the tracer and the extracellular specific activity of the cation (Method 1). When the cells were shrunken in hyperosmolar media, the flux of Na (but not of K) increased greatly, and in these conditions back diffusion of ^{24}Na had to be allowed for in the estimation of the Na flux from the curves representing the exchange of ^{24}Na between the bathing medium and the cells. When the cells were shrunken with sucrose (but not with additional NaCl; unpublished results) the exchange of ^{24}Na was well described by a single exponential function at temperatures between 38 and 4° C. The flux of Na was calculated from the time constant for the exchange according to the standard equations describing tracer movements in a two-compartment system (Sheppard, 1962). The flux, measured in m-equiv/l. measured cell volume.hr, was calculated from the following equation (Method 2):

$$\text{Flux} = \frac{KS_1S_2\,(100\text{-}h)}{S_1h+S_2\,(100\text{-}h)},$$

where K = the rate constant (hr^{-1}) for the exchange of ^{24}Na estimated by the least squares fit to the uptake data.

S_1 = intracellular Na content (m-equiv/l. measured cell volume).

S_2 = extracellular Na concentration (m-equiv/l.) and h = haemocrit (%).

(In a previous paper (Elford & Solomon, 1973), a form of Sheppard's equation

valid only at 50 % haematocrit was used to calculate the Na flux in cells shrunken in 100 mM sucrose. The fluxes in fresh and partially substrate-depleted cells should have been 273 ± 23 (6) and 139 ± 16 (10) respectively instead of 171 ± 13 and 89 ± 10 m-equiv/l. initial cell volume.hr.)

It should be noted that the experimental conditions do not exactly comply with the assumptions and requirements of the model. In shrunken cells, as the exchange of ^{24}Na went to completion, there was a continuous depletion of ^{24}Na from the bathing medium so that at 'equilibrium' the extracellular specific activity was about 7 % less than at zero time (the suspensions of shrunken cells had haematocrits of 10–12%). Furthermore, there was a continuous net loss of Na from cells shrunken in media containing sucrose at concentrations greater than about 50 mM. At 38° C, the initial loss of Na amounted to about 5 m-equiv/l. cells.hr but during the uptake of ^{24}Na, which started after a pre-incubation period of 60–90 min, the loss fell to about 2 m-equiv/l. cells.hr; this in turn resulted in a small but continuous decrease in cell volume during the uptake of tracers and so the value of h was not constant (see Results). However, errors introduced by the changes in haematocrit during an experiment were well within the limits of the other experimental errors as a relatively large decrease in h (12 to 10 %) produced a small increase (2 %) in the calculated value of the flux. Values of the Na flux were computed from the above equation using a value of the intracellular Na concentration averaged over the time course of the uptake of ^{24}Na. The flux was finally expressed in terms of the original volume of the cells estimated from the average haemoglobin content of the cells in the different media. It should be pointed out that when the half-time for the exchange of ^{24}Na was increased by lowering the temperature to below 10° C, it was possible to use both methods for calculating the Na fluxes since the uptake of the tracer was linear in both normal and shrunken cells. However, in this situation the value of the flux estimated by Method 1 was about 30% less than that given by Method 2; Na fluxes in shrunken cells were always calculated by the latter method.

It was thus possible, after allowing for the extracellular tracer in each sample of packed cells, to estimate (1) ^{24}Na or ^{42}K uptake directly in terms of activity per unit cell volume (2) relative cell volume from the haemoglobin content of the cells and (3) intracellular cation contents from which net fluxes could be estimated.

RESULTS

^{24}Na influx into cells of normal volume at 38° C

Fig. 1 illustrates the linear uptake of ^{24}Na into cells during incubation for 4 hr under isotonic conditions at 38° C. [^{131}I]albumin and [^{51}Cr]EDTA were the extracellular markers in two suspensions of red cells from one animal. A summary of intracellular Na and haemoglobin concentrations and the Na influxes in dog red cells under control conditions is given in Table 1. Measurements of the haemoglobin content of the cells throughout the influx period indicated that the volume of the cells remained constant as were the intracellular concentrations of Na and K. The pH of the cell suspensions usually did not change during 3–6 hr incubation at 38° C and haemolysis was usually negligible except after prolonged incubation in 125 mM sucrose at 38° C.

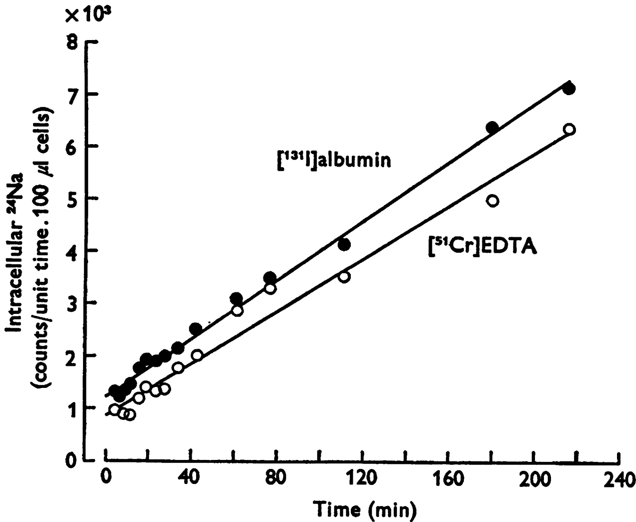

Fig. 1. Time course of the uptake of ^{24}Na (counts/unit time. 100 μl. measured cell volume) into dog red cells suspended at 38° C in isotonic phosphate-buffered media containing [^{131}I]albumin (●) or [^{51}Cr]EDTA (○) as extracellular markers. Unlabelled EDTA (0·1 mM) was present in the bathing medium containing [^{51}Cr]EDTA, and bovine serum albumin (1 % w/v) was present in both bathing media. Each point has a counting error of about ± 100 counts/unit time. 100 μl. cells. Cells were spun down within 15–20 sec of sampling in an Eppendorf 3200 centrifuge. Linear regression analysis of the influxes gave a best fit of $y = (27{\cdot}6 \pm 0{\cdot}5)x + (1236 \pm 46)$ for ●—● and $y = (24{\cdot}7 \pm 1{\cdot}0)x + (876 \pm 83)$ for ○—○, the errors are standard errors of the slope and intercept of each regression line. The extracellular specific activity of sodium was $5{\cdot}43 \times 10^6$ counts/unit time.m-equiv Na and was identical within experimental error in the two suspensions. The Na influxes calculated from the slopes of the two regression lines were 3·06 ± 0·07 (●) and 2·72 ± 0·11 (○) m-equiv/l. cells.hr and were not significantly different ($0{\cdot}05 < P < 0{\cdot}1$). The mean haemoglobin contents of the cells were 335·1 ± 2·0 (15) (●) and 330·6 ± 2·4 (15) (○) g/l. cells, this indicated that cells in the former group were slightly (1·4 %) but not significantly more shrunken than the latter ($0{\cdot}1 < P < 0{\cdot}2$). The cation contents (m-equiv/l. cells) in the two groups (● and ○ respectively) were 99·0 ± 0·3 (15) and 97·2 ± 0·6 (15) for Na, and 3·24 ± 0·12 (15) and 3·18 ± 0·11 (15) for K (± 1 S.E. with the number of determinations in brackets). After allowing for the slight difference in cell volume, the Na content of the cells in the two groups was identical within experimental error. The difference in the intercepts on the y-axis was significantly different ($P < 0{\cdot}001$) and represented penetration of ^{24}Na into spaces beyond that accessible to [^{131}I]albumin and [^{51}Cr]EDTA equivalent to 1·48 ± 0·06 μl. (●) and 1·05 ± 0·10 μl. (○) per 100 μl. measured cell volume; this difference could have arisen from steric exclusion effects at the surface of the cells or could merely indicate that 1 % of the cells were very leaky to Na. Unit time in this and subsequent Figures refers to the actual counting time for each sample, this varied between experiments from 100 to 300 sec.

TABLE 1. Na fluxes in dog red cells at 38° C ± 100 mM sucrose in the bathing medium

Hb_i (g/l. cells*)		Na_i (m-equiv/l. cells*)		Na flux† (m-equiv/l. isosmolar cell volume.hr)		Volume reduction (%)
Isosmolar	Hyperosmolar	Isosmolar	Hyperosmolar	Isosmolar	Hyperosmolar	
335·1 ± 2·0 (15)‡	459·7 ± 3·0 (11)	99·0 ± 0·3 (15)	109·6 ± 1·4 (11)	3·06 ± 0·07	253 ± 11	27·1 ± 0·6
323·5 ± 4·5 (5)	453·9 ± 10·6 (5)	105·8 ± 0·8 (5)	107·6 ± 1·9 (5)	2·85 ± 0·28	184 ± 7	28·7 ± 1·9
312·2 ± 3·8 (5)	429·0 ± 7·9 (6)	96·6 ± 0·7 (5)	107·2 ± 2·2 (6)	3·96 ± 0·10	—	27·2 ± 1·6
304·5 ± 1·4 (6)	427·9 ± 3·0 (6)	95·9 ± 0·4 (6)	102·7 ± 1·4 (6)	3·23 ± 0·04	212 ± 5	28·8 ± 0·6 } §
301·4 ± 2·2 (4)	416·9 ± 3·7 (5)	95·0 ± 0·2 (4)	102·7 ± 1·5 (5)	3·10 ± 0·28	222 ± 6	27·7 ± 0·8 } §
327·1 ± 3·6 (6)	442·8 ± 2·5 (6)	89·0 ± 0·6 (6)	94·7 ± 1·7 (6)	2·74 ± 0·10	224 ± 16	26·1 ± 0·8 } ‖
320·0 ± 1·7 (6)	442·6 ± 6·8 (6)	87·4 ± 0·6 (6)	95·2 ± 1·1 (6)	2·40 ± 0·27	236 ± 8	27·7 ± 1·2 } ‖

* Measured cell volume.

† Corrections associated with a small amount of apparently inexchangeable Na in shrunken cells have not been made.

‡ ± 1 S.E. with the number of determinations during the influx of ^{24}Na in parentheses.

§ Data from two successive aliquots of packed cells from samples taken at various times during the uptake of ^{24}Na in a single cell suspension.

‖ Data from duplicate suspensions of red cells from one animal.

^{24}Na and ^{42}K uptake at 38° C in cells shrunken in hyperosmolar solutions containing sucrose

^{24}Na uptake. The volume-dependence of the rate of exchange of ^{24}Na at 38° C in cells shrunken to varying degrees in hyperosmolar sucrose solutions is shown in Fig. 2. ^{24}Na uptake was usually measured under control isotonic conditions and in 100 mM sucrose; however, in two experiments, which are summarized in Table 2, the fluxes were also measured at

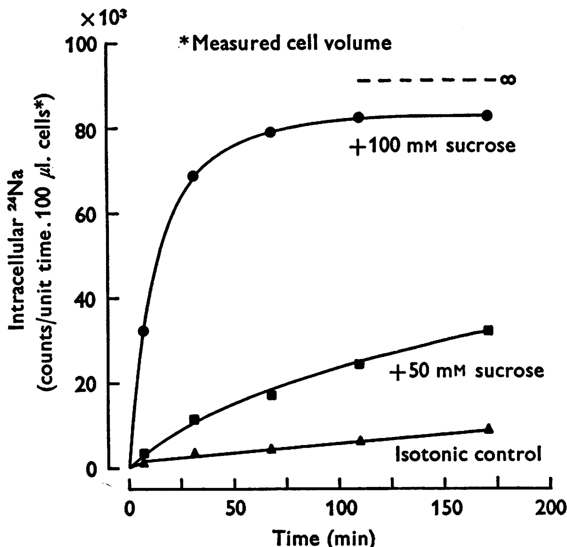

Fig. 2. Uptake of ^{24}Na in dog red cells at 38° C under isotonic conditions (▲) and in the presence of 50 mM (■) and 100 mM (●) sucrose. The specific activity of ^{24}Na was the same in each cell suspension at time zero. The slight initial curvature in the uptake of ^{24}Na in cells of normal volume was disregarded and the Na flux was estimated from the slope of the linear part of the curve. The mean intracellular concentrations (m-equiv/l. measured cell volume) of Na and K respectively were $97{\cdot}0 \pm 0{\cdot}8$ (5) and $2{\cdot}7 \pm 0{\cdot}1$ (5) ▲, $108{\cdot}0 \pm 0{\cdot}4$ (5) and $3{\cdot}05 \pm 0{\cdot}06$ (5) ■, $103{\cdot}3 \pm 1{\cdot}5$ (5) and $3{\cdot}8 \pm 0{\cdot}1$ (5) ●. Further data from this experiment are given in Table 2, experiment *A*. Cell volume was constant both in control isotonic conditions and in media containing 50 mM sucrose, but continued to decrease during the uptake of ^{24}Na in 100 mM sucrose from 73 to 69 % of the initial volume of the cells. The degree of cell shrinkage calculated from the changes in the K content agreed with that calculated from the changes in the Hb content of the cells. The theoretical intracellular activity of ^{24}Na, estimated on the basis of equal intra- and extracellular specific activity in cells at true equilibrium in 100 mM sucrose, is shown by the dashed line (–– ∞); the difference between this line and the asymptotic value of ●—● represents a difference between the intra- and extracellular specific activities of 8·3 and $9{\cdot}2 \times 10^6$ counts/unit time.m-equiv Na.

intermediate concentrations of sucrose (50 and 75 mM). In eight experiments, cells shrank on average by $27{\cdot}4 \pm 0{\cdot}6$ % in 100 mM sucrose and the Na flux increased by a factor of 83 ± 5 from $2{\cdot}78 \pm 0{\cdot}12$ to 226 ± 8 m-equiv/l. cells.hr. Individual estimates of haemoglobin and cation contents and Na fluxes in shrunken cells at 38° C are listed in Table 1.

TABLE 2. Increase in Na flux with reduction in cell volume at 38° C

Experimental conditions	Na flux (m-equiv/l. cells*.hr)	Average Hb_i (g/l. cells†)	Relative cell volume	
Isotonic control	$2{\cdot}36 \pm 0{\cdot}04$	$312{\cdot}1 \pm 0{\cdot}9$ (5)	1·00	*A*
+ 50 mM sucrose	$13{\cdot}5 \pm 0{\cdot}9$	$358{\cdot}9 \pm 0{\cdot}6$ (5)	$0{\cdot}870 \pm 0{\cdot}003$	*A*
+ 100 mM sucrose	234 ± 13	$435{\cdot}7 \pm 4{\cdot}5$ (5)	$0{\cdot}716 \pm 0{\cdot}008$	*A*
Isotonic control	$3{\cdot}11 \pm 0{\cdot}08$	$297{\cdot}1 \pm 1{\cdot}5$ (6)	1·00	*B*
+ 75 mM sucrose	132 ± 6	$384{\cdot}7 \pm 2{\cdot}0$ (5)	$0{\cdot}772 \pm 0{\cdot}006$	*B*
+ 100 mM sucrose	211 ± 10	$421{\cdot}7 \pm 4{\cdot}0$ (6)	$0{\cdot}704 \pm 0{\cdot}008$	*B*

* Fluxes are referred to 1 l. cells under control isotonic conditions.
† Measured cell volume in isosmolar or hyperosmolar media.

The uptake of ^{24}Na was measured simultaneously in each cell suspension within the two groups *A* and *B*. Cells were pre-incubated for 95 min at 38° C in a shaking water-bath before the addition of ^{24}Na. The values of the fluxes in Group *A* were estimated from the uptake curves in Fig. 2. The S.E.s of the fluxes arise mainly from the S.E.s of the slopes of the regression lines. Other errors are S.E. of the mean of *n* determinations. The order of magnitude difference between the fluxes in cells exposed to 50 and 75 mM sucrose would seem to indicate a co-operative increase in the permeability to Na over a relatively small decrease in cell volume.

^{42}K uptake. The relationship between cell volume and K flux was investigated using blood from three dogs. The experimental procedure was similar to that in the ^{24}Na uptake experiments except that owing to the relatively low influx of K, which was about 0·1 m-equiv/l. cells.hr, the uptake period was extended to about 6 hr at 38° C in order to reduce the error in the estimation of the flux from 3 to 5 points on an influx plot to less than about ± 10 %. In each experiment, simultaneous measurements were made of ^{42}K influx in cells suspended in isotonic medium and in media containing 50, 75, 100 and 125 mM sucrose.

In contrast to the volume dependence of the rate of ^{24}Na exchange in shrunken cells, the rate of ^{42}K uptake was not greatly affected even when the cells were shrunken by as much as 30 % (Fig. 3). Details of the results are listed in Table 3 from which it can be seen that changes in cell volume did not affect K influx in a consistent manner, and in experiment (2) the flux was not significantly altered over the entire range of cell volume (0 to −31 %).

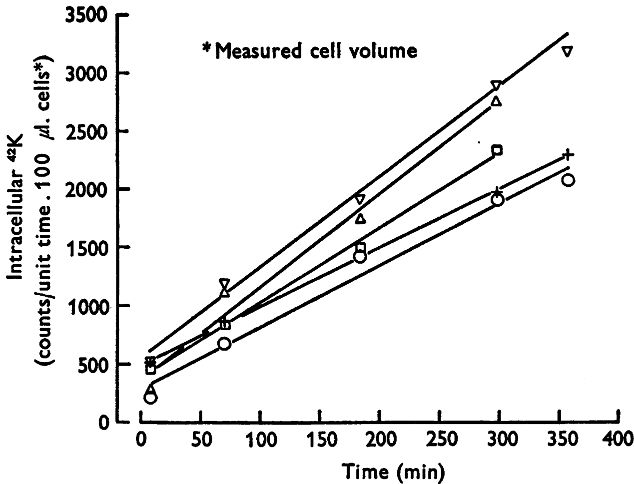

Fig. 3. Influx of ^{42}K into dog red cells at 38° C under isotonic conditions (+) and in the presence of 50 (○), 75 (△), 100 (□) and 125 (▽) mM sucrose. The relative volume of the cells and the values of the K fluxes are given in Table 3, experiment 2. The counting error for each point was about 60 counts/unit time. 100 μl. red cells. The lines drawn through the data points are the least-squares regression lines. Values of the extracellular specific activity of K in the different suspensions were $46{\cdot}3 \pm 1{\cdot}3$ (+), $50{\cdot}8 \pm 1{\cdot}4$ (○), $46{\cdot}1 \pm 0{\cdot}7$ (△), $48{\cdot}1 \pm 0{\cdot}3$ (□) and $47{\cdot}3 \pm 1{\cdot}0$ (▽) $\times 10^6$ counts/unit time. m-equiv K. Values of the fluxes calculated in Table 3 were reduced by about 3% if the influx plots were normalized with respect to unit haemoglobin concentration within the cells (instead of 100 μl. measured cell volume) in order to compensate for the continuous slight shrinkage (*ca.* 0·8% hr^{-1}) of the cells throughout the uptake of tracer in the presence of 75, 100 and 125 mM sucrose.

Effects of temperature on cation fluxes: temperature-dependence of Na fluxes in isosmolar and hyperosmolar media

The uptake of ^{24}Na into dog red cells under control isosmolar conditions and in hyperosmolar sucrose (100 mM) solutions at different temperatures was studied using red cells from a different animal in each of three experiments. There were marked differences in the temperature-dependence of the Na fluxes in normal and in shrunken cells as shown in Figs. 4 and 5*a* respectively. In cells of normal volume it was consistently found that the rate of uptake of ^{24}Na was increased on cooling from 38 to 22° C, at which temperature the flux reached a well defined maximum value $3{\cdot}1 \pm 0{\cdot}8$ ($n = 3$) times greater than at 38° C (Fig. 4). By contrast, the rate of exchange of ^{24}Na in cells shrunken in media containing 100 mM sucrose fell as the temperature was lowered from 38 to 4° C as illustrated in Fig. 5*a* and *b*. At each temperature in this range the exchange of ^{24}Na was well

described by a single exponential function (Fig. 5*b*) with a half-time that increased as the temperature was reduced as shown in Table 4. The disparity between the effects of temperature on Na fluxes in normal and shrunken cells are summarized in the Arrhenius plots in Fig. 6, from which it can be seen that the characteristic maximum in the influx in cells at normal volume disappeared when the cells were shrunken, but there was some curvature in the Arrhenius plot which was due in part to differences in the

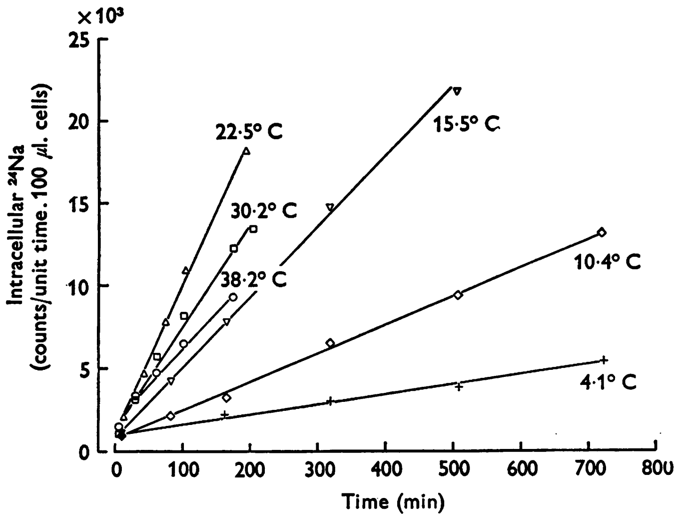

Fig. 4. Variation with temperature of the rate of ^{24}Na influx into dog red cells suspended in isotonic media at 38·2° C (○), 30·2° C (□), 22·5° C (△), 15·5° C (▽), 10·4° C (◇) and 4·1° C (+). Cells were from a single donation of blood from one animal. Temperature was controlled to about ±0·1° C. Extracellular specific activity was 10^7 counts/unit time.m-equiv Na, and did not vary by more than 2 % in the different cell suspensions. Values of the intracellular concentrations of Na and Hb, and the Na fluxes estimated from the slopes of the regression lines fitted to the influxes, are given in Table 4, experiment 2.

volume of the cells suspended in identical media (100 mM sucrose) at the various temperatures (Table 4). These differences resulted from the variation with temperature of the net loss of Na from shrunken cells (Fig. 7). For example, when four groups of cells from different animals were exposed for up to 6 hr at 38° C in media containing 100 mM sucrose (410–420 m-osmole/kg water), the average volume of the cells was reduced by 26·4 ± 1·1 % (n = 4), yet when the cells were exposed for up to 15 hr in identical media at 4° C, cell volume was reduced by only 20·5 ± 0·5 % (n = 4) and remained virtually constant throughout this time. Apart from minor

TABLE 3. Variation of K influx with reduction in cell volume in hyperosmolar sucrose solutions at 38° C

Experimental conditions	K influx (m-equiv/l. cells*.hr)	Average Hb (g/l.† cells	Relative cell volume	Flux ratio hyperosmolar: isosmolar	Expt. no.
Isotonic control	0·128 ± 0·016	302·6 ± 2·8 (5)	1·00	1·00	
+ 50 mM sucrose	0·071 ± 0·003	343·2 ± 1·0 (5)	0·882 ± 0·009	0·56 ± 0·07	1
+ 75 mM sucrose	0·092 ± 0·010	382·4 ± 2·4 (5)	0·791 ± 0·009	0·72 ± 0·12	
+ 100 mM sucrose	0·093 ± 0·003	423·4 ± 2·8 (5)	0·715 ± 0·008	0·73 ± 0·09	
+ 125 mM sucrose	0·082 ± 0·011	436·6 ± 5·2 (4)	0·693 ± 0·015	0·64 ± 0·12	
Isotonic control	0·065 ± 0·002	335·5 ± 2·0 (4)	1·00	1·00	
+ 50 mM sucrose	0·060 ± 0·005	381·2 ± 4·2 (5)	0·880 ± 0·011	0·93 ± 0·08	
+ 75 mM sucrose	0·068 ± 0·003	404·4 ± 6·0 (4)	0·830 ± 0·013	1·06 ± 0·06	2
+ 100 mM sucrose	0·072 ± 0·007	468·6 ± 5·0 (5)	0·716 ± 0·009	1·11 ± 0·12	
+ 125 mM sucrose	0·069 ± 0·005	484·4 ± 13·9 (5)	0·693 ± 0·020	1·07 ± 0·08	
Isotonic control	0·068 ± 0·005	319·0 ± 1·4 (4)	1·00	1·00	
+ 50 mM sucrose	0·083 ± 0·011	363·8 ± 0·9 (4)	0·877 ± 0·004	1·22 ± 0·18	3
+ 75 mM sucrose	0·091 ± 0·004	390·7 ± 5·7 (4)	0·817 ± 0·012	1·33 ± 0·11	
+ 100 mM sucrose	0·089 ± 0·008	425·8 ± 6·8 (3)	0·749 ± 0·012	1·31 ± 0·15	
+ 125 mM sucrose	0·096 ± 0·001	456·0 ± 7·5 (4)	0·700 ± 0·012	1·41 ± 0·10	

Cell suspensions were incubated at 38° C for 100 min (Group 1), 75 min (Group 2) and 65 min (Group 3) prior to the addition of ^{42}K. The measured extracellular concentrations of potassium were 8·9 mM (1), 4·5 mM (2) and 4·8 mM (3). * † See Table 2.

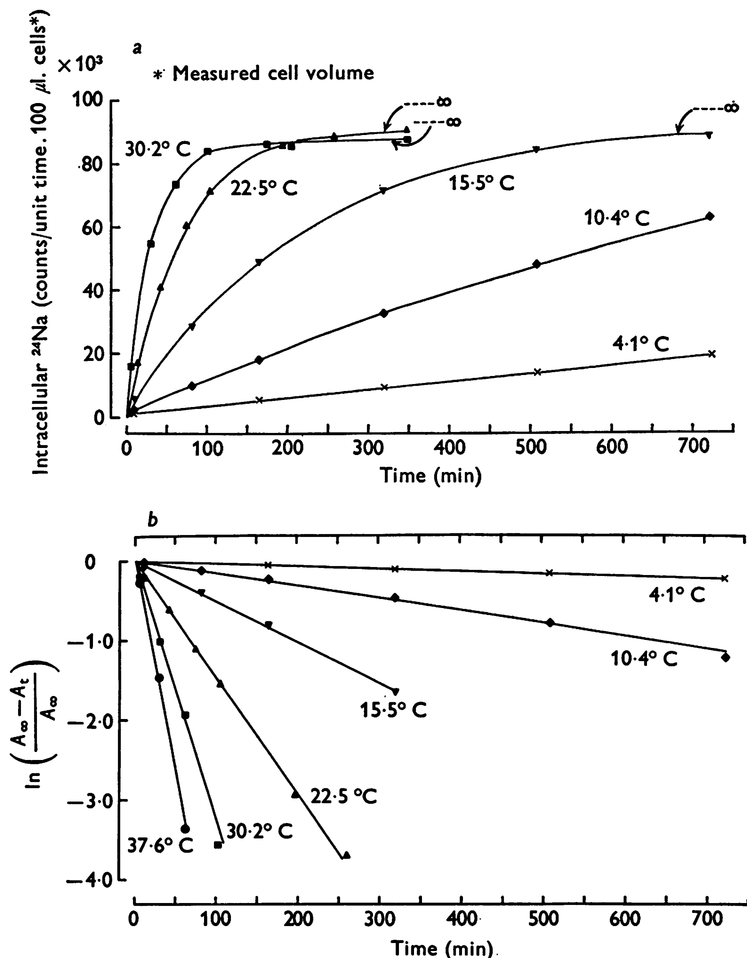

Fig. 5 *a*. Temperature-dependence of ^{24}Na exchange in dog red cells suspended in hyperosmolar solutions containing sucrose (100 mM). The experiments were carried out at the same time and with cells from the same animal as in Fig. 4. At 30·2, 22·5 and 15·5° C, the theoretical levels of ^{24}Na in the cells at equilibrium, based on a uniform distribution of tracer throughout the cells, are marked by dashed lines (−−∞).

b. Logarithmic representation of the curves in Fig. 5*a*. The straight lines show that at each temperature the exchange curve was adequately fitted by a single exponential function. The ordinate is ln (fraction of ^{24}Na not yet exchanged). At 10·4 and 4·1° C the activity of ^{24}Na (A_{∞}) in the cells at equilibrium was estimated from the Na content of the cells on the assumption that the amount of inexchangeable Na was independent of temperature, which it was at temperatures of 15·5° C and above. Owing to a technical failure with one of the cell suspensions, data from a separate experiment at 37·6° C (●—●) has been included. The half-times of the exchange, the Na fluxes and the relative volume of the cells are given in Table 4, experiment 2.

changes that could have been within the limits of experimental error, as they were not consistent between the separate groups, the cells in isosmolar media remained in the steady state throughout the uptake of ^{24}Na at the

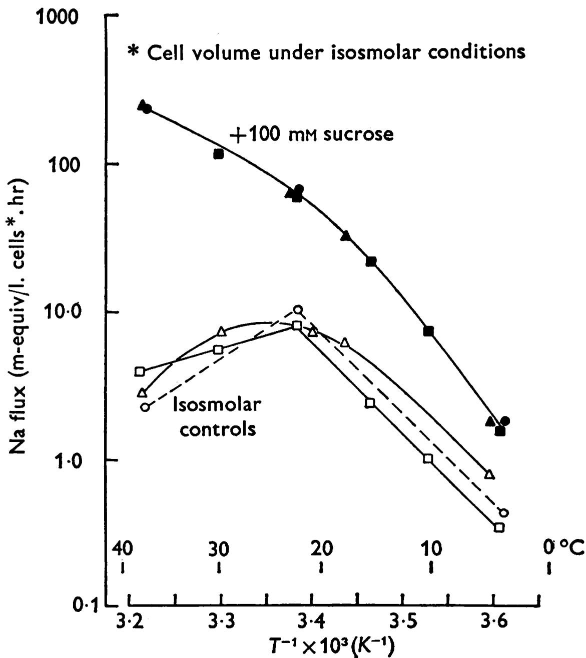

Fig. 6. Arrhenius plots of Na fluxes in dog red cells under isosmolar conditions (open symbols) and in 100 mM sucrose (filled symbols). At temperatures above about 22° C the apparent activation energy for Na influx in cells of normal volume was negative. The value given by the slopes of the least-squares fit to the ascending limb of (□—□) was $-8{\cdot}7 \pm 0{\cdot}3$ kcal/mole. The apparent activation energy from the descending limb of the same plot was $28{\cdot}2 \pm 0{\cdot}6$ kcal/mole. In shrunken cells, the regression line fitted to the six data points above 20° C yielded an apparent activation energy of $14{\cdot}8 \pm 0{\cdot}8$ kcal/mole, below 20° C this increased to $35{\cdot}0 \pm 1{\cdot}5$ kcal/mole; this increase was due in part to the lesser degree of cell shrinkage at lower temperatures (see text). In one series of experiments (△ and ▲) the cells were not pre-incubated at 38° C after the standard washing procedures at 4° C, but were left on ice for a few hours and then gently shaken for 1–2 hr at the temperature of the influx of ^{24}Na before the tracer was added.

different temperatures. The slight, but in some cases significant, variations with temperature in the volume of the cells and their cation contents under control isosmolar conditions are given in Table 4, in which the values of the Na fluxes are also shown.

Temperature-dependence of K influxes in normal and shrunken cells

Influxes of ^{42}K into dog red cells at different temperatures under control isotonic conditions and in media containing sucrose (100 mM) are shown in Figs. 8 and 9. The Arrhenius plot of the K fluxes in cells of normal volume was characterized by a distinct minimum between 11 and 13° C

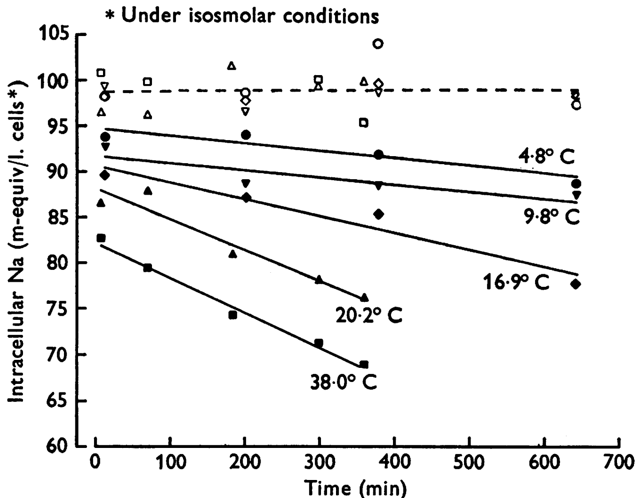

Fig. 7. Sodium content of dog red cells during suspension at various temperatures in isosmolar and hyperosmolar (+100 mM sucrose) solutions (open and filled symbols respectively). All groups were incubated at 38° C for 30 min after the washing procedures in the appropriate iso- or hyperosmolar media at 4° C. Paired cell suspensions were then gently shaken for further periods of 45 min at 38·0° C, 75 min at 20·2° C and 98 min at 16·9, 9·8 and 4·8° C before the addition of tracer. The Na content of the shrunken cells has been referred to cell volume under isosmolar conditions in order to compensate for the slight but continuous changes in cell volume. In isotonic media, cell sodium was essentially independent of temperature and did not change with time (dashed line). The average rates of loss of Na from shrunken cells were given by the regression lines as follows (in m-equiv/l. initial cell volume.hr): 2·3 ± 0·2 (■ 38·0° C), 2·0 ± 0·3 (▲ 20·2° C), 1·1 ± 0·2 (◆ 16·9° C), 0·5 ± 0·2 (▼ 9·8° C) and 0·5 ± 0·1 (● 4·8° C).

(Fig. 10). In two groups of experiments with blood from different animals, the fluxes at temperatures between 38 and 15° C were hardly affected by shrinking the cells, and the apparent activation energy for the K influx in normal and shrunken cells was not significantly different; however, at temperatures below about 12° C, K influx was significantly reduced when the cells were shrunken. From the data available at present it is not possible to offer an explanation for the finding that in only one of the two

TABLE 4. Na- and Hb-contents and Na fluxes in dog red cells suspended in isosmolar and hyperosmolar (100 mM sucrose) solutions at different temperatures.

	Isotonic controls			+ 100 mM sucrose				
Temp. (°C)	Na_i m-equiv/l.† cells)	Hb_i (g/l.† cells)	Na influx‡ (m-equiv/l. cells†. hr)	Na_i (m-equiv/l.† cells)	Hb_i (g/l. cells)	$t_{\frac{1}{2}}$ ^{24}Na exchange (min)	Na flux§ (m-equiv/l. cells*. hr)	Expt. no.
37·6	99·2 ± 1·4 (6)	333·8 ± 2·5 (6)	2·28 ± 0·35	103·5 ± 1·9 (6)	439·3 ± 7·3 (6)	12·7 ± 0·7	236 ± 16	
22·3	99·9 ± 1·1 (7)	330·1 ± 5·0 (7)	10·48 ± 0·30	107·9 ± 1·8 (7)	434·5 ± 3·1 (7)	44·7 ± 2·9	69·5 ± 4·9	1
3·7	97·2 ± 0·6 (5)	329·0 ± 3·7 (5)	0·44 ± 0·02	116·1 ± 0·7 (5)	410·2 ± 3·5 (5)	1902 ± 78	1·84 ± 0·09	
38·2	96·6 ± 0·7 (5)	308·8 ± 2·1 (4)	3·96 ± 0·10	107·2 ± 2·2 (6)	429·0 ± 7·9 (6)	—	—	
30·2	94·8 ± 0·5 (6)	304·5 ± 1·2 (6)	5·60 ± 0·31	101·9 ± 2·2 (6)	441·8 ± 5·2 (7)	22·5 ± 0·7	118 ± 5	
22·5	97·6 ± 0·7 (6)	300·9 ± 3·0 (6)	8·36 ± 0·34	107·7 ± 1·8 (6)	429·7 ± 4·4 (7)	46·8 ± 0·5	60·7 ± 1·5	
15·5	90·7 ± 0·2 (5)	301·8 ± 3·7 (6)	2·42 ± 0·08	105·0 ± 1·8 (5)	402·2 ± 5·7 (6)	135·3 ± 2·9	22·0 ± 0·8	2
10·4	90·4 ± 0·8 (6)	304·1 ± 4·8 (6)	1·03 ± 0·03	109·5 ± 1·5 (6)	393·6 ± 4·6 (6)	418 ± 15	7·6 ± 0·3	
4·1	95·0 ± 0·7 (5)	298·8 ± 2·3 (5)	0·35 ± 0·02	111·2 ± 1·0 (5)	382·8 ± 0·8 (5)	2063 ± 50	1·58 ± 0·04	
38·0	103·3 ± 1·1 (5)	319·1 ± 1·8 (5)	2·87 ± 0·25	111·3 ± 2·6 (5)	430·8 ± 3·0 (5)	12·8 ± 0·4	242 ± 10	
29·9	104·1 ± 0·6 (6)	317·4 ± 3·7 (6)	7·48 ± 0·35	—	432·3 ± 3·5 (6)	—	—	
21·0	106·4 ± 1·0 (5)	313·4 ± 4·0 (5)	7·51 ± 0·21	123·2 ± 2·2 (5)	402·9 ± 2·0 (5)	55·3 ± 1·3	64·5 ± 2·1	3
17·9	104·1 ± 1·1 (6)	311·5 ± 2·8 (6)	6·38 ± 0·15	119·8 ± 3·1 (6)	427·7 ± 4·8 (6)	96·5 ± 1·0	33·7 ± 1·1	
5·0	104·5 ± 0·9 (5)	321·8 ± 2·2 (5)	0·80 ± 0·17	128·4 ± 1·6 (5)	400·9 ± 6·7 (5)	2040 ± 144	1·9 ± 0·1	

† Measured cell volume at a particular temperature.
‡ Fluxes in cells under isotonic conditions have not been adjusted to compensate for the slight variation of cell volume with temperature.
§ Fluxes in shrunken cells have been referred to 1 l. cells under isotonic conditions at the various temperatures.
* Corrections for inexchangeable Na have not been made.

The extracellular concentrations of Na and K were nominally 153·4 and 5·0 mM respectively. The K content of the cells was between 2·7 and 4·8 m-equiv/l. cells. Each series of experiments at different temperatures was carried out using red cells from one dog. In experiment 3, washed cells were not pre-incubated at 38° C before further equilibration at the temperature of ^{24}Na influx, but were left on ice for a few hours and then shaken gently for 1–2·5 hr at the appropriate temperature before the addition of ^{24}Na. Suitably controlled experiments indicated that no systematic change in cell volume was introduced during the short time that cells from all groups were cooled on ice while 100 μl. volumes of the packed cells were taken. Arrhenius plots of this data are shown in Fig. 6. Where there is no number in brackets after ± S.E., the error either represents the S.E. of the slope of the appropriate regression line or has been calculated from the S.E. of the slope together with smaller contributions from the S.E.s of the other parameters in the flux equations.

experiments (Fig. 10*B*) was the 'elbow' in the Arrhenius plot of K influx removed by shrinking the cells.

'Inexchangeable' Na in shrunken cells

In this and in previous work (Elford & Solomon, 1973, 1974*b*), it was found that a significant fraction of the intracellular sodium in shrunken cells apparently did not exchange with ^{24}Na in the bathing medium. Figs. 2 and 5*a* show that the intracellular activity of ^{24}Na reached a well defined

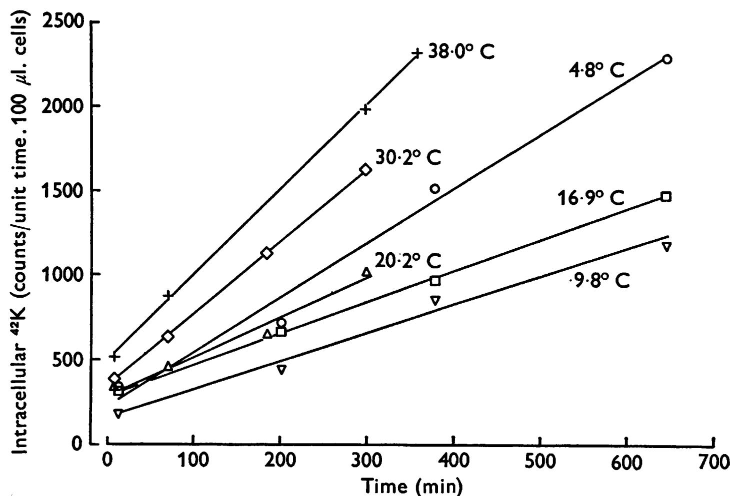

Fig. 8. The effect of temperature on the uptake of ^{42}K into dog red cells suspended in isosmolar media. + 38·0° C, ◇ 30·2° C, △ 20·2° C, ▽ 16·9° C, ▽ 9·8° C and ○ 4·8° C. The extracellular specific activity of ^{42}K was almost identical in each cell suspension (46·1 to 48·0 counts/unit time.m-equiv K). Note the relative position of the influx plot at 4·8° C, and the shift produced by shrinking the cells in hyperosmolar (100 mM) sucrose solutions (● in Fig. 9). The slopes of the least-squares regression lines (± 1 S.E.) were, in order of decreasing temperature, 4·98 ± 0·09, 4·25 ± 0·05, 2·2 ± 0·3, 1·81 ± 0·03, 1·6 ± 0·1 and 3·2 ± 0·3 (counts/unit time. 100 μl. cells.min^{-1}). The haemoglobin contents (g/l. cells) were (in the same order) 335·5 ± 2·0 (4), 329·3 ± 1·1 (4), 322·6 ± 1·3 (4), 327·5 ± 3·2 (4), 327·8 ± 1·1 (4) and 331·0 ± 1·4 (4). The K content of the cells varied from 2·1 to 2·7 m-equiv/l. cells.

asymptotic value, which was constant for several hours at temperatures between 38 and 30° C, and which was consistently below the theoretical equilibrium level based on equal intra- and extracellular specific activity. The calculated amount of intracellular Na that did not exchange with ^{24}Na during incubation for up to 5 hr at 38° C in the presence of 100 mM sucrose was 8·7 ± 1·6 (n = 8) m-equiv/l. shrunken cells; this value was about half the figure from previous work (Elford & Solomon, 1973, 1974*b*).

If an allowance is made for this amount of sequestered Na in the calculation of the Na flux from the half-time for the exchange of ^{24}Na, the Na flux in cells shrunken in 100 mM sucrose is reduced by 7 % to 208 ± 7 (n = 8) m-equiv/l. cells at their isosmolar volume. No experiments were carried out to see if a sequestered Na-compartment was present in cells of normal volume or whether it was a result of partially dehydrating the cells.

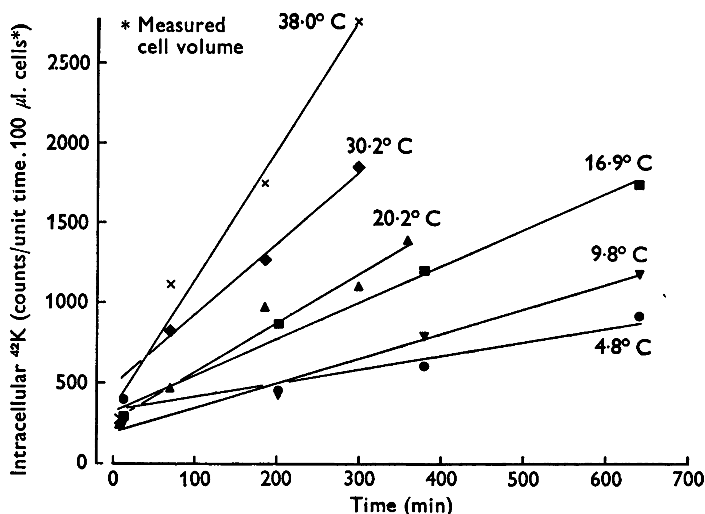

Fig. 9. Temperature dependence of ^{42}K influx in dog red cells suspended in hyperosmolar media containing 100 mM sucrose. The experimental conditions were identical to those in Fig. 8. Filled symbols have been used in the same convention as in Fig. 8 except for the influx at 38·0° C (×). Slopes (± S.E.) of the regression lines were 7·9 ± 0·7 ×, 4·4 ± 0·4 ◆, 3·1 ± 0·3 ▲, 2·2 ± 0·2 ■, 1·5 ± 0·1 ▼ and 0·84 ± 0·16 ●. The average haemoglobin contents of the cells were, in order of decreasing temperature, 465·9 ± 5·4 (4), 463·8 ± 8·4 (3), 439·0 ± 5·3 (5), 421·3 ± 4·9 (4), 417·9 ± 2·6 (4), 411·1 ± 2·5 (4) g/l. measured cell volume. K content of the cells ranged from 3·1 to 3·9 m-equiv/l. measured cell volume.

DISCUSSION

Na fluxes in dog red cells

The present data on the uptake of ^{24}Na in both normal and shrunken cells are compatible with a simple two compartment system (bathing medium and cells) with a small inaccessible fraction of intracellular Na in shrunken cells. The exchange kinetics for ^{24}Na found in this study are therefore not in agreement with the work of Lange, Lange & Solomon (1970) who found that the kinetics of Na transport in dog red cells were not determined by a single exponential process. Differences in experimental

procedure for the measurement of the influxes could well account for these discrepancies.

Values of the steady-state influx of Na in normal dog red cells range from about 3 m-equiv/l. cells.hr (in the present work) to 20 m-equiv/l. cells.hr (Sorenson *et al.* 1962; Parker & Hoffman, 1965; Hoffman, 1966; Streeten & Moses, 1968; Romualdez *et al.* 1972; Elford & Solomon, 1973). However, this variation is not surprising when the sensitivity of the fluxes to changes in cell volume is considered; for this reason it is essential to measure directly the relative volume of the cells from their water or haemoglobin content under the same conditions that the flux measurements are made.

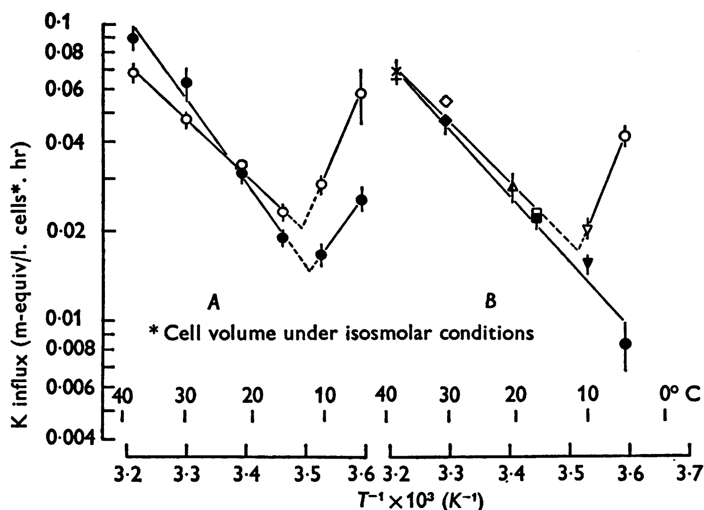

Fig. 10. Arrhenius plots of potassium influxes in dog red cells under isosmolar and hyperosmolar (+100 mM sucrose) conditions. The fluxes in shrunken cells have been referred to 1 l. cells under isosmolar conditions. Blood from a different animal was used in each series of experiments *A* and *B*. Open and filled symbols refer to isosmolar and hyperosmolar conditions respectively. The vertical bars, where large enough to be shown, represent ± 1 S.E. The data in *B* were calculated from the plots in Figs. 8 and 9 and the same symbols have been used in this Figure. It can be seen that shrinkage of the cells in 100 mM sucrose solutions had no significant effect on K influx except at temperatures below about 12° C when K influx was significantly reduced by shrinking the cells; in *B* this reduction was sufficient to remove the characteristic 'elbow' in the Arrhenius plots of the fluxes in cells of normal volume. The apparent activation energy for K influx increased slightly but not significantly between 38 and 15° C when the cells were shrunken: 8·5 ± 0·3 (○—○) to 12·6 ± 1·1 (●—●) kcal mole^{-1} in *A*, and 9·6 ± 1·2 to 10·0 ± 0·9 kcal mole^{-1} in *B* (the latter value was derived by including all six points in the calculation of the slope of the regression line). At temperatures below the 'elbow' the apparent activation energy for K influx in normal cells was about −22 kcal mole^{-1}.

The finding that there is a significant amount of inexchangeable Na in shrunken red cells of the dog was quite ancillary to the main purpose of the work and requires further study, especially as experiments on the net efflux of Na from human and bovine red cells has indicated that there is no difference between the specific activity of Na lost from the cells and the intracellular specific activity of Na (Garrahan & Glynn, 1967; Motais, 1973).

Changes in cation permeability with cell volume

Davson (1942) first suggested that the behaviour of dog red cells suspended in isosmolar solutions of various K salts could be explained on the basis of a reciprocal relationship between cell volume and the permeability of the cells to Na and K. This was confirmed by Parker & Hoffman (1965), who found that for a reduction in cell-water content from 73 to 59 %, Na influx increased by a factor of ten while the K influx decreased by approximately the same factor. However, in a later paper (Hoffman, 1966) it can be seen that the K flux is far less sensitive to cell shrinkage than the Na flux. Similarly, Sha'afi & Hajjar (1971) have shown that, in cat red cells, whose cation transport characteristics are in many respects similar to those of the dog, the K flux was reduced by only 50 % for a 25 % reduction in cell volume whereas the Na flux increased by more than a factor of 4 for only a 10 % reduction in cell volume. In fact, their work indicated there was no significant further reduction in K influx when the cells were shrunken from 95 to 75 % of their initial volume. Hence apart from the preliminary report by Parker & Hoffman (1967), the present work is in agreement with the earlier studies and demonstrates quite clearly that as the cells shrink there is little change in the permeability of the cells to K but there is a profound increase in the permeability of the cells to Na. It should be pointed out that, although the values of the steady-state fluxes of Na and K are markedly different, it can be shown, using the equations given by Katz (1966), that the permeability coefficients (cm . sec^{-1}) for Na and K in cells of normal volume are approximately equal ($P_{Na}/P_K = 1{\cdot}4$).

Na:Na exchange process in shrunken cells

It is clear from the relative magnitude of the steady-state fluxes of Na and K that a Na:K exchange mechanism in dog red cells can account for only a small proportion of the Na flux in both normal and shrunken cells. Also, as the K flux is essentially unaffected by cell shrinkage, it follows that the enhanced rate of ^{24}Na exchange in cells shrunken in media containing sucrose can be produced only by a Na:Na exchange process. It seems unlikely, however, that this process is controlled by a carrier-mediated mechanism of exchange-diffusion first proposed by Ussing (1947) and which seems to operate in Na-rich red cells of the cow (Motais, 1973).

The existence of such a process is indicated if the efflux of Na falls as the extracellular concentration of Na approaches zero (Motais, 1973). However, when dog red cells are treated in this way in $MgCl_2$ or KCl solutions there is a loss of intracellular Na in exchange for extracellular cation and a high proportion of the cells lyse (Davson, 1942; Sorenson *et al.* 1962; Lee, Brown, Hutzler & Auvil, 1972). Furthermore, the carrier mechanism of exchange diffusion cannot, by definition, be involved in net movements of cations since the carrier is supposed to shuttle across the membrane only when associated with an ion. However, when dog red cells are shrunken in hyperosmolar media containing additional NaCl instead of sucrose there is a large net uptake of Na, and the degree of cell shrinkage is less than that expected on the basis of the tonicity of the bathing solution (unpublished results). Hence it can be argued that cell shrinkage distorts the cell membrane thereby opening a Na-specific gate through which both net and exchange fluxes of Na take place, the direction of the former being dependent on the concentration gradient of Na across the membrane.

Effects of temperature on cation fluxes

The major finding of the present study is the disparity between the effects of temperature on the fluxes of Na and K in cells of normal volume, and the changes in these effects introduced by shrinking the cells in solutions made hyperosmolar with sucrose. It is interesting to discuss these results in relation to the recently developed concepts of the fluid mosaic model of cell membranes (Singer & Nicolson, 1972). There is some consensus for the view that the lipids in many types of cell membrane, especially those containing cholesterol, are in a fluid state under physiological conditions and that lateral movement of the membrane components occur (Taylor, Duffus, Raff & de Petris, 1971; Singer & Nicolson, 1972). It has also been demonstrated by a variety of techniques that lipids extracted from membranes undergo temperature-dependent phase transitions (Gulik-Krzywicki, Rivas & Luzzati, 1967; Ladbrooke, Jenkinson, Kamat & Chapman, 1968; Chapman & Urbina, 1971). By contrast, there is little direct evidence to show that phase transitions can occur in the lipids of intact cell membranes or membrane fragments except when the cholesterol content is low (Ladbrooke *et al.* 1968; Wilkins, Blaurock & Engelman, 1971), although there are several reports that the Arrhenius plots of a variety of processes associated with membrane transport systems or membrane bound enzymes exhibit abrupt changes in slope at certain temperatures, which have been associated with changes in the fluidity of the fatty acid chains of the membrane lipids (Wilson & Fox, 1971; Charnock, Cook & Casey, 1971; Esfahani, Limbrick, Knutton, Oka & Wakil, 1971; Lacko, Wittke & Geck, 1973). It is also interesting that the temperature

dependence of K (but not Na) influx in human red cells is similar to that found in the present work (Wieth, 1970; Dalmark & Wieth, 1970). However, it is difficult to explain the observed temperature-dependence of the cation fluxes in dog red cells in terms of phase transitions within the membrane. For instance, at about 22° C when the Na influx in cells under isotonic conditions is at a maximum, and there is a change in the apparent activation energy for Na transport in shrunken cells, there is no effect, within experimental error, on the K transport mechanism. Similarly at about 12° C at which temperature there is a well defined 'elbow' in the Arrhenius plot of the K influx there is no interference with the Na-transport system. These results, coupled with the observation that the activation energy for the transport of water across the dog red cell is constant from 37 to 7° C (Vieira, Sha'afi & Solomon, 1970) makes it improbable that any long-range order-disorder reorganization of the membrane lipids occurs at the temperatures at which the fluxes of Na and K are maximal and minimal respectively. In this context the present work supports one of the tenets of the fluid-mosaic model in that there should be no long-range order in a mosaic membrane with a lipid matrix (Singer & Nicolson, 1972).

Papahadjopoulos, Jacobson, Nir & Isac (1973) consider that their observation of a maximum at 35–40° C in the rate of self-diffusion of ^{22}Na through phospholipid vesicles is associated with the enhanced movement of Na through boundaries between domains or microcrystals of lipid formed by a phase transition. In support of this they showed that the permeability of the vesicles to [^{14}C]sucrose also increased along with that of ^{22}Na. However, in the present work the lack of any obvious coupling between the effects of temperature on the transport of Na and K would seem to indicate that the domain concept of Papahadjopoulos *et al.* (1973) is not relevant to the dog red cell membrane.

It is established that the dependence on cell volume of the Na permeability of dog and cat red cells is controlled by the metabolic state of the cell (Davson, 1942; Hoffman, 1966; Sha'afi & Hajjar, 1971; Romualdez *et al.* 1972; Sha'afi & Pascoe, 1973; Elford & Solomon, 1973). Recently, Elford & Solomon (1974*b*) argued from the effects of various anions on dog red cells partially depleted of substrates that the response of the Na flux to cell shrinkage was mediated by phosphoglycerate kinase, which is probably a membrane-bound enzyme (Schrier, 1966; Parker & Hoffman, 1967). Hence it seems likely, especially in view of the effects of temperature on the activation by phospholipids of enzymes involved with active transport processes (Priestland & Whittam, 1972; Kimelberg & Papahadjopoulos, 1972), that the changes in slope in the Arrhenius plots of Na and K influxes (Figs. 6 and 10) are associated with temperature dependent con-

formational changes of membrane-bound protein complexes specific for Na and K.

A detailed analysis of the effects of temperature on this system is likely to be extremely complex for two reasons: (1) temperature can influence the function of enzymes either directly by altering their quaternary structure or by changing the properties of the matrix in which they are located, and (2) the activation energy reflects the temperature dependence of only the rate limiting step in the over-all process of ion-translocation through the membrane, and this is undoubtedly a multistage process involving a number of rate constants.

Preliminary experiments on the effects of temperature on the dependence of sodium fluxes on cell volume in dog red cells were carried out at the Biophysical Laboratory, Harvard Medical School, Boston, Mass. during the tenure of a Travelling Research Fellowship from the Medical Research Council. An indication that the sodium influx in cells under isotonic conditions was greater at room temperature than at 38° C was first found by Dr Yvonne Lange at the Biophysical Laboratory. I thank Dr A. K. Solomon for stimulating my interest in dog red cells and for helpful discussion at various stages of the work. I also thank Colin Green of the Clinical Research Centre for supplying the dog blood for these experiments, and members of the Division of Radioisotopes for their help with the handling and counting of ^{24}Na and ^{42}K.

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