Cation Movements in the High Sodium Erythrocyte of the Cat

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ABSTRACT The uptake of ^{42}K and ^{24}Na by cat erythrocytes was investigated. Under steady-state conditions, the nontransient component of ^{42}K influx was found to be 0.18 ± 0.01 meq/liter RBC/hr and insensitive to ouabain (100 μM); the corresponding value for ^{24}Na was 17 ± 1 meq/liter RBC/hr. A study was made of the effects of anions upon cation movements in these and other mammalian red cells. Iodide was found to inhibit markedly (>50%) Na inward movements in cat and dog but not in the other erythrocytes. An increase (15-30%) in K uptake in the presence of iodide was noted in all the mammalian cells studied.

The majority of mammalian erythrocytes investigated maintain appreciable gradients of both K and Na between their interiors and the bathing plasma (Bernstein, 1954). Increasing evidence indicates that these gradients are maintained at the expense of metabolic energy, usually through the hydrolysis of ATP by a membrane-associated ATPase which is activated by Na and K but inhibited by ouabain (Dunham and Glynn, 1961; Tosteson et al., 1960). It is thus of considerable importance that a mammalian red cell has been found to deviate from this usual pattern. The cat erythrocyte maintains only slight gradients of K and Na (Hegnauer and Robinson, 1936); it also appears to possess no membrane ATPase which is activated by Na and K and inhibited by ouabain (Chan et al., 1964; Greeff et al., 1964). The transport properties of such an unusual cell are of considerable interest. This paper is concerned with the transport of ^{24}Na and ^{42}K across the cat erythrocyte membrane.

THEORETICAL SECTION

In order to analyze the steady-state influx kinetics of ^{42}K and ^{24}Na for cat erythrocytes, a model system must be postulated. The experimental data to

be presented later are incompatible with a simple two compartment system. With certain assumptions based upon experimental results, the three compartment model system of Fig. 1 was found to be sufficient to describe our data.

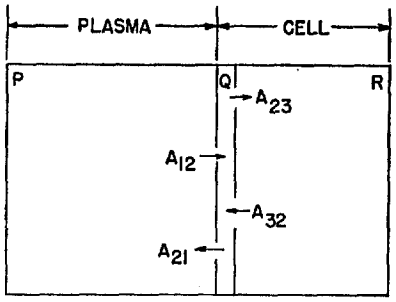

FIGURE 1. Three compartment model.

To describe the analysis of this model system, it is necessary to introduce and define the following symbols:

C^+ = the particular cation (either K or Na) under consideration.
a, b, c = concentrations (meq C^+/liter suspension) in compartments P, Q, R.
$\bar{p}, \bar{q}, \bar{r}$ = specific activities (relative cpm/meq C^+) in P, Q, R.
$\bar{s}$ = specific activity (relative cpm/meq C^+) in compartment $(Q + R)$.
t = time after addition of labeled C^+ to P.
$\bar{p}_0$ = value of $\bar{p}$ at $t = 0$.
$\bar{s}_\infty$ = value of $\bar{s}$ as $t \to \infty$.
A_{ij} = fluxes (meq C^+/liter suspension/hr) between the various compartments as shown in Fig. 1.
M_{ij} = fluxes (meq C^+/liter RBC/hr) corresponding to A_{ij}.
$(C^+)_0$ = concentration (meq C^+/liter plasma) in P.
$(C^+)_{in}$ = concentration (meq C^+/liter RBC) in $(Q + R)$.
H = hematocrit value (liter RBC/ liter suspension).
I = value of the function $(1 - \bar{s}/\bar{s}_\infty)$ at $t = t_1$.
$-m$ = slope (sec^{-1}) of the graph of $\ln(1 - \bar{s}/\bar{s}_\infty)$ vs. time for $t \geq t_2$ where $t_2 > 15$ min.

The equations governing the behavior of the model system can be written as follows (Solomon and Gold, 1955):

$$a d\bar{p}/dt = -A_{12}\bar{p} + A_{21}\bar{q} \tag{1}$$

$$b d\bar{q}/dt = A_{12}\bar{p} - (A_{21} + A_{23})\bar{q} + A_{32}\bar{r} \tag{2}$$

$$c d\bar{r}/dt = A_{23}\bar{q} - A_{32}\bar{r} \tag{3}$$

This set of equations cannot be solved exactly in terms of measurable quantities only. However, approximate solutions involving only measurable parameters can be obtained using the following assumptions: (1) For times t such that $0 \leq t \leq t_1$, where $t_1 \leq 15$ min, $\bar{r} \simeq 0$. (2) The fluxes between compartments P and Q are much greater than those between Q and R; *i.e.*, A_{12}, $A_{21} \gg A_{23}$, A_{32}. (3) Compartment Q contains only a small fraction of the total C^+; *i.e.*, $b \ll a$, c.

A straightforward but lengthy analysis yields the following results (Sha'afi, 1965):

$$M_{12} = M_{21} = \frac{(1 - I)\,(C)_0}{t_1[H/(1 - H) + (C)_0/(C)_{in}]} \tag{4}$$

$$M_{23} = M_{32} = \frac{m(C)_0}{H/(1 - H) + (C)_0/(C)_{in}} \tag{5}$$

Equations 4 and 5 were used to calculate the fluxes reported in this paper. For experimental convenience only, the time t_1 was taken as 15 min. The value of $\bar{s}_\infty$ used for plotting the function $\ln(1 - \bar{s}/\bar{s}_\infty)$ was calculated from:

$$\bar{s}_\infty = \frac{\bar{p}_0}{1 + [H(C)_{in}/(1 - H)(C)_0]} \tag{6}$$

MATERIALS AND METHODS

Blood was withdrawn into heparinized syringes from anesthetized (Diabutal) cats, dogs, and rabbits by heart puncture and from sheep via the jugular vein. The blood was immediately centrifuged, after which the plasma and buffy coat were removed by suction. In steady-state experiments, the red cells were then washed three times by alternate resuspension and spinning in incubation medium. The composition of this medium (pH 7.4) was (in mM): NaCl, 150.0; KCl, 5.0; $MgCl_2$, 1.0; $CaCl_2$, 2.5; Na_2HPO_4, 5.0; NaH_2PO_4, 1.0; glucose, 11.1. The washed red cells were resuspended in the incubation medium; at zero time the radioactive tracer was added. After thorough mixing for approximately 60 sec, aliquots were removed for hematocrit determinations and the medium radioactivity analyses. The suspension was then incubated on a shaker at 38°C. At specified intervals thereafter, aliquots of suspension were removed. The erythrocytes were separated from the labeled medium by centrifugation followed by three washes; each wash consisted of an alternate resuspension and spinning of red cells in unlabeled medium followed by removal of the supernatant by suction. At the end of the third wash, the uncorrected packed cell volume was determined by centrifuging the cells for 10 min at 1700 *g*. The error in red cell activity measurements ($\bar{s}$) due to trapped plasma was less than 1%.

A similar protocol was followed in those experiments designed to study the effect of anions on cation transport. For each anion a modified medium was prepared which was identical with the incubation medium except for the replacement of the monovalent chloride salts by salts of this anion. The red cells were washed twice in the incubation medium and then once in the modified medium. These cells were then re-

suspended in the latter solution to a hematocrit of about 30%. This suspension was shaken at 38°C for 15 min to allow equilibration of the anions to occur. Radioactive tracers were then added. Procedures for the sampling of the medium and red cells were identical with those of the steady-state experiments except that the cells were washed in the modified medium and the packed cell volumes were corrected for any shrinking or swelling due to the presence of the foreign anion.

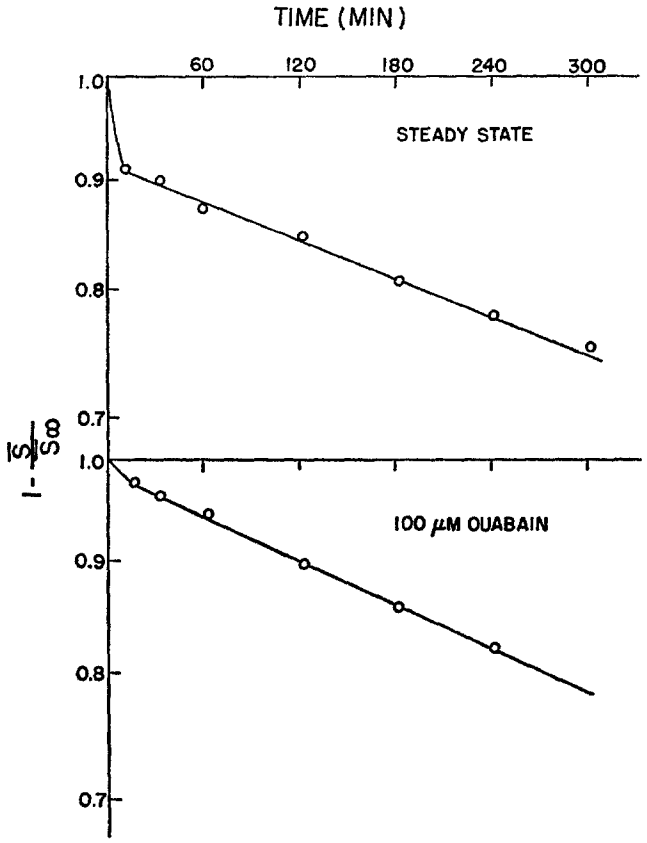

FIGURE 2. ^{42}K uptake by cat erythrocytes.

Radioactivity was measured with a well scintillation counter. All samples were counted consecutively at the end of each experiment; appropriate corrections were made for decay. ^{24}Na and ^{42}K were produced each week by irradiating Na_2CO_3 and K_2CO_3 respectively with neutron flux at the University of Illinois nuclear reactor. The irradiated salts were neutralized with HCl. In the early experiments, half-life determinations over a period of at least four half-lives were made on the radioactive tracers; since the measured half-lives agreed with those reported in the literature for ^{24}Na and ^{42}K, the tracers were considered to be free of radioactive contaminants in all subsequent work.

Hematocrit determinations were made by centrifuging suspension samples in

Wintrobe hematocrit tubes for 10 min at 1700 *g*. Trapped fluid space was estimated using ^{42}K according to the method of Tosteson and Hoffman (1960); the mean value of 8% obtained from 10 experiments was used to convert the experimental hematocrit readings and packed cell volumes into the values used for calculations.

Na and K concentrations were measured with an internal standard type flame photometer using lithium as the internal standard. In some of the early steady-state experiments, erythrocyte concentrations of Na and K were determined at various times during the experiments; the fact that the concentrations were found to be time-independent indicated that steady-state conditions had been maintained.

RESULTS

Steady-State K Fluxes

The uptake of ^{42}K by cat erythrocytes under steady-state conditions exhibits two phases. A rapid component which reaches equilibrium within 15 min and exchanges with less than 10% of the cellular K is followed by a much slower and more sustained component which exchanges with the bulk of the cellular K. The results of a typical K influx experiment are shown in Fig. 2. Fluxes were calculated according to equations 4 and 5. Representative values of these fluxes are given in Table I; the upper and lower limits are included. The mean value of M_{23} and the standard error of the mean obtained from 30 experiments on 15 different cats are 0.18 ± 0.01 meq/liter RBC/hr. The fast component of influx (M_{12}) was generally about six times greater than M_{23}.

TABLE I

REPRESENTATIVE VALUES OF K INFLUX

		Steady-State		Ouabain (100 μM)	
Experiment No.	Cat No.	*M_{12}	*M_{23}	*M_{12}	*M_{23}
2	5	1.16	0.20	0.43	0.16
6	5	1.16	0.19	0.52	0.21
10	6	1.16	0.20	0.66	0.20
15	6	1.00	0.18	0.44	0.20
20	10	1.21	0.16	0.44	0.19
25	8	1.10	0.15	0.50	0.16
30	10	1.04	0.15	0.48	0.17

* Expressed as milliequivalents per liter red blood cells per hour.

It should be noted that the major component of K influx is only 11% of that obtained for the human erythrocyte (Solomon, 1952).

Steady-State Na Fluxes

The uptake of ^{24}Na by red cells under steady-state conditions resembles that of ^{42}K in that two phases are observed. However, the magnitudes of the fluxes

differ considerably. Fig. 3 shows the results of a typical Na influx experiment. Na fluxes were calculated in exactly the same manner as K fluxes. Table II lists representative values of these fluxes; the upper and lower limits are included. It should be noted that there exists considerable variation between animals and even between samples taken from the same animal. This variation is probably due to extreme sensitivity of the Na transport system to small perturbations in environmental conditions. The average value of M_{23} and the standard error of the mean obtained from 44 experiments on 26 different cats are 17 $\pm$ 1 meq/liter RBC/hr. The fast component of influx (M_{12}) was usually slightly less than twice M_{23}.

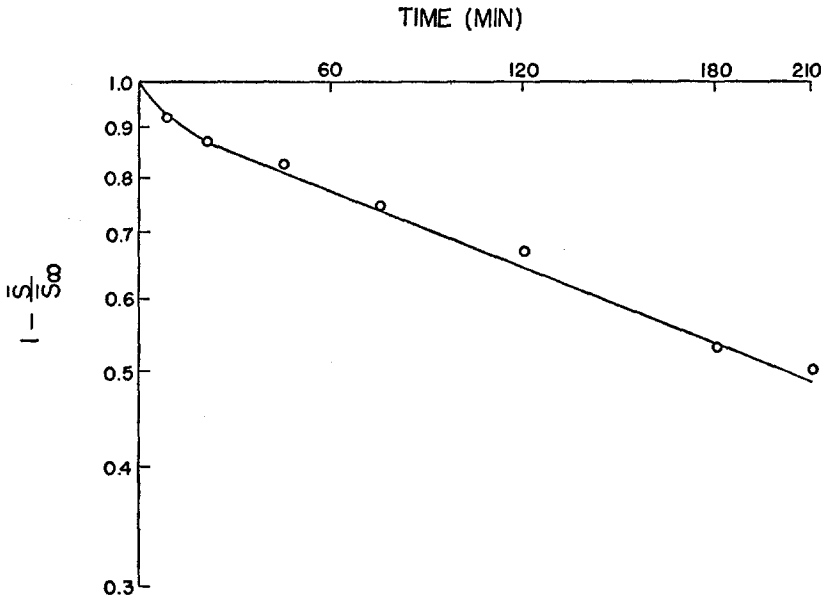

FIGURE 3. ^{24}Na uptake by cat erythrocytes under steady-state conditions.

At first it was suspected that the large values of M_{23} were ascribable to exchange diffusion. To test this hypothesis, cells were suspended in the incubation medium in which all the Na ions had been replaced by K ions; net Na outflow was measured. The results are shown in Fig. 4. Note that the net Na flux is even larger than the above values of M_{23}. If it is assumed that such nonequilibrium conditions do not drastically alter the transport mechanism, it follows from the definition given by Levi and Ussing (1949) that exchange diffusion can be eliminated as an important factor in our measurements.

Thus the steady-state Na flux in these cells is considerably larger than in most other mammalian red cells. In particular, the major component of Na flux has a magnitude over five times as great as that of the human erythro-

cyte (Solomon, 1952). From the preceding values of steady-state fluxes and plasma concentrations, it can be calculated that for inward movement the cat red cell prefers Na to K with a competition factor of 3. This is in distinct

TABLE II
REPRESENTATIVE VALUES OF Na INFLUX

Experiment No.	Cat No.	M_{is}	M_{ss}
			meq/liter RBC/hr
2	13	30	21
4	20	25	15
6	14	30	14
10	20	25	13
15	25	34	18
20	13	30	20
35	14	31	18
40	20	32	17
42	36	—	9
44	40	—	19

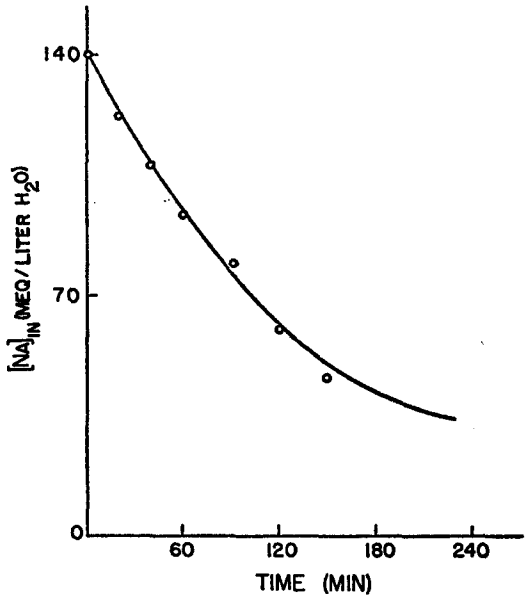

FIGURE 4. The time course of sodium escape from cat erythrocytes. The cells were suspended in isotonic potassium chloride.

contrast to the human erythrocyte, for which inwardly moving K is preferred to Na with a competition factor of 15 (Solomon, 1952).

Effect of Ouabain on K Uptake

It is generally accepted that cardiac glycosides act as competitive inhibitors of K influx in most mammalian erythrocytes (Glynn, 1957). In fact, that portion of the K uptake which can be inhibited by these compounds is often

taken as a measure of the active component of K influx (Tosteson and Hoffman, 1960).

The effect of the cardiac glycoside ouabain on K uptake in the cat erythrocyte is clearly demonstrated in Fig. 2 and Table I. The mean value of M_{23}

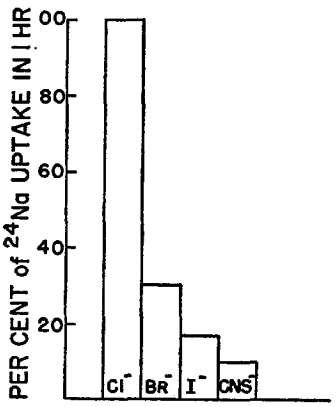

FIGURE 5. Effect of various anions on ^{24}Na uptake by cat erythrocytes. The amount of ^{24}Na entering the cells bathed in chloride solution in 1 hr was taken as 100%.

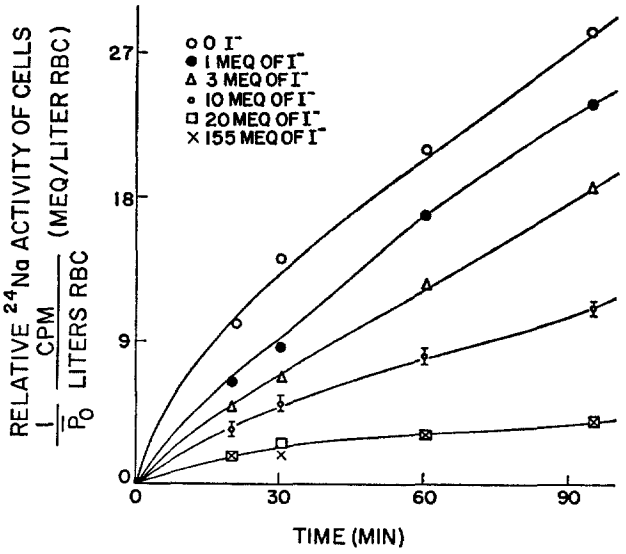

FIGURE 6. Effect of varying levels of iodide on ^{24}Na uptake by cat erythrocytes.

and the standard error of the mean obtained from 20 experiments on 10 different cats in the presence of 100 μM ouabain are 0.19 $\pm$ 0.01 meq/liter RBC/hr. This value is seen to be statistically indistinguishable from the value obtained in the absence of ouabain. On the other hand, this concentration of ouabain did inhibit the fast component (M_{12}) of K uptake by a factor of 2 $\pm$ 0.5. No adequate explanation for this effect can be given at the present time. However, if the fast component of influx can be partially

accounted for by the adsorption of labeled cation to the erythrocyte membrane, it is possible that ouabain exerts its effect by decreasing the magnitude of this adsorption.

The major component of K influx is unaffected by ouabain concentrations in the range from 50 to 300 μM. The action of this cardiac glycoside on K uptake by cat red cells differs from that on most other mammalian erythrocytes (Glynn, 1957; Tosteson and Hoffman, 1960). For example, 100 μM ouabain was found to inhibit 90% of the movement of ^{42}K into rabbit red cells (see Fig. 9).

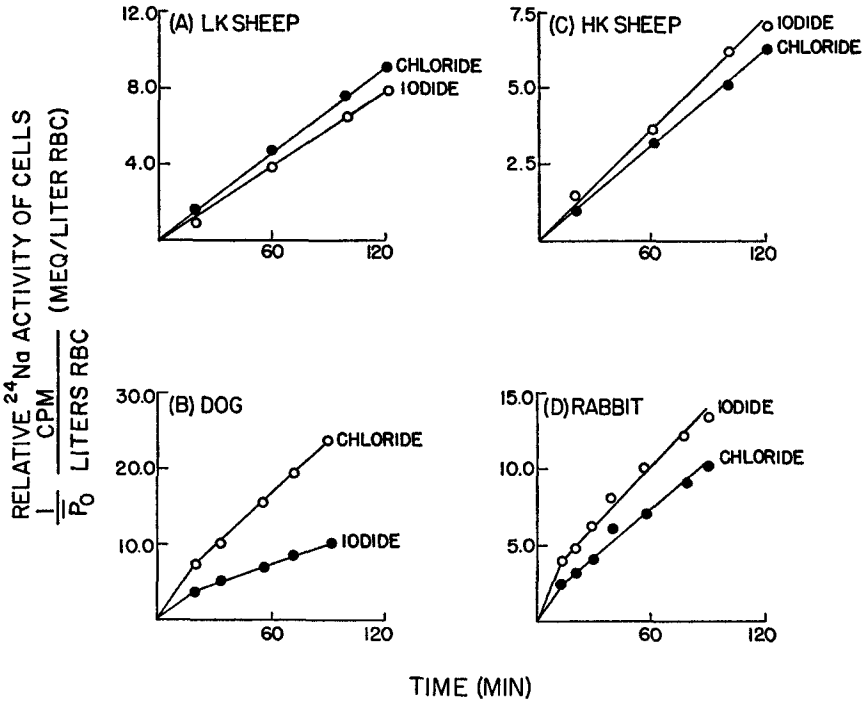

FIGURE 7. Effect of iodide on ^{24}Na uptake by red blood cells of various mammalian species.

Effect of Anions upon Na Movements in Cat Erythrocytes

To further compare the cation transport system of the cat with the systems of other mammalian erythrocytes, the effects of various anions upon cation movements were investigated. In these experiments, red cells were suspended in a solution identical to the incubation medium except for the replacement of the monovalent chloride salts by salts of the anion under study. Since the cells were not in a steady-state condition, the flux equations derived earlier in this paper are no longer applicable. A measure of the inward movement of cation was obtained by determining the amount of tracer entering the cells during the first hour.

The results of a typical set of experiments on Na uptake in cat red cells are shown in Fig. 5. It can be seen that the order of effectiveness of the various anions in inhibiting the uptake of Na is $CNS^- > I^- > Br^- > Cl^-$. This sequence follows the Hofmeister, or lyotropic, series. Similar results have been obtained for the net outward movement of Na from these cells (Davson-1940). Interestingly, this lyotropic series also describes the degree of inhibi, tion of excitability of internally perfused squid axons; such an effect on excitability is often not accompanied by any significant change in membrane resting potential (Tasaki and Singer, 1965).

To facilitate the further investigation of anion effects, it was decided to

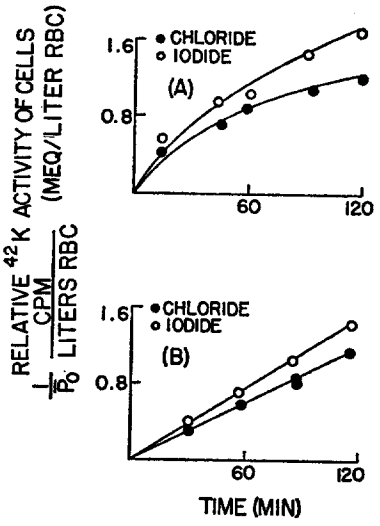

FIGURE 8. Effect of iodide on ^{42}K uptake by red blood cells of cat (A) and HK sheep (B).

focus our attention upon only one member of the lyotropic series; iodide was chosen for this purpose. To determine the range of effectiveness of this anion in inhibiting Na movements, a series of experiments was carried out in which various degrees of iodide substitution for chloride were employed. The results of a typical experiment are shown in Fig. 6. Note that maximal inhibition is achieved when as little iodide as 20 meq/liter plasma is present.

Effects of Iodide on Na Movements in the Erythrocytes of Other Mammals

The preceding results raised the question of whether or not the iodide inhibition of Na transport is a feature peculiar to the cat red cell. To answer this question, a series of experiments was carried out on erythrocytes of various other mammals (dog, human, rabbit, LK and HK sheep). Some of the results are shown in Fig. 7 A, B, C, D.

Na influx in red cells of the dog, which have internal cation concentrations similar to those of the cat, was markedly (>50%) inhibited by the presence

of iodide. This suggests that some similarities exist between the Na transport systems of the erythrocytes of these two species. On the contrary, the movements of Na into the red cells of the human, rabbit, LK and HK sheep were only slightly (10–20%) affected by the presence of iodide; accelerations were observed for the human, rabbit, and HK sheep cells whereas an inhibition was found for the LK sheep cells. These observations may indicate that cat and dog red cells regulate their Na movements in a different manner than do most other mammalian erythrocytes.

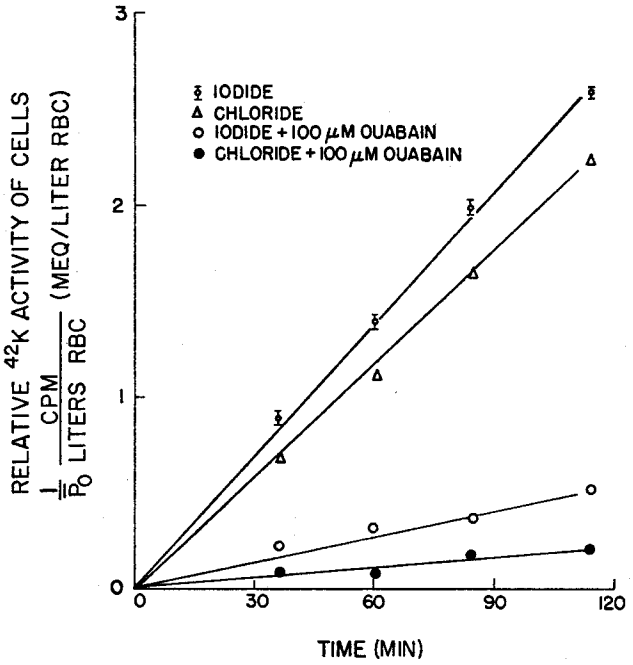

FIGURE 9. Effect of iodide on ^{42}K uptake by rabbit erythrocytes in the presence and absence of ouabain.

Effect of Iodide on K Movements in the Erythrocytes of Various Mammals

The effect of iodide upon the uptake of ^{42}K by erythrocytes of cat, rabbit, and LK and HK sheep was also investigated. Some of the results are shown in Fig. 8 A, B. In all cases a slight (15-30%) increase was observed. Certain of these cells have been shown to possess both an active and a passive K uptake. To determine upon which component iodide is acting, the active component of K uptake by the rabbit red cell was blocked by ouabain (100 μM). The results are shown in Fig. 9. Note that the same absolute increase in K uptake occurs whether or not the active component is inhibited. This implies that the action of iodide is mainly upon the passive component.

DISCUSSION

Most of the mammalian erythrocytes which have been studied appear to take up ^{42}K both actively and passively. The actively transported fraction, which is inhibited by ouabain, constitutes the bulk of this uptake. This portion is thought to be energized through the hydrolysis of ATP by a membrane-associated ATPase which is activated by Na and K but inhibited by ouabain. As a result of this energy-dependent process, the concentration of K inside the cell far exceeds that of the bathing plasma.

Conversely, the major component of ^{42}K uptake by the cat erythrocyte is exceedingly small and is not affected by ouabain. The distribution of K across this membrane closely approximates that predicted by the Donnan equilibrium. Furthermore, this cell appears to possess no (Na + K)–sensitive ATPase. On the basis of these observations it seems reasonable to postulate that these cells lack any measurable active component of K influx.

When the actions of anions of the lyotropic series upon cation transport by the cat red cell were first discovered, the effects were ascribed to diffusion potentials (Davson, 1940). Such a conclusion was based upon experiments performed without the assistance of radioactive tracers. Thus it was not known at that time that many of the anions studied have equilibration half-times of less than a second; also, the relative rates of entry of these anions were not accurately known (Tosteson, 1959). Furthermore, exclusive reliance upon net flux experiments limited the investigation to only the influx of K and the efflux of Na in these cells. On the basis of observations made under such experimental constraints, the idea that diffusion potentials could explain the anion effects upon cation transport was very reasonable.

Application of the tracer technique to the study of cation movements in the cat erythrocyte has enabled us to reject this early idea on the basis of the following reasons. (*a*) A diffusion potential would have the same qualitative effect on all cations moving in the same direction. However, the preceding results have shown that in the presence of iodide the inward movement of Na is inhibited while that of K is accelerated. (*b*) In the experiments reported here, the cells were preincubated for 15 min in the modified medium before the addition of tracer. This allowed the anions sufficient time to equilibrate. Another explanation which might seemingly account for these effects is that the foreign anions affect the Donnan potential across the cell membrane in a different manner than do chloride ions. This hypothesis, however, is inconsistent with the experimental observation cited above under (*a*).

Unfortunately, although some explanations for the anion effect can be ruled out, no conclusive hypothesis can be put forward at the present time. Nonetheless, one interesting observation which may partially account for

our findings is that the lyotropic series describes the relative adsorbabilities of the various anions (Harris, 1956). Thus it could be predicted that all the anions used in this work adhere to the cell membrane to a greater extent than would chloride. This adhesion might well cause a weakening of certain electrostatic interactions important for the stability of the membrane. Such an instability might result in a loosening of membrane structure and an increase in passive transport rates. Such considerations may explain the slight increases in both Na and K inward movements in the presence of iodide which have been observed in the majority of the red cells studied here.

The striking inhibition of Na transport caused by lyotropic anions, the extremely rapid turnover of Na, and the absence of a (Na + K)-sensitive ATPase in cat erythrocytes all argue in favor of the existence of a type of Na transport system in these cells qualitatively different from that in most other mammalian red cells. The rapid movement of Na, contrasted with the much slower turnover of K, suggests the presence in cat erythrocyte membranes of a mechanism specialized for the facilitation of Na transport. This mechanism could occur in the form of a carrier or a specialized pore system.

In accordance with the previous considerations of the action of lyotropic anions, their inhibitory action on Na transport may be due to any combination of the following reasons. (*a*) The anions may cause a generalized alteration in membrane configuration, resulting in changes in the shape and charge characteristics of specialized pores sufficient to inhibit the passage of Na ions through them. Alternatively, such a distortion of membrane structure might so alter the immediate environments of sodium-specific carriers as to make their operation extremely inefficient. (*b*) The anions may adsorb directly to the inside of a specialized pore, making the passage of Na ions physically improbable. (*c*) The anions may attach directly to sodium-specific carriers, thus impeding their movement or decreasing their affinity for Na.

Since the partial free energy of Na is somewhat lower inside the cell than outside, it is reasonable to postulate that the outward movement of Na is an energy-requiring step. Experimental attempts to investigate this aspect have been complicated by the extreme sensitivity of the Na transport system to its environment. Thus it has proven difficult to demonstrate that an inhibition of Na efflux by a glycolytic poison is due simply to a blockage of glycolytic energy production; the inhibition could also be caused by a direct action upon the transport system of either (*a*) the poison itself or (*b*) a glycolytic intermediate whose cellular levels have increased because of the metabolic block.

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