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Potassium Uptake by the Dog Erythrocyte

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The characteristics of potassium exchange by human erythrocytes have been the subject of intensive investigation for many years. The erythrocytes of various animal species, including the dog, differ from those of humans by maintaining a high intracellular sodium and low potassium, and it is a problem of interest to determine whether this difference in cellular ionic structure is associated with differences in the way these cells exchange potassium.

Sheppard, Martin, and Beyl (1) have shown that the formed elements of dog blood take up K^{42} in a manner which can be described as the sum of a fast and a slow rate process. The latter is identified with the erythrocytes, the former with the other formed elements. Our results confirm this finding of at least two cellular compartments with respect to potassium. Since we wished to study erythrocyte exchange, we have employed a centrifugation method of separating the red and white cells, suspending the erythrocytes in an artificial buffer, the composition of which resembles plasma. The use of this buffer permits adjustment of the ambient potassium concentration without alteration of total osmolarity or the concentration of any other ion except sodium.

Using this preparation, the inward transport of potassium by the dog erythrocyte has been studied at concentrations of potassium in the medium from 2.9 to 25.0 milliequivalents per liter, and at 38.0 and 33.0°C.

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METHODS

In general, the experimental methods were those used by Solomon (2).

Blood was withdrawn from mature mongrel male and female dogs which were bled no more often than once in two weeks. All apparatus in contact with the blood was coated with a siliconing compound. Separation of red and white cells was accomplished by immediate centrifugation of the heparinized blood at 10,000 g for 30 minutes. Positive pressure was applied to the plasma to expel the bottom layers of erythrocytes from the centrifuge tube through a blunt 15 gauge needle. The erythrocytes thus collected were then suspended in the buffer.

From the standpoint of experimental design, it was felt desirable to measure the increase in cell radioactivity rather than the decrease in plasma or buffer radioactivity, since the former method would permit the measurement of relatively large changes in small numbers of counts. By centrifuging the dog whole blood at 10,000 g for 30 minutes, the concentration of the white cells relative to the erythrocytes could be reduced to 0.3% of the normal value. This exclusion of the very rapidly exchanging white cell fraction from the experimental preparation permitted the use of measurements of cell radioactivity as a valid index of erythrocyte potassium exchange.

There is evidence that centrifuging alters the permeability characteristics of erythrocytes (3). In an effort to control this variable, dog whole blood with tracer amounts of $K^{42}Cl$ added was incubated without centrifuging but with otherwise similar handling for periods of eight to sixteen hours and the decline in plasma radioactivity measured.

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Incubation for a period of this length permits a determination of the rate of the slow fraction exchange notwithstanding the presence of the white blood cells. In a series of eight such determinations over a range of ambient potassium concentration from 5 to 20 milliequivalents per liter, the variation in inward transport of potassium with alterations in plasma potassium concentration was found not to differ significantly from that in the separated red cells preparation.

A second control is furnished by the work of Sheppard, Martin, and Beyl (1). Their results on two experiments with dog red blood cells separated from the white cells by repeated centrifugation at low g are similar to the present results with either whole blood or separated erythrocyte preparations at the same concentration of ambient potassium.

The phosphate-bicarbonate buffer used in the separated cell experiments is a modification of that used by Raker et al (4). Its composition is given in Table 1. Alterations in potassium concentration were obtained by substituting the proper amount of a potassium salt for the analogous sodium salt.

Hematocrits were determined in standard Wintrobe tubes at the beginning and end of each experiment. In the whole blood experiments, the tubes were centrifuged at 2170 g for 50 minutes, the radius measurement being made to the middle of the packed cell column. The volume of plasma trapped with the erythrocytes was measured by I^{131} labelled albumin from which the diffusible iodide had been removed previously by means of an anion exchange resin or by dialysis. The tracer was added

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to the whole blood suspension and the mixture shaken for 3 minutes. Centrifugation in 3 mm inside diameter tubes was begun within 5 minutes of the initial mixing. Following centrifugation, the tubes were cut 1 mm below the buffy-coat-erythrocyte interface, and the radioactivity of the packed cell column was determined in the cut tube. A sample of the supernatant was also taken for counting. It has been shown by Chaplin and Mollison (5) that at constant angular velocity, the percentage of trapped plasma decreases with increasing distances of the cells from the center of the centrifuge, hence our determinations err on the low side because of the exclusion of the 1 mm upper portion of the column which contains a relatively high proportion of trapped plasma. This error is small, and much less than the one which would result from attempting to cut the cell column at the plasma-cell interface, where contamination with only small amounts of plasma would be sufficient to make the determination of the trapped plasma in error by a large factor. The packed cells were then washed out of the tube and mercury was weighed in the tube to determine the volume of cells whose radioactivity had been measured. The ratio of counts per unit volume of packed cells to counts per unit volume of supernatant gives the fraction of trapped supernatant per unit of packed cells, (if the I^{131} labelled albumin is distributed like normal albumin) and the function $(1 - \%$ trapped) gives a correction factor for converting observed hematocrit to "true" hematocrit.

For the separated erythrocytes experiments, hematocrits were centrifuged at 2440 g for 50 minutes and the "true" hematocrit

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correction factor determined in similar fashion using I^{131} labelled albumin.

Table 2 gives the results of these experiments. In dog whole blood, with hematocrits of the order of 45 to 50, the percentage of trapped plasma relative to the volume of the packed cell column is 1.84 (± 0.05)%. In the separated erythrocytes preparations, with hematocrits of 20, the figure is 1.55 (± 0.02)%.

Hemolysis determinations were made at the beginning and end of all experiments. In the 5 hour separated erythrocytes experiments, the rate of hemolysis averaged 1.86 (± 0.30)% per hour; in the 8 to 16 hour whole blood experiments, the mean value was 0.035 (± 0.007)% per hour.

Samples for pH and potassium concentrations of whole blood or buffer suspension and plasma or buffer were taken at the beginning and end of each experiment. In the separated erythrocyte and the early whole blood experiments, glucose determinations were also made at the beginning and end of each experiment. The methods used were those previously described by Solomon (2).

In the separated erythrocyte experiments, samples for counting were removed at 30 to 60 minute intervals. The samples were centrifuged immediately at 1280 g for 3 minutes, the supernatant removed, the cells resuspended in 50 to 60 times their own volume of isotonic saline at room temperature and centrifuged again for 3 minutes. This washing procedure was carried out a total of 3 times. Following the final washing, the cells were hemolyzed in distilled

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water and counted in a Texas Co. gamma ray counter.* Appropriate dead time corrections were made; due to the use of gamma rays, no self-absorption corrections were required. Before counting, plasma or buffer samples were pipetted into a volume of distilled water approximately equal to that of the cells in order to equalize the geometry of the samples. In the whole blood experiments, samples were removed at intervals of one half to four hours, centrifuged immediately, and the plasma samples removed with Misco micro pipettes for counting. Samples of $K^{42}Cl$ were checked for radiochemical purity by determining the half-life of the radioactive material present in each experiment.

Experiments were performed in order to determine the losses of cell radioactivity due to repeated washings in isotonic saline at room temperature. In Figure 1, the results of these experiments are plotted as the ratio

$$\frac{\text{cell radioactivity at any washing}}{\text{cell radioactivity after the third washing}}$$

against the number of washings. From the figure, two conclusions may be drawn. First, there is an appreciable change in the slope of the curve between the second and third washing, from which it may be concluded that most of the measurable radioactivity in the supernatant has been removed in three washings. Second, for the third washing and beyond, the curve is a straight line describing a 1.77% loss of cell radioactivity per washing, on the basis of a calculated value for cell radioactivity before the first washing. Thus the observed values for cell radioactivity in the separated cell experi-

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*We should like to express our thanks to the Texas Co. for their kindness in making this counter available to us.

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ments are 94.7% of the "true" value of cell activity existing before the washing procedure was carried out. This figure has been used to correct the raw data on cell counts prior to the calculation of the influx constants.

Rates of inward transport of potassium in the separated erythrocyte experiments were determined by two methods. Initially, the cell counts were plotted against time and a smooth curve drawn through the points by inspection. Let us take as the defining equation the following expression:

$$(1) \quad \frac{dP}{dt} = -k_1 \frac{v_{q_0}}{v_q} (1 - \rho) v_q p + k_2 \frac{v_{q_0}}{v_q} (1 - \rho) v_q q$$

where P = counts in the plasma of 1 ml of whole blood

p = concentration of K^{42} in plasma (counts/ml plasma)

q = concentration of K^{42} in cells (counts/ml cells)

t = time

v_q = hematocrit; $v_p = 1 - \text{hematocrit}$

ρ = fraction of cells hemolysed

The subscript o refers to initial time as in v_{q_0}

The tangents to the curve of P against t were obtained by inspection and equation (1) was solved using tangents at several independent points. K influx and efflux could be obtained from k_1 and k_2 . Referred to initial conditions,

$$(2) \quad K \text{ influx} = k_1 [K]_{p1} \text{ and } K \text{ efflux} = k_2 [K]_{\text{cell}}$$

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where $[K]_{pl}$ and $[K]_{cell}$ refer to total (stable plus radioactive) K concentrations in plasma and cell respectively.

After all of the separated cell experiments were calculated in this fashion, they were recalculated utilizing the method of Solomon (2) making the assumption that the system was in a steady state, with inward transport equal to the outward transport of potassium.

In the form applicable to cellular data, the equation reduces to

$$(3) \ln(1 - q/q_{\infty}) = -(k_{ab} + k_{ba} v_p/v_q)t$$

where q_{∞} refers to the value of q at infinite time and is given by

$$(4) q_{\infty} = p_o / ([K]_{pl} / [K]_{cell} + v_q/v_p)$$

The mean value of $[K]_{pl}$ and $[K]_{cell}$ during the experiment are used to determine q_{∞} . k_{ab} and k_{ba} are defined so that

$$(5) K \text{ influx} = k_{ab}(v_p/v_q)[K]_{pl} \text{ and } k_1 = k_{ab}(v_p/v_q)$$

$$K \text{ efflux} = k_{ba}(v_p/v_q)[K]_{cell} \text{ and } k_2 = k_{ba}(v_p/v_q)$$

In the steady state influx equals efflux and

$$(6) k_{ab}(v_p/v_q)[K]_{pl} = k_{ba}(v_p/v_q)[K]_{cell}$$

so that K influx and efflux may be obtained from equations (3) and (6).

The method of calculation of inward potassium flux for the whole blood experiments also is that of Solomon (2) and is the analogue of that used in the separated erythrocytes experiments, as adapted for plasma measurements.

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Although the graphical method does not itself involve any assumptions about the total exchangeability of all the intracellular K, such an assumption is made as soon as the results of the experiments with K^{42} are interpreted in terms of the stable isotope as is done in equation (2). Previous experiments in man (4) have shown that all the intracellular K is exchangeable and that the process can be described with a single rate constant. A single experiment in which dog cells were incubated under sterile conditions at 38° C. for 93 hours supports a similar assumption in the case of the dog erythrocyte, for at the termination of this experiment 94 percent of the cellular K was found to have exchanged with the K of the plasma. Under the conditions of this experiment, which involved the initial addition of large amounts of penicillin and streptomycin as an alteration of our customary procedure, there was no indication that the process of K exchange was described by more than a single rate constant, after allowance for the fast white cell component. Further assumptions inherent in the use of the graphical methods of solution, such as lack of discrimination between stable and radioactive K, mixing, and the effect of the beta radiation from K^{42} have been discussed previously (2).

The values of inward K flux as calculated by the tangent method do not differ significantly from those computed by the method involving the assumption of the steady state. In view of the appreciable hemolysis observed in these experiments, together with the marked changes in cellular K concentrations, which varied on the average (excepting experiment 15 with an ambient K concentration of 25 meq/l) by 15 percent from beginning to end of the experiment, it is surprising that the steady state method of calculation gives results

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not significantly different from those obtained by the graphical method. Although theoretically the graphical method would be preferred, we have adopted the steady state method because it provides an analytical solution that does not require graphical estimations of slopes.

RESULTS

Table 3 and Figure 2 gives the results of experiments with separated erythrocytes at 38.0°C, showing the dependence of inward potassium flux on ambient potassium concentration. On the assumption that the relationship is linear, it can be expressed by the following equation fitted by the method of least squares:

$$\text{K influx (meq/l cells hr.)} = 0.028 (+0.002) [\text{K}]_{\text{amb}} - 0.003 (+0.013),$$

where $[\text{K}]_{\text{amb}}$ is the ambient potassium concentration in meq/l medium.

At the physiological concentration of external potassium which, in the dogs we have used, is 4.06 (± 0.07) meq/l medium, the inward potassium flux is 0.11 meq/l cells hour. Utilizing our measured value for the concentration of cellular potassium, the half-time for complete exchange of cell potassium is 36.6 hours. In molecular terms, this rate of inward flux is equivalent to the transport of 1 potassium ion per hour for each 2230 square Angstroms of red cell surface, or a flux of 2.08×10^{-15} mols/(cm² sec). As shown in figure 2, the above value for inward potassium flux compares well with that determined from the data on two experiments of Sheppard, Martin, and Beyl (1) of 0.067 meq/l of cells per hour at an average ambient potassium concentration of 3.85 meq/l.

The results of determinations of the relationship between ambient potassium concentration and inward potassium transport by

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erythrocytes in uncentrifuged preparations are presented in Figure 3 and Table 4. Assuming the relationship to be a linear one and using least squares, the dependence can be expressed as:

$$K \text{ influx (meq/l cells hour)} = 0.022 (+0.007) [K]_{\text{amb}} - 0.024 (+0.043).$$

The slope of this line is within one standard error of that obtained in the separated cells experiments, and on the basis of a "t" test there is a probability of greater than one in twenty that the difference is due to chance alone.

The average value for cellular potassium concentration in the uncentrifuged cell experiments on the basis of eight determinations was 7.72 (+0.43) meq/l, while that in the separated cell experiments with 21 determinations was 5.85 (+0.91) meq/l. The difference is due to the absence of white cells.

Determinations of the apparent activation energy of inward potassium transport were made in seven of the separated erythrocyte experiments over a temperature range of 33.0 to 38.0°C. In two determinations at 4.8 meq/l external potassium concentration, the average value was found to be 14,100 calories per mol. Determinations were also made at values up to an ambient potassium concentration of 25.3 meq/l. The results are shown in Table 5. There is some indication, as can be seen from the arrangement of the table, that the apparent activation energy is a function of ambient K concentration, decreasing with increasing K concentration. Such behavior would not be unexpected if inward K transport were a result of more than a single process. However, the data show considerable scatter, and it is not possible to settle this point without further experimental evidence.

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DISCUSSION

The data on glucose consumption by separated erythrocytes indicates an average utilization of 2.0 mM of glucose per liter of red cells per hour at the physiological range of external potassium. This is to be compared with the utilization in man (2) of 2.33 mM glucose/hr 1 cells and in the rat (6) of 2.46 mM/hr 1 cells. In these three cases the glucose utilization appears to be species independent. Furthermore, by comparison of the dog with its low intracellular K with the other species with high intracellular K, it becomes apparent that the intracellular K concentration, at least, does not determine the rate of glucose utilization.

In man (1, 2, 4) K influx is independent of extracellular K concentration, and takes place at a rate (1.67 meq/1 red cells hr) approximately 17 times as fast as in the dog. The apparent activation energy for K influx in the human red blood cell is $12,500 \pm 1300$ cal/mol, and approximately 1 equivalent of K enters the human red cell for each mol of glucose consumed. In the dog, the K influx depends on K concentration, and the apparent activation energy is 14,100 cal/mol at physiological ambient potassium concentration. The mol for mol relationship between glucose utilization and K transport does not hold in the dog (nor in the rat as has already been pointed out by Weller and Taylor (6)). Until further information is available about the detailed mechanism of K transport in man, it is not possible to say whether K transport in the dog red cell is mediated by a different process, or by some variant in the human process perhaps resulting from the difference in intracellular K content.

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REFERENCES

1. Sheppard, C. W., Martin, W. R., and Beyl, G., J. Gen. Physiol., 1951, 34, 411.
2. Solomon, A. K., J. Gen. Physiol., 1952, 36, 57.
3. Davson, H., and Danielli, J. F., Biochem. J., 1938, 32, 991.
4. Raker, J. W., Taylor, I. M., Weller, J. M., and Hastings, A. B., J. Gen. Physiol., 1950, 33, 691.
5. Chaplin, H., and Mollison, P. L., Hematology, 1952, 7, 1229.
6. Weller, J. M. and Taylor, I. M., Proc. Soc. Exp. Biol. Med., 1951, 78, 780.

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TABLE 1
Composition of Buffer**

	gm/l	mM/l
NaCl	5.913	101.16
MgCl ₂ .6H ₂ O	0.203	1.00
CaCl ₂	0.056	0.50
Na ₂ HPO ₄	0.242	1.70
NaHCO ₃ *	2.350	27.97
KCl	0.298	4.00
KH ₂ PO ₄	0.057	0.42
Glucose	2.500	13.88

*Added as Na₂CO₃ (1.484 gm/l) and converted to NaHCO₃ by passing 100% CO₂ through the solution.

**Before using the buffer 5% CO₂-95% air or oxygen is passed through it to bring it to pH 7.4. Potassium concentration is varied by substituting the appropriate potassium salt for one of the sodium salts.

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TABLE 2

Percentage of Trapped Plasma in Hematocrit Determinations
Using I-131 Labelled Albumin

Experiment Type	Trapped Plasma or Buffer		Average
	Experiment	ml plasma/100 ml packed cells	
Whole Blood	1	1.89	1.84 (± 0.05)
	2	2.06	
	3	1.99	
	4	2.04	
	5	1.93	
	6	1.75	
	7	1.96	
	8	1.91	
	9	1.68	
	10	1.59	
	11	1.60	
	12	1.70	
Separated Erythrocytes	1	1.49	1.53 (± 0.02)
	3	1.58	
	5	1.47	
	7	1.55	
	11	1.54	

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TABLE 3

The Dependence of Inward Potassium Flux at 38.0°C on Ambient Potassium Concentration in the Centrifuged Dog Erythrocyte

Experiment	Mean [K] _{amb} meq/l medium	Mean [K] _c meq/l cells	k _{ab}	Inward Flux meq/l cells hr.
9-A	2.88	5.12	0.0087	0.096
9-B	2.92	5.07	0.0083	0.093
18-A	4.75	5.95	0.0035	0.076
12-A	4.78	5.34	0.0111	0.204
12-B	4.81	5.34	0.0101	0.197
9-D	7.63	4.45	0.0078	0.228
9-C	7.65	4.28	0.0084	0.243
14-B	8.08	7.13	0.0048	0.162
14-A	8.11	7.23	0.0046	0.153
19-B	8.38	6.62	0.0058	0.171
19-A	8.39	6.49	0.0055	0.168
13-A	10.5	6.64	0.0050	0.223
13-B	10.5	6.82	0.0050	0.221
10-A	11.6	5.12	0.0092	0.426
10-B	11.7	4.87	0.0086	0.402
21-C	15.8	5.66	0.0081	0.447
21-D	15.8	5.95	0.0081	0.453
18-F	20.0	4.39	0.0051	0.550
18-E	20.2	3.51	0.0061	0.632
15-A	25.0	8.50	0.0067	0.644
15-B	25.1	8.42	0.0068	0.704

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TABLE 4

The Dependence of Inward Potassium Flux at 38.0°C on Ambient Potassium Concentration in the Uncentrifuged Dog Erythrocyte

Experiment	Mean [K] _{amb} meq/l medium	Mean [K] _c meq/l cells	k _{ab}	Inward Flux mEq/l. cells hour
23-A	5.14	7.67	0.0135	0.079
22-B	5.19	6.27	0.0205	0.088
22-A	5.21	6.26	0.0131	0.056
21-3	13.8	7.37	0.0187	0.255
21-A	13.9	7.45	0.0246	0.344
22-C	17.7	8.27	0.0307	0.508
22-D	17.7	8.42	0.0292	0.468
23-C	20.2	10.1	0.0088	0.216

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TABLE 5

The Apparent Activation Energy of Inward Potassium Flux
Between 38.0 and 33.0°C. in the Dog Erythrocyte

Experiment	Mean [K] amb meq/l medium	App. E _{act} (cal/mol)
18	4.75	13,610
12	4.79	14,590
14	8.10	8,450
19	8.38	3,950
13	10.6	4,300
18	19.9	9,350
15	25.3	0

Average 7,750 ($\pm 2,010$)

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LOSS OF CELL K^{42} BY WASHING

Figure 1

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VARIATION OF K INFLUX WITH $[K^+]_{amb.}$
IN SEPARATED DOG ERYTHROCYTES

Figure 2

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VARIATION OF K INFLUX WITH $[K^+]_{amb.}$
IN UNSEPARATED DOG ERYTHROCYTES

Figure 3

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