THE PERMEABILITY OF THE HUMAN ERYTHROCYTE TO SODIUM AND POTASSIUM

By A. K. SOLOMON

(*From the Biophysical Laboratory of Harvard Medical School, Boston*)

(Received for publication, March 13, 1952)

The present experiments have been undertaken to measure the permeability of the human erythrocyte to sodium and potassium; to determine the temperature coefficient of these two permeabilities; and to investigate the relationship between external concentration of sodium and potassium and their relative permeabilities. In addition, thermodynamic constants of these two processes have been measured where possible. It is in all cases desirable to make experimental observations when the cell is in a steady state, so that the system remains as independent as possible of the disturbance caused by the observation. The radioactive isotopes of sodium and potassium, Na^{24} and K^{42}, are admirably adapted for studies of this nature, as has been previously pointed out by Raker, Taylor, Weller, and Hastings (1) and Sheppard, Martin, and Beyl (2–4) in their studies on the permeability of the human erythrocyte to these elements.

I

Experimental Method

The experiments were carried out with fresh heparinized whole blood to which the necessary small quantities of isotonic solutions of glucose, $Na^{24}Cl$, and $K^{42}Cl$ were added. In some cases, the blood was diluted with a buffer of the composition given in Table I as used by Raker *et al.*, except for the addition of glucose. In cases in which the effect of Na concentration was studied, isotonic sucrose (or melezitose or raffinose) with the requisite admixture of isotonic NaCl (or other chlorides) served as diluent. For the K studies, the cells were incubated in a shaker under 5 per cent CO_2 (with oxygen or air) at temperatures in the neighborhood of 37°C. for periods of 4 hours. For the Na studies, the incubation continued for 10 or 12 hours. The incubation vessels were especially designed to keep the whole system including the vapor phase under the surface of the water bath. The gas mixture was saturated with water vapor first at room temperature by flowing through a bubbler filled with water at room temperature, and second at bath temperature by passing through another bubbler which was totally immersed under the surface of the bath. The gas did not emerge above the surface of the bath until it was exhausted from the reaction chamber. These precautions were sufficient to insure that the blood did not gain or lose any water, as indicated by measurements of the Na and K concentrations of the blood

at the beginning and end of the experiment. The maximum excursion between these figures was within the error of the method of measurement.

In the K series plasma samples were taken at 30 minute intervals for counting. Glucose concentration, pH, hematocrit values, and K concentration (whole blood and plasma) were measured at the beginning and end of the experiment. The Na uptake experiments were initially similar, but later were extended to 10 to 12 hours, with counting samples taken from the cellular fraction. Initial samples were taken at 45 minute spacing, later ones at 1 to 2 hour spacing.

For the initial runs the temperature of the water bath was held constant to ±0.5°C. After run 32, the temperature control was improved so that it could be maintained constant to ±0.2°C.; by run 43 it could be kept constant to better than 0.02°C. Tem-

TABLE I

*Composition of Buffer**

	gm./l.	mM/l.
NaCl	5.913	101.16
$MgCl_2 \cdot 6H_2O$	0.203	1.00
$CaCl_2$	0.056	0.50
Na_2HPO_4	0.242	1.70
Na_2CO_3	1.484	14.00
KCl	0.298	4.00
KH_2PO_4	0.057	0.42
Glucose	2.500	13.88

* 5 per cent CO_2-95 per cent air or oxygen is passed through the buffer to bring it to pH 7.4.

perature was measured by mercury thermometers calibrated against a Bureau of Standards certified thermometer, and in some cases temperature differences were measured by paired Beckman thermometers.

Hematocrit Determination.—

Since the determination of the hematocrit is so crucial to calculations of cellular concentrations of Na and K, special precautions were taken to obtain accurate values. From data given by Parpart and Ballentine (5), it can be seen that cells continue to shrink under increasing acceleration, even up to 190,000 *g*. Consequently, the hematocrit value as observed at any acceleration cannot be said to provide the "true" value. The variance of the results of the determination of "true" hematocrit values obtained by the different investigators mentioned in Ponder (6), and in later results obtained by others, has made it desirable to make independent measurements in connection with this investigation. Since it will be shown that Na^{24} enters the red cell very slowly, the "true" hematocrit reading can be obtained by the addition of a trace amount of isotonic $Na^{24}Cl$ to whole blood, followed by measurement of the radioactivity of the sample of blood, and of a sample of plasma separated from it. This ratio gives directly the amount of plasma in the blood. Two sources of error need to be considered. First, it is necessary to show that the rate of entrance of Na^{24} into the

cells is negligible in the time limit allowed. Since on the average 2.21 per cent of the plasma Na enters the cells per hour, and in general less than 10 minutes elapsed between the addition of the Na^{24} and the beginning of centrifugation, this error may be assumed to be less than 0.4 per cent. Furthermore, it is in the direction of overestimation of trapped plasma, and from comparison with the results of others, our values appear to be lower, not higher. A second source of error may arise from differential self-absorption of the Na beta particles in the blood and plasma; controls were carried out on each experiment to determine the self-absorption correction factor.

It is, of course, necessary to spin the hematocrit tubes long enough to insure complete packing at the centrifugal forces employed. 50 minutes suffices in the range of 1600 to 2100 *g* used in these experiments. An International Equipment Company clinical centrifuge was used at voltages from 100 to 105 volts D.C. A conical head (No. 802) at 100 volts D.C. gives an acceleration of 1690 *g* measured to the midpoint of the hematocrit tube; a normal head (No. 213) using small tube shields (No. 301) gives an acceleration of 1610 *g* at 105 volts D.C. at the same position. Speeds were measured with a Jagabi tachometer, as calibrated by a stroboscope, or by a Jagabi revolution counter and stop watch (chronometric tachoscope).

Experiments H-1 through 3, carried out at 1690 *g* using the indirect method described above, give a value as shown in Table II of 2.4 ± 0.6 ml. plasma trapped per 100 ml. packed red cells. Experiments H-13 through H-16 were carried out by direct measurement at 1610 *g* of the amount of Na^{24} trapped by the red cells in a breakable Pyrex tube of inside diameter 3 mm. equal to the inside diameter of the standard Wintrobe tube. After centrifugation the tubes were broken, and the red cell radioactivity measured directly by a dip counter, thus obviating the self-absorption correction. Although the error in the counting is greater in a dip tube than in an end window tube, this procedure had the advantage of direct measurement, that is the amount of trapped plasma was measured directly rather than obtained from the ratio of two nearly equal numbers. The value obtained from this set was 2.97 ± 0.34 ml. plasma trapped per 100 ml. packed red cells.

In connection with experiments to determine the Na content of red cells, it was necessary to measure the amount of plasma trapped by the cells in a tube of special design: a 10 mm. inside diameter tube drawn down to a thin waist of about 3 to 5 mm. inside diameter just below the top of the red cell portion. The results of experiments H-5 through H-12B give the amount of plasma retained in these tubes at 1610 *g*. The average value obtained 2.89 ± 0.24 ml. plasma retained per 100 ml. of packed red cells is not significantly different from that obtained by the 3 mm. I.D. straight tubes, and consequently both sets were averaged to give the figure of 2.92 ± 0.27 ml. plasma retained per 100 ml. of packed cells which has been used for all the experiments at 1610 *g*. The three experiments, H-12, A, and B, show that the addition of standard buffer to reduce the viscosity of the suspending medium does not affect the amount of plasma retained.

The error of about 0.3 ml. per 100 ml. in the trapped plasma figures, is, of course, a much smaller relative figure when considered as per cent of the hematocrit value. If one expresses the correction factor, "*r*," as the ratio of the true hematocrit value to the observed hematocrit value, the value at 1610 *g* is 0.971 ± 0.003 or 0.3 per cent.

Measurements were not made at 1850 *g*; an approximate figure can be obtained

TABLE II

Trapped Plasma in Hematocrit Tubes and "Waisted" Tubes

Experiment No.	Donor	Centrifugal force in	Plasma trapped	Head	Tube	Comments
		g	*per cent*			
H-1	—	1690	1.8	Conical	Wintrobe	
H-2	—	1690	2.7	"	"	
H-3	S	1690	2.6	"	"	
Average.....			2.4 ± 0.6			
H-5	Fra	1610	3.33	301 shield	Waisted	
H-6	All	1610	2.98	" "	" 10 mm.	
H-7	Mal	1610	2.95	" "	" " "	
H-8	Lie	1610	2.77	" "	" " "	
H-9	Ald	1610	2.54	" "	" " "	
H-10	Mey	1610	2.72	" "	" " "	
H-11	Ham	1610	3.10	" "	" " "	
H-12	Fra	1610	2.73	" "	" " "	Hematocrit value = 0.402
H-12A	Fra	1610	3.08	" "	" " "	Hematocrit value = 0.276 buffer added
H-12B	Fra	1610	2.72	" "	" " "	Hematocrit value = 0.151 buffer added
Average (H-5 through H-12).			2.89 ± 0.24			
H-13	Gar	1610	2.95	301 shield	3 mm. I.D.	
H-13A	Gar	1610	2.71	" "	" "	
H-14	Har	1610	3.56	" "	" "	
H-15	Bra	1610	2.72	" "	" "	
H-16	Mur	1610	2.91	" "	" "	
Average (H-13 through H-16).			2.97 ± 0.34			
Average (H-5 through H-16).			2.92 ± 0.27			

from measurements giving the hematocrit value of the same blood centrifuged at 1850 *g* and at 1690 *g*. The experimental value of the ratio $\frac{1690\ g\ \text{hematocrit}}{1850\ g\ \text{hematocrit}} = 1.008$, agrees very well with the value of 1.007 which is obtained from measurements of Millar (7) giving relative hematocrit value as a function of centrifugal force.

Sodium, Potassium, and Other Determinations.—

Na and K concentrations were measured on a Perkin-Elmer Model 52A flame photometer. In order to determine the accuracy of these determinations, quantitative graded amounts of NaCl and KCl were added to samples of whole blood and plasma. Determinations were taken in quintuplicate, and the average error in recovery for the whole blood samples was 1.25 per cent for Na and 1.38 per cent for K. For plasma, the average error in recovery was 1.50 per cent for Na and 1.58 per cent for K. Under our conditions of dilution, the additional contribution of 150 m.eq./liter of Na (reagent grade or spectroscopically pure) to the measured value of K in the range of 5 m.eq. K/liter, was 1 per cent of the K value. In view of the fact that this correction is so close to our experimental error, it has not been subtracted from the observed plasma K concentrations.

Plasma Na and K were determined directly on diluted samples (measured in a dilution of 1:50). Cell K was determined from the whole blood K (measured in a dilution of 1:100) and the corrected hematocrit reading. Cell Na was initially determined in the same fashion. However, following Experiment 73, it appeared desirable to measure cell Na directly owing to the large errors inherent in the indirect method in a system of high extracellular Na and low intracellular Na. A procedure was adopted in which the cells were spun down for 50 minutes at 105 volts D.C. in the special constricted tube already described in the hematocrit determination section. In these tubes the constriction fell just below the top of the red cell layer. After centrifugation, the tube was broken at the constriction, and the cell Na determined directly (at a dilution of 1:50).

The figure of 0.0292 ± 0.0027 ml. plasma trapped per ml. of packed cells has been used in estimating the correction. The following expression has been used to obtain the "true" Na concentration in the red cell:

$$\text{"True"}\ [\text{Na}]_{\text{cell}} = \frac{[\text{Na}]_{\text{cell observed}} - (1 - r)[\text{Na}]_{\text{plasma}}}{r}$$

in which r is the ratio of the true hematocrit determination to the observed hematocrit determination.

Glucose was measured by the Somogyi-Nelson method (8). pH was measured on a Cambridge meter and usually dropped by about 0.2 pH unit over a 4 hour period in whole blood. Hemolysis was measured initially in a Klett photometer, and later in a Beckman model B spectrophotometer at 416 mμ. In general, final hemolysis was less than 0.4 to 0.5 per cent (in the later experiments less than 0.2 per cent) and hence no corrections have been made for it. Hemolysis measurements were carried out as a control on all but a few of the experiments, although the human eye is itself a remarkably sensitive control, and visual estimation will, in general, suffice.

Purification of Radioactive Materials.—

Two physical methods were adopted to check on the purity of the isotopes as supplied from Oak Ridge, measurement of half-life and of beta ray absorption in aluminum. It is a routine procedure in our laboratory to subject all isotopes received

to a single point absorption analysis, in order to detect any gross mistake in labelling, and this procedure has been followed for all experiments.

K^{42}.—This isotope was received from Oak Ridge each week on Tuesday and usually decayed on a straight line curve with a proper half-life until Friday. Aluminum absorption curves showed a negligible shift from day to day. K^{42} was purified by absorption on a Dowex-50 column and subsequent elution by 0.15 N HCl according to the method of Cohn and Kohn (9). The absorption curve of the purified isotope was in agreement with that obtained with the non-purified material, and the half-life was in good agreement with the value of 12.44 hours given by Siegbahn (10). Rough half-life checks were made as routine in the course of most of the experiments.

Na^{24}.—This isotope as initially received from Oak Ridge was quite impure, containing a longer half-life contaminant whose identity is unknown. As a consequence, Na^{24} was purified as routine by absorption on a Dowex-50 column and subsequent elution with 0.15 N HCl according to the method of Cohn and Kohn (9). In order to free the eluate from any anionic contamination, and also to neutralize it without any addition of alkali, the eluted material was passed through a column of Amberlite IR-4b. The half-life of the resultant purified material which followed a logarithmic course for 13 half-lives appeared to differ significantly from the accepted value of 14.8 hours determined by Van Voorhis (11). Consequently, the half-life was redetermined on purified material. The half-life obtained (12), 15.04 $\pm$ 0.06 hours, agrees within its probable error with the more recent determination of 15.10 $\pm$ 0.04 hours obtained by Cobble and Atteberry (13) and that of 15.060 $\pm$ 0.039 obtained by Sreb (14).

The purification of Na^{24} by cationic and anionic exchange was continued until the samples from Oak Ridge were so pure that no significant difference could be observed between their absorption curve and that of the pure material. More recently, Na^{24} has been obtained from the Brookhaven National Laboratory. These samples appear to be pure as tested by the above criteria.

Counting Techniques.—

0.05 and 0.1 ml. samples of plasma and whole blood are pipetted into the center of small aluminum counting cups and counted directly. In the early stages, counts were made on a standard end window Geiger counter and appropriate dead time corrections were made. In the later stages, all plasma and whole blood counts were made on the proportional windowless counter developed by Robinson (15), which is free from dead time corrections. In general, counts were made to a 1 per cent probable error. Some samples were counted only to 2 per cent probable error, but this figure was never exceeded unless the exigencies of work with the short half-life material demanded it. No self-absorption corrections were necessary since only samples of equivalent self-absorption were compared. The reproducibility of potassium samples as measured by determining the standard deviation of sets of 8 to 10 duplicates was about 1.5 per cent. Since the Na^{24} beta is much softer than the K^{42} beta, it was expected that self-absorption in the plasma would make the Na results less reproducible than the K ones, as was found to be the case (standard deviation about 2 per cent in sets of 10 duplicates).

Na^{24} determinations in the red cell fraction were made using the dip counter previously described (16). Duplicate samples could not be obtained since sufficient blood was not available; in general, the accuracy was poor compared with the plasma determinations, being of the order of 3 per cent (standard deviation 2.9 per cent in 15 duplicates). Error in direct determination of red cell Na^{24} is of much less importance than error in plasma Na^{24} determination, since only 2.21 per cent of the plasma Na^{24} enters the red cell each hour, whereas 60 per cent of the freely exchangeable cell Na^{24} is exchanged with plasma every hour. Consequently an error of 3 per cent in red cell Na^{24} determination is equivalent to an error of less than 0.2 per cent in plasma Na^{24}.

Sodium and Potassium Concentration in the Normal Red Blood Cell.—

Na and K concentrations of the normal red cell were measured as routine in the early experiments and sporadically thereafter. Cell K and Na were measured by difference through Experiment 73; following this experiment, cell Na was measured directly. The results are given in Table III. The mean cell Na obtained by difference, 9.16 ± 4.98 m.eq./liter of red cells, agrees well with the mean value of 8.66 ± 1.40 obtained in the direct measurement. The standard deviation of the figures provides a good index of the increased accuracy of the direct method. If one arbitrarily assigns standard deviations of 2 per cent to whole blood Na and plasma Na, and 1 per cent to the hematocrit reading, one can calculate that the standard deviation of the cell Na determined by the indirect method should be 4.86 m.eq./liter which accounts satisfactorily for the large standard deviation obtained by this method.

Keitel (17) has also measured cell Na concentration directly. His figure of 15.9 m.eq./liter cells, uncorrected for trapped plasma, is higher than our comparable figure of 12.64. He has pointed out that the top fraction of cells is richer in Na than the lower fraction. In our estimations we usually discarded the top 10 per cent or so of the red cells, and hence some of the difference between the uncorrected cell Na figures may be ascribed to our having taken a non-uniform sample.

II

Experiments on K Transport

Equations Governing the Transport of K into the Red Cell.—

The general form of the equations that follow has already been put forward (18). The equations used in describing the transport of potassium in the steady state do not differ formally from those already presented by Raker *et al.* (1), and by Sheppard and Martin (2). Let us define the system by the following equation:

$$dp/dt = -k_{ab}p + k_{ba}q \tag{1}$$

TABLE III

K and Na Concentration for Normal Blood—(0.05 Ml. Heparin Added)

Experiment No.	Donor	Concentration, *m.eq./l. cells or plasma*						Hematocrit reading
		K		Na		Uncorrected Na New method	Corrected Na New method	
		Cells	Plasma	Cells	Plasma	Cells	Cells	
								per cent
33	Mal	91.4	4.75	6.3	146.0			48.3
34	Ott	90.6	5.27	13.0	143.0			48.3
35	Mal	94.6	4.40	—	—			47.7
36	Ott	96.6	5.15	0	152.2			45.1
37	Ott	90.9	4.90	8.3	143.1			47.2
38	Mal	90.2	4.60	7.2	143.3			48.7
39	Ott	89.2	4.71	—	—			46.6
40	Ott	91.1	4.91	15.6	147.1			44.9
41	Eis	93.8	5.05	9.5	143.3			44.7
42	Ott	92.4	4.55	11.4	147.4			45.8
43	Mal	97.8	5.09	5.1	149.6			49.0
44	Ott	94.0	4.10	17.9	138.4			46.4
45	Mal	94.0	4.80	10.8	139.0			48.2
46	Ott	91.3	4.64	13.0	141.7			48.4
47	Mal	97.0	5.40	9.8	145.2			48.9
50	Ott	95.0	4.50	6.6	142.0			47.7
51	Kr	94.1	4.19	12.4	140.7			48.0
52	Ott	92.9	3.98	0	147.5			46.8
53	Ga	100.9	3.90	8.4	142.4			49.4
55	Hug	100.4	3.88	2.2	137.6			—
70	Fra	—	—	6.8	143.4			—
73A	Mal	98.9	4.85	10.5	143.5			—
74	Mal	—	—	13.8	144.5			—
75	Sic	—	—	—	142.4			—
76	Katz	—	—	—	—	11.7	7.75	—
77	VanS	—	—	—	144.4	14.8	10.90	—
79	Mal	—	—	—	143.8	11.74	7.77	—
80	Fra	—	—	—	—	13.08	9.17	—
81	Cal	—	—	—	—	10.15	6.15	—
84	Roth	—	—	8.5	143.1	13.0	9.08	43.5
85	Nor	—	—	—	143.3	15.15	11.29	—
86	Ozer	—	—	—	142.1	13.60	9.73	—
87	Mav	—	—	—	142.4	13.24	9.35	—
Apr. 10	x	—	—	20.1	141.0	10.5	6.58	44.7
Apr. 11	x	—	—	7.6	—	12.75	8.56	47.7
Apr. 12	x	—	—	4.1	—	12.2	7.79	49.2
May 4	Mal	—	—	—	144.0	12.3	8.34	49.5
May 16	Ste	—	—	—	142.6	12.73	8.83	—
Average		94.1	4.65	9.2	143.6	12.64	8.66	47.3
Standard deviation		±3.3	±0.44	±5.0	±3.0		±1.40	

in which p is the concentration of K^{42} in the plasma, q its concentration in the cells, and t is time. This is the normal form for chemical equations in which the driving force for the reaction is the concentration gradient. Before defining the two transfer coefficients,[1] k_{ab} and k_{ba}, it is helpful to rewrite the equation. By definition, $p = P/v_p$, and $q = Q/v_q$, in which v_p and v_q are the respective volumes of the plasma and cell compartments, P is the amount of K^{42} in the plasma compartment, and Q is the amount of K^{42} in the cellular compartment. Therefore:

$$dP/dt = -k_{ab}v_p p + k_{ba}v_p q \tag{2}$$

We have now transferred the equation into one which gives us the amount of radioactive material leaving the plasma per unit time as a function of the concentration gradient across the cell membrane.[2] This transformation is necessary because we must compare systems of widely differing hematocrit values. This step frees the system from dependence on the size of the plasma compartment. In order to normalize the system so that it is also independent of the cell volume, we may divide by v_q and obtain:

$$dP/v_q\,dt = -k_{ab}(v_p/v_q)p + k_{ba}(v_p/v_q)q \tag{3}$$

in which $dP/v_q dt$ represents the amount of radioactive material leaving the plasma per unit time per unit volume of cells, $k_{ab}(v_p/v_q)$ is the fraction of the plasma K^{42} concentration leaving the plasma per unit time per unit volume of cells, and $k_{ba}(v_p/v_q)$ is the fraction of the cell K^{42} concentration leaving the cell per unit time per unit volume of cells.

The solution to equation (1) is:

$$p = \frac{k_{ba}\,p_0(v_p/v_q)}{k_{ab} + k_{ba}v_p/v_q}\left[1 + \left(\frac{k_{ab}v_q}{k_{ba}v_p}\right)e^{-(k_{ab} + k_{ba}v_p/v_q)t}\right] \tag{4}$$

in which p_0 is the initial concentration of K^{42} in plasma. Now when t goes to infinity, p will equal the term outside the brackets, or

$$p_\infty = k_{ba}\,p_0(v_p/v_q)/(k_{ab} + k_{ba}v_p/v_q) \tag{5}$$

and, transposing and taking the logarithm

[1] It must not be assumed that k_{ab} and k_{ba} are specific rate constants. In succeeding sections it will appear that k_{ab} is a function of $[K]_{pl}$; a possible relation between k_{ab} and certain specific rate constants will be derived in connection with the working hypothesis to be presented.

[2] Equation (2) can be transformed into a form analogous to Equation 3-2 of reference 18, since:

$$dP/dt = -k_{ab}P + k_{ba}(v_p/v_q)Q$$

so that k_{ba} in Equation 3-2 equals $k_{ba}(v_p/v_q)$ in our present terminology. For comparison, $k_{ba}(v_p/v_q) = k_2$ in the terminology of Raker *et al.* (1), and $k_{ba}(v_p/v_q) = P/S_2$ in the terminology of Sheppard and Martin (2).

$$\ln (p/p_\infty - 1) = -(k_{ab} + k_{ba}v_p/v_q)t - \ln (k_{ba}v_p/k_{ab}v_q) \tag{6}$$

To determine k_{ab} and k_{ba} we must plot $\ln (p/p_\infty - 1)$ against time and find the slope (which is negative), called S, and the zero time intercept, called I, of this straight line. Then:

$$k_{ba} = \frac{-S}{\frac{1-H}{H}(I+1)}$$
$$k_{ab} = -S - k_{ab}\frac{1-H}{H} \tag{7}$$

where H = hematocrit value = v_q and $1 - H = 1 -$ hematocrit value = v_p. To find p_∞, we take advantage of the observations of Raker *et al.* (1) and Sheppard and Martin (2) that all the red cell K is exchangeable under the conditions of experiments similar to ours. Then at infinite time the specific activity inside the cell will equal the specific activity in the plasma. Therefore, p_∞ will be given by

$$p_\infty = p_0/[1 + [\mathrm{K}]_{\mathrm{cell}}H/[\mathrm{K}]_{\mathrm{pl}}(1 - H)] \tag{8}$$

in which $[\mathrm{K}]_{\mathrm{cell}}$ and $[\mathrm{K}]_{\mathrm{pl}}$ are the total concentrations of labelled plus unlabelled K in cell and plasma respectively. Since the latter can be measured chemically, p_∞ can be found.

Certain assumptions are inherent in this mathematical treatment. It is assumed that the experimental system does not discriminate between radioactive and stable potassium, and further that the addition of the radioactive material does not materially disturb the system. The first assumption is supported by the fact that the relative K^{40} (the natural radioactive isotope of K) content of K derived from mineral and biological sources is identical within 0.5 per cent as measured by Mullins and Zerahn (19). The second assumption is supported by the small quantity of radioactive material added, in general about 0.2 mc. The radiation produced by 0.2 mc. in 100 ml. of solution is less than 50 rep. Sheppard and Martin (2) have irradiated one of their preparations with 1200 r of gamma radiation and observed no change from normal. Further, it is assumed that the concentrations of K in plasma and cell are uniform. The plasma is mixed by continual shaking, which may also contribute to mixing the cell contents. It is not absolutely necessary that the cells have a uniform concentration of K^{42}; it suffices that any diffusion effects within the cell be rapid compared to the particular step which we are studying, namely entrance into the cell. This assumption is supported by the fact that there is no evidence from the K^{42} exchange studies of more than a single rate-determining step.

The volume of the system enters the equations explicitly. As already mentioned, the volume of the system is measured by chemical determinations of

whole blood K concentration at the beginning and end of the experiment. These values agreed within experimental error of the determination. Changes in the relative volume of the cell and plasma compartments are given directly by the initial and final hematocrit values, which usually agreed within 2 per cent. The non-radioactive potassium concentrations of the cell and plasma enter the equations only through p_∞. It is possible to solve equation (6) with a knowledge of p_0, p_∞, and a single determination of p at any time t. The straight line obtained when $\ln (p/p_\infty - 1)$ is plotted against t is thus an average of data sufficient for many separate determinations of the constants k_{ab} and k_{ba}. By adjusting the p_∞ for each value of p to the concentrations, obtained by interpolation, at that specific time, it is possible to minimize errors

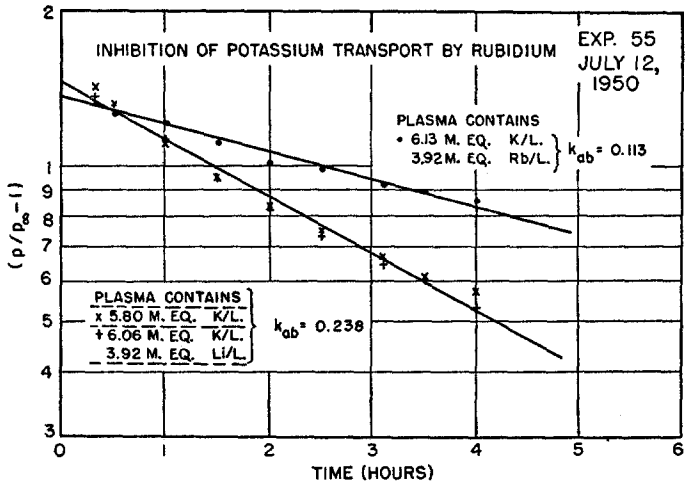


FIG. 1

arising from the changing concentrations of K in cell and plasma. In general, plasma potassium at normal levels fell by about 0.5 to 1 m.eq./liter over the course of the experiment, and cell K remained unchanged or gained about 1 m.eq./liter. Thus the percentage changes in cell K are negligible, while those of plasma K are equivalent to a drop of 10 to 20 per cent. Fig. 1 shows a typical graph of the data from Experiment 55, plotted according to equation (6).

Normal K Transport.—

The data for the normal transport of K across the red cell membrane at 37°C. are given in Table IV. Column 3, headed $k_{ab}(1 - H)/H$, gives the fraction of the cell potassium concentration transported per hour per liter of red cells. The average obtained from 18 experiments is 0.0195 ± 0.0019 or 1.95 per cent of the cell K. This figure is to be compared with a value of 0.0163 ± 0.0017 given by Raker *et al.* (1) and a value of 0.018 at 38°C. given by Shep-

pard and Martin (2). The two latter values agree very well among themselves. The former was obtained with cells incubated in an artificial medium, the latter with essentially whole blood, so it is difficult to account for the disagreement of the present results. However, the several values lie within about one and a half standard deviations.

TABLE IV

Transfer of K across the Normal Red Cell Membrane at 37°

Experiment No.	Hematocrit reading	$k_{ab}\frac{1-H}{H}$	$k_{ba}\frac{1-H}{H}$	Plasma K concentration	Inward K flux*	Glucose utilization
	per cent			*m.eq./l.*	*m.eq./l. cells hr.*	*mM/l. cells hr.*
30‡	48.6	0.110	0.0152	12.45	1.37	1.42
31	51.1	0.281	0.0189	5.83	1.64	1.62
32	49.9	0.242	0.0185	7.05	1.71	1.77
33	50.7	0.291	0.0214	6.40	1.37	1.78
34	48.7	0.229	0.0183	6.77	1.55	2.86
35	48.8	0.366	0.0235	5.80	2.10	3.55
36	46.9	0.303	0.0208	6.30	1.85	3.38
39	47.9	0.284	0.0207	5.99	1.71	2.08
43‡	48.4	0.278	0.0205	6.00	1.68	
44‡	46.9	0.328	0.0208	4.95	1.62	
45‡	48.4	0.277	0.0198	5.80	1.61	
50‡	48.1	0.196	0.0214	9.51	1.87	
54‡	40.3	0.289	0.0184	5.08	1.47	
55	48.2	0.256	0.0159	5.93	1.51	2.44
56‡	48.2	0.229	0.0189	6.59	1.76	2.18
57‡	41.9	0.266	0.0193	6.60	1.76	2.86
59	42.7	0.384	0.0186	4.50	1.73	2.43
71	49.6	0.302	0.0196	5.78	1.75	1.91
Average			0.0195		1.67	2.33
Standard deviation			±0.0019			

* Flux in this and subsequent tables is used in the sense of flux in one direction, unless otherwise stated.

‡ These experiments have been corrected to a temperature of 37°C.

The temperatures of Experiments 30, 56, and 57 lie within 1° of 37°; corrections to that temperature were made by use of the mean activation energy. Experiments 43, 44, 45, 50, and 54 comprise the set from which the activation energy was determined, and include pairs of observations taken a few degrees higher and a few degrees lower than 37°. The results in the table were obtained by graphical interpolation between the higher temperature and lower temperature values.

In molecular dimensions, we may calculate that 1 molecule is transported per hour per 131 square Angstroms of red cell surface, or we can state that

the K flux is 8.0×10^{-14} mols/cm.^{2} sec. The half-life for complete exchange of intracellular K is 35.5 hours. The average glucose consumption is about 2.33 mols per hour per liter of red cells, appreciably higher than the figure of 1.4 found by Raker *et al.* (1).

TABLE V

Apparent Activation Energy for K Transfer into the Red Cell

Experiment No.	Temperature	k_{ab}*	k_{ba}*	Activation energy	
				k_{ab}	k_{ba}
	°C.				
43	38.82	0.287	0.0212	9,906	10,810
	33.98	0.223	0.0160		
44	38.91	0.331	0.0207	12,641	11,628
	33.97	0.239	0.0153		
45	38.91	0.294	0.0211	11,883	12,890
	33.97	0.216	0.0151		
50	38.91	0.211	0.0227	13,410	13,000
	33.99	0.149	0.0162		
54	38.91	0.224	0.0142	13,219	13,520
	33.97	0.159	0.0100		
Average				12,211 ± 1400	12,369 ± 1300
Average (in and out)				12,300 ± 1300	

* Since the experimental conditions provided identical initial hematocrit values for the higher and lower temperatures, k_{ab} and k_{ba} need not be multiplied by $\frac{1-H}{H}$ for the determination of the activation energy.

Apparent Activation Energy for K Transport.—

The results of five experiments to determine the apparent activation energy of the processes of entrance and exit are given in Table V. Temperatures of 34 and 39° were chosen to be as close as practicable to the normal physiological range. The apparent activation energy in each experiment was obtained from the ratio of the transfer coefficients. Since the apparent activation energy for entrance and that for exit are identical within experimental error, the mean value of 12,300 ± 1300 calories/mol may be taken as the result of these experiments. This figure represents the activation energy that would be required for a hypothetical single step reaction which would transport the K into the cell, and another hypothetical single step reaction which would transport the K out.

The apparent activation energy is not directly related to Q_{10} which is a temperature-dependent constant. However, by specifying the temperature range as 27–37° the results of Raker *et al.* (1) ($Q_{10} = 2.2 \pm 0.2$) can be transformed into an apparent activation energy of 14,500 ± 1300 calories/mol. Sheppard and Martin (2), in a single experiment, give a Q_{10} of 2.35 which in the same temperature range is equivalent to an apparent activation energy of 15,800 calories/mol. Ponder (20) obtains a Q_{10} of 2.4, which is equivalent to an apparent activation energy of 16,200 calories/mol. Sheppard and Martin also find that the temperature coefficient for the process of entrance is equal to that for the process of exit at temperatures between 44° and about 22°. Below this temperature the two temperature coefficients diverge as would be expected in order to account for the many observations (21–23) of the loss of K from cells stored at low temperatures.

It has been pointed out by Raker *et al.*, and by Ponder that the activation energy for K transport is closely similar to the activation energy for glucose consumption. This is borne out by our observations in 15 experiments that the activation energy for glucose consumption is 13,300 ± 7,400 calories/mol. The high probable error arises from some of the earlier experiments in which the glucose measurements were not accurate.

Influence of External K Concentration.—

The results of several experiments to determine the effect of plasma K concentration on the transport of K into the red cell are presented in Table VI. These results, indicating that the amount of K transported per liter of cells is independent of the extracellular K concentration, are in essential agreement with those of Raker *et al.* (1) and Sheppard and Martin (2). The former found that extracellular concentrations as high as 75 m.eq./liter did not affect the amount of K transported into the cell.

From equation (3) it can be seen that the transfer coefficient for the amount of K leaving the plasma in unit time per milliliter of cells is $k_{ab}(v_p/v_q)$, or $k_{ab}(1 - H)/H$. If the amount of K transported in unit time is a constant, independent of external K concentration, then:

$$[K]_{pl} k_{ab}(1 - H)/H = \text{constant}$$

and $\dfrac{H}{k_{ab}(1 - H)}$ should lie on a straight line, when plotted against $[K]_{pl}$. Fig. 2 shows that this is indeed the case, and furthermore that the straight line can be extrapolated through the origin.

The fact that the K flux is independent of external K concentration indicates that K and Na are not transported into the cell by the same mechanism. This conclusion is only valid in the concentration range we have explored. If they were transported by the same mechanism, and only a limited total

cation flux were possible, Na and K would be expected to compete for the available places in the inward transport system according to their respective plasma concentrations. Doubling the plasma K concentrations which materially increases the fraction of K in the plasma, would be expected to increase the inward K flux by displacing some of the Na. Since this is contrary to our experimental results, it is likely that these two cations are transported by separate mechanisms.

TABLE VI

Effect of Plasma K Concentration on K Transfer into the Red Cell at 37°

Experiment No.	Hematocrit reading	k_{ab}	k_{ba}	K plasma concentration	$\frac{H}{k_{ab}(1-H)}$
	per cent			*m.eq./l.*	
35	48.7	0.349	0.0224	5.80	3.70
	50.1	0.166	0.0197	10.38	6.05
39	47.9	0.261	0.0190	5.99	3.51
	45.3	0.104	0.0163	14.00	7.97
56	48.2	0.248	0.0177	6.59	3.75
	48.1	0.213	0.0176	7.48	4.35
57	41.8	0.192	0.0139	6.60	3.75
	41.4	0.161	0.0153	8.10	4.39
	41.9	0.136	0.0142	9.35	5.30
	41.2	0.089	0.0112	11.35	7.87
59	42.7	0.286	0.0139	4.50	2.60
	41.2	0.188	0.0140	6.80	3.72
	40.5	0.116	0.0132	10.55	5.87
	41.0	0.071	0.0135	16.75	9.78

Effect of Competing Ions on K Transport.—

The results of two experiments carried out to determine whether other alkali ions competed with K for transport into the cell are given in Table VII. The results for Experiment 55 are shown in Fig. 1. It can be seen at once that Rb causes a decrease in K transport, whereas Li does not affect it. The relative effectiveness of Rb and K given in the last column of Table VII is obtained by finding from Fig. 2 the value of $[K]_{pl}$ corresponding to the observed $k_{ab}(1 - H)/H$. The observed $[K]_{pl}$ is subtracted from this value, and the difference (which represents the $[K]_{pl}$ which would have had to be added to depress the transfer coefficient) is divided by the actual initial concentration of Rb. It should be noted that the flame photometer used cannot differentiate between Rb and K, and that the values for this experiment are therefore less reliable than those for the other experiments.

The relative effectiveness of Rb can be illustrated by pointing out that a cell placed in an environment containing equal amounts of K and Rb, would transport 1.19 times as many Rb molecules as K. Neither Li nor Cs appears to compete with the K.

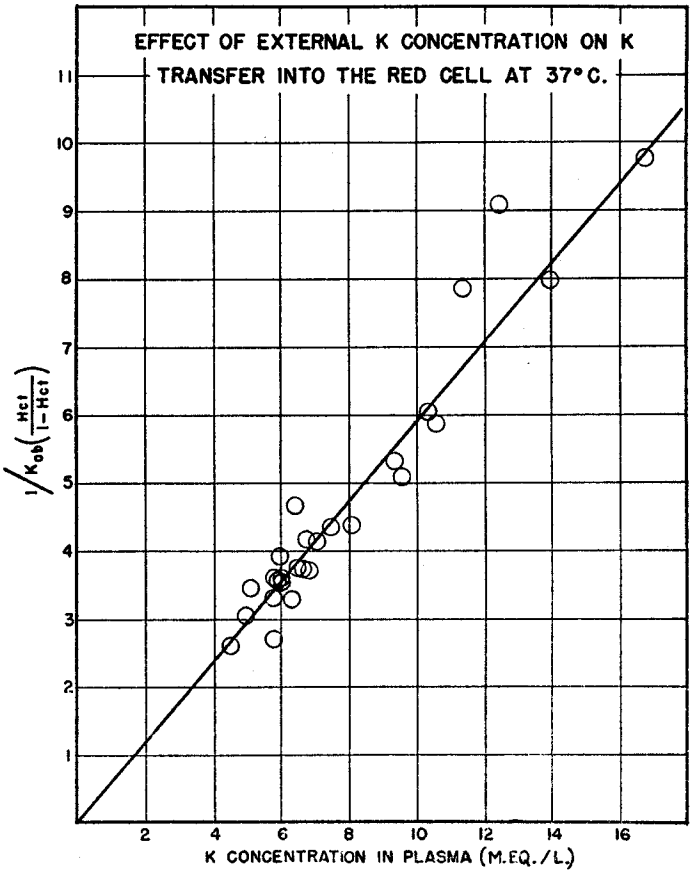


FIG. 2

III

Experiments on Na Transport

Equations Governing the Transport of Na into the Red Cell.—

In the early stages of the problem (before Experiment 60), it was not clear that all the cell Na could not be freely exchanged under our experimental conditions, and many experiments were carried out in a manner analogous to that used in determining the K transport. However, the data obtained in this

fashion are not accurate because there is no method for obtaining p_∞. It had been hoped that the approximation involved in equating

$$dp/dt = -k_{ab}p - k_{ba}q \cong -k_{ab}p \cong -k_{ab}p_0$$

would be small. However, the q term may not be neglected for, though q itself is small, k_{ab} is so large ($t_{\frac{1}{2}}$ for exchangeable q = 1.16 hours), that the equation $dp/dt = -k_{ab}p$ is only valid during the first few minutes of the experiment. Thus we cannot validly compare results obtained from the uptake of Na^{24} in the cellular fraction with those obtained by measurement of plasma Na^{24} loss. However, Sheppard, Martin, and Beyl (4) have presented results for plasma loss of Na^{24} in human cells. Their approximate value for "k_{ab}" of 1 per cent

TABLE VII

Competition of Other Alkali Ions with K for Entrance into the Red Cell at 37°C.

Experiment No.	Concentration			$k_{ab}\frac{1-H}{H}$	$k_{ba}\frac{1-H}{H}$	K flux	Relative effectiveness Rb/K
	K in plasma	Competing ion in plasma at $t = 0$					
	m.eq./l.	*m.eq./l.*	*m.eq./l.*			*m.eq./l. cells hr.*	
55	5.80	—		0.257	0.0160	1.49	
	6.06	Li	3.92	0.269	0.0167	1.62	
	6.13	Rb	3.92	0.128	0.0081	0.78	1.71
71	5.78	—		0.302	0.0196	1.75	
	5.80	Cs	3.84	0.286	0.0182	1.66	
	5.80	Rb	3.76	0.201	0.0123	1.16	0.67
Average							1.19

per hour as obtained from an equation similar to $dp/dt = -k_{ab}p$ agrees with our mean value of "k_{ab}" = 0.0105 ± 0.0051 obtained in the same manner by least squares from seven experiments—but neither is a valid index for k_{ab}.

As a consequence it was decided to measure the uptake by the red cells directly. The major disadvantage of this method was that it was necessary to wash the cells four times with buffer to make sure that no radioactivity was trapped in the fluid surrounding the cells. The loss of radioactivity attendant upon this procedure will be discussed below. Also the inherent accuracy of the dip counter used in the radioactivity measurement is only about 3 per cent. However, in spite of these difficulties, the method yielded results much more dependable than those obtained from the plasma observations.

The fact that all the Na did not exchange completely under our experimental conditions meant that p_∞ could not be obtained from flame photometer measurements. As a consequence these experiments were extended to periods

of 10 to 12 hours, until the cell radioactivity had reached a plateau, which was taken to represent q_∞.

Equation (1) can be transformed into:

$$dq/dt = k_{ab}\, p\, v_p/v_q - k_{ba}\, q\, v_p/v_1 \tag{9}$$

since $v_q\, dq/dt = v_p\, dp/dt$, in which q represents the concentration of radioactivity in the cells. The solution to (9) is:

$$q = \frac{k_{ab}\, p_0 v_p/v_q}{k_{ab} + k_{ba}\, v_p/v_q} [1 - e^{-(k_{ab}+k_{ba}v_p/v_q)t}] \tag{10}$$

At $t = \infty$, q_∞ = the term outside the bracket in equation (10). Transposing and taking the logarithm,

$$\ln (1 - q/q_\infty) = -(k_{ab} + k_{ba} v_p/v_q)t \tag{11}$$[3]

The function ln $(1 - q/q_\infty)$ lies on a straight line when plotted against time, as shown in Fig. 3, which represents a typical experiment. Since the error in $1 - q/q_\infty$ becomes quite large as q approaches q_∞, the first three or four points alone usually describe the straight line. In the following equations the slope of this line (which is negative) is denoted by T. We can determine k_{ab} and k_{ba} exactly[4] by:

$$k_{ab} = \frac{-Tq_\infty}{p_0 v_p/v_q}$$
$$k_{ba} = v_q/v_p(-T - k_{ab}) \tag{12}$$

in which q_∞ equals counts per minute per milliliter of cells at the level of the plateau, which we consider to be $t = \infty$.

The Steady State.—

The assumptions made for the K system equations are also implicit in the equations governing the Na transport. However, the assumption of the steady

[3] Equations 9, 10, and 11 can be transformed into Equations 3-3, 3-4, and 3-7 of reference 18, remembering that $Q = qv_q$, and that k_{ba} in reference 18 equals $k_{ba}v_p/v_q$ in our present terminology.

[4] An extrapolated value for k_{ab} may be found that is independent of q_∞. If the exponential term in equation (10) is expanded in an infinite series, and the whole resultant equation divided by t, the limit of q/t as t approaches zero becomes

$$\lim_{t\to 0} q/t = k_{ab} v_p v_q p_0,$$

from which k_{ab} can be determined.
In these experiments the initial k_{ab} did not agree well (within 10 to 20 per cent) with the final k_{ab} determined by equation (12), probably because of the loss of cell Na. Equation (12) has been used to determine the k_{ab} and k_{ba} values presented in the subsequent tables.

state which seems justifiable for the K studies is less acceptable for the Na ones. As was the case in the K studies, the system was checked for constant volume by Na whole blood analyses at the beginning and end of the experiments. The hematocrit reading remained constant within about 2 to 4 per

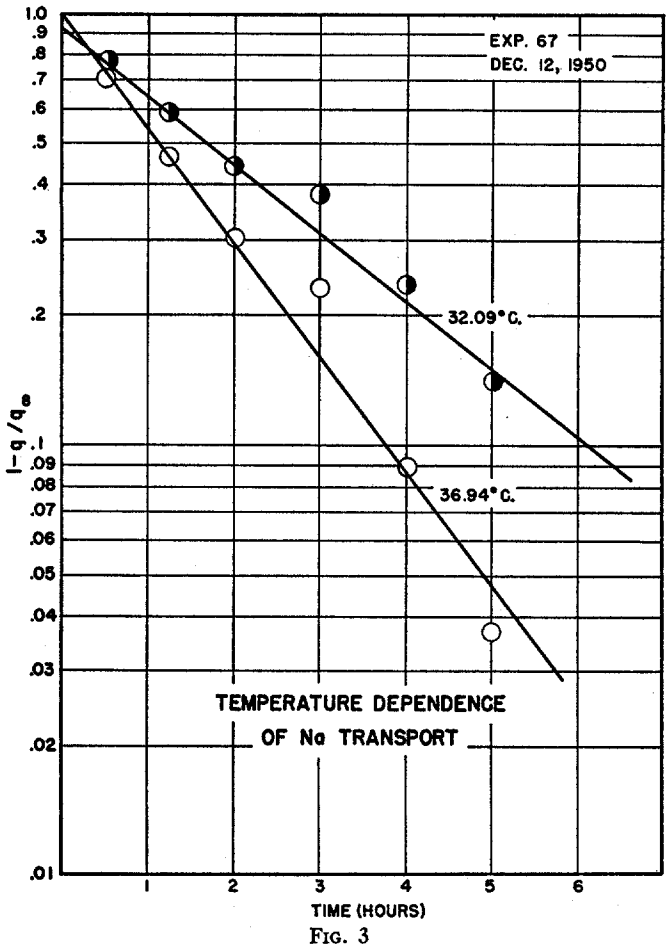


FIG. 3

cent, except in cases in which the external Na concentration was low or competing ions such as Li were present. These latter determinations are therefore less accurate than the other Na measurements. In general, the $[Na]_{pl}$ remained constant to better than 1 per cent. The cell [Na], however, dropped appreciably, usually from 1 to 2 m.eq. Na/liter lost from the cells (normal $[Na]_c$ = 8.6 m.eq./liter). Cell Na loss is even greater in cases in which the external Na concentration is low, or competing ions are present. Consequently, for Na it

must be realized that the equations, though themselves exact, apply to conditions which could not be experimentally realized completely, and that the data are therefore less accurate than could be desired.

It had been hoped that the washing procedure to free the cells from plasma would result in a negligible loss of Na^{24} from the cells. 1 ml. of cells was drawn from the sample boats, and spun at 1090 *g* for 10 minutes. The supernatant was sucked off, and 10 ml. of buffer was added. The cells were stirred, and centrifuged for 3 minutes. This procedure was repeated 4 times, by which time the radioactivity of the washings was negligible in comparison with that in plasma, and small compared with that in the cells.

There are two ways to lose Na from the cells in washing: direct loss of total cell Na, and indirect loss of radioactive Na by exchange with Na in buffer. The first process can account for as great a loss as the second, but no greater, because the loss of cell radioactivity must also include the loss of cell Na. The average of three determinations on the direct loss gives 3.9 per cent of the cell Na lost per washing. The average of six determinations of radioactive loss obtained on repeated washings following the four required to remove all plasma contamination, gives a value of 2.4 per cent of the cell Na^{24} lost per wash. Since the latter is smaller than the former we have averaged the two kinds of determinations to obtain a figure of 3.2 per cent lost per washing, or 12.8 per cent lost per set of four washings.

Fortunately, this loss in washing, if it is uniform, does not affect any of the constants derived from equation (10), since its effect is the same as though the counting efficiency were 12.8 per cent lower, or 12.8 per cent less radioactivity had been added to the system. However, it does affect the calculation of the freely exchangeable Na in the cell, and a correction has been made to the average figures to take account of this loss. Unfortunately, the loss is probably by no means uniform, and therefore may contribute appreciably to the scatter of the experimental data.

Normal Na Transport.—

The results of measurements of normal Na transport obtained from the cell determinations are presented in Table VIII. From the mean value of 0.597 ± 0.134 for $k_{ba}(1 - H)/H$ it can be calculated that the freely exchangeable Na in the red cell *in vitro* has a half-life of 1.16 hours. The amount of Na transported inward per hour by 1 liter of cells is 3.08 ± 0.57 m.eq., almost double the amount of K transported. In molecular dimensions, we may calculate that 1 molecule is transported per hour per 71 A^2 of cell surface, or we can state that the Na flux is 1.5×10^{-13} mol/cm.^{2} sec.

An observer on the outside of the cells could note that though there is 31 times as great a Na concentration as K concentration outside the cell, only 2.1 times as much Na is transported inwards per hour as K. That is, to the outside observer the cell prefers K to Na, and the competition factor is 15. If

the same observer now moves inside the cell, he will note that though there is 11 times as great a K concentration as Na concentration inside the cell, 2.1 times as much Na is transported out per hour as K. That is, to the inside observer, the cell prefers Na to K for outward transport, and the competition factor is 23.

Harris and Maizels (25) have recently measured the flux of Na across the red cell membrane by observing at 37°C. the transport of Na^{24} from cells previously incubated with Na^{24} at 37° overnight into a non-radioactive medium

TABLE VIII

Transfer of Na across the Normal Red Cell Membrane at 37°C.

Experiment No.	Hematocrit reading	$k_{ab}\frac{1-H}{H}$	$k_{ba}\frac{1-H}{H}$	Final plasma Na concentration	Na flux	Glucose utilization
	per cent			*m.eq./l.*	*m.eq./l. cells hr.*	*mM/l. cells hr.*
62	47.4	0.0247	0.790	143.3	3.54	2.80
64	40.1	0.0261	0.747	141.0	3.68	3.09
65	47.6	0.0279	0.712	135.8	3.79	2.97
67	41.4	0.0235	0.588	142.7	3.35	1.54
68	47.4	0.0172	0.561	142.7	2.45	2.06
72	10.8	0.0215	0.615	134.0	2.88	1.85
74	12.8	0.0162	0.400	134.0	2.17	
83	24.9	0.0226	0.528	139.5	3.15	2.29
84	22.8	0.0194	0.435	140.1	2.72	3.61
Average		0.0221	0.597		3.08	2.53
Standard deviation		0.0039	0.134		0.57	

Their mean value in five experiments of $k_{ab}\frac{1-H}{H}$ (their k_1) is 0.023 ± 0.006 in an external Na concentration of 135 mc./liter. This value is in excellent agreement with our figure of 0.022 ± 0.004. However, their value for the outward transport coefficient (their k_2) of 0.25 ± 0.09 does not agree with our value for $k_{ba}\frac{1-H}{H}$ of 0.597 ± 0.134. This discrepancy may arise from their assumption that all the intracellular Na is freely exchangeable, an assumption which is in disagreement with the results obtained under our experimental conditions. Sheppard and Beyl (24), who have also found a slowly exchanging Na fraction *in vitro*, have given a value of 3.3 m.eq./liter of cells per hour for the Na flux in agreement with our value.

Exchangeability of Intracellular Na.—

Since the indirect determinations of intracellular Na are not as reliable as the later direct ones, it has seemed best to arrive at the average figure for the

TABLE IX

Exchangeability of Na in the Red Cell

Experiment No.	Final plasma Na	Hematocrit reading	Freely exchangeable cell Na	Measured cell Na	Cellular Na exchanged	Comments
	m.eq./l.	*per cent*	*m.eq./l.*	*m.eq./l.*	*per cent*	
64	141.0	40.1	4.80	14.1	34.1	
	131.8	40.3	4.37	7.0	62.4	$[K]_{pl}$ = 14.4
65	135.8	47.6	4.79	11.6		
	140.3	47.7	4.34	9.1		32°
67	142.7	41.4	5.46	—	—	
	143.5	41.8	4.99	—	—	32°
68	142.7	47.4	4.79	8.0	59.9	
	144.3	47.6	4.29	8.0	53.6	32°
70	58.1	17.4	2.61	—	—	$[Na]_{pl}$ = 58.0
	78.0	18.6	3.12	—	—	" 86.9
	117.0	20.3	4.12	3.7	100	" 117.0
	150.5	20.3	4.92	2.0	100	" 147.5
72	134.0	10.8	4.64	3.2?	100	
	134.0	10.6	3.55	3.2?	100	27°
74	134.0	12.8	5.38	—	—	
	134.0	12.9	4.74	8.5	55.8	32°
75	147.0	24.1	4.23	5.6	75.6	
78	151.0	23.9	5.47	7.8	70.2	$[Na]_{pl}$ = 147.4
	125.2	22.8	4.19	7.1	58.9	" 124.2
	98.8	22.1	3.12	5.7	54.8	" 98.8
	71.4	20.5	2.29	4.7	48.8	" 71.4
79	150.0	23.8	5.33	7.0	76.2	
80	150.6	22.2	6.58	8.4	78.3	
	123.7	21.4	4.74	6.9	68.6	$[K]_{pl}$ = 102.3
	99.4	20.3	4.01	6.0	66.7	$[Li]_{pl}$ = 96.4
	77.7	19.9	3.24	5.8	55.8	$[Na]_{pl}$ = 50.1
81	147.3	22.7	4.06	5.9	68.8	
83	139.5	25.5	5.88	6.5	90.4	
	140.3	28.2	4.98	6.5	76.7	32°
	142.3	25.3	5.94	6.5	91.3	Low pH
84	140.1	22.9	6.15	9.3	66.1	
	144.0	25.2	5.81	8.3	70.0	Low pH

Average of freely exchangeable Na in normal cells	5.18 ± 0.72
Average total Na in cells 8.66 m.eq./l.—12.8 percent (washing loss)	7.55
Slowly exchangeable Na	2.37

freely exchangeable Na in a fashion independent of individual Na determinations. This can be done by calculating the concentration of freely exchangeable Na in the cell assuming equivalent specific activity between the plasma Na and the freely exchangeable intracellular Na at the plateau value of q_∞. The results are given in Table IX. In mathematical terms, $[Na]_{\text{exchangeable cell}} = q_\infty/(p_\infty/[Na]_{pl})$. Most experiments were carried out from 10 to 12 hours in order to determine the plateau value. This time is long compared with the

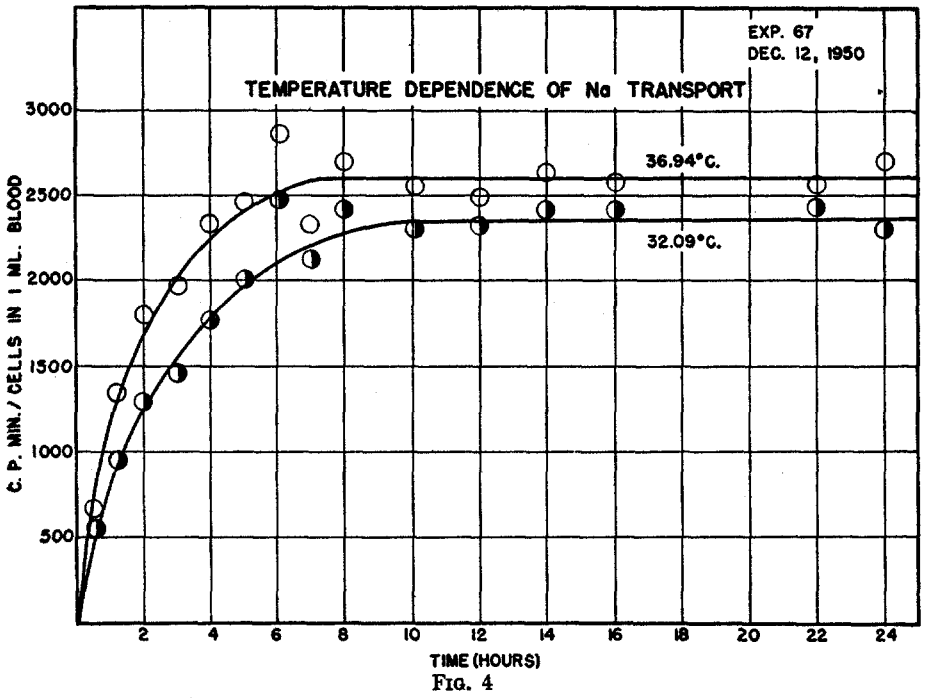


FIG. 4

mean half-life of freely exchangeable intracellular Na of 1.13 hours. The average normal freely exchangeable $[Na]_c$ is 5.18 ± 0.72 m.eq./liter uncorrected for washing. The washing correction may be arrived at from the normal [Na] in cells of 8.66 m.eq./liter by subtracting 12.8 per cent = 1.11 m.eq./liter leaving 7.55 m.eq./liter. This leaves a residue of 2.37 m.eq./liter of Na to be accounted for. Fig. 4 shows the data obtained for a 24 hour experiment. The relative flatness of the plateau may be taken as an indication that the slowly exchangeable Na does not leave the cell rapidly; if it were completely exchanged at the end of 24 hours, the final plateau value should be roughly half again as great as it is. At the end of one of the 24 hour experiments the cells were hemolyzed, and 91 per cent of the freely exchangeable Na was found in the hemolysate supernatant.

The difference in the final plateau value for a 5° temperature difference as shown in Fig. 4 is a general phenomenon observed in all the temperature coefficient experiments. It amounts to about 0.6 m.eq./liter. In Experiment 83, in which the Na analyses were done directly on the cells, there is no difference between the final cell Na concentration at the higher temperature and that at the lower temperature, indicating that a 5° temperature fall decreases the size of the slowly exchangeable compartment, and releases Na for free exchange. This result only adds to the difficulties of the situation since a 5° temperature fall apparently accomplishes more in freeing Na for exchange than 12 hours' incubation at the higher temperature. However, as shown in Experiment 78, placing the cell in a low Na environment does not reduce the size of the slowly exchangeable pool, and hence the per cent of Na exchanged decreases with decreasing cell Na concentration. No evidence has been presented that the slowly exchangeable Na is uniformly distributed among all the cells independent of their age. It may therefore be that the Na is bound tightly to old cells only. Indeed, Keitel (17) has found that lighter, younger red cells have a higher Na content than the heavier old ones.

It seemed desirable to investigate the possibility that the presence of white cells in the blood could account for either the freely or slowly exchangeable Na. For Na in human cells this possibility is unlikely under our conditions at the outset because the multiple washing procedure removes all visible evidence of the buffy coat from the red cells before they are counted. However, a Na^{24} uptake experiment was carried out on separated white cells;[5] the uptake of Na^{24} from Hank's solution which contains 146.1 m.eq. Na/liter was found to be 0.091 per cent of the supernatant Na per hour when normalized to a white cell population of 7,000 cells/c. mm. This can not account for any appreciable amount of the red cell exchange.

Since Edelman, James, and Moore (26) have shown that the specific activity of red cell Na equals that of plasma Na *in vivo* both in man and in dog at 24 hours following injection, the present results showing a more slowly exchangeable Na fraction *in vitro* must be accepted with reserve, and are certainly not indicative of the true state in nature.

Apparent Activation Energy for Na Transport.—

The apparent activation energy for the transport of Na into and out of the cell has been measured in five experiments. The results are presented in Table X. The method of calculation is identical with that used for the K transport activation energy. The apparent activation energy for entrance, 20,200 ± 2700 calories/mol, is significantly different from the figure of 12,300 ± 1300 obtained for the K process. This means that the processes for entrance of K

[5] Separated white cells were obtained by courtesy of Dr. J. L. Tullis of the Laboratory of Physical Chemistry of Harvard University.

and of Na are chemically different, and that one ion cannot call the tune which the other will follow.

Effect of pH on Na Transfer.—

It has already been shown by Raker *et al.* (1) that pH changes in the range of pH 7.5 to pH 7.0 do not affect the K transfer from the red cell. The results of similar experiments to determine the effect of pH on Na transfer are presented in Table XI. It will be seen that a change of about 0.5 pH unit (obtained by replacing the 5 per cent CO_2–95 per cent air mixture which passes

TABLE X

Apparent Activation Energy for Na Transfer into the Red Cell

Experiment No.	Temperature	$k_{ab}\frac{1-H}{H}$	$k_{ba}\frac{1-H}{H}$	Activation energy	
				k_{ab}	k_{ba}
	°C.				
65	36.94	0.0279	0.712	22,100	15,300
	32.04	0.0157	0.480		
67	36.94	0.0235	0.588	22,700	19,100
	32.04	0.0131	0.358		
68	36.94	0.0172	0.561	18,800	12,700
	32.04	0.0109	0.403		
72	36.94	0.0215	0.615	21,900	16,900
	27.01	0.00661	0.248		
74	36.94	0.0162	0.400	15,600	10,500
	32.04	0.0108	0.304		
Average				20,200	14,900
Standard deviation				2,700	3,400

over the cells in the bath with 20 per cent CO_2–80 per cent air) depresses the k_{ab} by about 23 per cent and the k_{ba} by about 15 per cent. The effect of pH on glucose consumption, however, is more marked, since the lower pH cuts the glucose consumption to about 55 per cent of the consumption at normal pH. These results are in agreement with those of Harris and Maizels (25) who find that changes of pH from their normal value of 7.4 to values of 7.0 and 6.8 decrease the flux of Na, and further that an increase to 7.8 is without effect.

Effect of Extracellular Na Concentration on Na Transport.—

The effect of external Na concentration on Na transport has been studied and the results are given in Table XII. Fig. 5 shows a graph of a typical ex-

TABLE XI

Effect of pH on Na Transfer in the Red Cell at 37°C.

Experiment No.	pH* Initial	pH* Final	$k_{ab}\frac{1-H}{H}$	$k_{ba}\frac{1-H}{H}$	$\frac{k_{ab}\frac{1-H}{H}\text{ low pH}}{k_{ab}\frac{1-H}{H}\text{ high pH}}$	$\frac{k_{ba}\frac{1-H}{H}\text{ low pH}}{k_{ba}\frac{1-H}{H}\text{ high pH}}$
83	7.53	7.39	0.0226	0.528		
	7.06	6.91	0.0162	0.427	0.72	0.81
84	7.56	7.39	0.0194	0.435		
	7.12	6.96	0.0159	0.389	0.82	0.89
Average					0.77	0.85

*pH measured at room temperature.

TABLE XII

Effect of External Na Concentration on Na Transfer into the Red Cell at 37°C.

Experiment No.	Sugar	$k_{ab}\frac{1-H}{H}$	$k_{ba}\frac{1-H}{H}$	Final external Na	Na flux
				m.eq./l.	*m.eq./l. cells hr.*
70	Sucrose	0.0266	0.587	58.1	1.55
		0.0206	0.569	87.0	1.80
		0.0170	0.479	117.0	1.99
		0.0158	0.387	150.5	2.38
78	Sucrose	0.0132	0.362	151.0	1.99
		0.0178	0.527	125.2	2.23
		0.0188	0.590	98.8	1.86
		0.0217	0.672	71.4	1.55
79	Raffinose	0.0139	0.371	150.0	2.09
		0.0219	0.648	122.2	2.68
		0.0244	0.777	98.2	2.40
		0.0273	0.841	73.9	2.02
80	Melezitose	0.0174	0.394	150.6	2.62
		0.0174	0.448	123.1	2.14
		0.0193	0.477	99.4	1.92
		0.0199	0.477	76.0	1.52

periment. To obtain the data in Table XII, the Na concentration was varied by diluting the blood with an approximate equal volume of NaCl, or mixtures of NaCl with various sugars. The sugars are all polysaccharides made up in concentrations of 310 mM/liter. The values at about 150 m.eq./liter of Na are necessarily obtained without any sugar, and hence the points are independent

of the inert diluent. In general, except for the lowest Na concentrations the hematocrit values did not change appreciably between the beginning and end of the experiments. At the lower concentrations of external Na the cell shrank and lost Na. The addition of an equal volume of the sugar reduced the initial pH values by 0.1 to 0.2 pH unit, with lower proportions of sugar making proportionately smaller changes in pH. The pH differences are not, as can be seen from Table XI, sufficient to account for the observed changes in k_{ab}.

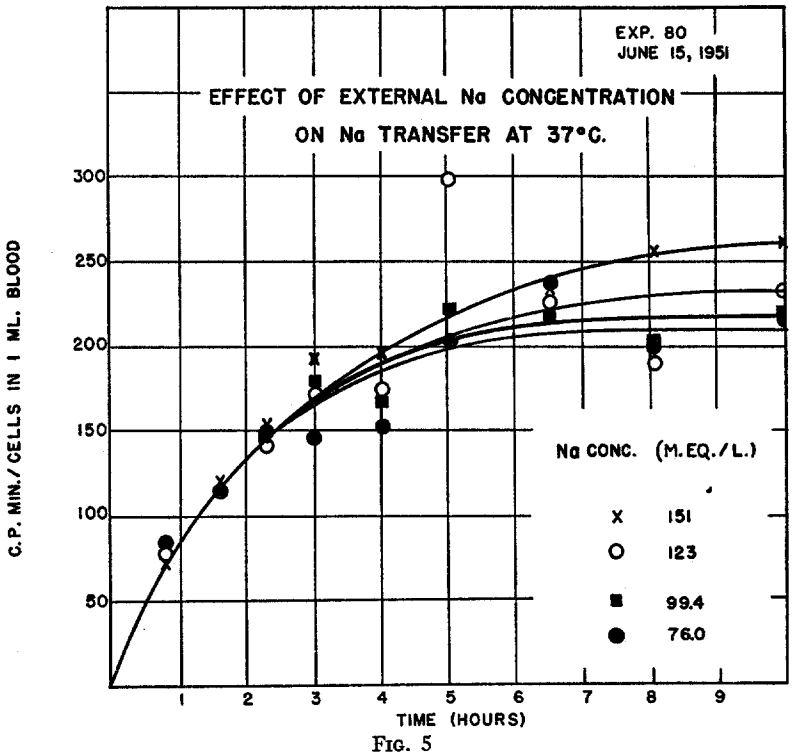


FIG. 5

It is possible to fit the data to a curve of the form

$$\mathrm{Na}_{(\text{transported per liter of cells})} = \frac{a[\mathrm{Na}]_{pl}}{1 + c[\mathrm{Na}]_{pl}} \tag{13}$$

as is usual in kinetic studies either of the Langmuir adsorption isotherm or the Michaelis-Menten enzyme-substrate system. The function $[\mathrm{Na}]_{pl}/\mathrm{Na}_{(\text{transported per liter of cells})}$ lies on a straight line when plotted against $[\mathrm{Na}]_{pl}$. The slope and intercept of the best straight line found by the method of least squares on the sucrose and melezitose data lead to values of $a = 0.0392$ and $c = 0.01046$. Fig. 6 shows a graph of Na transported per liter of cells against

$[Na]_{pl}$ in milliequivalents per liter. The curve is derived from equation (13) with the values of a and c above. It will be seen that the raffinose points fall

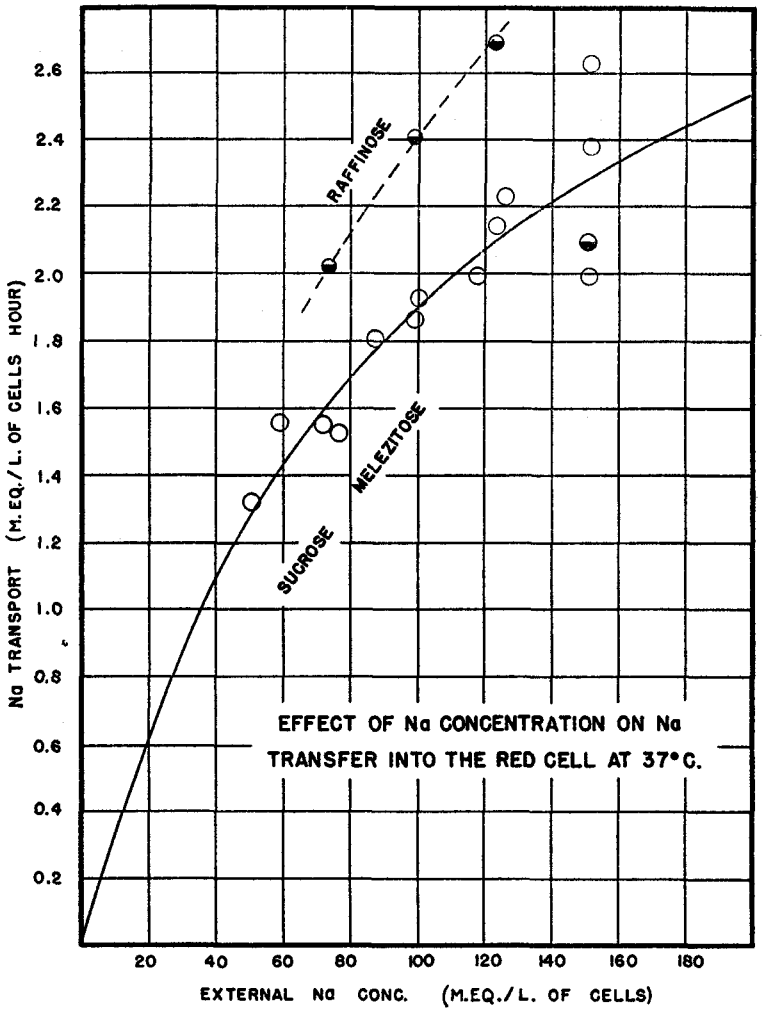


FIG. 6

on a different similar curve, indicating that the nature of the "inert" sugar affects the transport of Na. Also, neither the sucrose-melezitose[6] curve nor the

[6] The melezitose was kindly supplied by Dr. Hewitt G. Fletcher, Jr., National Institutes of Health.

raffinose data approach the value of normal Na transport (3.08 m.eq./liter at $[Na]_{pl}$ = 143.5 m.eq./liter), indicating that neither a NaCl nor sugar environment is truly physiological. However, since the sucrose-melezitose curve goes through the NaCl points, it serves as a basis for the examination of the effects of other ions.

It is also possible to fit the data to a straight line, for which the equation (in the case of sucrose and melezitose) is given in a later section. Since the experimental data do not provide an unambiguous choice between the two interpretations, it has been necessary to make an arbitrary decision, and we have chosen to use the form of equation (13).

Davson (27) has shown that human erythrocytes are sensitive to immersion in high concentrations of sucrose, and that they lose K at a rapid rate under these conditions. The loss is only 5 per cent at a NaCl concentration of 50 m.eq./liter which is as low as we have gone, but rapidly increases with further decreases in NaCl concentration. He has also shown that the K loss is not dependent on the pH change, and ascribes it to an alteration of the surface of the erythrocyte. Certainly, the difference we have observed between the Na transport in the presence of melezitose and of raffinose, both trisaccharides, indicates that the non-electrolyte has some action on the cell. Consequently equation (13) may reflect the action of the polysaccharide rather than that of external Na concentration. The question can only be settled by finding a truly inert diluent. However, one point may be raised in favor of considering equation (13) to be due to changes of Na concentration. In the case of frog sartorius, Harris and Burn (28), using dextrose as the inert solvent, have found that the permeability of muscle to Na is unaffected up to the point of half-replacement of NaCl by dextrose. Of course, since this is an effect which presumably occurs on the surface of the cell, we must be doubly careful in comparing one tissue with another.

Effect of Other Ions on Na Transport.—

The results of five experiments to examine the effects of other monovalent ions on Na transport are given in Table XIII. Experiments 62 and 64 show that increasing the normal external K concentration by a factor of 2 to 3 does not depress the Na transport. The 10 per cent depression at 10.43 m.eq. K/liter may be considered to lie within experimental error, particularly because the depression at the higher K concentration of 14.4 m.eq./liter of Experiment 64, is only 5 per cent. It had been hoped that this phenomenon of independence of external K concentration would be exhibited over the entire range of K concentration, but massive concentrations of K do affect the Na transport rate as shown by Experiments 75 and 81. Fig. 7 shows a typical plot of the effects of other ions on Na transport. The increase in q_∞ for K is typical of all such experiments, as is the lack of any real plateau, which re-

sults from the continued loss of cell Na in the abnormal low Na, high K environment. Since the cell Na is not constant, the system is no longer in a steady state. Hence the conditions requisite for equation (11) are not fulfilled,

TABLE XIII

Competition of Other Alkali Ions with Na for Entrance into the Red Cell at 37°C.

Experiment No.	Other ion or neutral material		External Na	$k_{ab}\frac{l-H}{H}$	$k_{ba}\frac{l-H}{H}$	Na flux	Na flux in presence of competing ion / Na flux in control	Approximate increase in Li content of cells	Approximate Li transport
		Initial concentration							
		mM/l.	*m.eq./l.*			*m.eq./l. cells hr.*	*m.eq./l. cells hr.*	*m.eq./l. cells*	*m.eq./l. cells hr.*
62			143.3	0.0247	0.790	3.26*	0.90		
	K	10.43	135.0	0.0216	0.808	2.81			
64			141.0	0.0261	0.747	3.68‡	0.95		
	K	14.40	131.8	0.0258	0.747	3.40			
75	Li	98.6	49.1	0.00883	0.239	0.434	0.42	21.4	2.25
	K	102.1	50.8	0.0133	0.239	0.676	0.66		
			147.0	0.0124	0.431	1.82§			
81			147.3	0.00874	0.319	1.29			
	K	102.3	56.0	0.0169	0.371	0.947	1.39		
	Li	96.4	52.3	0.0102	0.274	0.534	0.78	16.4	1.85
	Sucrose	188.0	50.1	0.0136	0.373	0.681‖			
82	Rb	97.2	50.9	0.0134	0.250	0.682			
	K	101.3	52.8	0.0128	0.251	0.676			
	Li	97.6	51.4	0.00838	0.222	0.431		29.1¶	2.60
	Sucrose	202.4	50.0	0.0263		1.32			2.23

* The value observed for Na transport at one concentration may be transformed to a value at another concentration by multiplying the observed value by the ratio obtained from Fig. 6. This value corrected to 3.16 at 135.0 m.eq./liter.

‡ Corrected to 3.57 at 131.8 m.eq./liter.

§ Corrected to 1.03 at 50 m.eq./liter.

‖ This value used as control.

¶ Calculated from change in hematocrit value, approximately 18.2 by direct measurement.

and the conclusions from this set of experiments can only be accepted as tentative. As has been shown following equation (10), q_∞ can increase either due to an increase in the rate of entrance (k_{ab}) or a decrease in the rate of exit (k_{ba}), or both. Unfortunately, the data do not permit a choice between these two processes, since the former is operative in Experiment 81, and the latter

in Experiment 75. Experiment 82 (in which the normal control is not dependable) shows that the effect of Rb is equal to that of K. Harris and Maizels (25) (in general agreement with our results) find that lowering the external K concentration to 1.9 m.eq./liter does not affect the Na flux, but that K concentrations in the range of 0 to 1 m.eq./liter depress the flux.

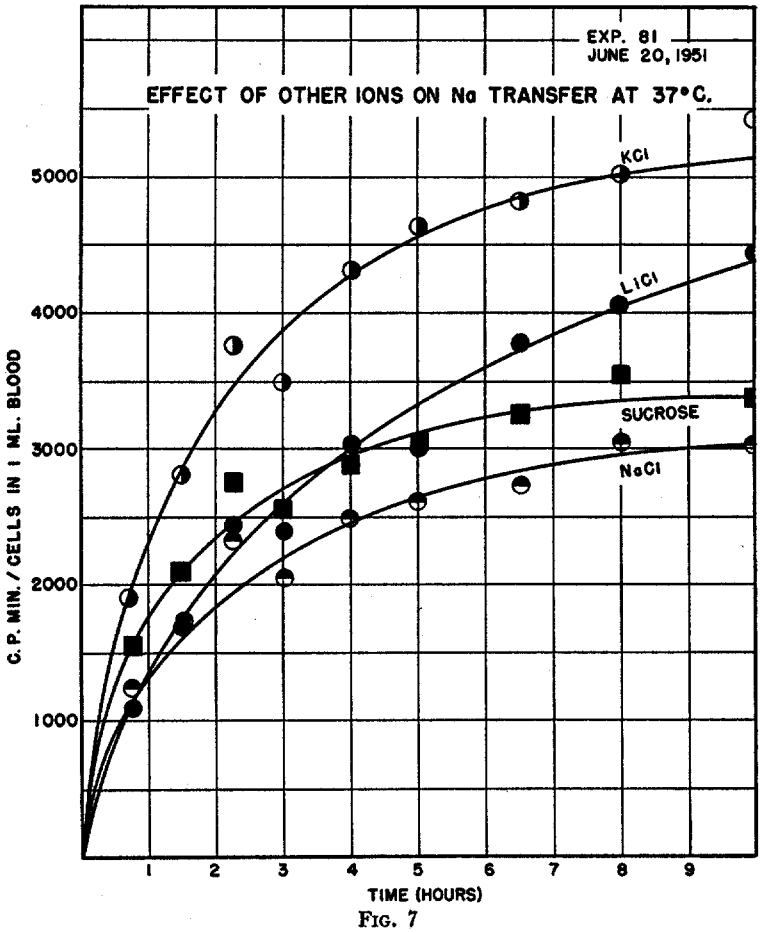


FIG. 7

The effect of Li is much more clear cut than that of K. It inhibits the transport of Na, cutting it down to about 60 per cent of normal, when twice as much Li is present in the external medium as Na. Also, the cells swell appreciably in the presence of Li, presumably due to its entrance into the red cell. If the swelling is considered to arise solely from entrance of Li (assuming no

outward transport of Li), and if correction is made for the measured loss of Na, one can arrive at a rough estimate of the total amount of Li which has entered the cell. This estimate is given in column 9 of Table XIII. In Experiment 82, in which the final Li concentration was measured directly, it appears that the estimate may be high by a factor of 1.5. However, we can say as an order of magnitude that 2.23 m.eq. Li enter the cell per hour per liter of cells in an external concentration of 97 m.eq. Li/liter. If the amount that enters depends directly on the external concentration, 3.28 m.eq. Li would enter per hour per liter of cells, in an external medium containing 143 m.eq. Li/liter. This is to be compared with the normal transport in the NaCl medium of 2.25 m.eq. Na/liter cells hour. The Li estimate is probably a maximum, and may be high by a factor of 1.5.

It is possible to arrive at an estimate of the amount of Li that is transported by the Na mechanism directly. From Experiments 75 and 81, we can calculate that 97 m.eq. Li/liter is sufficient to inhibit the transport of Na at a concentration level of about 50 m.eq./liter, reducing the total Na transport to 60 per cent of normal. If we add 96.4 m.eq./liter of an imaginary ion which is just as effective as Na, and competes with it on an equal basis for entrance into the cell, Fig. 6 will serve as a guide to the total transport. Under these conditions, we would transport 2.3 m.eq./liter hour of both ions. The Na transport would equal $50/146.4 \times 2.3$ or 0.73 m.eq./liter hour. Then 96.4 m.eq./liter of imaginary ion cuts the Na transport from 1.29 to 0.73 m.eq./liter hour or to 56 per cent of normal. To cut the Na transport to 60 per cent of normal or 0.77 m.eq./liter hour, we would need about 90 m.eq./liter of imaginary ion. We accomplish this with 96.4 m.eq./liter of Li, so Li competes with Na on a not quite equal basis. It is 90/96.4 or 93 per cent as efficient as Na as far as normal flux into the cell is concerned.

This result can serve as a guide from which we can estimate the Li transport by the Na mechanism. We can estimate that $0.93 \times \frac{96.4}{146.4} \times 2.3 = 1.4$ m.eq. Li/liter cells hour are transported in the Na ferry. If under these conditions a maximum of 2.2 m.eq. Li and a minimum of 1.5 m.eq. Li enter a liter of red cells in an hour, we have a possible maximum deficit of 0.8 m.eq. Li/liter cells hour which cannot be carried by the Na mechanism.

Since it is experimentally observed that the cells swell more in high Li solutions than in any other alkali ion solution of equal concentration, it is necessary either that more Li enter than can be carried by the Na mechanism, or that the Na mechanism operates to take Li in, but not to transport it out. Harris and Maizels (25) have suggested in accord with the second possibility that the reversal of Li transport is incomplete and that the outward passage of Li from red cells proceeds very slowly if at all.

IV

DISCUSSION

The Energetics of the System.—

The system involved in the transport of potassium from the outside of the cell to the inside; and conversely, the return of the potassium from the inside of the cell to the outside, is under our experimental conditions a steady state process, and not a reversible process at equilibrium. However, it is well known and has been pointed out explicitly by Hearon (29), and Tolman and Fine (30), that it is possible to evaluate thermodynamic constants for irreversible processes by taking advantage of the fact that free energy, heat content, and entropy are functions of state. For clarity in the ensuing discussion we will also consider the changes that take place in an imaginary energy reservoir, which we will consider to be associated with the cation transport and which can serve as a sink or source of energy. The first process of interest is the transport of 1 mol of K from the plasma to the cell. Energy is necessarily consumed by this process, which requires a supply of glucose. If we restrict ourselves to the K transport alone, we may evaluate the free energy required for the entrance of 1 mol of K from the cell following the equation given by Ussing (31):

$$\Delta F = RT \ln \frac{[K]_c}{[K]_{pl}} + RT \ln \frac{\gamma_{K_c}}{\gamma_{K_{pl}}} + F(\psi_c - \psi_{pl}) \tag{14}$$

in which $[K]_c$ is the K concentration in the cell, $[K]_{pl}$ is the K concentration in the plasma, γ_{K_c} and $\gamma_{K_{pl}}$ are the respective activity coefficients, R the gas constant, T the absolute temperature, F, Faraday's constant, ψ_c the electric potential of the cell, and ψ_{pl} that of the plasma. To simplify this expression we may postulate that the activity coefficient of the K ion is the same in the cell as it is in the plasma, buttressing this argument with the observation of Raker *et al.* (1) that the cellular K is totally exchangeable and is characterized by a single rate constant, thus indicating that none of the K is bound tightly enough to another molecule to affect the transport process. A second piece of supporting evidence can be taken from the observation of Hodgkin and Keynes (32) that the K inside a sepia nerve fiber has a diffusion constant very similar to that in an ordinary aqueous solution, and hence is not bound to any large molecule.

The electrical potential may be approximated by observation of the behavior of the Cl ion which we believe to be freely diffusible into the cell and not a participant in any active transport process. If we can assume that the Cl activity coefficient is identical inside and outside the cell, we may write, at equilibrium, which is the usual steady state condition for Cl ions:

$$\Delta F = RT \ln \frac{[Cl]_e}{[Cl]_{pl}} + F(\psi_e - \psi_{pl}) = 0 \tag{15}$$

The ratio $[Cl]_e/[Cl]_{pl} = 0.69$ has been determined by Hastings, Sendroy, McIntosh, and Van Slyke (33) for normal human beings. Using this value, we may say that

$$F(\psi_e - \psi_{pl}) = -226 \text{ calories/mol}$$

or that the potential of the cell is 9.9 mv. negative to the plasma. Equation (14) then becomes:

$$\Delta F = RT \ln \frac{[K]_e}{[K]_{pl}} - 226 \text{ calories/mol} \tag{16}$$

ΔF in this equation can then be evaluated from the figures of 4.65 m.eq. K/liter plasma and 94.15 m.eq. K/liter cells taken from Table III, and the water content of plasma of 93.5 per cent and of cells of 66.1 per cent, taken from Ponder (34). At 37°C., ΔF is equal to 1644 calories/mol.

Thus far, we have limited our attention to the inward process. However, to maintain the steady state, we must also transport 1 mol of K from the cell back to the plasma. Now we can write:

$$\Delta F = RT \ln \frac{[K]_{pl}}{[K]_e} + 226 \text{ calories/mol} \tag{17}$$

and thus obtain a ΔF which is equal in magnitude and opposite in sign to that obtained for the inward transport. ΔF in a cyclic system is zero for the round trip journey of one mol of ions. However, some energy must be supplied by the reservoir, because the free energy for outward transport gives only the maximum net work that the system may perform, and the actual net work derived is less than this by an amount depending on the difference of the system from reversibility. Let us, however, assume that the K outward transport system is not coupled energetically with the inward transport system, that is, that the net energy supplied by the K concentration gradient is not used in the inward K transport. With this assumption, it is possible to set an approximate maximum value for the energy required for the round trip of 1 mol of K ions. The minimum value, which would obtain in a reversible system is 0 calories/mol, the maximum value, which we take to equal the inward free energy expenditure is 1644 calories/mol. It would seem likely, however, in view of the over-all economy of biological processes, and the aptitude of living cells for carrying out physical processes with a considerable conservation of energy, that the true value for the energy required is smaller than this.

In the case of Na transport, it is possible to substitute Na for K in all the relevant terms in equation (14). However, since all the Na in the cell is not freely exchangeable under our experimental conditions we may not assume that the activity coefficients are identical within and without the cell. For

purposes of this calculation, we will take the figure of 6.29 m.eq. Na/liter cells obtained from Tables IX and III as representing the freely exchangeable Na in the cell, and 143.55 m.eq. Na/liter plasma from Table III as the plasma Na. With the figures already given for cell and plasma water, this leads to a value of -1720 calories/mol as the energy available from the inward transport of Na according to the first term in the Na equivalent of equation (14). In the absence of further information, we will arbitrarily assume that, after having taken these concentration figures, the activity coefficient for the Na within is equal to that for the Na without. Therefore, taking account of the potential we may say that $\Delta F = -1720 - 226 = -1946$ calories/mol. Using an argument similar to that developed in the case of K, we note that the ΔF for round trip transport of 1 mol of Na ions is 0 calories/mol. The maximum energy required to be supplied by the reservoir which we take to be equal to the free energy expenditure for outward transport is 1946 calories/mol.

From Table IV, we see that 1.67 m.eq. K is transported per liter of cells per hour, requiring an approximate maximum energy consumption of 2.8 calories, and from Table VIII, we see that 3.08 m.eq. Na is transported per liter of cells per hour requiring an approximate maximum energy consumption of 6.0 calories leading to a total approximate consumption of 8.8 calories per liter of cells per hour. Since according to Meyerhof (35) the energy available from the anaerobic metabolism of glucose is 48,000 calories/mol, and since (Table IV) 2.33 mM of glucose per liter of cells are consumed per hour, we have 110 calories energy available, of which we require only 8.8 for maintenance of the cation balance. It is clear that the transport of Na and K requires but a small fraction of the total energy released by the glucose, only 3800 of the 48,000 calories/mol available.

Flynn and Maizels (36) have suggested that in the red blood cell Na outflow is active and K inflow is passive. As pointed out by Ussing (31), the best criterion for active transport is the sign of the free energy change. Any process in which the free energy increases can be considered to be active; any process in which the free energy decreases can be considered as passive. Such a definition is in agreement with thermodynamic practice. However, Flynn and Maizels infer that, in the steady state, free energy from the Na transport out can serve to provide energy for the K transport in. As has been pointed out above, the free energy required in a reversible system for the round trip transport of 1 mol of Na is zero. Furthermore, we can be sure that the round trip does not go by a process which is thermodynamically reversible since no such biological systems have yet been observed. Thus the Na round trip transport should result in an energy deficit in the reservoir, a deficit which is only reduced to zero in a reversible system. Under no circumstances, therefore, can any energy be made available from the round trip of 1 mol of Na ions in the steady state.

The data on activation energy given in Tables V and X can be coupled

with those given above in order to construct an energy level diagram of the transport system as is shown in Fig. 8. The interpretation of such a diagram, however, is most difficult, particularly because we cannot be sure whether the

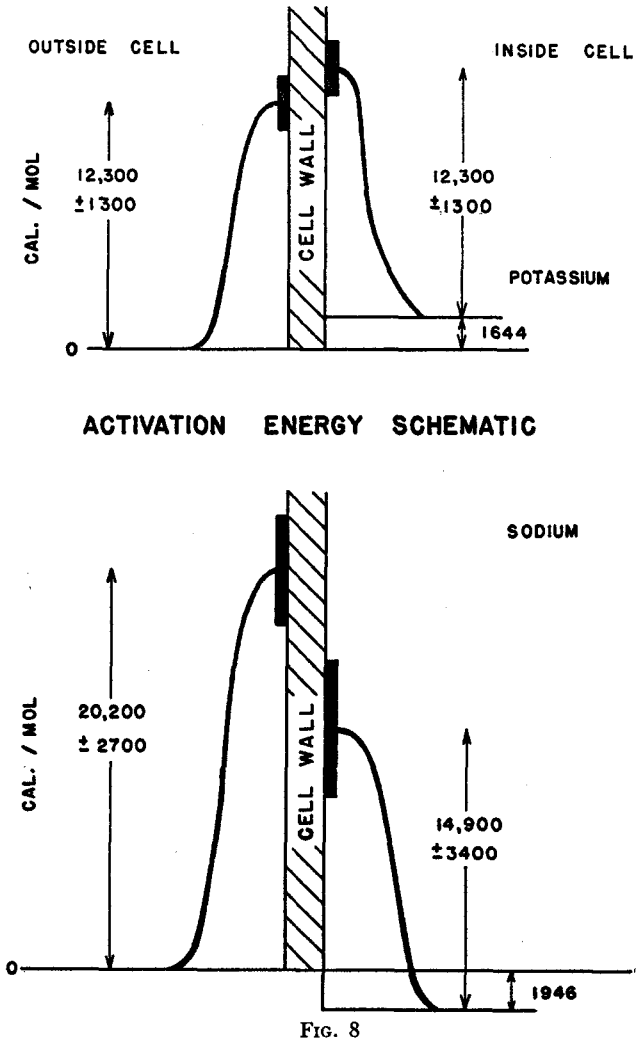


FIG. 8

activation energy diagram refers to the actual transport process or to a different reaction which is energetically coupled to the transport. Furthermore, as has been pointed out the activation energies shown in Fig. 8 are the activation energies of hypothetical single step reactions for the entrance and exit of each ion. Although such processes are unlikely, we may nonetheless draw two

limited conclusions from the data. First, neither of the cations is transported in either direction by a process like simple diffusion, since diffusion in water solution is characterized by an activation energy of the order of 4500 calories/mol. Second, it appears likely that the process of entrance and the process of exit of Na are different, since they may well proceed through activated complexes of different energy. Although our measurements do not provide evidence for a similar statement with regard to K transport, Sheppard and Martin (2) have shown that at temperatures of 15°C. and lower the apparent activation energy for the K entrance process is quite different (different by more than 1650 calories/mol) from that of the exit process, and hence at these temperatures the two processes are probably different. Data from a different source support this conclusion since Taylor and Weller (37) have shown that cholinesterase inhibitors decrease the rate of uptake of K^{42} by human red cells *in vitro*, and that choline acetylase inhibitors increase the rate of loss of K^{42} by the cells, thus indicating that two separate reactions are involved.

Relation between Glucose Utilization and Cation Transport.—

Having set approximate limits to the energy required for Na and K transport, we will now investigate the energy source to see whether the steps in which the energy is made available can be localized. The relationship between metabolic activity and cation transport has been known since Wilbrandt's (38) work on human erythrocytes, in which he showed that both NaF and iodoacetate destroyed the normal permeability characteristics of the cell with respect to K. The NaF inhibition was reversed by pyruvate, whereas no substrate was found to reverse the iodoacetate inhibition. Harris (22) in his subsequent experiments pointed out that the absence of glucose did not affect the K transport until some hours (perhaps 8) after the glucose was exhausted, although the exhaustion of the glucose affected the Na permeability more rapidly. This is in accord with our observation that the inadvertent exhaustion of the glucose in one instance was followed at once by an abrupt rise in the inward Na transport. Danowski (21) has shown that the K loss which accompanies NaF inhibition does not cause a change in cell volume, and hence that the K is probably exchanged for another cation.

Raker *et al.* (1) have shown that the increase of glucose utilization by red cells as the temperature is raised is paralleled by an increase of K transport, an observation confirmed in the present experiments. They have further shown that much of the glucose is consumed to produce lactate. In the case of monkey red cells, it has been shown by McKee, Ormsbee, Anfinsen, Geiman, and Ball (39) that glucose goes nearly quantitatively to lactate, thus supporting the view that the only important energy source for the red cell is the anaerobic metabolism of glucose. It should be pointed out that the coupling between glucose utilization and K transport is not direct, for we could

find no exact correlation between the amount of glucose consumed in an experiment with the amount of K transported in the same experiment. Neither could we find any correlation between the initial glucose level and the amount of K transported. However, as soon as the system is subjected to a stress, as for example a change in temperature, the glucose utilization and K transport respond equally.

Since it appears that the anaerobic consumption of glucose supplies the energy required for the maintenance of the cation steady state, it is desirable to investigate the known steps of anaerobic glucose metabolism to see whether the energy providing step or steps can be localized.[7] When NaF is added to the system, it prevents the formation of phospho-enol pyruvate, and hence stops the whole glycolytic cycle, since the accumulation of any one product must affect the earlier members of the reaction chain. The reversal of NaF inhibition by pyruvate provides a hydrogen acceptor to let the coenzyme I cycle continue. Presumably the conversion of 1:3 diphosphoglycerate to 3 phosphoglycerate continues in part, so that energy may be made available from the high energy phosphate bond formed at this step. In the presence of fluoride, it would not seem possible that the dephosphorylation of phosphopyruvate could proceed, and no energy could be supplied by the 2 high energy phosphate bonds normally made available at this step. Thus on the basis of the information at present available, we may infer that the free energy for maintenance of the cation steady state may come either from the high energy phosphate bonds formed from 1:3 diphosphoglycerate, or from the steps involving the coenzyme I system. Since, as has been shown, the maximum energy required for the transport of Na and K is small, 3800 calories/mol glucose consumed, we need not necessarily implicate a high energy phosphate bond as the energy source, for any reaction or reactions which yield the requisite energy may be involved. The possibility exists that the coenzyme I H transport system is linked to the K and Na transport; no evidence is available for or against this mechanism.

Competition by Other Ions.—

Studies on competition between the alkali ions were carried out in order to determine whether Na and K were transported by the same mechanism, and also whether the mechanism could discriminate between Na and K and the other alkali ions. From the data presented in Tables VII and XIII, it is possible to sketch a diagram which is given as Fig. 9 showing the results of these experiments. This diagram can be employed as a qualitative guide only, in view of the uncertainties involved in its preparation, as for example the disregard of the effect of massive external K concentrations on Na transport; and the rather uncertain measurements of Li transport into the cell. The heights

[7] I am indebted to Dr. E. G. Ball for helpful discussions on these points.

of the various blocks represent the approximate ease of entrance of the several ions; numerical data may be found in the tables. The height of the Na block in the middle section and that of the K block in the bottom section have both been normalized to a value of unity, so that only relative heights in each sec-

APPROXIMATE COMPETITION AMONG ALKALI IONS FOR ENTRANCE INTO THE HUMAN RED CELL

	Li	Na	K	Rb	Cs	
Hydrated Radius	10.0	7.9	5.3	5.09	5.05	A
Naked Radius	0.68	0.98	1.33	1.49	1.65	A

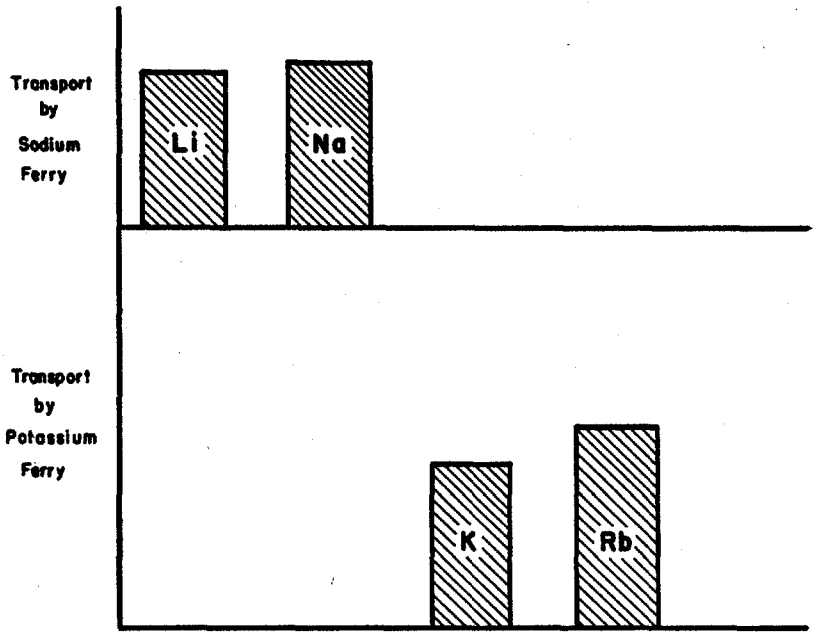


FIG. 9

tion are significant. The radius data come from Wiklander, as reproduced by Kunin and Myers (40). The absence of a block indicates that no competition has been found.

The red cell is not unique in its inability to distinguish between Na and Li. As early as 1902, Overton (41) showed that the irritability of muscle could be maintained in solutions of LiCl, as well as it could be in the normal NaCl en-

vironment. No other ion he tried, including K, Rb, and Cs would substitute for Na. Evidence from another tissue supports the similarity between K and Rb and the difference between Rb and Cs, since Huf and Wills (42) in their studies of the accumulation of Na at the chorion layer of frog skin have found that K and Rb separately each cause an increase in the accumulation, larger that that caused by Cs.

Since the electronic configuration of all the cations is similar and since their charge is identical, we must look to ionic size as an important factor in enabling the cell to distinguish between the ions which it transports across the membrane. In the case of muscle and nerve, Conway (43) has suggested that the hydrated ionic radius is an important factor governing permeability. On the contrary in these studies with the red cell the naked radius appears more likely to be the important factor. From Fig. 9 and Tables VII and XIII, we can make two observations. First, there is a sharp cleavage between Na and K for K cannot enter by the Na ferry, nor can Na enter by the K ferry. Second, there is a sharp cleavage between the behavior of Rb and that of Cs, for Rb enters the cell on the K ferry more readily than K, and Cs enters it very little, if at all. In connection with the first observation, there is a large difference between both hydrated and naked radius for Na and K, and either would suffice to explain the different methods of entry. In connection with the second observation it seems essential to find a difference between Rb and Cs to account for the high transport of the former and the very low transport of the latter. Since the hydrated radii of both atoms are almost identical whereas the naked radii differ by 10 per cent, it seems that the naked radius should be an important factor in enabling the cell to differentiate between these two ions. It should be mentioned that investigators and tables differ with regard to absolute values of naked, and particularly, hydrated radii. However, absolute values are unimportant in a discussion of this kind and relative values are more important. In this respect, fortunately there is general agreement, and the values given in Fig. 9 may be taken as representative.

If, therefore, we may conclude that the behavior of these ions is significantly different from that to be expected of free ions in a watery medium, we must look to the possibility of their entering into a reaction on the surface of the cell, of their transport through a non-aqueous medium, or both. These suggestions are in substantial agreement with the working hypothesis which will be set forth below.

Since the competition between ions for transport across the red cell membrane is apparently governed by ionic size, there are bound to be resemblances, superficial or real, between these mechanisms, and any other process which is sensible of ionic size, that is any process whose rate is in accord with the Hofmeister alkali ion series, either direct or inverse. For example, it has

been suggested[8] that the relative competition among these ions is similar in some respects to ion exchange. However, according to Kunin and Myers (44), the activation energy of cation exchangers is low, 4 to 5 kilocalories per mol, and the kinetics are in general accord with the diffusion theory. As we have seen neither of these statements can be made with respect to the red cell cation transport mechanism. Therefore we may conclude that the ion exchange mechanism as exemplified by resins is not a completely satisfactory model for red cell cation transport, though of course we cannot draw any valid conclusions about ion exchange mechanisms involving proteins in place of resins.

The Cation Barrier in the Red Cell Membrane.—

The hypothesis which will be put forward in the next section is based on an assumption that the cell is impermeable to free cations. Therefore it is necessary to examine the information available about the red cell membrane to see whether such an assumption is tenable. First, let us consider the charge on the outside of the membrane. Abramson and Moyer (45) have shown from mobility measurements that the red cell in man has an approximate zeta potential of −16.8 mv., which they have calculated to be equal to 15 million excess electrons on each red cell. They further estimate that about 1 per cent of the red cell surface is occupied by these 15 million electrons. The electronic charge is independent of the physical state of the cell, since, as Abramson, Furchgott, and Ponder (46) have shown, cells lysed by a variety of methods exhibit a mobility unchanged from the normal.

Waugh (47) in a review concerning the ultrastructure of the red cell membrane suggests that the membrane consists of a layer of platelets of proteins tangentially oriented on the red cell envelope and a second layer, either single or double, of lipoid oriented radially. It is tempting to associate the negative zeta potential in part with the protein layer in view of the usual tendency for proteins to have a negative charge at physiological pH, although this association is by no means complete as pointed out by Ponder (34). Abramson, Furchgott, and Ponder have, however observed that chloroform, a lytic agent which they thought might dissolve, or at least disorient the lipoid layer, did not change the mobility of the lysed cells from that of the normal cells. Now, if we combine the figure of Abramson and Moyer of 1.5×10^7 excess charges per cell and the mean value given by Ponder for cell area as 1.1×10^8 A^2, we obtain a figure of one net negative charge for every 750 A^2 of cell surface. This figure may be compared with values estimated by Edsall (48) for fibrino-

[8] I am indebted to Dr. F. D. Carlson for pointing out the resemblance of Na and K transport to ion exchange mechanism.

gen and albumin both of which have one net negative charge for approximately 700 A^2 of area at a pH of 7.3.

The negative zeta potential gives no evidence about the charges deeper in the membrane, but merely states that the resultant charge at the outside of the cell is negative. We are therefore free to assume that there exists a set of dipoles associated with the lipoid layer within the cell so arranged that the positive charge is outermost, and that any particles passing through the protein layer have next to penetrate a positive charge barrier. Fig. 10 shows a schematic drawing of a possible arrangement of charges. Such a positive

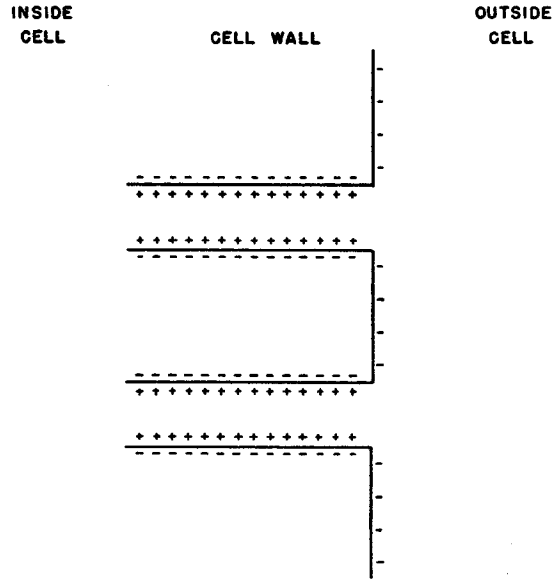


FIG. 10

charge barrier in the cell would account for the relative cellular impermeability to cations.

Furthermore, it would fit in with the theory of Teorell (49) and Meyer and Sievers (50) concerning passive selective diffusion across a membrane.[9] Briefly the theory supposes that in cases of passive transport, increased transport of ions of one sign is caused by the presence of ions of the opposite sign fixed in the membrane. Meyer and Sievers give the equation $\frac{n_A}{n_K} = \frac{U_A (y + A)}{U_K y}$ for the ratio of the transport of anions to cations in a membrane in which the concentration of fixed cations is A. n_A and n_K are the respective "transport num-

[9] I am grateful to Dr. T. Rosenberg for pointing out the relevance of these theories to this problem.

bers" of anions and cations in the membrane, U_A and U_K are the respective mobilities of these ions in the membrane, and y is the concentration of salt in the membrane given by $y(y + A) = c^2$ where c is the external concentration. Since it has been shown by Fetcher (51) and Sollner and Carr (52) that the predictions of this theory are not quantitatively verified, it is profitable only to examine it in a qualitative fashion to see whether it may yield any valid information concerning the red cell membrane.

The relative permeability of anion to cation through the membrane can be obtained from a comparison of the half-time for cellular K exchange of 35.5 hours obtained in this study and that for cellular Cl exchange of 1 to 3 minutes in the rabbit at 0° obtained by Hahn and Hevesy (53). In view of the much more rapid exchange of HCO_3 for Cl shown by Dirken and Mook (54), the figure of 1 minute is probably an overestimate. However, the ratio of 2000 between these two permeabilities is probably correct within one to two orders of magnitude. In view of the difficulties associated with the quantitative interpretation of the Teorell-Meyer-Sievers theory, and the lack of information about the relative "mobility" of K and Cl in the membrane, we can only say that this theory supports the assumption of a positive charge region in the membrane. Waugh has pointed out that the water content of the membrane is only about 25 per cent and hence the Teorell-Meyer-Sievers requirements for a large fixed cation concentration within the membrane are somewhat reduced. Incidentally, if the 25 per cent water content may be considered to be associated with the protein, a not improbable assumption, the lipoid layer may be expected to be relatively water-free, supporting the possibility of the presence of a lipoid-soluble non-dissociable K carrier.

The presence of a non-conducting membrane which restricts the free passage of ions as such is supported by the measurements of Fricke (55), who estimated the capacity of the red cell membrane as 0.80 microfarad/cm². This is to be compared with values for nerve membranes of the order of 1 microfarad/cm². These values of membrane capacity have been interpreted by Cole (56) and more recently by Katz (57) as describing an insulating membrane with a few scattered gaps representing a small portion of the total area through which water and ions can pass.

If the membrane were a simple charge barrier alone, we would expect that except for modifications due to size, all small anions would penetrate very fast, within a few orders of magnitude of the rate of chloride penetration. However, it has been shown by Mueller and Hastings (58) that phosphate, probably as $HPO_4^=$, penetrates the cell slowly; the net phosphate transfer (8.49 μM P/minute liter plasma) being less indeed than that observed for K. However, this picture is complicated by the manifold reactions into which the phosphate enters. The high temperature coefficient observed by Gourley and Gemmill (59) for phosphate transfer is typical of a metabolically linked proc-

ess rather than simple diffusion. Further there may be considerable competition on the cell surface for any free $HPO_4^=$ ions. Therefore the slow penetration of $HPO_4^=$ through the membrane, relative to Cl^-, points to a metabolic factor in erythrocyte anion permeability.

Further support for the view that the behavior of phosphate is anomalous can be found in the observations of Gray and Sterling (60) on the permeability of the erythrocyte to Cr^{51}. Chromium is only present in the red cell in trace quantities, and it is unlikely that the inward permeability to Cr^{51} is seriously modified by metabolic factors. These authors found that the erythrocyte was permeable to Cr^{51} as Na_2CrO_4, but that Cr^{51} would not enter the cell as $CrCl_3$. When added *in vitro*, the half-time for exchange of $CrO_4^=$ was 38 minutes. When $CrCl_3$ was added, almost all of it (94 to 99 per cent) remained in the plasma. As would be expected, the $CrO_4^=$ exchange is faster than the $HPO_4^=$ exchange, for the half-time for the latter, as determined by Mueller and Hastings, is 109 minutes.

Although it is not possible from the evidence at hand to state that there is a positive charge barrier deep in the erythrocyte membrane, such an assumption would in no way be contrary to the published data. Indeed the assumption is in harmony with the evidence presented, and provides an explanation for the startling difference between cationic and anionic permeability. In the range of size of inorganic ions discussed, steric effects, though not absent, would seem to be secondary, both to the charge barrier and to metabolic effects, so marked in the case of $HPO_4^=$.

Working Hypothesis for the Transport of Na and K.—

We can now sum up the requirements for a working hypothesis concerning the Na and K transport across the red cell membrane. First, the normal mechanisms for Na and K transport should be different, though both their energy sources are probably linked to glucose metabolism. Second, their transport across the membrane should be in non-ionic form, presumably in attachment to a carrier. Third, the mechanism should include a feature which accounts for the fact that the K transport into the cell is independent of extracellular K concentration, and that the Na transport is not linearly dependent on extracellular Na concentration. Fourth, the hypothesis should include a method for regulating intracellular K and Na concentration. Finally, the hypothesis should explain how cold-stored or glucose-deprived cells can lose K and gain Na, and can then reverse this process when restored to a normal environment.

It is clear that any hypothesis which meets the third requirement involves the assumption of a limiting substance or set of reactions that regulate the transport. The data in Fig. 2 show that the K ferry is limited in its capacity; there is no standing room, and the number of passengers is limited by the

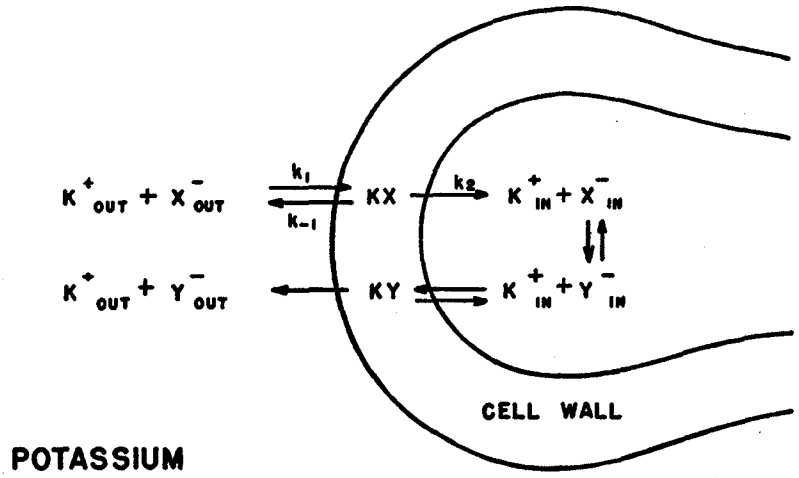
number of seats, and the number of trips per hour. The Na ferry, on the other hand, is not full, and so is sensitive to the number of passengers waiting on the shore. The number carried is a function not only of the passengers waiting, but also of the number of vacant seats, and the number of trips per hour. This observation implies that we accept the Na concentration effect as real, which is not necessarily true. However, the working hypothesis will fit any situation from a linear dependence of Na transport on external concentration, to independence of Na transport of external concentration, and hence may be advanced without more conclusive evidence on the Na concentration effect. In the treatment which follows we will accept the Na concentration effect, but always with the implicit reservation that it is open to later proof.

The two most likely models for the ferries are the Michaelis-Menten model in which the number of vacant seats represents the amount of free enzyme waiting for a suitable substrate to which it may couple, and the Langmuir adsorption isotherm, in which the number of vacant seats represents the number of unfilled adsorption sites on the cell surface. Of course, if we assume as is not unlikely that the enzyme, or enzymes, responsible are located on the cell surface, these two models become the same and the difference disappears. However, it must not be assumed that the treatment which follows implies any evidence against any model system which is carrier-limited.[10]

Let us assume as shown in Fig. 11 that the K^+ ion is transported through the lipoid membrane by forming a lipoid-soluble, lipoid–non-dissociable complex on the cell surface, and let us call this complex KX. KX, in response to its local concentration gradient, diffuses into the cell, and in the watery medium there, dissociates into K^+ and X^-. We must destroy X^- at once in order to block the back reaction, and hence we assume that X^- is metabolized into Y^- in one step or a number of steps. Then to complete the cycle and to transport K^+ out of the cell, we let K^+ unite with Y^-, forming KY, a lipoid-soluble, lipoid–non-dissociable complex, which is then free to diffuse through the membrane to the plasma where it dissociates and Y is lost to the system. Now it is necessary further to assume that the consumption of X at 37° is very rapid, but that its consumption and consequent transference to Y do not represent the rate-limiting step. It is not possible at this time to decide whether X^- goes to Y^- and then diffuses out of the system, as would probably be the case were X^- and Y^- members of the glucose-lactate system, or whether X^- is conserved by regeneration from Y^- outside the cell.

The question now arises as to why X^- does not serve as the normal high temperature escape route. We know it is necessary to postulate a mechanism for the regulation of intracellular K, which, as shown in Table III, is remarkably constant. We have shown that it is not regulated by the external K

[10] A full discussion of this point can be found in papers by Burton (61) and Hearon (29).



WORKING HYPOTHESIS FOR CATION TRANSPORT IN HUMAN RED BLOOD CELLS

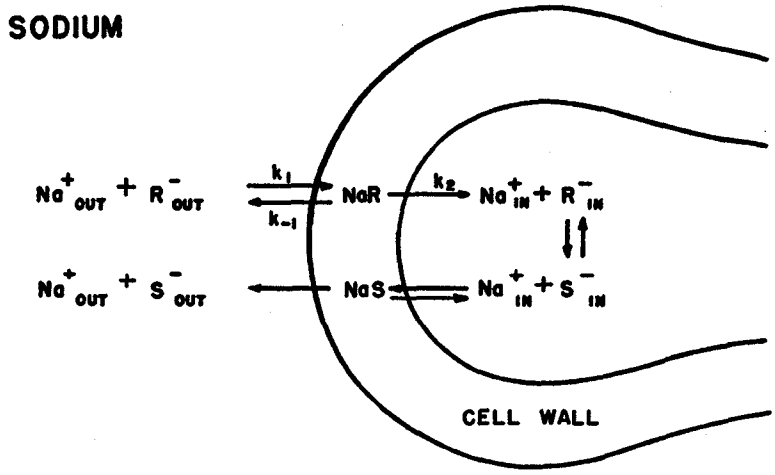


FIG. 11

concentration, and we may therefore hypothesize that it is regulated by the internal K concentration. If X^- is brought in only as KX, there will be no excess X^- within the cell to allow for the removal of excess K^+. But if Y^- contains a few more sites for outward transport than does X^- for inward

transport, or if the $X^- \rightarrow Y^-$ reaction rate is dependent on intracellular K concentration, the cell can regulate itself by the law of mass action applied to its internal K concentration.

Using a steady state treatment similar in many respects to that of Laidler (62), we may attempt to evaluate the specific rate constants in the first two reactions in Fig. 11. Consider the following three reactions which include the reaction for the formation of X^- from the unknown reactants C_0 and C_1.

$$C_0 + C_1 \underset{k_{-0}}{\overset{k_0}{\rightleftharpoons}} X_p^- \tag{18 a}$$

$$K_p^+ + X_p^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} KX \tag{b}$$

$$KX \xrightarrow{k_2} K_c^+ + X_c^- \tag{c}$$

in which the subscript p refers to plasma and c to cell.

In the steady state,

$$\frac{d[X^-]_p}{dt} = k_0[C_0][C_1] - k_{-0}[X^-]_p - k_1[X^-]_p[K^+]_p + k_{-1}[KX] = 0 \tag{19 a}$$

$$\frac{d[KX]}{dt} = k_1[K^+][X^-]_p - k_{-1}[KX] - k_2[KX] = 0 \tag{b}$$

Solving these equations for $[KX]$, we obtain:

$$[KX] = \frac{k_0 k_1 [K^+]_p [C_0][C_1]}{k_{-1}k_{-0} + k_2 k_{-0} + k_1 k_2 [K^+]_p} \tag{20 a}$$

$$[KX] = \frac{k_1[K^+]_p[X^-]_p}{k_{-1} + k_2} \tag{b}$$

The rate of reaction is then:

$$-\frac{d[K^+]_p}{dt} = k_2[KX] = \frac{k_0 k_1 k_2 [K^+]_p [C_0][C_1]}{k_{-1}k_{-0} + k_2 k_{-0} + k_1 k_2 [K^+]_p} \tag{21}$$

and if $[K^+]k_1 k_2 \gg k_{-1}k_{-0} + k_2 k_{-0}$

$$-\frac{d[K^+]_p}{dt} = k_0[C_0][C_1] \tag{22}$$

so that the reaction rate is independent of plasma $[K^+]$, in accord with our results. From Fig. 2, we have seen that $k_{ab}\frac{(1-H)}{H}$ for K uptake is not a constant independent of $[K^+]_p$ but moves inversely with it, so that the prod-

uct $k_{ab} \frac{(1 - H)}{H} [K^+]_p$ is a constant equal to 1.67 m.eq./liter cells hour, which is the reaction rate. From 20 b and 21, we can see that

$$k_{ab} \frac{(1 - H)}{H} = \frac{k_1 k_2 [X^-]_p}{k_{-1} + k_2} \tag{23}$$

Hence k_{ab} is not a specific rate constant, but according to the present hypothesis includes not only the three specific rate constants k_1, k_{-1}, and k_2, but also the concentration of the unknown intermediate $[X^-]$. Therefore, the apparent activation energy derived from the temperature coefficient of K transport may reflect any combination of activation energies of reactions 1 (*a*), (*b*), and (*c*) as well as any temperature-induced change of the concentration of X^-. However, it is interesting to note that the reaction rate gives a straight line Arrhenius plot over the temperature range 22 to 44°C. as shown by Sheppard and Martin (2). Further, the apparent activation energy that we have obtained appears to be independent of $[K]_p$. These observations support the assignment of the activation energy to a single rate constant in Equation 23, but they do not in any sense prove it.

An exactly similar hypothesis for Na can be formulated, according to the reaction scheme shown in Fig. 11. The carrier, of course, is different from that for K, and is formed by the reaction:

$$D_0 + D_1 \underset{k_{-0}}{\overset{k_0}{\rightleftharpoons}} R_p^- \tag{24}$$

All the constants k_0, k_{-0}, k_1, k_{-1}, and k_2 are different from those for K. A set of equations equivalent to the K set would lead to

$$-\frac{d[Na^+]_p}{dt} = \frac{k_0 k_1 k_2 [Na^+]_p [D_0][D_1]}{k_{-1} k_{-0} + k_2 k_{-0} + k_1 k_2 [Na^+]} \tag{25}$$

which can be rearranged to the form of equation (13)

$$-\frac{d[Na^+]_p}{dt} = \frac{a[Na^+]_p}{c[Na^+]_p + 1} \tag{26}$$

in which

$$a = \frac{k_0 k_1 k_2 [D_0][D_1]}{k_{-1} k_{-0} + k_2 k_{-0}} = 0.0392 \text{ and } c = \frac{k_1 k_2}{k_{-1} k_{-0} + k_2 k_{-0}} = 0.0105$$

using the numerical values already obtained. Now, the fraction of the carrier filled, θ, is given by

$$\theta = \left(\frac{a[Na^+]_p}{c[Na^+]_p + 1} \right) \Big/ \frac{a}{c} = 0.60 \tag{27}$$

at $[Na^+]_p$ = 143.5 m.eq./liter. That is, the normal Na transport mechanism, accepting our experimental results, operates at 60 per cent of capacity.

More detailed information about the relative values of the constants for both Na and K must await identification and estimation of the amount of carrier present in each case.

Now let us examine the requirement that the hypothesis account for the behavior of red cells in the cold and in the absence of glucose. It has been pointed out that cells at 4°C. exchange intracellular K for Na, an exchange which is reversed when the cells are returned to 37°. Ponder (63) has shown that in the absence of glucose at 37° a K—Na exchange, similar to that at 4°C., takes place. These glucose-free cells do not swell by more than 10 or 20 per cent indicating that the absence of glucose does not damage the cellular cation barrier in such a way as to allow free cation transport. Thus the charge on which this impermeability presumably depends is fixed in the membrane and is not the result of a glucose-limited metabolic process.

This behavior can be accounted for in our working hypothesis by assuming that the step in which the carrier X^- is metabolized to Y^- (and R^- metabolized to S^-) has a high activation energy, but is not the rate-limiting step at 37°. Then as the temperature is lowered, this step becomes limiting, X^- and R^- accumulate, and the back transport of K^+ and Na^+ from the cell goes on by means of exchange diffusion. Further, we would assume that the $X^- \rightarrow Y^-$ and $R^- \rightarrow S^-$ step is dependent upon a supply of glucose, which is a most likely assumption, so that the absence of glucose would also cause an accumulation of X^- and R^-.

Since at 37° in the presence of glucose, the Na and K mechanisms are demonstrably independent, the existence of a species-selective (Na or K) carrier would still not account for the observed K depletion and Na uptake. It is necessary to require that the trapped carrier always takes a cation with it as it crosses the membrane, for otherwise it would short-circuit the membrane cation barrier and provide cationic equilibrium inside and outside the cell. Since this does not occur, the trapped carrier must only cross the membrane with a cation, and it becomes necessary to assume that the absence of glucose, or incubation in the cold, abolishes the species selectivity of the carrier. Under these conditions, the non-selective carrier would be trapped in the cell wall and would ferry Na and K back and forth until the concentration gradient for each ion disappeared, although the cation barrier still remained. Increase in temperature (or addition of glucose) would cause the disappearance of the carrier and the regaining of species selectivity. Thus the cell would gradually return to a normal intracellular cationic composition.

Next let us examine the requirement that the cell composition be regulated, particularly in respect to K, for which more evidence is available. Krogh (64) has considered the possibility that the volume of the red cell was the factor

which regulated the intracellular K concentration. In his Croonian lecture, he reported that experiments undertaken in his laboratory had failed to show that red cell volume exercised any regulatory effect. Harris and Maizels (25) have suggested that the intracellular K determines the rate of K transport. Ponder's (20) observations on the 3 hour accumulation of K at 37° as a function of the time red cells have previously been cold-stored provide clear indication that this is likely. Since red cells when cold-stored lose K, the uptake of K when cold-stored cells are brought to room temperature, is a function both of the red cell K content, and the length of storage of the cells. Ponder finds that cells cold-stored for 1 day have a K concentration 89 per cent of normal and that cells cold-stored for 3 days have a K concentration 68 per cent of normal. When these cells are incubated for 3 hours with glucose at 37°, the cells with the lower K concentration accumulate K more than half again as fast as those with the higher K concentration, a further indication that intracellular K content regulates net cellular uptake of K. Cells cold-stored for periods longer than 3 days have a lower rate of K accumulation, possibly due to deterioration of the cells.

Such a mechanism is in accord with the working hypothesis, for an increase in intracellular K would cause the reaction $K^+ + Y^- \rightarrow KY$ to proceed more quickly, and hence transport more K out of the cell. With a constant rate of K transport into the cell, regulation could be achieved by adjustment of the rate of exit alone.

However, one disturbing fact remains. Ponder (20) finds that the uptake of K at 37° by cells previously cold-stored for 60 hours is 3 m.eq./liter cell H_2O hour or approximately 4.5 m.eq./liter cells hour This almost 3 times as great as the normal flux of 1.67 m.eq./liter cells hour observed by us at 37°. Hence even complete inhibition of outward transport is not sufficient to account for Ponder's observation. The possibility exists, therefore, that the rate of K entrance increases when the K content of the cells is very low, which does not fit our present working hypothesis. This may arise either from the unphysiological condition of the cell, or may be a real feature of cation transport under normal conditions. This point must await further experimental results.

The question may arise as to whether there is further evidence beyond that from the activation energy measurements that the passive transports, K out from the cell and Na into the cell, cannot be accounted for on the basis of simple diffusion, or rather diffusion through a potential barrier. Should this be the case, the amount of K leaving the cell in unit time would bear a linear relationship to the external K concentration. In the steady state the amount of K leaving the cell is equal to the amount entering the cell, and this we have seen is independent of external concentration. Hence simple diffusion through a potential barrier will not account for the outward transport of K.

In the case of Na, we find from the data presented in Table XII that the

amount of Na entering the cell bears an approximately linear relationship to the external Na concentration. Fitting the data of Experiments 70, 78, 80, and 82D by the method of least squares, we find that the data can be expressed by the following equation:

$$\frac{\text{Milliequivalents of Na transported into cell}}{\text{Liter of cells, hour}} = 0.72 + 0.019\,[Na]_{ext}$$

Fick's diffusion equation may be paraphrased thus:

$$\frac{\text{Milliequivalents of Na transported into cell}}{\text{Liter of cells, hour}} = \text{constant}\,\{[Na]_{ext} - [Na]_{cell}\}$$

These two expressions may be compared by their behavior at low $[Na]_{cell}$. The second equation would have a negative intercept at $[Na]_{ext} = 0$, and the amount of Na transported per hour per liter of cells would be zero when $[Na]_{ext} = [Na]_{cell}$. This is not in accord with the experimental facts, which in so far as they can be trusted in view of the artificial medium in which they were obtained, indicate that Na transport into the cell is not a process of diffusion through a potential barrier, or indeed one of simple diffusion of any kind.

There are many similarities between the present hypothesis and others advanced for a wide variety of tissues, including the requirement that the carrier itself be metabolized, or at least transformed inside the cell, so that back diffusion is prevented (31, 65–67). It is interesting to note that the theory of nerve conduction of Hodgkin and Huxley (68) requires two phases of changed permeability in the nerve sheath during activity, one associated with a sudden increase in Na permeability, and a second, later in time, associated with an increase in K permeability. The independence of Na and K transport which we have observed in the red cell seems to have a counterpart in the nerve. Hodgkin and Katz (69) also have postulated a lipoid-soluble carrier to account for the movement of Na in nerve.

In the cat erythrocyte, Davson and Reiner (70) have found that Na and K permeability are affected very differently by a wide variety of poisons, from which they conclude that there is a special mechanism for Na penetration from which K is more or less excluded. Ponder (63) has also recently suggested in a general way that "an outwardly directed Na transfer mechanism and inwardly directed K mechanism" would be satisfactory as an explanation for the transport phenomena in red cells, another suggestion that is in good accord with the present hypothesis.

The present hypothesis has been put forward to serve as a working hypothesis, in the hope that a kinetic system consisting of a set of concrete reactions would be of help in fashioning experiments to test the validity of the assumptions that have been made. It is believed that the hypothesis conforms

in general to the experimental facts observed in measurements of red cell cation flux, but it is realized that a considerable number of additional experiments are required before any of the specific reactions proposed may be considered as established.

The research has been supported in part by the Atomic Energy Commission. The author is particularly indebted to Dr. I. M. Taylor and Dr. J. M. Weller for their explanation of the flame photometer and the care of red cells. The flame and other analyses which have been so essential to the problem have been carried out successively by Mrs. Preston Gifford 3rd, Miss Brita Rolander, and Mr. Huntington Mavor, all of whom have offered invaluable assistance in the laboratory. The counting was kindly done by Mrs. Frances Newton. The author is grateful to Dr. F. Brink, Dr. J. Friedenwald, and Dr. A. B. Hastings for their helpful suggestions.

SUMMARY

Measurements have been made on the permeability of the human erythrocyte to Na and K *in vitro*, using radioactive tracers to observe the system in the steady state. The average inward K flux is 1.67 m.eq./liter cells hour, and the apparent activation energy is 12,300 ± 1300 calories/mol. The inward K flux is independent of the external K concentration in the range of concentrations studied (4 to 16 m.eq. K/liter plasma). Rb appears to compete with K for transport into the cell, whereas Na and Li do not. The average inward Na flux is 3.08 ± 0.57 m.eq. Na/liter cells hour, and the apparent activation energies are 20,200 ± 2700 calories/mol for inward transport, and 14,900 ± 3,400 calories/mol for outward transport. The inward Na flux is dependent on the external Na concentration, but not in a linear fashion. Li appears to compete with Na for inward transport, whereas K and Rb do not.

An approximate maximum estimate shows that the energy required for cation transport is only 8.8 calories/mol liter cells hour of the 110 calories/mol liter cells hour available from the consumption of glucose. A working hypothesis for the transport of Na and K is presented.

BIBLIOGRAPHY

1. Raker, J. W., Taylor, I. M., Weller, J. M., and Hastings, A. B., *J. Gen. Physiol.*, 1950, **33,** 691.
2. Sheppard, C. W., and Martin, W. R., *J. Gen. Physiol.*, 1950, **33,** 703.
3. Sheppard, C. W., and Martin, W. R., AECD-2249, Atomic Energy Commission, 1949.
4. Sheppard, C. W., Martin, W. R., and Beyl, G., *J. Gen. Physiol*, 1951, **34,** 411.
5. Parpart, A. K., and Ballentine, R., *Science*, 1943, **98,** 545.
6. Ponder, E. Hemolysis and Related Phenomena, New York, Grune & Stratton, 1948, 53 *ff.*
7. Millar, W. G., *Quart. J. Exp. Physiol.*, 1925, **15,** 187.
8. Nelson, N., *J. Biol. Chem.*, 1944, **153,** 395.

9. Cohn, W. E., and Kohn, H. W., *J. Am. Chem. Soc.*, 1948, **70,** 1986.
10. Siegbahn, K., *Ark. Mat. Astron. Fysik.*, 1947, **34B,** No. 4.
11. Van Voorhis, S. N., *Physic. Rev.*, 1936, **49,** 889.
12. Solomon, A. K., *Physic. Rev.*, 1950, **79,** 403.
13. Cobble, J. W., and Atteberry, R. W., *Physic. Rev.*, 1950, **80,** 917.
14. Sreb, J. H., *Physic. Rev.*, 1951, **81,** 469.
15. Robinson, C. V., *Science*, 1950, **112,** 198.
16. Solomon, A. K., and Estes, H. C., *Rev. Scient. Instr.*, 1948, **19,** 47.
17. Keitel, H., private communication.
18. Solomon, A. K., *J. Clin. Inv.*, 1949, **28,** 1297.
19. Mullins, L. J., and Zerahn, K., *J. Biol. Chem.*, 1948, **174,** 107.
20. Ponder, E., *J. Gen. Physiol.*, 1950, **33,** 745.
21. Danowski, T. S., *J. Biol. Chem.*, 1941, **139,** 693.
22. Harris, J. E., *J. Biol. Chem.*, 1941, **141,** 579.
23. Scudder, J., Drew, C. R., Corcoran, D. R., and Bull, D. C., *J. Am. Med. Assn.*, 1939, **112,** 2263.
24. Sheppard, C. W., and Beyl, G. E., *J. Gen. Physiol.*, 1951, **34,** 691.
25. Harris, E. J., and Maizels, M., *J. Physiol.*, 1951, **113,** 506.
26. Edelman, I. S., James, A. H., and Moore, F. D., private communication.
27. Davson, H., *Biochem. J.*, 1939, **33,** 389.
28. Harris, E. J., and Burn, G. P., *Tr. Faraday Soc.*, 1949, **45,** 508.
29. Hearon, J. Z., *Bull. Math. Biophysics*, 1949, **11,** 29, 83; 1950, **12,** 57, 85.
30. Tolman, R. C., and Fine, P. C., *Rev. Mod. Physics*, 1948, **20,** 51.
31. Ussing, H. H., *Physiol. Rev.*, 1949, **29,** 127.
32. Hodgkin, A. L., and Keynes, R. D., *Abstract XVIII Internat. Physiol. Cong.*, 1950, 258.
33. Hastings, A. B., Sendroy, J., Jr., McIntosh, J. F., and Van Slyke, D. D., *J. Biol. Chem.*, 1928, **79,** 193.
34. Ponder, E., Hemolysis and Related Phenomena, New York, Grune & Stratton, 1948, 119, 121, 135.
35. Meyerhof, O., *Ann. New York Acad. Sc.*, 1944, **45,** 377.
36. Flynn, F., and Maizels, M., *J. Physiol.*, 1950, **110,** 301.
37. Taylor, I. M., and Weller, J. M., *Biol. Bull.*, 1950, **99,** 311.
38. Wilbrandt, W., *Tr. Faraday Soc.*, 1937, **33,** 956.
39. McKee, R. W., Ormsbee, R. A., Anfinsen, C. B., Geiman, Q. M., and Ball, E. G., *J. Exp. Med.*, 1946, **84,** 569.
40. Kunin, R., and Myers, R. J., Ion Exchange Resins, New York, John Wiley and Sons, Inc., 1950, 63.
41. Overton, E., *Arch. ges. Physiol.*, 1902, **92,** 346.
42. Huf, E. G., and Wills, J., *Fed. Proc.*, 1951, **10,** 67.
43. Conway, E. J., *Irish J. Med. Sc.*, 1947, **6,** 593, 654.
44. Kunin, R., and Myers, R. J., Ion Exchange Resins, New York, John Wiley and Sons, Inc., 1950, 24.
45. Abramson, H. A., and Moyer, L. S., *J. Gen. Physiol.*, 1936, **19,** 605.
46. Abramson, H. A., Furchgott, R. F., and Ponder, E., *J. Gen. Physiol.*, 1939, **22,** 545.

47. Waugh, D. F., *Ann. New York Acad. Sc.*, 1950, **50,** 835.
48. Edsall, J. T., private communication.
49. Teorell, T., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33,** 282; *Proc. Nat. Acad. Sc.*, 1935, **21,** 152; *J. Gen. Physiol.*, 1937, **21,** 107.
50. Meyer, K. H., and Sievers, S. F., *Helv. Chim. Acta*, 1936, **19,** 649, 665, 987. Meyer, K. H., Hauptmann, H., and Sievers, S. F., *Helv. Chim. Acta*, 1936, **19,** 948. Meyer, K. H., *Helv. Chim. Acta*, 1937, **20,** 634. Meyer, K. H., and Straus, W., *Helv. Chim. Acta*, 1940, **23,** 795.
51. Fetcher, E. S., Jr., *J. Physic. Chem.*, 1942, **46,** 570.
52. Sollner, K., and Carr, C. W., *J. Gen. Physiol.*, 1944, **28,** 1.
53. Hahn, L., and Hevesy, H., *Acta physiol. scand.*, 1941, **3,** 214.
54. Dirken, M. N. J., and Mook, H. W., *J. Physiol.*, 1931, **73,** 349.
55. Fricke, H., *Physics*, 1931, **1,** 106.
56. Cole, K. S., *Cold Spring Harbor Symp. Quant. Biol.*, 1940, **8,** 110.
57. Katz, B., Symposia of the Society for Experimental Biology (Great Britain) in press.
58. Mueller, C. B., and Hastings, A. B., *J. Biol. Chem.*, 1951, **189,** 869.
59. Gourley, D. R. H., and Gemmill, C. L., *J. Cell. and Comp. Physiol.*, 1950, **35,** 341.
60. Gray, S. J., and Sterling, K., *J. Clin. Inv.*, 1950, **29,** 1604.
61. Burton, A. C., *J. Cell. and Comp. Physiol.*, 1936, **9,** 1.
62. Laidler, K. J., Chemical Kinetics, New York, McGraw-Hill Publishing Co., Inc., 1950, 280 *ff.*
63. Ponder, E., *J. Gen. Physiol.*, 1951, **34,** 359.
64. Krogh, A., *Proc. Roy. Soc. London, Series B*, 1946, **133,** 176.
65. Rosenberg, T., *Acta chem. scand.*, 1948, **2,** 14.
66. Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1949, **35,** 548.
67. Osterhout, W. J. V., *Biol. Bull.*, 1950, **99,** 308.
68. Hodgkin, A. L., and Huxley, A. F., *Abstract XVIII Internat. Physiol. Cong.*, 1950, 36.
69. Hodgkin, A. L., and Katz, B., *J. Physiol.*, 1949, **108,** 37.
70. Davson, H., and Reiner, J. M., *J. Cell. and Comp. Physiol.*, 1942, **20,** 325.