PERMEABILITY OF ERYTHROCYTES TO RADIOACTIVE POTASSIUM

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It appears to be well established that the red cells are impermeable to cations, but there have nevertheless been some experiments which have demonstrated slow changes in the potassium content. The availability of radioactive isotopes makes possible a re-examination of this question, and some reports based upon this method have already been published.

Cohn and Cohn (1939) showed that radioactive sodium exchanged with sodium in dog erythrocytes *in vivo* so that half the sodium had exchanged in 12 hours. They found that the sodium exchanged as if there were a simple diffusion, taking into account the difference in concentration of sodium between dog cells and plasma. In this laboratory Manery and Bale (1940) have also found evidence of penetration of sodium into dog cells *in vitro*, but their results with rats and rabbits *in vivo* were inconclusive on account of the small amounts of sodium normally present in the cells of these animals.

On the other hand, Hahn, Hevesey, and Rebbe (1939 *a* and *b*) using the radioactive potassium isotope K^{42} found that only about 3 per cent of the potassium in red cells of the frog or rabbit exchanged with the plasma. Joseph, Cohn, and Greenberg (1938 and 1939) working with the rat measured activities in whole blood only, but their data are consistent with a fall in plasma radioactivity followed by a rise in corpuscular activity. Even so they find quite low penetration of radioactive potassium into the red cells. Eisenmann, Ott, Smith, and Winkler (1940) working with human red cells reported no exchange of potassium or sodium. In view of the extremely low sodium content of human cells, it is doubtful whether penetration of sodium could be measured by a radioactive method in this material.

A preliminary abstract of our experiments with radioactive potassium and red cells has already been published (Noonan, Fenn, and Haege, 1940). In this paper they are presented in greater detail.

Methods

Radioactive potassium chloride was prepared by bombarding 100–200 mg. of pure crystals of KCl with a neutron beam of 4.5 m.e.v. for 3 to 4 hours. The crystals were then dissolved in a little water, and the potassium was precipitated as $KClO_4$ by the addition of a saturated solution of NH_4ClO_4. Powdered MnO_2 was added and the mixture was filtered in a porous crucible. The precipitate was washed with alcohol followed by ether and dried. The crucible was then ignited either in a muffle furnace at 500°C. overnight or in a quartz crucible over a Bunsen burner for 1 hour which was found to be sufficient time to reduce the $KClO_4$ to KCl. The crucible was then cooled and weighed. The KCl was dissolved out in a little hot water, transferred to a volumetric flask, and made up to 10 ml. The crucible was dried and weighed again and the quantity of KCl in solution was calculated from the loss in weight. This procedure insured that radioactive sodium which might have contaminated the potassium as well as any activated chlorine was eliminated. There was also sufficient time for radioactive chlorine to disintegrate before the potassium was counted. The original potassium solution was diluted 1/500 for counting and was counted at least every 4 hours while counting of the experimental samples was in progress. Background counts were taken with the same frequency.

In the *in vivo* experiments a portion of the KCl solution (which was approximately isotonic) was injected directly into the animal, intraperitoneally or subcutaneously without anesthesia. Blood from the rabbit was taken from the ear vein into a beaker containing a dry mixture of sodium and ammonium oxalate. It was centrifuged at once. The plasma was drawn off and measured in a graduated centrifuge tube. The cells were washed once with saline (0.85 per cent NaCl) and centrifuged in a graduated centrifuge tube. The volume of cells was recorded and the saline removed. Nitric acid and a drop of caprylic alcohol were then added to the tubes, which were placed in a steam bath until the solution was a clear yellow. The tubes were cooled, the volume of digest recorded, and a 3 ml. aliquot was placed in the cup of a Geiger-Müller counter (Bale, Haven, and LeFevre, 1939) for counting. Afterwards aliquots of the digest were taken for potassium analysis by the method of Shohl and Bennet as modified by Wilde (1939).

Blood for *in vitro* experiments was obtained from rats and rabbits by cutting the throat and in humans by venous puncture. Either heparin or oxalate was used as an anticoagulant. The cells were washed twice in unbuffered mammalian Ringer that was 0.005 molar in potassium (0.037 per cent KCl). For rat and human cells it contained 0.8 per cent NaCl, 0.008 per cent $MgCl_2$, and 0.016 per cent $CaCl_2$, and for rabbit cells 1.0 per cent NaCl, 0.01 per cent $MgCl_2$, and 0.02 per cent $CaCl_2$. The cells were suspended in Ringer containing radioactive potassium or ordinary potassium depending upon the experiment so that 20 cc. of the mixture contained about 1 cc. of cells. The suspension was agitated in a water bath at 37.5°C. and was also aerated by a stream of air. Aliquots were taken at suitable intervals which were measured to the time of starting the centrifuge. The supernatant Ringer was sucked off and counted after a tenfold dilution with water. The cells were washed once with Ringer and treated just as the cells from the *in vivo* experiments. Because of the errors introduced into potassium analyses by large quantities of sodium Ringer was not analyzed for potassium, but the change in the K content was calculated from the change in cell potassium.

In some experiments the change in radioactivity of the Ringer was similarly calculated.

The counts of the standard radioactive potassium solution were plotted on semilogarithmic paper, and activities at times corresponding to sample counts were interpolated from straight lines connecting the plotted points. Ordinarily the semilogarithmic graph had the theoretical slope for a half-life of 12.5 hours. Any deviation from this slope indicated a change in the efficiency of the counter which was thus automatically corrected for by this procedure. All counts were expressed as a fraction of the standard at the time of counting. By dividing this fraction by the ratio of the potassium concentration in the nitric acid digest to that in the standard, solution counts were reduced to a molar basis (*i.e.* number of counts per unit of potassium) which expresses the fraction of the active potassium present in the potassium of the sample.

This figure is referred to as the "activity" of the sample or the relative number of counts per mol of potassium on the basis of 1000 for the number of counts in the standard solution per mol of potassium.

RESULTS

The penetration of radioactive potassium into human cells suspended in Ringer at 37.5°C. is shown in Table I and Fig. 1 as measured in two experiments A and B. The data for cells were fitted empirically by a curve of the type, $x = mt^n + c$, and the slope of this curve at each point calculated as $mnt^{(n-1)}$. The accuracy of each point does not exceed 5 per cent, and this method was considered adequate to give the slope to the possible limits of accuracy. The diffusion coefficient[1] is calculated as the quotient of this slope divided by the difference in relative activity of cells and Ringer. The diffusion coefficients average 0.20×10^{-3} and 0.24×10^{-3} in two cases. The differences are probably not significant.

The penetration of radioactive potassium into the red blood cells of a rabbit *in vivo* is shown in Table II and Fig. 2. The diffusion coefficient has been calculated in the same way as for the human cells, and it is observed to be larger immediately after injection. This might be correlated with the high potassium concentration in the plasma at this time.

Radioactive potassium was put into rabbit erythrocytes by suspending them in Ringer containing active potassium for 10 hours at room temperature (23°C.). A portion of the same cells received identical treatment except that the Ringer contained nonradioactive potassium. Both lots of cells were centrifuged and washed once with plasma. The inactive cells were suspended in radioactive Ringer and the active cells in inactive Ringer. Both lots were aliquoted in 20 ml. portions into 50 ml. Erlen-

[1] This is not strictly a diffusion coefficient in the usual sense, for it has the dimensions of minutes^{-1} and its value depends upon the area and thickness of the diffusing surface as well as the actual speed of penetration.

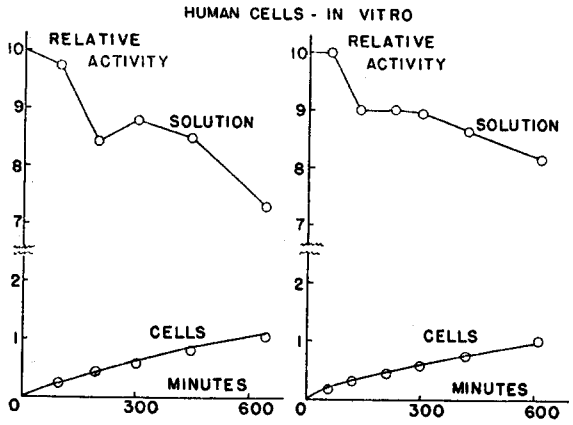

FIG. 1 *a* and 1 *b*. Two experiments on human red cells suspended in Ringer's solution with radioactive potassium (*a*) cells of R.B.D. (*b*) cells of L.F.H. Ordinates, relative activity $\times 10^{-2}$. Abscissae, minutes from time of suspension of the cells in the solution. Curves for cells follow the empirical equations given in Table I.

TABLE I

Human Erythrocytes—in Ringer

A

Time	Hemato-crit	K concentration		Relative activity		Empirical curve cell activity	Slope cell activity	Diffusion gradient	Diffusion coefficient $\times 10^{-3}$
		Ringer	Cells	Ringer	Cells				
min.	*per cent*								
0	5.0	5.00	(77.8)	(1000)	0			1000	
95	4.9	5.00	77.8	975	25	24	0.211	950	0.222
195	4.85	5.23	76.6	842	43	43	0.170	799	0.213
300	4.6	5.35	75.5	880	59	65	0.150	821	0.183
445	4.85	5.30	74.4	850	81	86	0.133	769	0.175
640	4.45	5.70	70.4	730	106	110	0.121	624	0.194

Average diffusion coefficient = 0.197×10^{-3} min.$^{-1}$
Empirical curve for cell activity $x = 1.32t^{0.7} - 5.1$

B

0	5.0	5.00	(84.6)	(1000)	0			1000	
60	5.0	5.00	84.6	1000	19	23	0.274	981	0.280
120	4.95	5.07	84.0	900	33	31	0.240	867	0.277
210	4.85	5.13	83.0	900	44	44	0.213	856	0.249
300	5.0	5.13	82.0	896	58	59	0.199	838	0.239
420	4.9	5.30	81.0	863	75	76	0.186	788	0.236
610	5.0	5.55	74.1	817	101	99	0.172	716	0.241

Average diffusion coefficient = 0.254×10^{-3} min.$^{-1}$
Empirical curve for cell activity $x = 0.778t^{0.8} + 3.5$

meyer flasks which were agitated at 37.5°. At suitable intervals pairs of flasks were removed and the cell suspensions treated exactly as in the

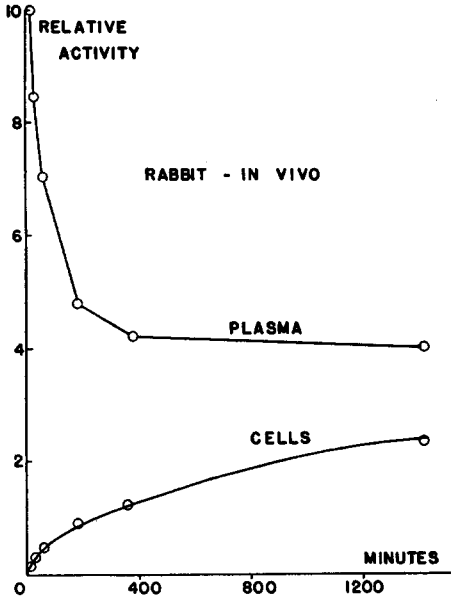

FIG. 2. Relative activity $\times 10^{-2}$ of the potassium (ordinates) in the plasma and blood cells of rabbits drawn at various times (abscissae) after injection of radioactive potassium. Figures in Table II. The curve for the cells is drawn to follow the empirical equation given in Table II.

TABLE II

Rabbit Erythrocytes in Vivo

Time	Hematocrit	K concentration		Relative activity		Empirical curve cell activity	Slope cell activity	Diffusion gradient	Diffusion coefficient $\times 10^{-3}$
		Plasma	Cells	Plasma	Cells				
min.	*per cent*								
10	45.0	6.50	85.6	1000	18	20	1.010	982	0.970
30	40.7	6.15	92.6	846	32	34	0.578	814	0.710
60	40.4	6.34	94.8	705	50	48	0.412	655	0.630
180	37.7	5.60	81.2	480	91	85	0.236	389	0.607
360	34.0	4.94	91.4	422	124	120	0.170	298	0.571
1440	31.6	4.59	93.2	403	236	240	0.084	167	0.503

Average diffusion coefficient = 0.665×10^{-3} min.$^{-1}$
Empirical curve for cell activity $x = 632t^{0.5}$

other experiments. Table III and Fig. 3 show the penetration into and out of the cells. In this case the slopes were estimated graphically. They are less reliable because of the few points which are rather erratic. This may

be due in part to excessive hemolysis. There seems to be a difference between the diffusion constants in the two cases. At present we see no

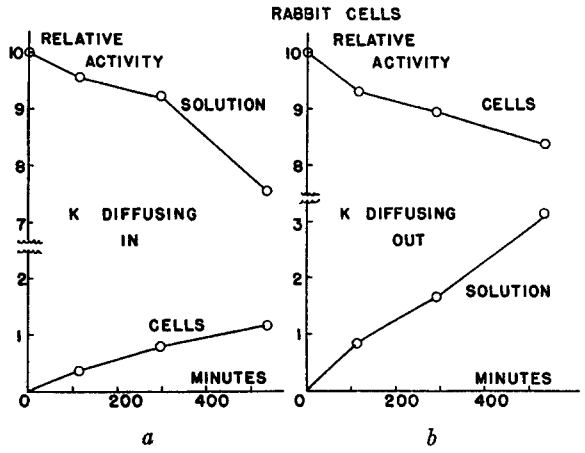

FIG. 3. Rabbit cells *in vitro*. In (*a*) radioactive K is in the Ringer's solution diffusing into the cells; in (*b*) it is in the cells diffusing out into the solution. Ordinates, relative activity of the potassium $\times 10^{-2}$; abscissae time from beginning of diffusion. All curves drawn through experimental points as given in Table III.

TABLE III

Rabbit Erythrocytes in Ringer

A. Diffusion in

Time	Hematocrit	K concentration		Relative activity		Slope cell activity	Diffusion gradient	Diffusion coefficient $\times 10^{-3}$
		Ringer	Cells	Ringer	Cells			
min.	*per cent*							
0	3.0	5.00	80.4	1000	0	0.30	1000	0.30
115	3.1	5.11	73.0	956	38	0.46	918	0.50
295	3.2	5.20	67.7	922	80	0.19	742	0.26
535	2.2	6.42	45.0	756	119	0.15	635	0.23

Average diffusion coefficient = 0.32×10^{-3} min.$^{-1}$

B. Diffusion out

0	3.0	5.00	80.4	0	1000	0.59	1000	0.59
115	3.0	5.11	73.0	84	931	0.36	847	0.42
295	3.2	5.21	67.5	167	895	0.24	728	0.33
535	2.6	5.98	55.3	315	839	0.20	524	0.38

Average diffusion coefficient = 0.40×10^{-3} min.$^{-1}$

justification for considering this difference as significant in view of the inaccuracy of the measurements. The coefficient, however, is probably significantly lower than for the rabbit erythrocytes *in vivo*, but of course

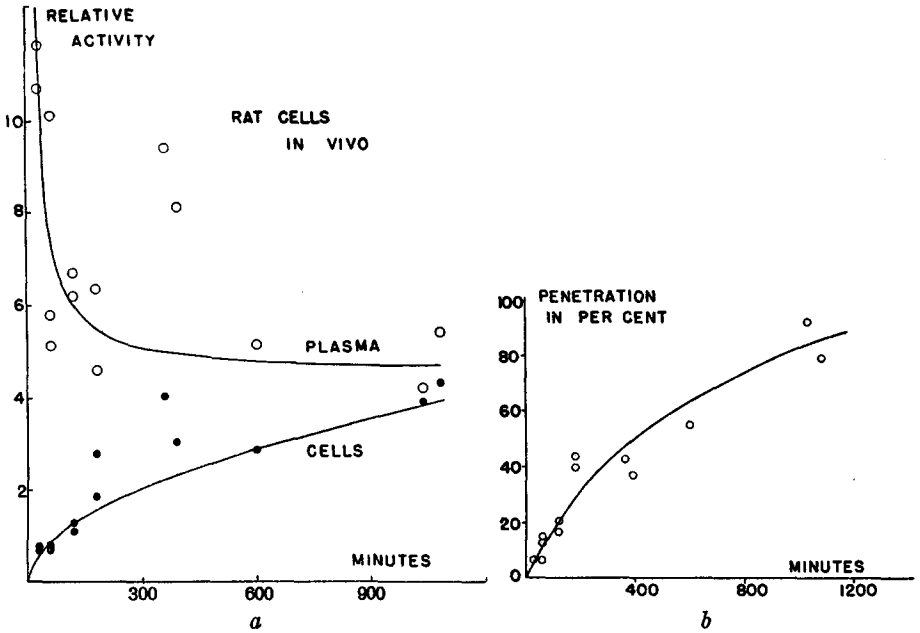

FIG. 4*a*. Results of injections of radioactive potassium into 14 rats. Ordinates, relative activity $\times 10^{-2}$ of the potassium in plasma and cells where the activity of the injected dose in per cent of the body weight is 1000. Data of Table IV.

FIG. 4*b*. Cell activity in per cent of plasma activity or per cent penetration of radioactive K into cells of rats (ordinates) at different times (abscissae) after injection. Calculated from data of Table IV.

TABLE IV

Rat Erythrocytes in Vivo

Time	K concentration		Relative activity		Empirical plasma	Curves cells	Slope cells	Diffusion gradient	Diffusion constant
	Plasma	Cells	Plasma	Cells					
min.									
30	9.1	86	1071	69	1150	64	1.08	1086	1.00
30	8.8	103	1164	76					
60	12.6	84	528	80		92	0.76	658	1.15
60	12.0	95	580	77	750				
60	8.4	96	1030	69					
120	8.2	100	673	113	600	130	0.54	570	0.95
120	8.4	95	622	128					
180	7.6	86	459	182	550	159	0.44	391	1.12
180	9.6		633	278					
360	7.4	78	944	404	500	225			
390	11.0	97	815	304	495	234			
600	11.0	101	514	281	480	290	0.23	290	0.80
1035	6.5	110	421	390	468	382	0.20	86	0.23
1080	7.7	97	544	431	467	390	0.20	57	0.35
						Actual gradient		(113)	(1.77)

Average diffusion coefficient 30 to 600 minutes = 1.0×10^{-3} min.$^{-1}$
Empirical curve for plasma $P = 450 + 18{,}000/t$
Empirical curve for cells $x = 11.9t^{0.5}$

the cells *in vitro* were in poor condition. Penetration of radioactive potassium at 23°C. during the loading process took place at a mean rate of 0.1 counts/mol per minute with a gradient of 1000 counts/mol so that the coefficient of diffusion is of the order of 0.1×10^{-3}. This value is 0.27 times the mean rate at 37.5°C. which corresponds to a Q_{10} of about 2.4.

In the course of other experiments a number of rats were injected with radioactive potassium intraperitoneally or subcutaneously. Each animal received 1–3 cc. of 0.1 or 0.2 M KCl, a large dose. The animals were sacrificed after various intervals, blood was collected, and the tissues were analyzed for radioactivity and potassium. The results of these analyses will be reported elsewhere.[2] After centrifuging the blood, plasma was removed as completely as possible and the cells were dissolved in nitric acid without washing. A known volume of plasma was similarly digested in nitric acid, counted, and analyzed for K. There is unfortunately a very large variability in the results as shown in Table IV and Fig. 4, probably due to variations in the experimental procedure and to variations in the ability of the different animals to dispose of the large dose of potassium in the various tissues of the body. Nevertheless, if we discard the points at 6 and 6.5 hours, it is possible to make a fair approximation to the data as shown in the curves of Fig. 4 *a*. Then taking the slope of the cell curve by differentiation as before, we have calculated the diffusion coefficient for different points. This value shows very little variation except at 18 hours, and if at this time the actual gradient experimentally observed between cells and plasma is used for calculation instead of the difference between the empirical curves, even this point falls more nearly in line. It is significant, we believe, that the diffusion coefficient is higher in rat than in rabbit or human erythrocytes.

Although these data show great irregularity when plotted as in Fig. 4 *a* they are somewhat more regular if the per cent penetration (*i.e.* cell activity $\times$ 100 $\div$ plasma activity) is plotted against time as in Fig. 4 *b*. In about 30 hours the exchange may be expected to be complete. The interpretation of this curve, however, is somewhat difficult because of the widely varying gradient which was exceedingly high immediately after the injection and because an apparent increase in penetration can be caused by decreased plasma activity due to exchange with other body tissues. The data of Table IV were obtained from experiments on fourteen different rats and they afford therefore most convincing evidence of the cation permeability of rat cells under normal physiological conditions in the body.

Radioactive potassium was loaded into rat cells by injecting active KCl

[2] *Am. Jour. Physiol.*, in press.

into the rat 5 hours before taking the blood. Table V and Fig. 5 show how the active potassium left the red cells when they were suspended in Ringer. There was considerable hemolysis which accounts for the high activity in the Ringer. In this case the points are too erratic to justify fitting an em-

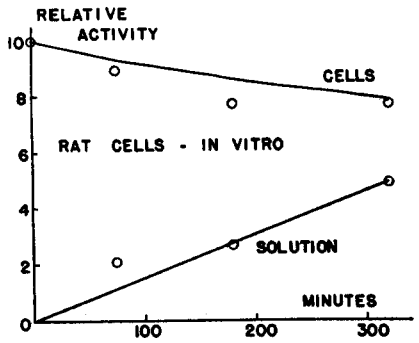

FIG. 5. The diffusion of radioactive potassium from rat red cells into the Ringer's solution in which they are suspended. The graph for the solution follows the empirical formula while the graph for the cells is the calculated theoretical curve (see text). Ordinates relative activity $\times 10^{-2}$; abscissae, time from the beginning of diffusion.

TABLE V

Rat Erythrocytes in Ringer

Time	Hematocrit	K concentration		Relative activity		Empirical curve Ringer activity	Theoretical curve cell activity
		Ringer	Cells	Ringer	Cells		
min.	*per cent*						
0	0.051	5.00	96.5	0	1000	0	1000
75	0.047	5.48	95.0	213	894	115	931
180	0.045	5.83	90.3	264	773	275	860
320	0.032	7.10	89.4	489	769	489	793

Diffusion coefficient $b = 1.0 \times 10^{-3}$

Empirical curve for Ringer activity $R = at = 1.53t$

Theoretical curve for cell activity $x = \left(1000 + \frac{1.53}{0.001}\right)e^{-.001t} + 1.53t - \frac{1.53}{0.001}$

pirical curve. However, if we assume a linear rise in the Ringer activity $R = 1.53\ t$, and a diffusion coefficient of 10^{-3} the value obtained in the *in vivo* experiment, we can calculate the fall in activity of the cells.[3]

[3] Assuming the law of diffusion

$$\frac{dx}{dt} = b(R - x)$$

and a linear relation for the Ringer activity $R = R_0 + at$ on solving the differential

The upper graph in Fig. 5 is the curve calculated in this way, and it appears to be a reasonable approximation to the experimental points. There seems to be no justification in this data for assuming a different diffusion coefficient for *in vitro* than for *in vivo* exchange in rat erythrocytes.

DISCUSSION

If a cell is permeable to a given ion K and does not change its content of that ion over a period of time, we can say that the numbers of K ions crossing its membrane in each direction in unit time are equal. Now let the fractions x_i and x_o represent the ratios of radioactive to normal K ions inside and out and let p equal the number of K ions crossing in each direction per minute. Then the number of active ions crossing per minute in the two directions is $x_i p$ and $x_o p$.

The change in the number of active ions inside after unit time is $x_o p - x_i p$ or $p(x_o - x_i)$. The rate of change in the ratio of active to total K ions (K_i) is accordingly $\frac{p}{K_i}(x_o - x_i)$. We can let $\frac{p}{K_i} = b$, since K_i must be a constant for a given cell that is not gaining or losing ions, and in differential form we write $dx = b(x_o - x_i)\, dt$. This is identical with the ordinary diffusion equation if we take the relative concentration of radioactive ions to total ions of the kind under consideration. b equals the fraction of ions leaving the cell per minute and is equal to the diffusion coefficient for K ions leaving the cell. The experiments reported here show that the radioactive potassium ions behave as if the membrane were allowing a constant fraction of the potassium inside to cross in each direction per minute. These results are all based on the assumption that the potassium remains in the cells and therefore in so far as they fit diffusion curves they give no evidence of changes in the concentration of potassium due to leakage. Some of the deviations of the diffusion coefficient from a constant value may well represent net movements of potassium. For example, a movement of KCl into the cells increasing their potassium content when the plasma potassium is high would account for the apparently higher rate of uptake of the rabbit cells *in vivo* soon after injection.

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equation we get

$$x = \left(x_0 - R_0 + \frac{a}{b}\right) e^{-bt} + R_0 + at - \frac{a}{b}$$

where x and R are the activities per mol of potassium for the cells and Ringer respectively. x_0 and R_0 are their activities at time 0. t is the time, a the slope of the Ringer activity, and b the coefficient of diffusion.

If the activity of the cell potassium is expressed as a percentage of the activity of the plasma potassium the result also indicates the percentage of the cell potassium which has exchanged with the plasma potassium. Thus in Table I it is seen that after 10 hours *in vitro* 12.4–14.5 per cent of the potassium of human cells has exchanged with the plasma potassium. For rabbit cells the exchange is 29 per cent complete after 6 hours *in vivo* (Table II) and 59 per cent complete after 7.9 hours *in vitro* (Table III). Possibly human cells also would show better exchange *in vivo*. The exchange in rat cells *in vivo* (Table IV) is 55 per cent complete in 10 hours and 79 per cent complete in 18 hours. The comparative constancy of the diffusion constant indicates that in human cells the exchange would be complete if sufficient time were allowed. There is no evidence as yet that the exchange is limited to a certain fraction of the cell K and that this diffusible fraction is larger in rats than in men. It appears instead that the rat cells are more permeable than human cells. The calculated diffusion constants represent a better measure of this permeability than the percentage exchange in unit time because in the latter no account is taken of the varying diffusion gradients.

It should be mentioned that sodium can cause serious contamination in radioactive potassium. K^{41} accounts for less than 7 per cent of the element, and yet it is the only isotope which can be activated to K^{42}. All the sodium atoms can be activated somewhat more easily than the K^{41} isotope. Since radioactive sodium and potassium have very similar half lives, it is easy to see how a small percentage impurity of sodium would cause serious trouble. Metallic potassium which has been used by most other workers as a source of radioactive potassium is notoriously the most difficult form of the element to purify. It may be suggested as a possibility that the low penetration observed by Hahn, Hevesy, and Rebbe (1939 *b*) was due to sodium contamination. The large ratio of plasma to cell sodium would make the counts in plasma very high compared to those in cells and give a very low calculated penetration.

Eisenmann, Ott, Smith, and Winkler (1940)[4] concluded from their measurements with radioactive potassium that there was no free penetration of potassium into human red cells. Actually there is no experimental conflict with our data. Their figures show an average of 4.4 per cent (maximum 8 per cent) penetration in 4 hours whereas our figures show 15 per cent penetration in 10 hours. There are some important differences of tech-

[4] The full report of this work appeared after this manuscript had been accepted for publication. This paragraph was added later by permission of the editors.

nique. Their cells were left in plasma while ours were immersed in Ringer's solution. On the other hand, they added dry potassium to plasma and in such large amounts (up to 60 mEq. per liter of whole blood) that the solution was quite hypertonic. Both of these factors would probably decrease the permeability in their experiments. In our experiments the solution was, if anything, slightly hypotonic which may account for the slight hemolysis observed. Such hemolysis introduced no error into the measurement of the penetration into the cells which remained intact, but the permeability of those cells may have been abnormally high.

The actual penetration of potassium into human cells was not large in our experiments (only 15 per cent). The shape of the curve indicates, however, no great diminution in rate of penetration even after 10 hours. The belief that all the potassium is eventually exchangeable has, therefore, some justification.

It may be supposed that the permeability of the cells may have been modified by the radioactivity of the solutions. According to the results of Mullins (1939) with *Nitella*, the effect if any would be a decrease of permeability. We do not have as yet observations at a sufficient variety of radiation intensities to permit an experimental answer to this question. The actual radioactivity of the solutions in our *in vitro* experiments estimated in terms of the count given by a saturated solution of potassium acetate was about 7 microcuries per liter. This would seem to be low enough so that no isotope effect would be anticipated.

In rats at least there seems to be no escape from the conclusion that the red cells are normally more or less permeable to potassium. Possibly, however, rat cells are impermeable to sodium. Otherwise it would be difficult to understand why potassium does not normally exchange for sodium.

SUMMARY

The diffusion coefficients for the exchange of potassium across the membrane of erythrocytes of humans, rats, and rabbits have been determined by the use of artificially radioactive potassium, both into and out of the erythrocytes both *in vitro* and *in vivo*.

The diffusion coefficients found in minutes^{-1} were 0.2 to 0.25 $\times$ 10^{-3} for human, 0.32 to 0.665 $\times$ 10^{-3} for rabbits, and 1.0 $\times$ 10^{-3} for rat erythrocytes. Rabbit erythrocytes appear to be more permeable *in vivo*.

Reasons are advanced to explain the failure of earlier workers to demonstrate appreciable exchange of potassium in erythrocytes.

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