XCIV. STUDIES ON THE PERMEABILITY OF ERYTHROCYTES.

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Kerr [1929] concluded that in solutions outside the physiological range erythrocytes were permeable to Na^+ and K^+. He also observed that ox erythrocytes suspended in an aqueous solution containing 200 mg. K per 100 ml. showed a greater increase in their K content than did cells suspended in serum of which the K content had been raised to 200 mg. per 100 ml. and hence assumed that normal serum lowered the permeability of erythrocytes. In view of the well recognised depressing effect of Ca^{++} on the permeability of a number of animal membranes, *e.g.* in the case of *Arbacia* eggs as described by Lucke and McCutcheon [1928], it was thought to be not improbable that the serum-Ca played an important rôle in determining the impermeability of the erythrocyte membrane to Na^+ and K^+ which is normally observed. The experiments to be described were carried out primarily to test this theory.

Experimental.

Freshly collected ox blood was filtered through muslin, centrifuged, the serum and top layer of corpuscles were removed and 25 ml. of corpuscles suspended in 25 ml. of saline in a corked flask. After a given time the suspension was centrifuged, the saline removed and 5 ml. of corpuscles measured out into a beaker and diluted with distilled water. The proteins were precipitated with 10 ml. of trichloroacetic acid, filtered off and the filtrate was evaporated to dryness in a silica flask. Ashing was completed by Kutz's method [1931]. The residue was taken up with water and made up to 10 ml.

Sodium determination. The volumetric method of Kahane [1930] was tried but found to be quite unsatisfactory. Balint's [1924] modification of the Kramer-Tisdall method gave good results and was employed throughout.

Potassium determination. The method of Kramer [1920] was used.

Blood-volume changes. These were determined by blood counts on the centrifuged corpuscles and by haematocrit determinations on the suspension.

Results.

In Table I are shown the results of suspending ox corpuscles in various aqueous solutions using this procedure. Exp. 1 shows a large decrease in the potassium content of the corpuscles when suspended in 0·9 % NaCl. Addition of $CaCl_2$ up to 100 mg. of Ca per 100 ml., which is roughly ten times as much as occurs in normal serum, produces no significant effect on this decrease. Exp. 2 shows that there is little difference in K content between the control cells and those suspended in NaCl, Ringer solution and buffered Ringer. Now these two experiments differ in the way in which the controls were determined. In Exp. 1, as

Table I.

No.	Washing fluid	Time	K mg./ 100 ml.	Na mg./ 100 ml.	Vol. chge. %	Corr. K	Corr. Na
1	Serum	—	87·9	—	—	87·9	—
	0·9% NaCl	Overnight	78·9	—	−5	75·0	—
	0·9% NaCl + 20 mg. Ca/100 ml.	,,	79·1	—	,,	75·1	—
	0·9% NaCl + 40 mg. Ca/100 ml.	,,	79·5	—	,,	75·5	—
	0·9% NaCl + 60 mg. Ca/100 ml.	,,	78·9	—	,,	75·0	—
	0·9% NaCl + 100 mg. Ca/100 ml.	,,	80·0	—	,,	76·0	—
2	Serum*	Overnight	58·5	—	+3	60·2	—
	0·9% NaCl	,,	63·0	—	−6·3	59·6	—
	Ringer	,,	67·4	—	−13	58·6	—
	Buffered Ringer	,,	68·5	—	−12	60·3	—
3	Serum*	6 hours	61·1	—	+3·2	63·1	—
	NaCl oxalate	,,	61·8	—	−2·9	60·0	—
	Oxalated serum	,,	64·0	—	−7·3	59·3	—
	Ringer oxalate	,,	64·8	—	−9·4	58·7	—
4	Serum	Overnight	86·1	180	—	86·1	180
	Serum*	,,	77·6	196	12·3	68·0	175
	1·5% KCl	,,	192	155	−6	181	146
	1·5% KCl + $CaCl_2$	,,	198	154	−9	180	140

* Corpuscles left in contact with their serum.

in Kerr's experiments, the control corpuscles were not left in contact with their serum but were immediately ashed and analysed for potassium, whilst in the second experiment the control corpuscles were left suspended in serum for the same period as the others were left in their respective aqueous solutions. If the corpuscles are impermeable to Na and K when in contact with their serum, then there should be no difference between the values obtained by either method. The fact of the small difference between the control corpuscle-potassium and that of corpuscles suspended in NaCl in the one case and the large difference in the other case would point to the possibility of potassium leaking out of the corpuscles almost as well when in contact with serum as when in contact with an aqueous NaCl solution. Exp. 4 of Table I, in which controls were determined in both ways, confirms this, the K content of corpuscles left overnight in contact with serum in the ice-chest decreasing by 18 mg. per 100 ml. Returning to Exp. 2 it is seen that it is very difficult to draw any definite conclusion as to the effect of suspending corpuscles in Ringer or the effect of buffering the solution, since the differences are small and the error introduced by the volume-change correction would probably cover these differences. Again in Exp. 3 it appears that oxalated serum approximates more closely in behaviour to NaCl solution than to normal serum but the differences are small and come within the maximum error. In 1·5% KCl an increase of over 100% in the K content and a decrease of about 30% in the Na content are observed. The Ca^{++} seems not to affect the permeability of the corpuscle membrane to K^+ or Na^+.

It was now felt necessary to develop a technique in which the error introduced by the blood-volume correction, amounting on the average to about 5 %, could be removed. In the next series of experiments 5 ml. of corpuscles were placed in a centrifuge-tube and 10 or 5 ml. of the saline added. After a given period the corpuscles were centrifuged down, and the supernatant fluid was carefully and completely sucked off, great care being taken not to remove any corpuscles. The whole of the corpuscles in the tube were then laked, ashed and analysed for Na and K. This method is independent of the volume changes, as the whole of the corpuscles are analysed and not an aliquot as previously. Table II shows some of

Table II.

No.	Washing fluid	Time hrs.	K mg./ 100 ml.	Na mg./ 100 ml.
1	Serum	—	71·2	—
	Serum*	4	68·3	—
	0·9 % NaCl	4	65·0	—
	Buffered Ringer	4	67·9	—
2	Serum*	3	84·4	182
	1·5 % KCl	3	146	135
	1·5 % KCl + $CaCl_2$	3	145	136
3	Serum	—	64·7	190
	Glucose	3	63·1	145
4	Serum	—	75·4	174
	84 % serum; 16 % H_2O	2	67·2	136
5	1·5 % KCl	2	151	125
	1·5 % KCl; 0·9 % NaCl	2; 0·5	85·4	163
	1·5 % KCl; 0·9 % NaCl	2; 1	83·7	163
6	Serum	—	—	208
	Glucose	0·5	—	151
	1·5 % KCl	0·5	—	153
	Glucose: KCl, 9 : 1	0·5	—	150

* Corpuscles left in contact with their serum.

the results obtained by this method. Exp. 1 shows that in 0·9 % NaCl alone the corpuscles lose more K than they do in serum, whilst in buffered Ringer the losses are approximately the same. Hence the losses of K in NaCl observed are probably due to a general increase of permeability consequent upon certain changes due to degradation characteristic of dead tissue and not to the removal of any specific factor in the serum.

Losses of Na and K are experienced on suspending the corpuscles in isotonic glucose or dilution of the serum by about 20 %. The large increase in K content and the large decrease in Na content observed on suspending corpuscles in KCl solution might quite reasonably be interpreted as adsorption and desorption phenomena. It may be recalled that the average contents of ox-serum are 20 mg. K and 300 mg. Na per 100 ml. Hence it would be expected that if adsorption occurred at the serum-interface Na would be the chief constituent of this adsorption layer. Thus suspending corpuscles in a medium containing very little or no Na, *e.g.* in glucose or KCl should produce a large drop in their Na content. The succeeding experiments represent attempts to solve this problem.

In Exp. 5 of Table II three lots of corpuscles were suspended for 2 hours in 1·5 % KCl. One lot was then analysed for K whilst the two others were suspended in 0·9 % NaCl. One lot was immediately centrifuged down and the other after half an hour. If the potassium were actually permeating the membrane one might expect to find a greater amount in the lot immediately centrifuged off than in the lot centrifuged half an hour later. The results show no appreciable difference between the two. (It is to be noted that the centrifuging was allowed to continue for half an hour, that time being thought necessary; it was later discovered that from a slightly hypertonic aqueous solution corpuscles could be centrifuged off within 2–3 minutes.) A comparison of the losses of Na in glucose and KCl shows no difference. If the loss were due to desorption one would expect that the desorption would occur more readily in KCl where there are ions to exchange with the adsorbed Na than in glucose. However it must be pointed out that we are not here measuring relative velocities but the amounts

lost in one specified time. In Table III the results of a series of kinetic experiments are shown. Exps. 1 and 2 show the effect of suspending corpuscles for times varying from 8 to 60 minutes in 1·5 % NaCl. As is observed the corpuscle-Na

Table III.

No.	Washing fluid	Time (mins.)	K mg./100 ml.	Na mg./100 ml.
1	Serum	—	83·2	159
	1·48 % NaCl	8	79·7	197
	,,	30	79·6	218
	,,	60	76·0	213
2	Serum	—	77·5	185
	1·53 % NaCl	7	74·2	232
	,,	15	74·2	247
	,,	22	74·0	255
	,,	26·5	73·8	278
	,,	31·5	73·8	276
3	Serum	—	93·7	176
	1·5 % KCl	4·5	261	131
	,,	7·5	265	129
	,,	15·5	271	126
	,,	30·0	288	126
	1·5 % KCl; 0·9 % NaCl	30; 5	148	200
	,,	30; 9	138	174
	,,	30; 10	137	188
	,,	30; 31	136	176
4	Serum	—	—	160
	1·5 % NaCl	8·5	—	226
	,,	11·0	—	228
	,,	2·85	—	231

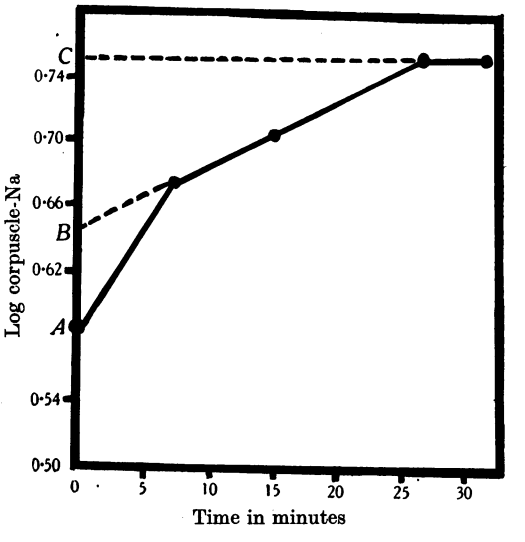

Fig. 1.

increases with time arriving at a stationary value some time between 8 and 30 minutes. The results of Exp. 2 are shown diagrammatically in Fig. 1 where the log of the Na content of the corpuscles is plotted against time. If the process is one of permeation a straight line should result; the graph shows a sharp initial

rise and then a slower one to equilibrium. Exp. 3 shows similar behaviour in 1·5 % KCl and subsequent suspension in 0·9 % NaCl.

Two explanations of Fig. 1 are possible. Either (*a*) the initial sharp increase is due to adsorption, and the slower increase due to penetration, in which case the adsorption would be represented by *AB* and the permeation by *BC*; or (*b*) the effect may be due to inefficient stirring, in which case the whole increase might be due to adsorption or permeation or both. To test this last hypothesis Exp. 4 was performed using twice as much saline for suspending the corpuscles as in Exps. 1, 2 and 3, and the suspension was vigorously stirred for at least 2 minutes before being centrifuged. As the Table shows the phenomenon of the secondary increase has disappeared under these conditions.

In Table IV and Fig. 2 are shown the results of suspending corpuscles in mixtures of NaCl and KCl, the K varying in concentration from 40 to

Table IV.

Washing fluid		Corpuscles	
Na mg./100 ml.	K mg./100 ml.	K mg./100 ml.	Na mg./100 ml.
Serum		84·8	203
39·2	439·5	85·7	207
58·8	427·7	86·1	207
78·4	217·5	89·3	207
117·6	393·0	96·0	207
176·6	358·0	105·0	205
225·0	324·0	113·0	197
298·0	287·0	118·0	189
588·0	116·0	145·0	154

Corpuscles suspended for 1 hour in washing fluid.

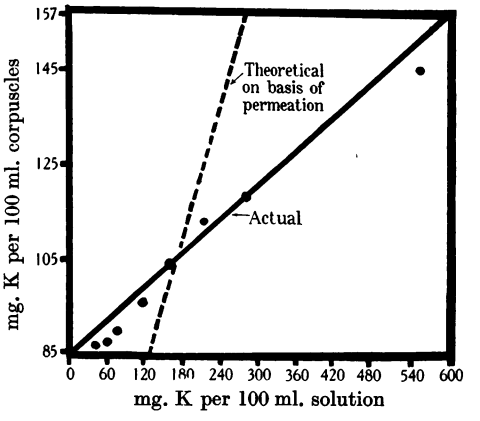

Fig. 2.

600 mg./100 ml. and Na from 440 to 136 mg./100 ml. The total molar concentration of the two ions Na^+ and K^+ was kept constant to maintain identical tonicity of the different solutions and so prevent differences in volume.

Applying the Freundlich adsorption equation:

$$c = kC^{1/n}$$

this may be written:

$$\frac{kC}{C^{\left(\frac{n-1}{n}\right)}} = c,$$

the denominator of the left-hand side representing the factor introduced to account for the limited area of the surface available for adsorption. Applying this to the case where we have both Na^+ and K^+ present:

$$c_{K^+} = \frac{kC_{K^+}}{(C_{K^+} + C_{Na^+})^{\frac{n-1}{1}}},$$

assuming that n is the same for both Na^+ and K^+. Now under the conditions of the experiment $(C_{K^+} + C_{Na^+})$ is constant, so that the adsorption isotherm of either component should be a straight line. It is unfortunate therefore that the shape of the curve of corpuscle-K against concentration in the aqueous solution will not help to distinguish between adsorption and permeation; however, the slope of the curve will tell us whether anything like a simple equilibrium is being established. The actual curve, which has been drawn as a straight line, is certainly rather ambiguous, and one must hesitate before deciding how to characterise the phenomenon it represents. The theoretical curve, assuming that K^+ permeates and is divided equally between the two media, is seen to be very much steeper than the actual curve, showing that if the increases are due to permeation they must occur in accordance with some very obscure equilibrium.

Discussion.

The serum-factor of Kerr appears not to be Ca^{++}. The apparent difference in behaviour between corpuscles in serum and those in NaCl solution has been shown to be illusory, depending on the method of determining the control. K leaks out of the corpuscles into the serum at about the same rate as into NaCl solution. The results of suspending corpuscles in isotonic glucose and diluted serum do not agree with those of Hamburger [1910]. Hamburger found no change in glucose solution and penetration of Na in diluted serum. A point which bears very strongly against the explanation of the losses of Na in KCl and glucose solutions as being due to desorption is the following. If the total molar concentration of Na + K in the corpuscles is compared with that of the serum we find that they are equal; *e.g.* the average of seven different lots of blood gives a total conc. of 14·9 *mM*/100 ml. for the corpuscles and 14·9 *mM*/100 ml. for serum. This of course is to be expected. Now if we assume that approximately 25 % of the corpuscle-Na is adsorbed on the surface, as Exps. 2, 3, 5 and 6 of Table II would indicate, then the average total molar concentration in the corpuscles becomes 13·7 *mM*/100 ml., *i.e.* there would be an osmotic difference between the cells and serum at equilibrium, which of course is impossible. It must therefore be concluded that the losses of Na in KCl and glucose solutions are due to permeation. Hence we are confronted with the phenomenon of a rapid permeation (within 5 minutes) of some 50 mg. Na per 100 ml. to an equilibrium state in which a high concentration gradient is still maintained. From Table IV it is observed that the concentration of Na in the washing fluid may be decreased from 440 to 360 mg. per 100 ml. without producing any significant change in the corpuscle-Na, which seems to indicate that there is a concentration gradient which has to be exceeded before any permeation can occur.

As far as the K^+ ion is concerned a definite but small loss of K occurs in NaCl solution which must be due to permeation. All that can be said of the behaviour of corpuscles in KCl solution is that if permeation of K^+ does occur it takes place within 5 minutes, and an equilibrium position is reached remotely different from that expected on the basis of free permeation.

A possible explanation of these phenomena might be arrived at on the assumption that treatment of the cells with the solution examined itself affects the permeability of the corpuscles, increasing the proportion of those which may be called "dead cells." These "dead cells" will establish a perfect thermodynamic equilibrium with the surrounding fluid, whilst the remainder will maintain their normal impermeability. The net effect will then appear as a partial permeability such as is actually observed. Against this is the fact that only in rare instances was any haemolysis observed, so that a state of permeability to Na^+ and K^+ must be postulated which lies mid-way between those of the normal cell and the truly "dead" cell. Further, one would have to assume that the corpuscles are much more permeable to Na^+ than to K^+ in this hypothetical dead state, since in glucose solutions only about 2 % of the K^+ is lost compared with about 30 % of the Na^+.

Ponder and Saslow [1930; 1931] conclude from their measurements of corpuscular volume that in isotonic salt solutions a loss of osmotic substances occurs, and in hypertonic NaCl and KCl the two salts penetrate to such an extent as to produce an increase in volume after 5 minutes. The results described in this paper show the loss of only small quantities of K in isotonic NaCl and large quantities of Na in KCl accompanied, however, in the latter case by a larger increase in the K content. In 1·5 % NaCl the increases of Na observed are certainly not sufficient to demand an increase in corpuscular volume, and in actual fact an invariable contraction occurred in 1·5 % NaCl and KCl solutions lasting for hours. It must be borne in mind, however, that Ponder and Saslow were working with rabbit's blood, and such large differences in behaviour between species are not out of the question. Reference may here be made to the work of Woodhouse and Pickworth [1932], who measured the permeability of corpuscles to a large number of ions. These authors did not study the influence of K^+ ions as "it may be taken without further experimental proof that the erythrocyte is impermeable to K^+," yet if they had measured the permeability of K^+ they would have obtained a "permeability coefficient" of over 40, a value they obtained for Cl^- in NH_4Cl. Until some method has been devised which will decide unequivocally whether a given ion permeates or is adsorbed one must accept with reserve their statement that the NH^+_4 ion permeates readily.

Summary.

1. In 0·9 % NaCl ox erythrocytes lose K; this loss is unaffected by the presence of Ca^{++} ions and is about equal to the loss sustained in their normal serum under the same conditions.

2. In 1·5 % KCl the corpuscles lose 25–30 % of their Na within 5 minutes; no further appreciable losses occur after this time. The corpuscle-K increases by 100–200 % in the same time and remains constant.

3. In 1·5 % NaCl a considerable increase in corpuscle-Na occurs, but not sufficient to raise the corpuscle concentration to that of the external solution.

4. In glucose solution a large loss of Na and a small loss of K occur. The loss of Na is the same in glucose as in KCl or in mixtures of the two.

5. In diluted serum losses of Na and K are observed.

6. The corpuscle-K is plotted against the concentration of K in the suspending fluid when the latter is varied between 40 and 600 mg./100 ml. The results are not in accord with what would be expected on the basis of ready permeation and only fit approximately an adsorption curve.

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