V. NOTE ON THE PERMEABILITY OF ERYTHROCYTES TO CHLORIDE.

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WHEN investigating the permeability of red cells suspended in mixtures of potassium phosphate and potassium chloride, it was found that the presence of chloride greatly depressed the permeation of the phosphate. At the same time the concentration of chloride in the red cells was in excess of that in the external fluid, and appeared to balance the total osmotic pressure of the external chloride and phosphate. In order to determine how far the external osmotic pressure was able to modify the anion content of erythrocytes, these cells were suspended in mixtures of potassium chloride with glucose, to the latter of which they are relatively impermeable. At the same time, observations of the change in p_H of the external fluid were made, to see how this was affected by the redistribution of Cl^- .

Method.

A modification of the haematocrit was used in these determinations. Blood containing heparin as an anti-coagulant was centrifuged in tubes drawn out into sealed, graduated and calibrated capillaries for 30 min. at 4000 r.p.m., after which the cells had attained constant volume (*ca.* 0.05 cc.) and were translucent. The volume of red cells was read, the supernatant plasma washed away, and the cells resuspended in 7 cc. of the test fluid. After 10 min. the tubes were respun and the volume of cells again read. The approximate p_H of the test fluid was determined colorimetrically, and then the tube and capillary down to the upper face of the red cell column were carefully washed with a capillary pipette. Lastly, the red cells were removed and their chloride content estimated by the method of Claudius [1924].

Among the constituents of the red cell are H^+ , Cl^- and HCO_3^- which very readily permeate the cell membrane, and potassium and haemoglobin which do not. When such cells are placed in solutions of potassium chloride, the H^+ , Cl^- and HCO_3^- are redistributed according to the Donnan equation,

$$\frac{[H^+]_{cell}}{[H^+]_{fluid}} = \frac{[Cl^-]_{fluid}}{[Cl^-]_{cell}} = \frac{[HCO_3^-]_{fluid}}{[HCO_3^-]_{cell}}.$$

As the volume of the external fluid is relatively very great Cl^- will largely displace HCO_3^- , and the HCO_3^- will combine with an equivalent amount of potassium in the external phase, where its concentration will necessarily be very low. When the Cl^- content of the external fluid is raised, water flows out of the cell and cell Cl^- rises. If no Cl^- enters the red cell from the external fluid beyond that required to displace HCO_3^- , and if allowances are made for changes in cell volume, then the Cl^- calculated as a content of the original volume of cells should equal at all dilutions the sum of HCO_3^- and Cl^- , which existed in the original untreated cell. Cell $[Cl^-]$ varies between 48 and 54 mM, and according to Henderson [1928], the cell $[HCO_3^-]$ is about 13 mM, corresponding to a total concentration of 61 to 67 mM. Table I shows that if

allowances are made for changes in volume, the chloride content of red cells suspended in potassium chloride solutions at p_H 7.6, is fairly constant within wide limits of dilution, and is approximately equal to the sum of the normal Cl^- and HCO_3^- .

Table I.

Cells in KCl solution at p_H 7.6.

External fluid [Cl ⁻] mM	Cell [Cl ⁻] mM (A)	Final volume. Original vol. = 100 %		$\frac{A \times B}{100}$
		(B)	%	
89	46	140		64
124	58	113		65
145	63	102		64
216	85	82		70

If, as a result of dilution, the external $[Cl^-]$ fell very low, according to the Donnan equation Cl^- should leave the cell, and as Cl^- cannot diffuse alone, H^+ would have to leave with it; that is, a hydrolysis of KCl inside the cell should occur resulting in a decrease of cell $[Cl^-]$ and external p_H , and the combination of the corresponding cell K with haemoglobin. Owing to haemolysis, the direct observation cannot be made, but Coulter [1924-5] has shown that if cells are placed in a solution of sucrose, the latter becomes more acid. This experiment was confirmed by placing 0.1 cc. of cells in two tubes and adding 10 % glucose at p_H 7 to one, and 1.7 % KCl at p_H 7, to the other; the p_H of the glucose changed to 5.8, and that of the KCl to 7.6. At the same time the cells in glucose lost 10 mM of chloride, which corresponds to an external p_{Cl} of 4.2. There is thus some discrepancy between the external p_H and p_{Cl} .

Red cells were next placed in a solution of 24 mM KCl in 9 % glucose at p_H 7. The p_H of the external solution rose to 7.6 and the $[Cl^-]$ of the cells to 79 mM, the internal chloride was therefore much higher than the external chloride, and was nearly as great as the $[Cl^-]$ of cells exposed to a pure KCl solution of the same osmotic pressure as that of the glucose and KCl in the experimental solution.

When cells are placed in glucose solution containing HCl, a proportion of the H^+ and Cl^- enters the cells. If the HCl is kept constant and KCl is added to the outer phase, cell $[Cl^-]$ must increase correspondingly, in accordance with the Donnan equation. As the potassium of the KCl cannot permeate readily, cell Cl^- does not diffuse from the external KCl, but is mainly derived from, and limited by, the external HCl. It follows that with increasing concentration of external KCl, more and more HCl will pass into the red cell, and the external p_H will rise. This effect is shown in Table II.

Table II.

Exp. No.	External fluid				Cells	
	Cl as HCl mM	Cl as KCl mM	Glucose %	Final p_H	Cl mM	Volume (normal = 100 %)
1	1	0	10	3.6	86	78*
2	1	1	10	3.8	100	78*
3	1	4	10	4.1	124	83*
4	1	24	9	4.7	168	85*
5	1	115	5	5.0	174	99
6	1	230	10	5.0	240	71
7	1	230	0	5.2	176	104

* These volumes are approximate owing to cell agglutination and partial distribution on the wider part of the centrifuge-tube.

It will be seen that the external p_H increases with the addition of KCl, and that the $[Cl^-]$ of the cells is far in excess of the external $[Cl^-]$. If glucose diffused freely, then cell $[Cl^-]$ would depend only on the external $[Cl^-]$ and p_H , and the anomalous distribution of Cl^- actually found must be correlated with the observation that glucose permeates the red cell very slowly. Under these circumstances it appears that the $[Cl^-]$ in the cell depends on the p_H and the osmotic pressure of the external non-permeating substance, provided that a large excess of total chloride is available, and a certain relatively low minimum concentration is exceeded (Exps. 4, 5, 6 and 7). In interpreting cell Cl^- changes, it must be remembered that untreated red cells have a $[Cl^-]$ of 50 mM per litre and also that their final volume is less than 1/140 of the external fluid. Thus in Exp. 1 the gain in cell Cl^- is about 12 % of the external Cl^- and in Exp. 4, about 70 % of the external HCl and 3 % of the total Cl^- . The loss of external Cl^- in Exp. 1 is equivalent to a rise in p_H from 3 to 3.1 (approximately). The discrepancy between this and the actual p_H recorded (3.6), may be due in part to the buffering action of that fraction of HCO_3^- displaced from the cell by chloride and also to the red cell not being completely impermeable to cations.

In this type of experiment, the external osmotic pressure may be maintained by a non-electrolyte, like glucose, or by a slowly penetrating anion, such as phosphate. In mixtures of potassium phosphate and chloride, the presence of the Cl^- greatly depresses the permeation of phosphate, and excess of Cl^- enters the red cell to balance the external phosphate and chloride. This effect is best seen at p_H 5.1, where the cell is least permeable to phosphate, and is less evident at p_H 6, where the cell is more permeable. Cells were exposed for 5 min. and the temperature was kept constant, because it was found that phosphate diffusion has a high temperature coefficient (2.4 between 20 and 30°).

Table III. *Diffusion of chloride from phosphate-chloride mixtures of varying strengths.*

Depression of freezing-point, 0.42°.

Cl/phosphate by volume	External Cl^- mM	Internal Cl^- mM	External phosphorus mg./100 cc.	Internal P mg./100 cc.
1/0	124	112	0	0
1/1	62	110	200	22
1/6	21	102	330	39
0/1	0	40	400	170

The phosphate-chloride equilibrium is unstable, because with the lapse of time more phosphate enters the red cell and Cl^- is displaced.

Conclusion.

If red cells are exposed to solutions containing permeating anions and other anions or non-electrolytes which permeate less easily, the amount of permeating ion diffusing across the positively charged membrane into the red cell depends on the external p_H and the total osmotic pressure of the less diffusible substances—always provided that a certain absolute quantity and minimum concentration of the permeating ion is exceeded. The concentration of this ion in the cell may be many times greater than its equilibrium concentration in the external phase.

REFERENCES.

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