LXIII. STUDIES ON THE PERMEABILITY OF ERYTHROCYTES.

III. THE CATION CONTENT OF ERYTHROCYTES OF RABBIT'S BLOOD IN HYPER- AND HYPO-TONIC SERA.

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IN earlier papers of this series [Davson, 1934; Davson and Danielli, 1936] attention has been drawn to the work of Ponder and Saslow [1930; 1931] on the volumes of rabbit's erythrocytes. These authors observed that in hypotonic solutions the erythrocytes gained much less water than was expected and in hypertonic solutions lost far less; in fact an increase in volume of the erythrocytes was generally observed instead of the expected contraction. These anomalous results have been interpreted by Ponder and Saslow as being due to the penetration or loss of cations and have been considered as evidence that the erythrocyte may become completely permeable to cations. Although the author [1934] has found a certain penetration of K^+ and Na^+ in hypertonic solutions with the ox erythrocyte, none of the order of magnitude inferred by Ponder and Saslow was ever found. It was decided therefore to repeat some of the experiments of Ponder and Saslow determining both K^+ and Na^+ and volume changes. It was found that although the changes in volume are not so great as those to be expected on the basis of complete cationic impermeability, the changes are in the right direction and the anomalies are by no means so great as those described by Ponder and Saslow.

EXPERIMENTAL.

Hypotonic experiments. Defibrinated rabbit's blood taken from the carotid under ether anaesthesia was measured into centrifuge-tubes; the erythrocytes were centrifuged, water was added and the erythrocytes were mixed with the diluted serum. After one hour the suspensions were centrifuged again and 1 ml. of erythrocytes was weighed out for the determinations of cations and 2 ml. for the determination of the water content. A haematocrit determination on the original blood gave the proportion of serum to whole blood and thus enabled the calculation of the degree of hypotonicity. For the K^+ determination it was found impossible to haemolyse the erythrocytes completely by adding distilled water, so that in the ashed trichloroacetic acid filtrates low values for K^+ were obtained; the erythrocytes were therefore ashed whole.

Hypertonic experiments. No preliminary centrifuging was necessary; 2-5 ml. of a 4% solution of KCl were added to the blood. The rest of the procedure was as above.

RESULTS.

In Table I the results of representative experiments are shown. The relative volumes in column 4 are calculated from the changes in water content. In column 5 the cation contents are recorded as determined by comparing the

Table I.

Exp.	Tonicity compared with normal of rabbit serum	% H ₂ O	Volume		Cations (millimols.)	
			Found	Expected	Found	Expected
1	1	66.7	100	100	111	111
	1.76	62.1	88	76	112	143
	2.56	58.6	80	63	111	173
2	1	62.0	100	100	110	110
	0.84	64.3	109	110	110	103
3	1	68.7	100	100	111	111
	0.68	73.5	124	121	110	113

relative volumes of the test and control corpuscles: thus in Exp. 1 the cation content is 111 millimols. per 100 g. in the control, and 112 and 111 millimols. per 88 g. and 80 g. respectively in the test solutions. In this way the cation content is referred to a fixed number of corpuscles so that any changes in its value must be caused by penetration or loss of cations. In the last column is shown the cation content expected on the basis that the anomalies in the volumes are explicable by the penetration or loss of cations. It is evident that in hypertonic solutions the volume changes differ from those expected whereas in hypotonic solutions the changes are approximately in accordance with expectation. The close agreement between the cation contents in control and test experiments is illusory as considerable errors must be introduced by failure to pack the corpuscles thoroughly. Thus in an experiment (not shown) in which the hypertonicity was produced by addition of NaCl instead of KCl an apparent increase of nearly 6% in the K⁺ content of the erythrocytes occurred; as no extra K⁺ had been added to the serum this apparent increase must have been due to underestimation of the shrinkage; however, even when the shrinkage was corrected the discrepancy between the experimental and theoretical values was of the same order as that found in the experiments shown.

DISCUSSION.

Two facts emerge from the results described here. First that the abnormalities observed by the author are not so large as those described by Ponder and Saslow. Secondly that the abnormalities observed are not to be correlated with the penetration of K⁺ or Na⁺. In discussing a possible free penetration of both cations and anions into the erythrocyte the following point should not be neglected. The cells suspended in isotonic KCl solution may be represented thus:

Hb-	K ⁺
Cl-	Cl-
K ⁺	
Cells (1)	Serum (2)

If now the cells are freely permeable to both K⁺ and Cl⁻ the equation representing the Donnan equilibrium is

$$[\text{K}^+]_1 [\text{Cl}^-]_1 = [\text{K}^+]_2 [\text{Cl}^-]_2 \\ = [\text{Cl}^-]_2^2.$$

Or since $[\text{K}^+]_1 > [\text{Cl}^-]_1$ $[\text{K}^+]_1 + [\text{Cl}^-]_1 > 2 [\text{Cl}^-]_2.$

Hence there must always be a difference in osmotic pressure between cells and serum; to compensate this water will penetrate into the cells, but in virtue

of the assumed diffusibility of both ions more KCl will penetrate and so the process will go on until the cells burst. If on the other hand only one ion is diffusible no such relation is obtainable, in fact it may be shown that osmotic equality must exist between cells and serum. Thus it is evident that the erythrocyte maintains its integrity precisely in virtue of the indiffusibility of its cations. Hence Ponder and Saslow's contention that the erythrocytes may become completely permeable to cations in hypertonic solutions is untenable without further assumptions.

Takei [1921] states that the erythrocytes of the rabbit behave like perfect osmometers in hypertonic solutions up to 4 % NaCl and then behave anomalously in that they swell and haemolyse to a certain extent. Ege [1923] on the other hand disputes these conclusions both on theoretical and experimental grounds. Thus the state of affairs is still very confused, but it seems from the results described here that the anomalies in hypertonic solutions cannot be ascribed to the penetration of K^+ or Na^+ .

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