XLVIII. STUDIES ON THE PERMEABILITY OF ERYTHROCYTES.

II. THE ALLEGED REVERSAL OF IONIC PERMEABILITY AT ALKALINE REACTION.

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Under physiological conditions the erythrocyte membrane is impermeable to cations. The suggestion that this impermeability might be a function of the normal ionic environment of the cells, which might be reversibly converted into a state of permeability by a suitable change in this environment, was put forward in an earlier paper [Davson, 1934]; experiments, however, failed to confirm this hypothesis in respect of Ca^{++}. The work of Mond [1927], who found that at alkaline reactions the erythrocyte became impermeable to anions and permeable to cations, together with that of Ponder and Saslow [1930; 1931] whose results pointed to a complete cationic permeability in hypertonic saline solutions seemed to provide a basis for this view.

Mond's results however are by no means above criticism. In the first place the cells were placed in isotonic sugar solution to which *N* NaOH had been added and the final p_H was taken as the p_H at which the change was produced. Thus 5 ml. of corpuscles were added to 6·6 ml. of a glucose solution to which 0·4 ml. of *N* NaOH had been added, so that in actuality the cells were added to a solution of p_H 12·8 and not to one of 10·1, the p_H at which the mixture finally arrived. To attribute any change in the membrane thus produced to a change from p_H 7·4 to one of 10·1 is quite unwarrantable. As the effect of strong bases on living tissue is particularly destructive it is only to be expected that irreversible changes in the membrane would be produced by Mond's treatment and free permeability to cations would most likely follow from these essentially irreversible changes. Mond himself remarks that he had great difficulty in preventing haemolysis by his treatment. In the second place no attempt was made to demonstrate the reversibility of the changes produced. Thirdly, the impermeability to anions at $p_H > 8·3$ was not actually proved; all that was observed was that the equilibrium ratio of Cl^- in the cells to Cl^- in the sugar solution at p_H 8·3 was not markedly different from that at $p_H > 8·3$.

In this paper it will be shown that no change in the cationic permeability occurs between p_H 7·4 and 10·0 provided that the cells are brought to these p_H values by the use of buffers; the changes in the cell Cl^- concentration observed are to be explained on the basis of the Donnan equilibrium.

Experimental.

The procedure already described [Davson, 1934] was employed; ox blood, which was collected directly into our own vessels from the slaughtered animal was used. The saline solutions contained Na^+, K^+ and Ca^{++} in the proportions characteristic for ox serum and were diluted to the isotonic value with the buffer solutions. It was found necessary to wash the erythrocytes twice with the

alkaline buffer-saline solutions before they attained the desired p_H. In the first two experiments borate buffers were used but, as a certain amount of agglutination occurred under these conditions, the borate was replaced by glycine. K^+ was determined by the method of Kramer [1920] and Cl^- with a Ag-AgCl electrode. A few of the results are given in Table I.

Table I.

Exp.	Washing fluid	p_H	K^+ mg./100 ml. corp.	Cl^- mg./100 ml. corp.
1	Unwashed	7·4	64·8	—
	Ringer	7·4	56·0	—
	Borate/Ringer	9·0	56·0	—
2	Unwashed	7·4	67·6	—
	Ringer + KCl	7·4	88·0	—
	Borate/Ringer + KCl	8·3	88·0	—
3	Unwashed	7·4	72·7	234
	Glycine/Ringer	7·35	64·9	242
	Glycine/Ringer	9·10	65·0	153
	Glycine/Ringer	10·0	67·6	110
	Serum + Glycine/Ringer. 1 : 1	9·63	67·2	—

Results.

From Exps. 1 and 3 it is evident that the erythrocytes washed with buffer-saline solutions at p_H 8·3–10·1 lose no more K^+ than they do when washed at physiological p_H; the loss of K^+ in the latter case has been discussed in the earlier paper and is probably due to damage of the cell membrane. It is to be noticed that serum diluted to 50 % with buffer-saline at p_H 10·2 and showing a final p_H of 9·6 gives approximately the same result with regard to K^+ as ordinary buffer-saline solutions. In Exp. 2 the K^+ content of the Ringer solution was increased to 576 mg. per 100 ml. and it is seen that its penetration into the cells due probably to irreversible damage to the membrane is independent of p_H.

Returning to Exp. 3 it is seen that the Cl^- content of the cells decreases with increasing p_H; this is to be expected on the basis of the Donnan equilibrium since the haemoglobin in the cells is ionising as an acid and this ionisation increases with the alkalinity.

Discussion.

The permeability of the cell to ions has been considered to be a function of the electrical charge on its membrane [Höber, 1922]; if this were so the impermeability of the erythrocyte membrane to cations would be explained by assuming the existence of a positive charge on it. However, under the influence of an electric field the erythrocyte migrates to the positive electrode and thus there can be no doubt that its electrokinetic potential is negative. This charge is due chiefly to the layer of adsorbed proteins, since Fåhreus [1921] has shown that agglutination of the erythrocytes which probably depends on their charge is dependent on the nature of the protein in the fluid in which they are suspended. The degree of agglutination is greatest in fibrinogen, less in globulin and least in albumin solutions at physiological p_H and this is the order of increasing acidity of isoelectric point. To resolve this contradiction the work of Mond has been cited [Gellhorn, 1929] as showing that another protein must exist in a certain layer of the erythrocyte membrane with an isoelectric point of p_H 8·3 which would therefore be positively charged at physiological p_H; it is this hypothetical protein (supposed to be the globin of haemoglobin) which is said to determine the nature of the ionic permeability. Thus, if this were so, the membrane would be normally impermeable to cations and permeable to anions and

this relationship would be reversed at p_H 8·3. In actual fact the presence of free globin has never been established in the erythrocyte membrane and the theory is based on experimental facts which this paper has shown to be quite unreliable and, as will be shown later, on a misconception of the nature of the potentials which determine ionic penetration.

In the interpretation of the influence of the potential of a membrane on its permeability properties care has to be taken to distinguish between the electrokinetic ζ- and ϵ-potentials. The ϵ-potential across an interface is determined partly by the asymmetrical distribution of ions in the neighbourhood of the interface and partly by the non-ionic electrical dipoles of the molecules oriented at the interface. Of these two components only one, the ionic term, changes its sign at the isoelectric point; the other, the dipole term, has a sign and magnitude which are largely independent of p_H changes. The dipoles are definitely fixed at the interface, while the ions form a rather diffuse layer. Some of these ions are free to move independently of the interface, others are not. Thus when an electric field is applied to this system the two sets of ions will move separately. The potential difference between the set of ions fixed in the surface and the set in the bulk phase is known as the electrokinetic ζ-potential. The electrokinetic potential being dependent on the existence of ions changes its sign at the isoelectric point where it is zero. It will thus be clear that, since ϵ is due to the sum of two terms (dipole moment and lack of symmetry in ionic distribution) and ζ is due to another term, namely the independence of motion of some of the ions, and is not directly related to either of these other two, ϵ and ζ will not be

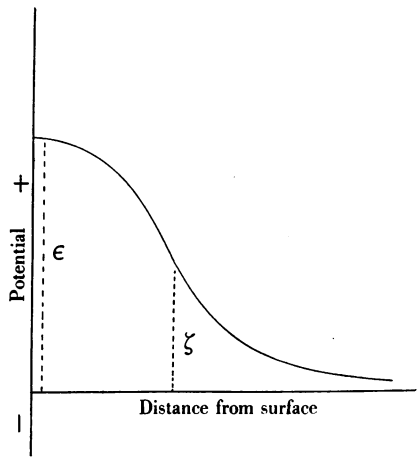

Fig. 1.

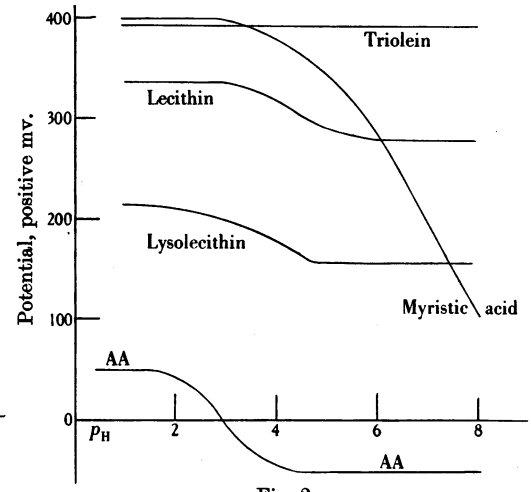

Fig. 2.

Fig. 1. Difference of ϵ- and ζ-potentials.

Fig. 2. ϵ- and ζ-potentials. Curve AA illustrates the potential changes with p_H for lecithin. The remaining curves are for ϵ, taken from Hughes [1935].

identical and may even be of different signs. This is illustrated diagrammatically in Fig. 1 and in Fig. 2 are shown ϵ and ζ curves for lecithin. It is evident from the figure that whilst ζ changes its sign at the isoelectric point, ϵ remains positive over the whole range of p_H investigated. In fact all the substances which are supposed to be in the erythrocyte membrane have positive ϵ-potentials at all p_H values, so far as has been investigated.

With regard to the penetration of ions through a membrane, there are three suggested mechanisms for the process.

(1) If the mechanism is that of the passage through a homogeneous lipoid layer, and not through a pore, then ϵ is the potential of interest. Since in the case of the erythrocyte ϵ is probably always positive and is not changed in sign by p_H changes, a reversal of ionic permeability with change of p_H is not to be expected.

(2) If penetration is through a pore in the membrane, as suggested by Michaelis [1925], ζ is the potential of interest. That this is so will be obvious from Fig. 3*a*. The ions which can penetrate through such a pore must be (*a*) free to

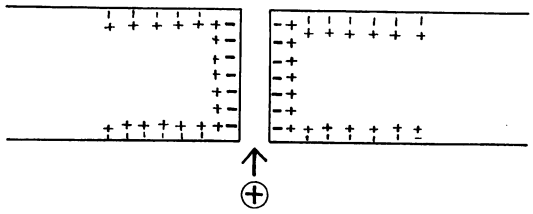

Fig. 3*a*. Pore mechanism of ionic penetration.

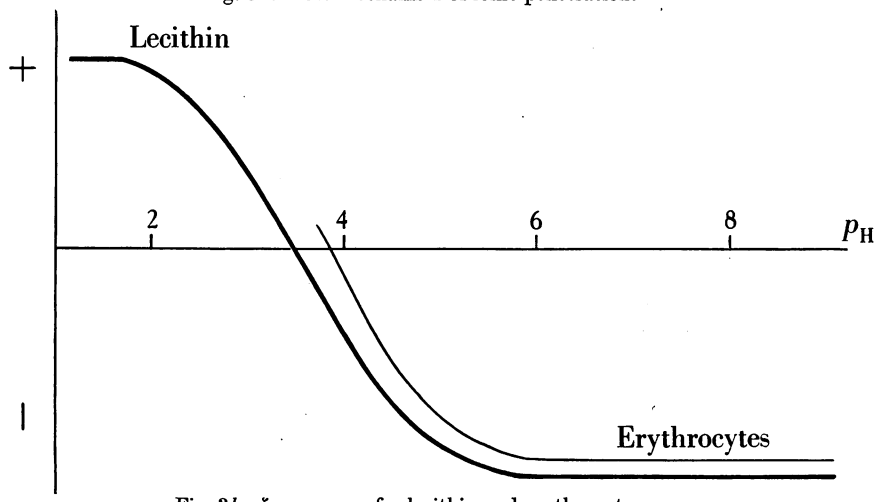

Fig. 3*b*. ζ-p_H curves for lecithin and erythrocytes.

move and (*b*) of opposite sign to the fixed ions of the pore walls. Hence when the walls ionise as an acid cations will penetrate, and when they ionise as a base anions will penetrate. Thus there should be a reversal of ionic penetration at the isoelectric point of the erythrocyte wall. The work of Coulter [1920], *e.g.*, shows that the isoelectric point of the erythrocyte is at about p_H 4·75 as is shown in Fig. 3*b*. Hence over the range of p_H 7–10 the pore surface should ionise as an acid and cations only should penetrate, which is not so.

(3) If the ions penetrate by combination with a component of the erythrocyte membrane followed by diffusion through the membrane as an ionic doublet, as suggested by Osterhout [1930], neither ϵ nor ζ is of direct importance. However, if a cation is to penetrate, the surface molecules must ionise as an acid. Thus only cations will be able to penetrate on the alkaline side of the isoelectric point and only anions on the acid side. Thus again there should be a reversal of the sign of ionic penetration at p_H 4·75.

In the present paper it has been shown that over the range of p_H 7–10 anions only penetrate the erythrocyte wall. Hence mechanism (1) is the only possible one for the erythrocyte. From the above considerations we are led to a picture of penetration through the erythrocyte membrane in which the potentials encountered by ions are as shown in Fig. 4. The form of the potential curve

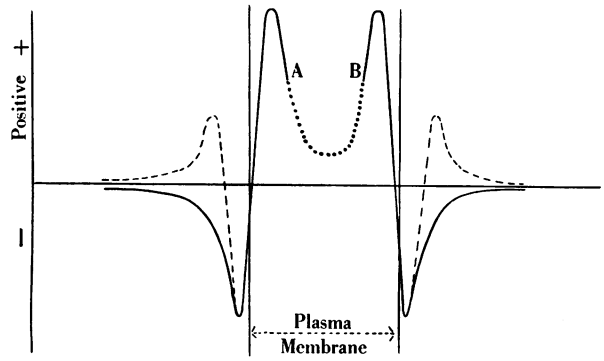

Fig. 4. Potential diagram perpendicular to the plane of thin lipoid membrane. Continuous line when ionising as a base; broken line when ionising as an acid. Dotted portion: potential undefined.

between A and B is difficult to define but is not of significance for this argument. The important point is that the plasma membrane has an excess of positive charge due to the oriented dipoles of the interfaces and hence the solubility of anions in the membrane will be much greater than that of cations. The establishment of equilibrium by ionic exchange should therefore be rapid in the case of anions and slow in that of cations, possibly of negligible speed as found here experimentally.

SUMMARY.

1. The work of Mond on the change in the ionic permeability of erythrocytes at p_H 8·3 is criticised. Using buffer solutions to bring the erythrocyte to p_H 8–10 no reversal of ionic permeability was obtained.

2. The potentials at the membrane interfaces are discussed in relation to theories of the mechanism of ionic penetration.

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