L. STUDIES ON THE PERMEABILITY OF ERYTHROCYTES

VI. THE EFFECT OF REDUCING THE SALT CONTENT OF THE MEDIUM SURROUNDING THE CELL

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THE effect of diminishing the electrolyte content of the medium bathing the cell, keeping the osmotic pressure constant, is generally recognized as an injurious one, whereby the normal selective permeability of the plasma membrane is disturbed; as an example of this the work of McCutcheon & Lucke [1928], who found that in glucose solution the permeability of *Arbacia* eggs to water is doubled as compared with eggs in sea water, may be quoted. In earlier papers [Davson, 1934; 1936; 1937; Davson & Danielli, 1936; 1938] some of the conditions which determine the erythrocyte's normal impermeability to cations, and changes in these conditions which lead to a breakdown of this impermeability, have been described. The material presented here is an extension of the work to the special case of the effect of an absolute decrease in the quantity of electrolytes in the suspension medium.

Joel [1915] has shown that if the erythrocytes of the ox are washed repeatedly with sugar solution and then suspended in the same medium, the latter shows an increase in electrical conductivity with time which is due, as he supposed, to the passage of salts from the cells; however, this treatment is drastic and there is no evidence that the changes observed were reversible and were not due to haemolysis. Jacobs & Parpart [1933], working under less extreme conditions, found changes in the fragility of the same erythrocytes on exposure to hypotonic solutions of non-electrolytes which they interpreted as being due partly to anionic exchanges and partly to the loss of cations from the cells. Maizels [1935] found, by direct chemical analyses, that the human erythrocyte loses K in glucose solution in a reproducible manner, the effect increasing with temperature.

The results of Joel and of Jacobs & Parpart are essentially indirect and, whilst the constructions placed upon them appear quite probable, experience has shown [Davson, 1936; 1937; Davson & Ponder, 1938] that indirect measurements as in conductivity and fragility studies are of questionable value in regard even to qualitative estimates of changes in cation permeability, whilst quantitative measurements are virtually excluded. Thus the results of Maizels remain as certain facts, inadequate in themselves, however, to allow of any explanation of the phenomenon of the induced cation permeability due to the reduction in the electrolyte content of the medium. Before entering into the experimental part of this paper, possible mechanisms for the cation permeability and the kinetics of the loss of K may be briefly touched upon.

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If an erythrocyte is placed in a non-electrolyte solution there will, as Jacobs & Parpart [1933] have shown, be a tendency for the Cl^- and HCO_3^- to leave the cell; this loss of anions will be replaced by OH^- from the non-electrolyte medium. Thus, owing to the rapid shift of anions across the membrane, there will be a difference of $p\text{H}$ (which may amount to three units if the dilution of the blood is great enough), the cells being alkaline in respect to the suspension medium. What seems not to have been realized hitherto is that in consequence of this $p\text{H}$ difference there will be a large potential difference between the inside and the outside of the cell, the latter being negative in respect to the former. The magnitude of this P.D. will be given by the formula:

$$E = RT/F \ln [\text{H}_0]/[\text{H}_i],$$

where $[\text{H}_0]$, $[\text{H}_i]$ are the respective H ion concentrations inside and outside the cell, and thus may be of the order of 150 mV. As a result it would not be surprising if positive ions, should they come under the influence of this P.D., were repelled from the inside to the outside of the cell so that the normal restraint on these ions, which inhibits their migration, is overcome. Thus the phenomenon of cation permeability might reasonably be expected on suspending erythrocytes in a non-electrolyte medium. As K escapes from the cell it will be accompanied by Cl^- and HCO_3^- and, to a lesser extent, by OH^- ; as a result of the migration of the HCO_3^- and OH^- ions the difference of $p\text{H}$ tends to be reversed, and also the sign of the P.D., as the loss of K proceeds. If the cation permeability is due entirely to the acceleration of the ions down a potential gradient, then it must cease as soon as the difference in $p\text{H}$ is neutralized; furthermore, the addition of salts at any time during the escape of K, in so far as it reverses the P.D. caused by the shift of anions, should inhibit the further loss of K.

If, however, the cause of the cation permeability is not the electrostatic acceleration of the ions but is due to the potential difference acting directly on the membrane, then the inhibition of the cation permeability due to the levelling out of the P.D. is only to be expected if the change in the membrane is a reversible one. If, further, it can be shown that addition of salts inhibits the migration of K after it has begun, and also that the latter process does *not* cease when the P.D. is neutralized or reversed in sign, it follows that the cation permeability is not due to the P.D. at all, and its cause must be sought in an actual change in the membrane due to the reduction in the salt content of the medium in contact with its exterior. The experimental work to be described may thus be considered in respect to these three general theories and it will be seen that by a process of exclusion one must adopt the last-mentioned in explaining the permeability to cations in non-electrolyte media.

The rate of loss of K will be given by the simple unimolecular equation:

$$\frac{dK_i}{dt} = k (K_i - K_0 - \beta),$$

where k is the permeability constant,¹ A the area of the cells, K_i and K_0 the concentrations of K inside and outside the cells respectively and the constant β equals the difference between the concentrations of K inside and outside the

¹ The use of a permeability constant for a single ion is not strictly correct, since the rate of permeation of a given ion will be a function of two probabilities, viz. those that a positive ion and a negative ion will pass the membrane at any given moment. However, in the case of the erythrocyte, which is so freely permeable to Cl^- and HCO_3^- , the use of a single constant for K^+ is probably justified, at least for the purposes of this paper. It will be seen that the experimental results deviate so greatly from the equation that this consideration is a minor one compared with the auto-inhibitory effect of the escape of K *per se*.

cells at equilibrium and is introduced into the equation to satisfy the condition that dK_i/dt equals zero when the K concentration inside the cell has dropped to its equilibrium value, which will be determined by the Donnan equilibrium, and will be greater than the concentration in the suspension medium; its absolute value will be small compared with the initial value of K_i owing to the large volume of suspension medium. *The equation assumes that the conditions determining the escape of K are unaffected by this escape.*

Now to a first approximation K_i , the concentration of K inside the cells, will be constant, since the loss of K plus its accompanying anions will result in a decrease in the osmotic pressure inside the cell causing the passage of H_2O into the suspension medium.¹ Under the conditions of the experiments K_0 may also be treated as a constant, and, owing to the disk shape of the cells, A will be approximately constant. Hence the equation becomes:

$$dK_i/dt = \text{Constant},$$

so that the loss of K should proceed linearly with time, and any gross deviation from linearity should be ascribed to a variation in k , the permeability constant, i.e. to a change in the properties of the membrane or the P.D. across it due to the loss of K.

EXPERIMENTAL

The non-electrolyte solution used was 0.32 *M* glucose, in glass-distilled water, for all species except human for which 0.32 *M* sucrose was used, since human erythrocytes are comparatively permeable to glucose. The whole blood was added to the non-electrolyte solution, contained in a centrifuge tube immersed in a thermostat at 25°; the ratio of the volumes of blood to suspension medium was 1 : 10. After the appropriate interval the suspension was centrifuged, the supernatant fluid was removed and the K or Na content of the cells determined as described earlier [Davson, 1934; 1937] using the method of Kramer [1920] for K and that of Barber & Kolthoff [1928] for Na. If the cells contained only a little K, as in the case of the cat, the dissolved ash was not made up to a definite volume from which aliquots could be taken but was evaporated to dryness in a silica beaker and the whole of the K in it determined; this procedure was also used for all Na determinations. The errors arising out of all steps in the procedure rarely amounted to more than 1 %. As controls, cells suspended in non-electrolyte medium and immediately centrifuged down were used so that losses due to the centrifuging of the cells *per se* were discounted. *pH* changes in the suspension medium were determined colorimetrically.

RESULTS

In Table I the variation in the K contents of the erythrocytes of the man, guinea-pig, rat, pig and rabbit with time of suspension in a non-electrolyte medium are shown; figures for three individuals of each species are presented to give some idea of the variability in the magnitude of the changes. The results are expressed as percentages of the control value. In Table II similar figures are given for the K and Na contents of the erythrocytes of the ox and cat; these

¹ Since about one-third of the K in the cells is in the form of a polyvalent haemoglobin salt, K_nHb , there will be an excess of osmotically active cations over anions, so that the loss of a pair of ions ($K + Cl^-$) or ($K + HCO_3^-$) reduces the osmotic pressure inside the cells by a greater amount than the fractional decrease in the K content. Hence, strictly speaking, the concentration of K should rise in the cells, although the amount per cell falls. This effect is compensated, however, by the increasing acidity inside the cell which reduces the amount of K_nHb .

Table I. *The variation of the K content of the erythrocytes of different species with time of suspension in a non-electrolyte medium. Temp. 25°*

Time	Man			Guinea-pig			Rat			Pig			Rabbit		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
Control	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1 hr.	74	80	82	84	91	98	95	93	92	99	—	99	98	100	101
2 hr.	57	69	69	76	79	94	89	87	86	99	99	99	98	99	100
3 hr.	52	60	59	69	74	93	86	83	—	93	—	99	98	99	96
6 hr.	—	—	—	—	—	—	—	—	—	—	96	98	—	—	—

Table II. *The variation of the K and Na contents of the erythrocytes of the ox and cat with time of suspension in a non-electrolyte medium. Temp. 25°*

Time	Ox						Cat					
	I		II		III		I		II		III	
	K	Na	K	Na	K	Na	K	Na	K	Na	K	Na
Control	100	100	100	100	100	100	100	100	100	100	100	100
1 hr.	95	98	95	96	100	100	99	98	94	97	95	92
2 hr.	95	98	92	91	97	98	92	85	88	94	87	71
3 hr.	92	95	91	90	93	96	89	66	87	90	78	34
5 hr.	70	77	—	—	—	—	—	—	—	—	—	—

species differ from those in Table I in that their erythrocytes contain more Na than K. From Table I it is evident that the erythrocytes of the man, guinea-pig and rat show a well-defined permeability to K when suspended in a non-electrolyte medium; the cells of the pig and rabbit, however, are sharply differentiated from the former group in that the loss of K in the first 3 hr. is very small and of the order of 1–2 %. This species difference will be taken up later.

With regard to the results in Table II it may be remarked that the cells of the ox and cat show irregularities in their behaviour which were not encountered with the species in Table I. Thus in Exp. 1 of the ox there is a loss of 5 % in the K content during the first hour, no loss during the second hour, a loss of 3 % during the third, and of 22 % during the last 2 hr.; the same anomalous course was followed by the Na values; again in Exp. 1 of the cat we have a loss of 1 % of the K during the first hour, 8 % during the second hour and 3 % during the third hour. Another sort of irregularity occurs most markedly with the cat erythrocytes; this is in respect of the relative rates of loss of Na and K. It is generally realized that although K has a larger atomic weight than Na, the size of the K ion in aqueous solution is smaller than that of the Na ion in virtue of the greater hydration of the latter; consequently, if size determines the relative rates of migration of the two ions across the membrane, K should penetrate more rapidly than Na. This appears to be the case with the ox, and on occasions with the cat, but Exps. 1 and 3 for the cat show a much greater percentage loss of Na than of K during the intervals 1, 2 and 3 hr. That these irregularities can be due to experimental errors is quite out of the question, so that it would appear that under certain conditions Na may penetrate a membrane more rapidly than K, unless, of course, the equilibrium conditions determining the cessation of the permeability process are more complicated than would generally be expected; thus if the K in the cells is, at the outset, much nearer its equilibrium value than the Na, then comparisons between the losses of K and Na in given times would not necessarily lead to the determination of comparative permeability constants. The acceleration in the rate of loss of cations with time mentioned above will be discussed more fully later.

To return to the behaviour of some of the species in Table I, the K contents of the human, guinea-pig and rat cells are plotted against time of suspension in a non-electrolyte medium in Fig. 1; it will be seen from Table I that the curves are probably typical of the species, the rate of loss being in the order: man > guinea-pig > rat. The curves show deviations from linearity, the deviation being more pronounced and showing itself sooner the greater the initial rate of

Fig. 1. Loss of K from the erythrocytes of the rat, guinea-pig and man in non-electrolyte solution at 25°. Ordinates: cell K content as a percentage of the control value. Abscissae: time of suspension in the non-electrolyte medium in hours. Figures in parentheses indicate the pH of the suspension medium after centrifuging down the cells.

loss. Furthermore, the curves tend to different stationary states; thus the rat cells tend to a value of 72.5, the guinea-pig to one of 60 and the human to 35 or less, these figures being, as before, percentages of the initial cell K contents. The figures near points on the curves are the values for the pH of the suspension medium after centrifuging and it is clear that the change over from an acid medium to an alkaline one mentioned in the introduction certainly occurs. However, the loss of K does not cease as soon as the medium becomes alkaline.

The reversibility of the induced permeability to K is brought out by the results of Table III and Fig. 2. In Table III, row (a) gives the loss of K during 1 hr. of suspension in the non-electrolyte medium followed by 2 hr. of suspension in the same medium to which NaCl had been added to bring the concentration of the latter up to 0.10 *M*. Row (b) gives the loss of K during 1 hr. of suspension in the non-electrolyte medium, and row (c) the loss during 2 hr. suspension in non-electrolyte solution plus NaCl. If the process is reversible (b) plus (c) should be equal to or greater than (a), and this is the case within the limits of experimental error. In Fig. 2, the curve *AB* gives the loss of human cells with time in sucrose solution; *AC* represents the loss in a sucrose plus NaCl solution (0.10 *M* with respect to NaCl). At the points *X* NaCl was added as before and the lines *XX'* show the loss of K subsequent to the addition of NaCl. The approximate parallelism of the lines again indicates reversibility, even after 2 hr. in the non-electrolyte medium alone. The slight loss of K from the cells in sucrose plus

Table III. *Experiments designed to show the reversibility of the induced permeability to K. (N.E. is abbreviation for non-electrolyte solution)*

Treatment	% loss of K		
	Man	Guinea-pig	Rat
(a) 1 hr. in N.E.; 2 hr. in N.E. + NaCl	20	23	13
(b) 1 hr. in N.E.	20	24	13
(c) 2 hr. in N.E. + NaCl	2	3	0
(b) + (c)	22	27	13

Fig. 2. Curves demonstrating the reversibility of the induced permeability to K. Temp. 25°.

NaCl indicated by the line *AC* is not due to the presence of sucrose since it occurs in isotonic NaCl alone [Maizels, 1935; Davson, 1937].

In Fig. 3 the loss of K from the human erythrocyte in 2 hr. is shown as a function of the electrolyte content of the medium and it is seen that the effect of diminishing the electrolyte content of the medium begins when the former has been reduced to less than 0.09 *M*. The extreme sensitivity of the rate of loss to salt content of the suspension medium at values of the latter below 0.016 *M* is another interesting point.

From the curves in Fig. 1, the coincidence of the retardation of the rate of loss of K with the increasing alkalinity of the suspension medium might lead one to suppose that the cause of this retardation was the alkalinity; this, however, would be in contradiction to Maizels' results which showed an increased rate of loss in an alkaline medium, although too much weight must not be placed on these results since the alkalinity was produced by addition of NaOH to the medium giving an initial reaction of the medium of about pH 10 which in itself is sufficient

Fig. 3. Variation in the rate of loss of K from human erythrocytes with salt content of the suspension medium. Ordinates: percentage loss of K in 2 hr. Abscissae: concentration of NaCl in the suspension medium in moles/litre. Temp. 25°.

Fig. 4.

Fig. 5.

Fig. 4. The effect of the addition of acid and alkaline buffers to the non-electrolyte medium on the loss of K from human cells. AB is the control curve for non-electrolyte medium plus NaCl. Temp. 25°.

Fig. 5. Comparison of the losses of K from washed and unwashed human cells in non-electrolyte media. Temp. 25°.

to produce irreversible damage. In Fig. 4 is shown the effect of the addition of the buffers NaH₂PO₄, NaHCO₃ and Na₂HPO₄ to the non-electrolyte medium on the loss of K from human cells. The control curve, AB, represents the loss of K in sucrose solution to which NaCl had been added to make the total ionic con-

The results shown in Table IV may now be briefly enumerated. Exp. 1 shows that the erythrocytes of the rabbit maintain their virtual impermeability to K in glucose solution for as long as 7 hr. of suspension; there is indeed a loss of 4 % by the end of this time but the figures show that it is distributed evenly over the whole period. Washing the cells of the rabbit twice with glucose before suspension in glucose solution for a definite time causes a fair degree of permeability to K (Exp. 2). Addition of copper to the non-electrolyte medium causes a rapid sedimentation of the rabbit cells but does not produce any marked permeability to K.

DISCUSSION

The theory that the permeability of the erythrocyte to cations, induced by the diminution of the electrolyte content of the suspension medium, is due simply to an electrostatic repulsion of K and Na by the potential gradient across the membrane must be abandoned, since the loss of cations does not cease when the suspension medium becomes more alkaline than the contents of the cell (in virtue of the buffering power of haemoglobin very little change in the pH of the inside of the cell will occur during the escape of K; any change which does occur will be to make the contents more acid). As the P.D. considered here is a thermodynamic one, depending on the relative concentrations of Cl^- and HCO_3^- in the cells and suspension medium, only small and irregular variations between species should occur in it. However, it is found that the rabbit and pig cells show little tendency to lose K under conditions where human cells lose as much as 70% in 7-8 hr. This again presents a difficulty in the way of interpreting the permeability as an electrostatic phenomenon, although it may be overcome by the assumption that the normal restraints on the migration of cations vary in strength among the species, being greatest with the rabbit and pig and least with the human cells. This assumption is not in accord, however, with previous work on species characteristics [Davson, 1937]. Passing, then, to the next possible mechanism, viz. that the P.D. acts on the membrane, possibly by a re-orientation of the dipole constituents of the membrane, then the loss of K must only be expected to cease when the P.D. is neutralized provided that the change in the membrane is reversible. Now it has been shown that the induced permeability to K is indeed reversible by addition of salts, so that it would, on this theory, be necessary that the permeability to K should cease as soon as the P.D. were neutralized. We must therefore reject the P.D. as a significant factor in causing the permeability to cations; hence the cause of the permeability must be sought in a reversible change in the membrane due to the decrease in the salt content *per se*, apart from any potential effects due to ionic exchanges across this membrane.

Attention should be drawn here to a correlation between the magnitude of the induced permeability to cations and the rate of sedimentation of the erythrocytes in the non-electrolyte solutions. The erythrocytes of all species generally sediment more rapidly when suspended in a non-electrolyte solution than in isotonic NaCl, but this difference is far more pronounced in the case of the human, guinea-pig, rat, ox and cat cells than with the rabbit and pig cells; in fact individual samples of pig blood have been obtained which showed no evidence of settling, when added to glucose solution, after an hour, whereas if human or guinea-pig blood is treated in the same way the cells drop to the bottom of the tube within 5 min. or less. The reason for the rapid sedimentation is, of course, the agglutination; the suspensions which sediment rapidly contain large aggregates of cells whereas a suspension of pig blood is found to contain only discrete cells.¹ Washing the rabbit cells, which normally show very little permeability to cations in a non-electrolyte medium, twice with glucose solution and then suspending them in a further quantity causes them to lose K fairly readily, and at the same time marked agglutination is observed. Thus there

¹ This correlation is not perfect; thus one specimen of rat blood gave no sign of sedimentation and yet lost K at a rate typical for this species. Similarly, a specimen of rabbit blood sedimented fairly rapidly with no loss of K; in this case, nevertheless, there was no observable agglutination. No specimen of human, guinea-pig, or cat blood has, however, been found which did not show marked agglutination.

seems to be some change in the surface of the erythrocyte, produced by the reduction of the electrolyte content of the medium, which allows agglutination to occur and at the same time induces a permeability to cations. That the agglutination itself does not cause the cation permeability but is only symptomatic of the changes which do produce it is shown by the fact that addition of Cu to the non-electrolyte medium, which causes rabbit cells to agglutinate to a tremendous extent, does not cause any marked loss of K; further, addition of acid phosphate to the medium decreases the rate at which K escapes from human cells whilst it increases the degree of agglutination. The theory that a change in the surface of the erythrocyte is responsible for the cation permeability would be in accord with the apparently anomalous behaviour of the ox cells; here we had an acceleration of the rate of loss as the time of suspension increased, and this could be accounted for by assuming that the change in state of the surface of the erythrocytes of this species takes time, so that during the first hour or so the cells will not be very permeable whereas later, when the change becomes more pronounced, the permeability increases.

To determine the nature of this surface change presents some difficulties. The effect of a reduction in the salt content of the medium on such systems as protein sols or thin films of lipoids has been studied and has been described as a "shift of the isoelectric point" [Michaelis & Rona, 1919] or a change of the "surface pH " [Danielli, 1937], and these descriptions agree in so far as the changes expected will be those occasioned by a shift of the pH of the medium to the acid side, if the dissociating groups in the systems are on the alkaline side of their isoelectric points. As the ionizing groups in the erythrocyte membrane are quite definitely on the alkaline side of their isoelectric point [see e.g. Abramson, 1934], if the phenomenon of the induced permeability to cations has any similarity with the changes described by Michaelis & Rona and by Danielli, then it would be expected that it would be inhibited by alkaline and accelerated by acid buffers, yet we have seen that the reverse is true; acid phosphate reduces the rate of loss of K far more than an equivalent quantity of a neutral salt such as NaCl, whilst alkaline phosphate or bicarbonate does not reduce the rate of loss as much as NaCl does. The change in the surface of the erythrocyte is, then, not one which could be simply ascribed to a change in the ionization of its acid groups, and it is interesting to note, if the correlation between the agglutination of the cells and their induced permeability to K means anything, that Abramson [1934] has shown that the charge on the surface of the erythrocyte, measured electrocataphoretically, is not the determining factor in rouleau formation and therefore, presumably, in agglutination. Further investigation of this point must be on a system which allows changes in the salt content of the medium being made independently of changes in the pH . With the intact erythrocyte this is impossible owing to the exchanges of anions. Such a system might be given by a spread film of the extracted lipoids from the erythrocyte [Gorter & Grendel, 1925] and work on this is under way [Danielli & Davson]; however, it may well be that the permeability to cations is a result of an asymmetry of the membrane, since the concentrations of salts inside and outside are, under the conditions of suspension in a non-electrolyte medium, widely different, so that studies on films may not throw light on the problem.

To return to the actual experimental data, there is a final point which requires attention, namely the falling off in the rate of loss of K with time, which occurs at quite an early stage in the process. The equation derived to suit the conditions of the experiments obviously does not fit the observed data, and it seems, in view of the large deviation from linearity, that the discrepancy is not

due to any of the approximations introduced in the derivation, but rather to the "permeability constant" being a function of the loss of K at any given moment. The alkalinity of the suspension medium caused by the migration of K is not the cause of this auto-inhibition since alkalinity favours the loss of K; similarly the increased salt content of the medium can have little influence owing to the large volume of the latter. A possible factor is the P.D. which will be built up by the migrating HCO_3^- ; as the loss of K proceeds, the inside of the cells will become acid whilst the suspension medium becomes alkaline, and this difference in reaction will be reflected in a difference in potential, and this latter may eventually become great enough to inhibit the further loss of K. Support is given to this view by the curves of Fig. 5, in which the losses of K from cells, out of which most of the HCO_3^- has been washed, are compared with those from cells with their normal contents. It was seen that the washed cells showed only a very little inhibition of the loss of K after 6 hr. of suspension whilst the unwashed cells showed marked inhibition after 2 hr. suspension. This difference in behaviour may be attributed to the difference in the HCO_3^- content of the cells; the passage of a given quantity of K will be followed by a greater pH difference when this K is accompanied by HCO_3^- than when it is accompanied by Cl^- . It was noted that the pH values for the suspension media agreed with this view. Thus although the P.D. due to an excess of OH^- in the cells over that in the suspension medium is not the chief factor in causing the migration of K, it may be of importance in determining its cessation. The slower the initial rate of loss, i.e. the greater the obstacle offered by the membrane to the passage of K, the smaller will be the P.D. required to inhibit the loss; hence the loss of K from the rat cells will be expected to cease at a smaller external pH than that from human cells, and this is so.

Two practical points following from this work may be touched upon. Many studies of the permeability of erythrocytes to non-electrolytes are made by adding a small quantity of blood to a large volume of the pure non-electrolyte solution, and using the time required for haemolysis as an index to the rate of penetration of the non-electrolyte [*vide e.g. Jacobs et al. 1935*]. The customary dilution of the blood in the non-electrolyte medium is 1 in 500, and it is quite clear from the present work that even at a much lower dilution (1 in 10) the erythrocytes of many species are in a definitely abnormal state, so that the results of this type of work, interesting and important as they are, may nevertheless not represent the behaviour of cells in their normal environment. Jacobs *et al.* [1937] have indeed studied the influence of salts on the permeability of ox erythrocytes to glycerol, and have found changes which, however, can be at least partly, if not wholly, ascribed to changes in the volume of the cells due to anionic exchanges. It would be interesting if this work were extended to the erythrocytes of species which show cation permeability in a non-electrolyte medium more markedly, e.g. human and guinea-pig cells, since the demonstration of a normal behaviour in respect to the permeability to non-electrolytes and an abnormal behaviour regarding cation permeability might lead to important conclusions relating to the structure of the erythrocyte membrane.

A more disturbing point is the effect of the escape of K on the rate of haemolysis when this is being used as an index to the rate of penetration of a non-electrolyte. From the curve in Fig. 3 it follows that with a dilution of blood of 1 : 500 the rate of loss of K from the erythrocytes of some species may be many times greater than that described here; as a result of this loss of K the amount of non-electrolyte which must penetrate a cell to cause it to burst will be greater, so that the time required for haemolysis will be no true index to the rate

of penetration of the non-electrolyte. This will be especially true in respect to the determination of temperature coefficients, since the effect of a rise in temperature will be two-fold: (a) an increase in the rate of penetration of the non-electrolyte, and (b) an increased escape of K. These effects will be antagonistic, and it is interesting to note that Jacobs *et al.* [1935] find with certain species an actual decrease in the rate of haemolysis in glycerol solutions on raising the temperature, and this may be due to the fact that the influence of (b) outweighs that of (a). Probably this consideration does not affect the main conclusions drawn by Jacobs *et al.* from their measurements of temperature coefficients, but it would be a serious factor in any calculations of the kinetics of penetration in which values of the Q_{10} determined under these conditions are included.

SUMMARY

The permeability of the erythrocytes of several species to cations, on suspending them in a non-electrolyte medium, has been investigated. The cells of the man, guinea-pig and rat are permeable to K, and those of the ox and cat to both Na and K, under these conditions; the latter species show irregularities in that there may be an acceleration of the rate of loss of cations with time, and in that Na may sometimes penetrate more rapidly than K. The cells of the man, guinea-pig and rat give smooth curves on plotting their K contents against time of suspension; however, there is a falling off with time in the rate of loss of K, so that a stationary state is reached at times remote from the equilibrium position. This auto-inhibition is ascribed tentatively to the building up of a potential gradient which eventually inhibits the further migration of K. The erythrocytes of the rabbit and pig show little tendency to lose K under the same conditions, the loss after 7 hr. of suspension may be as low as 4 % compared with a loss of nearly 70 % with human cells.

The change in the membrane causing the permeability is a reversible one in that the escape of K may be inhibited after it has begun by the addition of NaCl to the non-electrolyte medium.

Human cells are very sensitive to salt concentration of the suspension medium when this is less than 0.02 *M*; at a concentration of 0.09 *M* the non-electrolyte effect ceases.

Alkaline buffers added to the suspension medium reduce the rate of loss of K to a smaller extent than an equivalent quantity of NaCl; acid buffers have the reverse effect.

There is an apparent correlation between the tendency of the erythrocytes of different species to agglutinate in a non-electrolyte medium and the induced permeability to K; the agglutination itself is not the cause of the permeability to K, but may be symptomatic of a change in the surface of the cell which allows cations to cross the membrane.

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