110. THE INFLUENCE OF THE LYOTROPIC SERIES OF ANIONS ON CATION PERMEABILITY

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THE literature contains the results of a great deal of work on the influence of the lyotropic series of ions on physiological phenomena. The idea in these studies was that cells, including their membranes, constituted colloidal systems, and since the most characteristic feature of colloids is the influence of hydration on their physical state, it was argued that if certain physiological phenomena showed influences by ions which normally affected the degree of hydration of colloidal systems, then the systems determining the physiological phenomena were in themselves colloidal. Thus Höber [1909] has studied the influence of anions on the ciliary movement in the epithelium of the frog's stomach, and has found that if the anions are arranged in the order of their activities a lyotropic series is obtained. Similarly, e.g. Port [1910] has studied the effect of anions on saponin haemolysis, Raber [1920] changes in the conductivity of *Laminaria* to alternating currents, Gellhorn [1922] changes in the motility of spermatozoa and Loeb & Cattell [1915] the poisoning of *Fundulus* embryos by K and the facilitation of recovery. It is on the basis of work of this nature that Höber [1922] has developed the so-called "Colloid-chemical Viewpoint" in regard to physiological phenomena; according to this viewpoint, the membrane of the cell is a colloidal system and its permeability is determined by its degree of hydration. Dehydrating substances, such as Na_2SO_4 , were supposed to exert a thickening or packing effect on the micelles constituting the membrane, thereby decreasing permeability; hydrating substances, such as NaCNS , were supposed on the other hand to loosen the membrane and so increase permeability.

A careful investigation of the claim that SO_4 decreases permeability and that CNS increases it reveals that this is not a general rule; thus if attention is confined only to the work quoted by Höber [1922] it is found that Raber [1920] reports the reverse relationship and Loeb & Cattell [1915] show that whereas dehydrating substances such as SO_4 and tartrate apparently inhibit the penetration of K into *Fundulus* embryos, the same substances apparently accelerate the escape of this ion from the embryos. Again the evidence put forward by Port [1910] on the effect of ions on saponin haemolysis has not been confirmed by the more exact measurements of Ponder [1934], and Höber [1908] in his study of hypotonic haemolysis ignored the shrinking of the erythrocyte caused by the presence of a divalent anion, such as SO_4 , through ionic exchanges.

In view of these considerations it appears that the experimental basis for the colloid-chemical viewpoint is not strong, and it is therefore of interest to study the influence of the lyotropic series of anions on a comparatively simple system. Such a system is provided by the erythrocytes of the cat which show a well-defined and accurately measurable permeability to the cations Na and K [Davson, 1940].

The cat erythrocyte, unlike those of most other species, contains mainly Na as cation, so that if it is suspended in isotonic KCl solution there will be a gradient of K in the direction: suspension medium $\rightarrow$ cells, and a gradient of Na in the opposite direction. Hence if cat cells are suspended in isotonic solutions of KCNS, KI, KBr, KCl etc., and the rates at which K enters and Na leaves the cells are measured, influences of the anions on cation permeability may be determined. Before passing to the experimental results, it may be pointed out that a series of anions may exert influences on cation permeability merely in virtue of the different diffusion potentials created, provided that the anions themselves are permeable. Thus, provided that the chances of Na-K exchanges across the membrane are negligibly small in comparison with the chances of the migration of a pair of oppositely charged ions, the rate at which a K salt of a univalent acid will enter the cell will be given by Nernst's equation:

$$D = \frac{2UV}{U+V} RT,$$

where U and V refer to the mobilities of the cation and anion through the membrane respectively. If V varies with the different anions, it is clear that the observed rate of penetration of K when cells are suspended in solutions of the K salts of different acids will vary in accordance with the mobility of the anion of the salt. Hence an influence of a series of anions on cation permeability is not necessarily to be attributed to an influence on the structure of the membrane, i.e. by hydrating or dehydrating it, and it may be that this purely electrical effect was a factor in the many studies of the influence of the lyotropic series on physiological activity, since the activity of tissues and cells is bound up in some way, not clearly understood, with the migration of ions across membranes.

The assumption that Na-K exchanges will be infrequent in comparison with the migration of pairs of oppositely charged ions is justified in the case of the cat erythrocyte, since the permeability of the membrane to Na and K is very small in comparison with its permeability to anions; also earlier results indicate that the penetration of K is virtually independent of the speed of penetration of Na and *vice versa*.

EXPERIMENTAL

Blood was taken from the cat under ether anaesthesia. Since storage of the blood overnight has been found to affect Na permeability, leaving K permeability unchanged, the blood was used on the day of withdrawal for experiments on Na permeability; in general experiments on K permeability were carried out a day later. The experimental technique for studying cation permeability was essentially the same as that described earlier [Davson, 1934; 1937]; in experiments involving K permeability the cells after suspension in a solution of a K salt were washed once with isotonic NaCl solution to remove interstitial K. This procedure was necessary and was shown not to produce any significant errors. Since 1.4 ml. of whole blood were added to 10 ml. of the solution of K salt, the cell suspension was in general adequately buffered, except in the case of the K salts of slowly penetrating acids, e.g. SO_4 , where, owing to the exchange of anions from the interior of the cell with OH ions from the outside, the reaction shifted from pH 7.4 to pH 7.0; such a shift is sufficient to produce a serious change in Na permeability only, and this point has been discussed in the section on results. The solutions of the K salts of the fatty acids were prepared by titration of 0.165 *M* KOH with the purest specimens of the acids obtainable. All experiments were carried out in a bath at 25°.

RESULTS

Potassium permeability. If the quantity of K penetrating the erythrocytes in 1 hr., when suspended in an isotonic solution of the particular K salt, is used as an index to the rate of penetration, a typical experiment gives the following series of permeabilities of the cell membrane to K, the value for KCl being put equal to 100:

CNS	I	NO_3	Br	Cl	Acetate	Oxalate	Sulphate	Citrate	Tartrate
178	143	133	112	100	85	84	56	45	29

i.e. the series is that known as the lyotropic series. However, it would appear from the work of Ege [1922] and of Maizels [1934] that the anions penetrate the erythrocyte in the same order as a lyotropic series, so that the effects observed here may be due to the electrical diffusion effects discussed earlier.

Fig. 1.

Fig. 2.

Fig. 1. Increase in the K content of cat erythrocytes suspended in isotonic solutions of the K salts of various acids. Ordinates: percentage of the original content. Abscissae: time in hours.

Fig. 2. Increase in the K content of erythrocytes suspended in solutions of the K salts of various acids. Ordinates: percentage of the original content. Abscissae: time in hours.

In Fig. 1 curves for the penetration of K from the K salts of a number of univalent acids are given; the curves in unbroken lines refer to experiments carried out simultaneously on the same sample of blood, and are therefore strictly comparable among themselves; those in broken lines refer to experiments on different samples of blood, and in plotting the results on the same scale as the others the curve for the penetration of KCl in each instance has been used as the standard of reference. The curves for Cl, NO_3 , Br and acetate lie very

close together, whilst CNS, IO_3 and CN show very decided accelerations of K permeability in comparison with Cl. In Fig. 2 curves for multivalent anions are shown and it is evident that these anions retard K penetration in comparison with Cl. In these experiments the solutions were 0.165 *M* with respect to K and tonicity was maintained equivalent to that of a 0.165 *M* K salt of a univalent anion by addition of an appropriate amount of the Na salt of the anion investigated.

In an attempt to determine whether the effects of the anions observed here were due to alterations in the membrane or to the diffusion effects discussed earlier, the proportions of isotonic KCl and K_2 tartrate solutions in the suspension medium were varied, and the rates at which K entered the cells determined; similar experiments were carried out with Cl- SO_4 , Cl- IO_3 and Cl-CNS mixtures. If the effect of the anion, other than Cl, is one on the membrane, a linear relationship between the concentration of this anion and permeability would not necessarily be expected, whereas if the effect were due to diffusion potentials alone, a linear variation would be expected. The curves (Fig. 3) for

Fig. 3. Variation in the rate of penetration of K into the erythrocyte in mixtures of isotonic solutions of KCl and the K salts of different acids whose anions are referred to as X. The curves are not comparable one with another so far as the rates of penetration are concerned. Ordinates: rate of penetration of K in arbitrary units. Abscissae: proportions of KCl and KX solutions in the mixtures.

Cl- SO_4 , Cl-tartrate and Cl-CNS mixtures do show a fair approximation to linearity; the Cl- IO_3 curve, on the other hand, obviously does not, indicating that the action of the IO_3 is definitely on the membrane itself. This inference is borne out by the observation that cells suspended in isotonic NaIO_3 for a few hours are permanently damaged, in that subsequent washing of cells so treated with isotonic NaCl causes haemolysis. The almost linear relation between the proportions of the two anions in the suspension medium and the permeability to K does not, of course, preclude an explanation of the effects of the anions on the basis of a direct action on the membrane.

Sodium permeability. Curves for the escape of Na from the same sample of cat erythrocytes in isotonic solutions of K salts of different univalent acids are shown in Fig. 4. Here a striking difference between K and Na permeabilities is observed, anions at the hydrating end of the lyotropic series, e.g. CNS and I, causing an almost complete inhibition of Na permeability; acetate which retarded K permeability in comparison with Cl causes an acceleration of Na permeability. The behaviour of IO_3 is irregular and conforms with the results on K permeability; NO_3 and Br, which accelerated K permeability as compared with Cl, retard Na permeability.

Fig. 4.

Fig. 5.

Fig. 4. Decrease in the Na content of the cat erythrocyte when suspended in isotonic solutions of the K salts of various acids. Ordinates: percentage of the original content. Abscissae: time in hours.

Fig. 5. Decrease in the Na content of the cat erythrocyte when suspended in isotonic solutions of the K salts of various acids. Ordinates: percentage of the original content. Abscissae: time in hours.

In Fig. 5 representative curves for the escape of Na in solutions of isotonic KSO_4 , tartrate and citrate are shown compared with the escape in isotonic KCl solution. It is seen that the rate of escape in Cl solution is greatest, whilst the behaviour of tartrate is irregular in that a rapid acceleration of the rate of escape of Na occurs after the second hour. The SO_4 , tartrate and citrate anions penetrate the erythrocyte membrane very slowly in comparison with the univalent anions (it is possible that citrate does not penetrate at all), so that a suspension of cells in the isotonic K salts of these acids is found to be acid, due probably to the exchange of OH ions in the medium with Cl and HCO_3 ions in the cells; as SO_4 , tartrate etc. diffuse into the cell the reaction of the suspension medium becomes less acid. Since it has been shown earlier [Davson, 1940] that the permeability of the cat erythrocyte to Na is very sensitive to the pH of the medium, it was thought that the acidity caused by these ionic exchanges might retard Na permeability, so that it might quite possibly be that SO_4 , for example, would actually accelerate the rate of escape of Na as compared with Cl. To test

this, cells were suspended in isotonic Na_2SO_4 solution for 3 hr., by which time the complete exchange of anions had taken place, and the reaction of the supernatant fluid was pH 7.4. The cells were then centrifuged down and re-suspended in isotonic K_2SO_4 solution, and the rate of loss of Na was compared with that from cells similarly treated with first Na and then KCl. The results are shown in Fig. 6, and it is clear that in these circumstances SO_4 has a pronounced accelerating action. This effect may, of course, be due to the necessity for SO_4 to act on the inside of the cell, quite apart from the pH changes; whatever may be the cause, it is clear that if SO_4 and Cl are allowed to act under comparable conditions, the rate of escape of sodium is greater in the Na solution. It seems quite possible that the acceleration of Na escape with time in tartrate solution is due to the slow diffusibility of tartrate ions into the cell; the regular behaviour of citrate, on the other hand, may be due to the complete indiffusibility of this ion through the erythrocyte membrane.

Fig. 6.

Fig. 7.

Fig. 6. Decrease in the Na content of the cat erythrocyte when suspended in isotonic KCl and K_2SO_4 solutions after a preliminary treatment with NaCl and Na_2SO_4 solutions. Ordinates: percentage of the original content. Abscissae: time in hours.

Fig. 7. Decrease in the Na content of the cat erythrocyte when suspended in isotonic solutions of the K salts of straight-chain fatty acids. Ordinates: percentage of the original content. Abscissae: time in hours.

The effect of the series of straight-chain fatty acids is shown in Fig. 7; increasing the length of the hydrocarbon chain causes a small decrease in permeability until we pass from valeric to hexoic acids, in which case complete inhibition of Na permeability is observed.¹ Höber [1936], in attempting to explain

¹ An isotonic solution of the K salt of octoic acid, was found to be haemolytic; however, it was found that a mixture of isotonic KCl and K octoate in equal proportions was not haemolytic and inhibited Na permeability.

the haemolytic effect of Na salts of the higher fatty acids, suggests that the haemolysis is due to hypotonicity owing to the formation of micelles; on this basis it may be argued that the inhibition of Na permeability by hexoate is due to hypotonicity, which also inhibits Na permeability. That this explanation is untrue is shown by measuring the permeability in 0.20 *M* K hexoate, in which case inhibition is also found; furthermore the 0.165 *M* solution may be diluted to at least 0.13 *M* without producing any haemolysis.

The effect of varying the proportions of CNS and Cl and of I and Cl in the suspension medium on Na permeability is shown in Fig. 8, and it is clear that

Fig. 8. Variation in the rate of penetration of Na from the erythrocyte in mixtures of isotonic solutions of KCl and KI and of KCl and KCNS. Ordinates: rate of escape of Na as percentage of the original content per hour. Abscissae: proportions of KCl and KX in the mixtures.

the amount of CNS which must be present to produce complete inhibition is of the order of 0.045 *M*. The curves do not represent experiments on the same blood but comparative experiments under these conditions indicate that CNS is much more effective than I in inhibiting Na permeability. For convenience the effects of mixtures ranging from pure KCl to six parts of the latter to four of KCNS and KI only are shown; if the curves were extended to show the effects of richer mixtures the complete absence of any semblance to linearity in the relation between permeability and proportions of Cl and CNS and of Cl and I would be more evident.

DISCUSSION

The results described in this paper indicate a dual behaviour of anions according as K or Na permeability is being studied. The hydrating anions, e.g. CNS and I, cause an acceleration of K permeability and a retardation of Na permeability, and the hydrating anions, e.g. SO_4 , have the reverse effects in the two cases. This duality has already been observed in regard to the effects of alkaline earths, tonicity, ageing and narcotics [Davson, 1940], and it would seem that there is some fundamental difference between the two modes of penetration.

It has been suggested earlier that the influence of anions on permeability may at least in part be due to diffusion potentials; qualitatively the experiments on K permeability would appear to bear out this viewpoint. It must be realized, of course, that the equation of Nernst involves mobilities of the ions, and the assumption made in this discussion is that the mobilities are proportional to the penetrations of the ions through the membrane: if the penetration of K occurs through a pore in the membrane and if the number of pores available for K diffusion is very small in comparison with those available for anion penetration then it would be quite possible that the actual mobility of the K ion through the membrane is of the same order of magnitude as that of anions, in fact it may even be greater than that of SO_4 , for instance, whilst the relatively low penetration would be due to the limited number of pores available for K diffusion. If this were the case it seems possible that the simple equation for the diffusion of electrolytes in aqueous solution is not applicable without modification to the more complicated problem of permeability.

So far as Na permeability is concerned, the diffusion potential factor, if it is present, is obviously obscured by some other effect; the extraordinary inhibiting effects of CNS, I and hexoate are strongly reminiscent of the action of narcotic substances described earlier. With these substances it was found that Na permeability may be completely inhibited whilst under the same conditions K permeability was only accelerated. It has also been found (unpublished) that surface-active substances such as Na oleate will in very low concentrations (1 part in 10,000 in this case) also inhibit Na permeability, and it is interesting that CNS and I ions exert their lyotropic effects in virtue of their adsorbabilities at a protein-water interface. Again, the sudden transition in behaviour in passing from valeric to hexoic acids is suggestive that the anion is exerting its influence in virtue of its adsorbability at the membrane-aqueous interface, in fact the work of Schulman & Rideal [1937] on the penetration of mixed lipid-protein films would suggest that at a certain stage in a homologous series of fatty acids, the acid will actually penetrate the film, as opposed to a head-to-head adsorption with the hydrocarbon end of the acid remaining in the water phase. It is interesting, in this connexion, that Schulman & Rideal have shown that the strongly surface-active substance, saponin, will not penetrate a lipid-protein film, and unpublished work of Davson has shown that saponin has no influence on Na or K permeability, nor yet does it inhibit the penetration of glycerol into the rabbit erythrocyte, although this permeability process is affected by narcotic substances [Anselmino & Hoenig, 1930; Jacobs & Parpart, 1937]. Na permeability may thus be inhibited by narcotic substances such as amyl alcohol, ether, urethanes etc., by soaps and by adsorbable anions such as CNS and I. If there is a factor in common with all of these substances it is apparently not their ability to hydrate colloids, since ether is just as effective as CNS. The full explanation of the phenomena must obviously await further quantitative experimental work on the influence of narcotic substances and of other anions. The results of this paper show beyond doubt that the behaviour of the erythrocyte membrane, so far as ionic permeability is concerned, is not related in any simple manner, if at all, to hydration phenomena.

SUMMARY

The influence of the lyotropic series of anions on Na and K permeability in the cat erythrocyte has been studied. Anions at the dehydrating end of the series, e.g. SO_4 , retard K permeability and apparently accelerate Na permeability.

Anions at the hydrating end of the series, e.g. CNS, have the reverse effects in the two instances. If the permeability to Na is measured in isotonic solutions of the K salts of the homologous series of straight-chain fatty acids, a small decrease in permeability occurs with each step from acetate to valerate; on passing from valerate to hexoate permeability is completely inhibited. It is argued that the results show that the effects of anions on permeability phenomena are not predictable from a knowledge of their behaviour in respect to the hydration of colloidal systems.

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