CXXXIV. STUDIES ON THE PERMEABILITY OF ERYTHROCYTES

V. FACTORS IN CATION PERMEABILITY

BY HUGH DAVSON¹ AND JAMES FREDERIC DANIELLI

From the Departments of Physiology and Biophysics, University College, London, and the Biological Laboratory, Plymouth

(Received 25 April 1938)

EARLIER papers in this series [Davson, 1934; 1936; Davson & Danielli, 1936] have been devoted to testing certain published claims regarding the permeability of the erythrocyte to cations [Kerr, 1929; Mond, 1927; Ponder, 1934], some of which have been confirmed and others not. The present work is an attempt to define the conditions under which the erythrocyte may become permeable to K^+ ; so far no systematic investigation of this nature has been made, many observations hitherto described being of a casual or accidental nature, e.g. Ørskov [1935] has shown that the lead dissolved by acid salines from the piston of his syringe was sufficient to cause a rapid loss of K^+ ; Maizels [1935] has shown that human erythrocytes lose K^+ in glucose solutions; Kerr [1929] and Davson [1934] have shown that variations in the Na^+ and K^+ content of the erythrocytes of certain species may occur by varying the nature of the suspension medium appropriately. Of other investigations in the literature those of Yannet *et al.* [1935], Henriques & Ørskov [1936], Eisenman *et al.* [1937] and McCance [1937] are predominantly *in vivo* studies and consequently the range of variation of the nature of the suspension medium is limited to small changes.

The problem has been attacked along three general lines which may be summarized by the questions: (a) Is the high concentration gradient of K^+ maintained by most erythrocytes against their surroundings due to an active secretory process? (b) To what extent may the losses and gains of K^+ described in earlier work be due to mechanical damage caused by centrifuging the cells? (c) Is haemolysis, however produced, preceded by a preliminary stage of permeability to K^+ ?

Before passing to the experimental part of the paper it should be said that recent work by Davson [1937] indicates that the permeability of the erythrocyte to K^+ is to a certain degree a species characteristic, so that for a complete analysis of the conditions under which the erythrocyte becomes permeable to K^+ a large number of species should be studied. In view, however, of the wide scope of this paper such was not practicable, so that most of the detailed studies have been limited to the case of the rabbit erythrocyte except where special circumstances demanded the use of other species.

EXPERIMENTAL

The experimental method was approximately the same in all cases as that of Davson [1934]. A known quantity of blood was measured into a centrifuge tube and the solution with which it was to be washed was added. At the end of the treatment the suspension was centrifuged and the supernatant fluid discarded.

¹ Beit Memorial Research Fellow.

The cells were haemolysed by the addition of distilled water and the proteins precipitated with trichloroacetic acid. The centrifugate was evaporated and ashed and made up to 10 ml. Duplicate determinations of K^+ were made on this solution by the method of Kramer [1920]. The total errors in the procedure rarely aggregated to more than 1%. It is as well to emphasize that the results are independent of any volume changes which may occur in the erythrocytes, and the method consequently has great advantages over all the others described, and at present being used, by different workers.

RESULTS

(a) *Is there an active secretory process?* The very low oxygen consumption of the non-nucleated erythrocytes of, for instance, the rabbit indicates the absence of an active metabolic process maintaining the concentration gradient of K^+ between the cell and its surroundings. Nevertheless Halpern [1936] claims that the migration of inorganic phosphorus across the erythrocyte membrane is determined by the cell glycolysis; further, the observation of Ørskov mentioned earlier might be interpreted as the poisoning of a catalytic system. The effects of a number of catalytic poisons and accelerators on the K^+ content of the erythrocytes of the rabbit, goose and dogfish have been tried and the results are shown in Table I. The cells of the goose and dogfish were chosen as they are

Table I. *The effect of narcotic concentrations of certain catalytic poisons and accelerators on the K^+ content of erythrocytes expressed as a percentage of the control value. Temperature, unless otherwise stated, 25°. 1 ml. of blood to 10 ml. of suspension medium*

Species	Narcotic	Concentration	Time hr.	K content
Rabbit	NaF	0.05 M	1	100
	NaF	0.02 M	1	99
	NaF	0.01 and 0.005 M	1	100
	NaCN	0.01, 0.005 and 0.001 M	1	100
Goose	NaF	0.05 M	1	95.5
	NaF	0.02 M	1	95
	NaF	0.01 M	1	96
	NaF	0.005 M	1	98
	NaCN	0.01, 0.005 and 0.001 M	1	100
	Ph-urethane	0.003 M	20 (5°)	100
	CO	Saturated	20 (5°)	100
Dogfish	NaF	0.02 M	20 (5°)	100
	NaCN	0.01 M	10	101
	NaF	0.003 M	10	98
	Urethane	0.001 M	10	97
	Methylene blue	0.02 %	10	101
	Pyocyanine	0.02 %	10	101

nucleated and consequently might be expected to show a more active secretory process. From the table it is seen that of all the poisons studied only fluoride, in the cases of the goose and dogfish, has any significant effect on the K^+ content (the effect of urethane is not a narcotic effect but belongs to the phenomena described in the third section). That the effect of fluoride is only remotely, if at all, connected with the inhibition of glycolysis is shown by the extremely small loss of K^+ and the fact that the effect increases with increasing concentration of fluoride although all the concentrations studied were adequate to inhibit glycolysis completely. This effect of fluoride, which is very marked at higher temperatures, has been described by Wilbrandt [1937], who informs us that he is

investigating this point and that he is satisfied that it is not due to glycolysis *per se*.

Pyocyanine and methylene blue, in virtue of their effects in increasing metabolism, might be expected to increase the K^+ content of the cells suspended in Ringer solutions; actually they had no significant effect on the K^+ content of dogfish erythrocytes. Provisionally, then, it may be said that in all probability the high concentration of K^+ in the erythrocytes of most species is not maintained by an active metabolic process.

(b) *Can centrifuging cause losses of K^+ ?* Earlier studies of various workers have shown that the erythrocytes of several species lose K^+ when suspended in isotonic salt solutions; in the case of the ox the loss varies between 5 and 15%, appears to be almost instantaneous, and occurs to a lesser extent in serum [Davson, 1934]. In view of the discontinuous nature of this loss it occurred to us that it may be due to the centrifuging of the cell suspension. The erythrocytes of the dogfish, as opposed to those of the ox, have a very high sedimentation rate even in isotonic saline solutions, so that it was found to be quite practicable to wash them with saline or their own serum without centrifuging. In Table II, Exp. 1 it

Table II

Exp. no.	Species	Treatment	K^+ content
1	Dogfish	Cells allowed to settle once from serum	100
		Cells allowed to settle twice from serum	100
		Cells allowed to settle once from Ringer	100
		Cells allowed to settle thrice from Ringer	100
		Cells centrifuged once from serum	93
		Cells centrifuged thrice from serum	91
		Cells centrifuged once from Ringer	96
		Cells centrifuged thrice from Ringer	88
2	Ox	Cells centrifuged immediately	100
		Cells centrifuged after 3.5 hr.	100
		Cells centrifuged after 19 hr.	98
		Cells centrifuged after 24 hr.	96
		Cells centrifuged after 48 hr.	96
		Cells centrifuged after 68 hr.	95
		Cells allowed to settle from saline	95.5
		Cells centrifuged from saline	88.5
3 (a)	Ox	Cells centrifuged from serum once	100
		Cells centrifuged from serum twice	94
3 (b)	Ox	Cells centrifuged from serum once	100
		Cells centrifuged from serum twice	97
3 (c)	Ox	Cells centrifuged from serum once	100
		Cells centrifuged from serum twice	99
4	Horse	Cells allowed to settle from serum	100
		Cells centrifuged from serum once	100
		Cells centrifuged from serum twice	100
		Cells centrifuged from serum thrice	100
5	Ox	Cells centrifuged from serum	100
		Cells washed once in isotonic NaCl	79
		Cells washed twice in isotonic NaCl	72
		Cells washed three, four and five times	72
6	Rabbit	Cells allowed to settle from serum	100
		Cells centrifuged from serum	100
		Cells washed once in isotonic NaCl	98.5
		Cells washed twice in isotonic NaCl	95.5
		Cells washed thrice in isotonic NaCl	95.5

is seen that washing dogfish erythrocytes three times, either in their own serum or in Ringer solution, causes no loss of K^+ if the cells are allowed to

settle by the force of gravity alone between washings; on the other hand, considerable losses are sustained when the cell suspensions are centrifuged.

Thus in the case of the dogfish, the act of centrifuging the cells alone may cause an appreciable loss of K^+ . That there should be such a loss is not altogether surprising, for in the centrifuge the lipid constituents of the plasma membrane will have a centripetal force exerted on them as opposed to a centrifugal force on the erythrocyte as a whole, so that considerable internal stresses will be introduced. This type of leakage by mechanical effects is not of any great theoretical interest, but its practical applications are more noteworthy. Thus in the past, if it was desired to investigate the effect of, for example, time, or a salt solution, on the erythrocyte K^+ content, one lot of cells would be centrifuged from serum and ashed immediately for a control, whereas the test lot would be centrifuged from serum with the control lot, resuspended in the test medium and centrifuged a *second time* after the elapse of the desired interval. Obviously, now, the control is not a control, since it has been centrifuged once whereas the test sample has been centrifuged twice. With this in mind the problem of the loss of K^+ from the ox cells has been reinvestigated. In Exp. 2 of Table II cells of the ox were allowed to stand in their own serum for varying periods in the ice chest and each sample was centrifuged once only. It is evident that no loss occurs during the first 3–4 hr. and that even after 68 hr. the loss is only 5%. In the same experiment cells were allowed to stand 68 hr. in isotonic NaCl, at the end of which time they had sedimented well enough to remove most of the saline; the K^+ content of these cells is compared with that of another lot of cells allowed to stand for an equal time in saline but centrifuged finally. That there is a true escape of K^+ during the 68 hr. (4.5%) is evident from the table, but on the other hand the loss obtained when the cells were centrifuged (11.5%) is very much greater. In Exp. 3 (*a, b, c*) the K^+ contents of cells centrifuged once and twice from serum are compared, and here again the same point is brought out, although not so definitely, namely that centrifuging the cells from serum is sufficient to cause a significant loss of K^+ . In Exp. 4 the absence of a similar effect with horse erythrocytes, which sediment rapidly in their own serum, is shown. The effect of repeated washing of the erythrocytes of the ox and rabbit is shown in Exps. 5 and 6 respectively.

(*c*) *Is haemolysis preceded by a state of cation permeability?* Since this work was begun in 1935, Ørskov has shown that traces of heavy metals may cause a rapid loss of K^+ from the erythrocytes of certain species. As heavy metals are strong lysins this fact would appear to substantiate the above hypothesis at least in respect of heavy metal haemolysis; in the present section the effect of a number of different agents, all of which cause haemolysis, on the K^+ content of the rabbit erythrocyte is studied. The method adopted is best explained by describing a particular experiment, e.g. that using amyl alcohol. A series of concentrations of this alcohol in isotonic NaCl buffered with phosphate to about pH 7.4 were made up in centrifuge tubes and equilibrated in a thermostat at 25°. 1 ml. lots of blood were measured into each tube and after 1 hr. the suspensions were centrifuged and the degree of haemolysis was measured by comparing the colours in the supernatant fluids with those in tubes containing varying concentrations of haemoglobin prepared by diluting a laked specimen of the blood with water. Samples showing a degree of haemolysis above 10% were usually rejected, as the error in the determination of the degree of haemolysis in these cases produced too great an effect on the apparent change in K^+ content. Besides the haemolytic concentrations of these substances, just sublytic concentrations were used. If we suppose that 5% haemolysis has occurred in one

sample, then the change in the K^+ content of this sample will be 5% of that of the control if the agent has not caused some or all of the remaining 95% of the cells to lose K^+ ; if on the other hand the change in the K^+ content is greater than 5% it is concluded that the agent has caused a leakage of K^+ . In Table III

Table III. *The effect of lysins on the K^+ content of rabbit erythrocytes expressed as a percentage of the control values. Corrections have been made for haemolysis so that any value less than 100 indicates an escape of K^+ . Time 1 hr. Temperature 25°. (The concentrations of digitonin are expressed as fractions of a saturated solution; e.g. " $X/10$ " = $\frac{1}{10}$ saturated solution)*

Lysin	Concentration	% haemolysis	Cell K^+ content
Saponin	1 in 10,000	7	100
	0.9 in 10,000	4.5	100
	0.8 in 10,000	2	100
	0.7, 0.6, 0.5 in 10,000	None	100
Digitonin	4 $X/5$	7.5	97
	7 $X/10$	5	96
	3 $X/5$	3	99
	2 $X/5$	1	101
	$X/5$ and $X/10$	None	100
Na oleate	2.0 in 10,000	10.5	93.5
	1.8 in 10,000	8	95
	1.6 in 10,000	5	97
	1.4 in 10,000	3	97
	1.0 in 10,000	2	100
	0.5 in 10,000	1	100
Na valerate	0.2, 0.3, 0.4, 0.5 M	None	100
Na cholate	4.0 in 1000	10	96.5
	1.6 in 1000	5	100
	1.0, 0.9, 0.8, 0.7 and 0.6 in 1000	None	100
Quinol	0.075 M	c. 5	27.7
	0.050 M	c. 5	41
	0.025 and 0.01 M	None	100
Resorcinol	0.163 M	None	16.9
	0.145 M	None	33.8
	0.131 M	None	62.0
	0.091 and 0.036 M	None	100
Catechol	0.136 M	c. 5	9.1
	0.091 M	c. 5	51.5
n -AmOH	0.18 M	None	87
	0.09 M	None	97
	0.07 M	None	100
Guaiacol	0.04 M	None	84
	0.03 M	None	92
	0.02 M	None	96.5
	0.01 M	None	99
Guaiacol (dogfish)	0.01 M (pH 5)	None	85
	0.01 M (pH 6)	None	86
	0.01 M (pH 7)	None	83
	0.01 M (pH 8)	None	83
	0.01 M (pH 9)	None	85
	0.01 M (pH 10)	3	70

the effects of a number of substances which all, with the possible exception of Na valerate, cause haemolysis of the rabbit erythrocyte, are shown. In the final column the K^+ content of the erythrocytes has been corrected for the degree of haemolysis, so that any value less than 100 indicates a loss of K^+ over and above that due to the actual destruction of the cells. Saponin in both lytic and non-lytic concentrations causes no loss of K^+ ; digitonin, Na oleate and cholate only cause a small loss of K^+ when in lytic concentrations, and then only when

Table IV. *The effect of pH and hypotonicity on the K⁺ content of the erythrocyte expressed as a percentage of the control value. Temperature and time unless otherwise stated 25° and 1 hr. Values have been corrected for haemolysis, if any*

Species	Treatment	% haemolysis	Cell K ⁺ content
Rabbit	pH 6.5-9.0 c.	None	100
	pH 6.4	1.5	100
	pH 6.1	5	99
	pH 5.8	15	97
	pH 9.1 (c.)	5	99
	pH 9.2 (c.)	13	94
Dogfish	pH 5-9	None	100
	pH 10	3	100
Ox	0.12 M KCl 1 hr. (40°)	None	129
	0.12 M KCl 2 hr. (40°)	5	145
	0.12 M KCl 4 hr. (40°)	10	162
Rabbit	50°	None	86
	55°	20	86

the degree of haemolysis is high; of the remaining substances considerable losses may be obtained in both lytic and sublytic concentrations, with the exception of Na valerate.

In Table IV the effects of acidity and alkalinity, temperature and hypotonicity are shown. It is evident that pH changes *per se* over the whole of the non-haemolytic range are completely without influence on the K⁺ content of the rabbit and dogfish erythrocytes. This extends our proof that Mond's [1927] contention that there is a reversal of ionic permeability at alkaline pH is groundless [Davson & Danielli, 1936]. In the haemolytic range there seems to be a barely significant loss of K⁺ when the degree of haemolysis is large (5-15%), although in the case of a degree of haemolysis of 15% there is a possible error of about 3%. Temperature, it will be noted, has a considerable effect even when it is not so high as to cause haemolysis (50°). The effect of hypotonicity has been discussed in detail earlier [Davson, 1937], and the results shown in Table IV are some unpublished ones demonstrating that, besides an escape of K⁺, penetration of this ion into the cell may also occur from hypotonic KCl. In this experiment ox cells were kept at 40° in 0.12 M KCl for 1, 2 and 4 hr. After these periods they were washed in isotonic NaCl so as to remove all intercellular KCl and then the K⁺ contents were determined. As a result of the penetration of K⁺ the difference in osmotic pressure between the cells and their suspension medium was increased to such an extent that greater than 10% haemolysis occurred, whereas a hypotonicity of 0.12 M was not sufficient of itself to cause any lysis.

The effect of silver. The remainder of this section is devoted to an examination of the effect of the heavy metal silver on the permeability of the rabbit erythrocyte to K⁺. The silver ion has a strong haemolytic effect on rabbit's blood; concentrations of 1×10^{-3} to 1×10^{-8} g. per ml. of AgNO₃ were tried. After half an hour solutions containing more than 1×10^{-7} g. per ml. were found to be haemolytic at 25° so that the studies of this heavy metal have been confined to concentrations of this order. In Table V, Exp. 1, the influence of washing the cells in isotonic NaNO₃ on the subsequent effect of silver in a concentration of 1×10^{-7} g. per ml. is shown. By adding the unwashed blood directly to the solution containing silver only 7% of the K⁺ is lost in 1 hr., whereas after washing twice with isotonic NaNO₃ a loss of 66% of the K⁺ occurs. In the subsequent experiments the control value of 100 has been ascribed to the K⁺ content of cells washed twice in isotonic NaNO₃ and then suspended in a further

Table V. *The effect of silver on the erythrocytes of the rabbit. Unless otherwise stated 1 ml. of blood to 10 ml. of washing fluid; concentration of AgNO_3 1×10^{-7} g./ml.; temp. 25°*

Exp. no.	Treatment	K ⁺ content	Time min.
1	Control	100	60
	NaNO_3 - AgNO_3 added directly to blood	93	60
	" " to once washed cells	59	60
	" " to twice washed cells	35	60
	Control	100	60
2	Isotonic NaNO_3 2.5×10^{-8} g. AgNO_3 /ml.	96	60
	" 5.0×10^{-8} "	94	60
	" 7.5×10^{-8} "	83	60
	" 1.0×10^{-7} "	51	60
	" 2.5×10^{-7} "	46	15
	" 5.0×10^{-7} "	35	15
	" 7.5×10^{-7} "	27	15
	Control	100	45
3	Isotonic NaNO_3 - AgNO_3 : blood—20 : 1	56	45
	" " 10 : 1	91	45
	" " 5 : 1	100	45
	" " 2 : 1	100	45
	Control	100	60
4	Isotonic NaNO_3 - AgNO_3 unbuffered	82	60
	" NaCl - AgNO_3 unbuffered	100	60
	" NaHCO_3 - AgNO_3	91	60
	" NaNO_3 - AgNO_3 - Na_2HPO_4 pH 6.1	80	60
	" NaNO_3 - AgNO_3 - Na_2HPO_4 pH 7.0	93	60
	" NaNO_3 - AgNO_3 - Na_2HPO_4 pH 7.8	93	60
	" NaNO_3 - AgNO_3 - Na_2HPO_4 pH 8.1	79	60
	Control	100	60

lot of isotonic NaNO_3 for the same time as the test cells are suspended in their silver solutions. In Exp. 2 the effect of varying the concentration of AgNO_3 from 2.5×10^{-8} to 7.5×10^{-7} g. per ml. is shown, and in Exp. 3 the effect of varying the corpuscle:suspension medium ratio; this latter effect is very striking, since a concentration of silver which may cause 44 % of the K^+ to leak out of the cells in 45 min. when they are suspended in 20 ml. of fluid is quite ineffective in causing a leakage when the volume of the suspension medium is reduced to 5 ml. (It should be noted that with AgNO_3 in concentrations of greater than 1×10^{-7} g. per ml. a certain amount of haemolysis occurred, but in no case was the amount of haemolysis adequate to account for the K^+ loss observed.) In Exp. 4 the effect of varying the pH of the medium and also of pure isotonic NaCl and NaHCO_3 solutions is shown. In the pH studies the cells were washed once with isotonic NaNO_3 , once with the buffer mixture (consisting of four parts of isotonic NaNO_3 to one part of isotonic Na_2HPO_4 acidified with HNO_3) and then suspended in a new lot of the buffer mixture to which AgNO_3 had been added. It is clear that there is no definite pH in the range studied to the acid or alkaline side of which the effect of silver ceases, and this metal consequently differs from copper in its behaviour in this respect (Davson, unpublished).

DISCUSSION

The points brought out in section (a) need little discussion; it is quite clear that the high concentration of K^+ in the cells of the species studied is not maintained by a metabolic process which is normally poisoned by such agents as CN , CO or urethane, or accelerated by methylene blue or pyocyanine; consequently this high concentration gradient must be considered as a static phenomenon.

The effects of centrifuging the cells on their K^+ content described in section (b) may have an important bearing on the apparent effects of varying the concentrations of Na^+ and K^+ in the suspension medium on the concentrations of these ions in the cells; as an example Davson [1934] found a 30 % loss of Na^+ from the ox cells on washing once with isotonic glucose or KCl; Jacobs & Parpart [1933], using an indirect technique which did not involve centrifuging, found only very small losses of total cations so that it may very well be that the loss only occurs when the cells are centrifuged.

By far the most interesting results from the point of view of elucidating the nature of the plasma membrane surrounding the erythrocyte are those given in section (c); they also have a bearing on the mechanism of haemolysis. It has been shown that substances such as amyl alcohol, the dihydric phenols, guaiacol etc., cause large losses of K^+ in 1 hr. at 25° even in non-haemolytic concentrations; in higher concentrations all these substances are haemolytic, so that it is quite evident that so far as they are concerned a stage of cation permeability precedes the stage of haemolysis. Davson & Ponder [1938] have suggested that the essential element in the process of haemolysis consists in a free permeability of the cell to cations, and these results appear to substantiate the view. Of the remaining lysins studied Na oleate and cholate, digitonin and saponin, the first three show evidence of cation leakage only in haemolytic concentrations (to prevent confusion it should be repeated that in speaking of a cation loss under haemolytic conditions it is meant that there is a loss over and above that resulting from the haemolysis itself), whilst saponin shows no evidence of losses even under these conditions within the range in which it was practicable to study its effects. As this latter group of substances are much stronger haemolytic agents than the former group, there appears to be a contradiction; that this need not necessarily be the case is apparent if it is assumed that a substance like saponin has such a strong effect on the membrane that as soon as it is present in a sufficient concentration to exert its influence, this concentration is sufficient to cause not only a rapid permeability to cations but also haemolysis. In this event no other loss of cations than that due to the haemolysis will be observed. We have formed certain provisional ideas relating to the mechanism of the action of these lysins in causing a permeability of the erythrocyte to K^+ but it is not proposed to discuss them in detail until a projected study of the behaviour of thin lipid films, obtained by extraction of the cells with lipid solvents, has been carried out; however a bare indication of these ideas would not be out of place. There is a certain amount of evidence that the lipid constituents of the erythrocyte membrane exist in the surface of the erythrocyte as a solid film; the impermeability of this membrane to cations may therefore depend on the proper orientation of the lipid molecules, and hence a disturbance of this orientation may lead to a general alteration of the permeability characteristics of the membrane which may include a state of permeability to cations. The haemolytic agents studied above are all substances which might be expected to penetrate the lipid film and consequently tend to liquefy it, and it may be that the disorientation of the lipid molecules produced in this way is adequate to cause a permeability to K^+ . The large effects produced by the substances studied which contain a benzene nucleus, e.g. quinol, may also be correlated with their influence in inhibiting the penetration of glycerol into the erythrocyte; alcohols and other narcotics slow the rate of penetration of glycerol into the erythrocytes of many species; this effect is very much more pronounced with the dihydric phenols (Davson, unpublished); on the other hand substances such as saponin or Na cholate have a very small if any effect. Thus there seems to be

a direct relation between the influences on K^+ and glycerol permeability: whether they are causally connected remains to be seen, but it may be remarked here that if a cell is losing K^+ it will take longer to haemolyse in a solution of a penetrating non-electrolyte, so that the rate of penetration of the latter will appear by the methods usually adopted to measure it to be slower than it actually is. Before concluding the discussion of these haemolytic agents the effect of guaiacol should be considered more particularly; Osterhout [1930] has founded a theory of the mechanism of accumulation of cations in living cells on the possibility that K^+ may enter the cell combined with an organic acid in the undissociated form or as an ionic doublet; the substance used for his experimental models has been this substance guaiacol. At first sight it would appear that the behaviour of guaiacol in causing a loss of K^+ from the erythrocyte confirms in some measure Osterhout's theory. That it is wrong, nevertheless, in respect of the erythrocyte, is shown by the complete absence of any effect on the permeability to K^+ shown by Na valerate, which should fulfil the requirements demanded by Osterhout's theory more adequately than guaiacol; furthermore the strong effect shown by amyl alcohol, which is not acidic, would make it appear that the loss of K^+ is not associated with the power of forming K^+ salts, but is due to a more general property of the substances under consideration. The absence of any significant variation of the loss due to guaiacol with pH between the limits pH 5 to pH 9 in the case of the dogfish (Table III) also precludes this view (the increased loss at pH 10 is best interpreted as a combination of the separate effects of alkalinity and guaiacol, since a pH of 10 is very close to the range of alkaline haemolysis with the dogfish).

It is interesting to note that a change in pH of the suspension medium will not cause a loss of K^+ until the alkalinity or the acidity is so great as to cause haemolysis, so that the effect of H^+ and OH^- is similar to that of the powerful lysins such as digitonin. Temperature haemolysis is preceded by an escape of K^+ ; whether this escape results simply from the increased kinetic energy of the K^+ ion or from a change in the membrane as well would be difficult to determine.

In view of the absence of effect of narcotics on the permeability to K^+ it would seem that the effect of lead, silver and other heavy metals is not associated with the poisoning of a catalytic system responsible for maintaining a concentration gradient of K^+ , but may be attributed to a direct influence on the state of the erythrocyte membrane. The influence of heavy metals on negatively charged colloidal systems is, in extremely low concentrations, to favour the aggregation of initially discrete particles. Thus if the normal behaviour and integrity of the erythrocyte membrane are dependent on the maintenance of a certain arrangement of discrete micelles or large molecules, the aggregating influence of a heavy metal such as lead or silver might easily result in the formation of local clumps of molecules or micelles leaving leaky patches in the membrane at other places. On this basis, many degrees of change may be expected, from a mild packing of the molecules or micelles over the whole surface, which might be reflected in an actual decrease in the rate of penetration of certain solutes, to the absolute rupture of the cell membrane, which would be reflected in the phenomenon of haemolysis, according as the concentration and nature of the heavy metal are varied. Davson [1936] showed that should the erythrocyte become permeable to both Na^+ and K^+ , then, as a result of the new Donnan equilibrium conditions, a difference of osmotic pressure should pertain between cells and external medium, which would result in swelling and final haemolysis. Thus the primary mechanism of haemolysis by heavy metals might well be an

initial change in the permeability allowing Na^+ and K^+ to diffuse through, and resulting in an osmotic pressure difference leading to swelling and bursting of the cells. The consideration of this as a possible factor in haemolytic systems might assist in resolving the complexity of the problem. On the other hand the induced permeability to cations and the haemolysis may represent merely different stages in the same process, so that this mechanism should be looked for only with those low concentrations of heavy metal under the influence of which haemolysis occurs only after some time.

The behaviour of silver in causing a ready penetration of cations leads to some important practical conclusions. Williams & Jacobs [1931] discovered that isotonic solutions of certain "Reagent" brands of NaCl caused the haemolysis of the erythrocytes of certain teleostean fishes, and Ball [1933] showed that the impurity responsible was silver which was introduced during the purification of NaCl. The present work has shown that haemolysis by silver is preceded by loss of K^+ , so the danger of using such preparations of NaCl even when they produce no visible haemolysis is obvious. Jacobs & Corson [1934] have shown that the rate of haemolysis of certain erythrocytes in glycerol solutions is slowed by the presence of traces of copper (using distilled water from a copper still provides sufficient copper to show the effect) and since then Davson (unpublished) has shown that copper in the same concentrations may cause a leakage of K^+ ; hence in studies on erythrocytes, and presumably on nerve, muscle, marine eggs etc. as well, glass-distilled water and preparations of NaCl known to be free from traces of silver should be used. The practice of defibrinating blood by shaking it with mercury, employed by Yannet *et al.* [1935] and Eisenman *et al.* [1937], should be avoided as any mercury passing into solution would inevitably influence the permeability of the erythrocytes to cations.

SUMMARY

1. The possibility that the high concentration gradient of K^+ maintained by the erythrocytes of many species against their surrounding plasma is due to an active metabolic process has been investigated. Metabolic poisons such as CN, CO, urethane, and accelerators such as methylene blue and pyocyanine had no influence on the K^+ content of the non-nucleated erythrocytes of the rabbit and the nucleated cells of the goose and dogfish. Fluoride caused a small loss of K^+ but it was concluded that this is not associated directly with the inhibition of glycolysis produced by this substance.

2. The extent to which losses of K^+ may be caused by centrifuging the cells from serum or saline was studied. Direct proof of such losses was obtained with the erythrocytes of the dogfish and ox. The possible bearing of these effects on earlier work is discussed.

3. The question as to whether haemolysis, however produced, is preceded by a state of cation permeability was studied. Subhaemolytic concentrations of amyl alcohol, the dihydric phenols and guaiacol caused losses of K^+ from the rabbit erythrocyte; digitonin, Na cholate and oleate caused losses only when in haemolytic concentrations (these losses were over and above those due to the haemolysis itself); saponin produced no loss of K^+ in all the concentrations studied. Acidity and alkalinity were only effective under haemolytic conditions, whilst raising the temperature caused considerable losses of K^+ ; the penetration of K^+ into the erythrocytes of the ox from strongly hypotonic aqueous KCl was demonstrated. The haemolytic action of silver is preceded by an induced permeability to cations.

We heartily thank Prof. Drummond for his encouragement and advice; one of us (J. F. D.) is indebted to the Department of Scientific and Industrial Research for a personal grant and to the Royal Society and Dixon Fund for apparatus grants.

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