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SOME ASPECTS OF THE OSMOTIC LYSIS OF ERYTHROCYTES

II. DIFFERENCES IN OSMOTIC BEHAVIOUR OF ERYTHROCYTES AFTER TREATMENT WITH ELECTROLYTE AND NON-ELECTROLYTE SOLUTIONS

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SUMMARY

1. The delay of the lysis of pig erythrocytes in buffered glycerol solutions proved to be correlated to the intracellular anion content.

2. At physiological pH values the decrease in intracellular chloride content in buffered sucrose solutions appeared to be coupled only to a minor degree to an exchange with phosphate. This exchange was negligible in buffered NaCl solutions.

3. The exchange of chloride *versus* hydroxyl ions coupled to the buffering action of hemoglobin proved to deliver the most important contribution to the differences in the osmotic resistance in sucrose and in the lysis rate in glycerol solutions. The variations in total anionic charge, depending on extracellular and intracellular pH, showed a rather good correlation with the net charge of hemoglobin.

4. The time of lysis in isotonic solutions of permeant does not only depend on the permeability of the erythrocyte membrane, but also on the extent of increase of osmotic resistance due to chloride–hydroxyl ion exchange.

INTRODUCTION

In the preceding paper[1] it was shown that erythrocytes depending on their pretreatment, display differences in osmotic resistance properties in hypotonic solutions of non-permeating sucrose and in hemolysis behaviour in solutions of permeating glycerol. The anomalous lysis phenomena might be caused by rapid changes in osmotic properties of the red blood cells. Since water equilibrium is established rapidly[2–5], the critical erythrocyte volume will be exceeded almost instantaneously above a certain difference in initial intra- and extracellular osmotic activity. At smaller osmotic differences, however, the relatively slowly permeating glycerol molecules, accompanied by water molecules, have to pass the membrane. Thus it will take longer before the critical erythrocyte volume is reached. In the meantime compensatory processes may take place, either a shrinkage of the erythrocytes due to efflux of osmotically active material, or an increase of membrane area and thus a decrease of the volume/surface ratio. The result of these phenomena

would be a delay in lysis. The following possible explanations will be considered: (a) The influence of the extracellular electrolyte concentration on membrane area due to changes in membrane charge. (b) A rapid loss of osmotic active substances from erythrocytes in non-electrolyte solutions. Some authors[6-10] demonstrated that in non-electrolyte solutions human erythrocytes rapidly lose a considerable amount of cations and presumably also of anions. (c) Variations in intracellular content of monovalent and divalent anions due to exchange with extracellular anions. (d) The exchange of hydroxylions against other anions (*e.g.* chloride) coupled to the buffering action of hemoglobin[11].

In this paper it will be shown from chemical analyses that the lysis delay of NaCl-treated and untreated red blood cells is mainly due to a rapid chloride–hydroxyl ion exchange. Together with the buffering action of hemoglobin this exchange process results in a decrease of the intracellular amount of osmotically active substances, a decrease of the cellular volume and an increase of the hemolysis time. The influence of the intracellular chloride concentration on the lysis behaviour of red blood cells is also described.

MATERIALS AND METHODS

The erythrocytes were treated as described in the preceding paper[1], followed by two successive treatments with unbuffered 300 mM sucrose to remove extracellular electrolytes. The pH and buffer concentration were varied in each experiment. Lysis experiments were performed as described before[1]. Sodium and potassium were determined in hemolysates of the red blood cells as well as in serum with an Eppendorf flame photometer. Bicarbonate was estimated in these solutions with a Natelson microgasometer[12,13]. After precipitation of protein with trichloroacetic acid (5 % final concentration), inorganic phosphate was assayed with ammonium molybdate[14], chloride titrimetrically with $Hg(NO_3)_2$ (refs 12 and 13) and sulphate turbidimetrically with $BaCl_2$ in gelatin solution[15]. The intracellular pH was determined in hemolysates of red blood cells in a 5-fold volume of water, since these pH values differ slightly from the actual intracellular pH[16,17]. Extracellular pH changes were determined with a Philips pH meter, Type PW 9408 equipped with a combined glass electrode CA 13-NS and a Servogor recorder Type RE-511.

The average erythrocyte volume was calculated from the total volume, which was determined with the [^{14}C]inulin dilution method, and from the number of red blood cells determined with a Coulter Counter[1]. This figure was used in estimating the number of untreated erythrocytes in 1 l without medium. The concentration of the intracellular components was assayed in equal numbers of erythrocytes after the various treatments. Results were expressed in concentrations per l untreated cells, because the mean erythrocyte volume may change during treatment.

The net charge of hemoglobin at different pH values was determined by potentiometric titration of 1 % oxyhemoglobin in 0.1 M KCl solution at 25 °C with HCl and NaOH[18,19]

RESULTS

The difference in osmotic resistance between sucrose and NaCl-treated erythrocytes in hypotonic sucrose solutions seemed not to be due to adsorption of electro-

lytes to the membrane. This can be concluded from the 50% hemolysis values of erythrocytes either treated with an excess of buffered NaCl, or with an excess of buffered NaCl followed by two times unbuffered sucrose (Table I). Though in all cases a small increase in osmotic resistance was observed, the differences in osmotic resistance appeared to be maintained.

TABLE I

THE INFLUENCE OF DIFFERENT TREATMENTS ON THE OSMOTIC RESISTANCE OF PIG ERYTHROCYTES IN SUCROSE

Erythrocytes were treated with buffered (1 mM sodium phosphate, pH 7.5) solutions of 300 mM sucrose or 150 mM NaCl. Portions of these erythrocyte suspensions were washed thereafter with unbuffered 300 mM sucrose. The results on these four groups of erythrocytes are compared with the values on erythrocytes only washed with unbuffered 300 mM sucrose. 50% hemolysis values at 37 °C are given in mosmoles sucrose/l.

Treatment of erythrocytes	*50% hemolysis values*
Untreated	126
Untreated + two times unbuffered sucrose	123
Four times buffered NaCl	145
Four times buffered NaCl + two times unbuffered sucrose	139
Four times buffered sucrose	114
Four times buffered sucrose + two times unbuffered sucrose	110

The loss of osmotically active cations and anions from pig erythrocytes was studied after various treatments (Table II). The number of untreated red blood cells representing a 1-l volume was calculated from an experimentally determined mean cell volume of 61 μm^3, a value, which is in agreement with the literature[20]. Porcine red blood cells treated with unbuffered 300 mM sucrose only, showed approximately the same cation and anion composition and content as untreated red blood cells, only the bicarbonate content being lower. Treatment with unbuffered sucrose could therefore be used to remove extracellular electrolytes after the various treatments. When erythrocytes were treated with buffered solutions of sucrose or NaCl, considerable changes in anion composition were observed, while the cation composition was hardly altered. The intracellular magnesium and calcium contents have not been studied, because their contribution to the osmotic activity is negligible in view of their low intracellular concentration[21]. Since the loss of cations from pig erythrocytes after these extensive treatments is negligible, a rapid loss of cations with an equivalent efflux of anions, can not be responsible for the observed phenomena. Compared with untreated erythrocytes the chloride content increased in NaCl-treated cells but decreased in sucrose-treated red blood cells. The phosphate content increased markedly in sucrose-treated red blood cells, but not in NaCl-treated cells. Erythrocyte membranes of various mammalian species earlier appeared to be very well permeable to anions like chloride[22], bicarbonate[23], sulphate[24] and phosphate[25]. Exchange of chloride and phosphate may play a role in the observed lysis delay. Bicarbonate cannot contribute significantly to an exchange process, since the treatments with unbuffered and buffered solutions strongly diminished its intracellular concentration. The sulphate content appeared to be less than 1 mmole/ $16.4 \cdot 10^{12}$ red blood cells and its contribution can therefore be neglected.

TABLE II

ION CONCENTRATIONS AND pH IN BLOOD, SERUM AND PORCINE RED BLOOD CELLS AFTER VARIOUS TREATMENTS

Samples containing approx. 2 ml erythrocytes were used. One group was washed twice with unbuffered 300 mM sucrose only. A second group was washed four times with 8 ml 300 mM sucrose buffered with 1 mM sodium phosphate to pH 7.5, then two times with unbuffered 300 mM sucrose. A third group was washed four times with 8 ml 150 mM NaCl, buffered with 1 mM sodium phosphate (pH 7.5), then two times with unbuffered 300 mM sucrose. The concentrations of the ionic components in blood and serum are given in mmoles/l. The concentrations in erythrocytes are given in mmoles per $16.4 \cdot 10^{12}$ cells, the number of cells representing 1 l of untreated erythrocytes. The concentrations of the ions in untreated erythrocytes were calculated from the concentrations in serum and blood, and the total cell volume, determined by means of [^{14}C]inulin dilution (54.4 % ± 0.13). The values given are the means with standard errors for 6 analyses except for HCO_3^- which was only assayed in duplicate. The intracellular pH was determined in lysates in triplicate.

	Sodium	*Potassium*	*Chloride*	*Phosphate*	*Bicarbonate*	*pH*
Serum	141.9 ± 0.8	8.4 ± 0.1	105.7 ± 0.1	3.5 ± 0.1	17.1–18.3	7.59 ± 0.02
Blood	68 5 ± 0.6	72.4 ± 0.3	76.8 ± 0.1	6.3 ± 0.1	13.7–14.3	7.61 ± 0.01
Untreated erythrocytes	7.1 ± 0.3	128.1 ± 0.5	52.6 ± 0.2	8.7 ± 0.1	9.4–11.4	7.60 ± 0.01
Erythrocytes treated with unbuffered sucrose	8.5 ± 0.2	133.0 ± 0.5	51.5 ± 0.1	8.0 ± 0.1	6.5– 6.9	7.60 ± 0.01
Erythrocytes treated with buffered sucrose	7.0 ± 0.1	126.4 ± 0.8	29.7 ± 0.3	17.2 ± 0.1	3.4– 3.6	7.89 ± 0.03
Erythrocytes treated with buffered NaCl	10.2 ± 0.1	132.4 ± 0.4	77.5 ± 0.2	8.1 ± 0.1	3.3– 3.7	7.41 ± 0.02

The differences in anion composition were studied more extensively in experiments at 3 °C, 22 °C and 37 °C, combined with osmotic resistance determinations in hypotonic buffered sucrose solutions, in order to determine whether there would be a correlation between the changes in chloride and phosphate content. At all three temperatures almost the same differences in osmotic resistance of NaCl-treated and sucrose-treated erythrocytes were observed. In buffered NaCl solutions a considerable increase in chloride without change in phosphate content took place at all three temperatures. In buffered sucrose solution the intracellular phosphate content was increased at 22 °C and 37 °C, but not at 3 °C. This effect was also observed with red blood cells of man[25]. At all three temperatures we observed, however, a decrease in chloride content. This indicates that intracellular chloride is not exchanged against extracellular phosphate only.

Since the osmotic resistance and the permeability measurements were performed in sodium phosphate-buffered non-electrolyte solutions at 37 °C, the decrease in chloride content as well as the increase in phosphate content was studied in more detail in order to determine which kind of exchange is taking place. Therefore red blood cells suspended in unbuffered sucrose were added to buffered sucrose solutions of pH 7.5 and 5.5. A high buffer concentration was chosen in order to assure a nearly complete exchange of anions. After various time intervals samples were taken, and after treatment of the erythrocytes with ice-cold unbuffered sucrose, the intracellular chloride and phosphate content determined. We preferred addition to ice-cold unbuffered sucrose since it was observed that at low temperature no uptake of phosphate takes place. The results obtained at pH 7.5 (Fig. 1) indicate that the chloride content decreased by 32 mmoles in 10 s or less, while the phosphate content increased only by 1.9 mmoles per $16.4 \cdot 10^{12}$ red blood cells during this period. This rules out an equivalent exchange of chloride against secondary phosphate. After

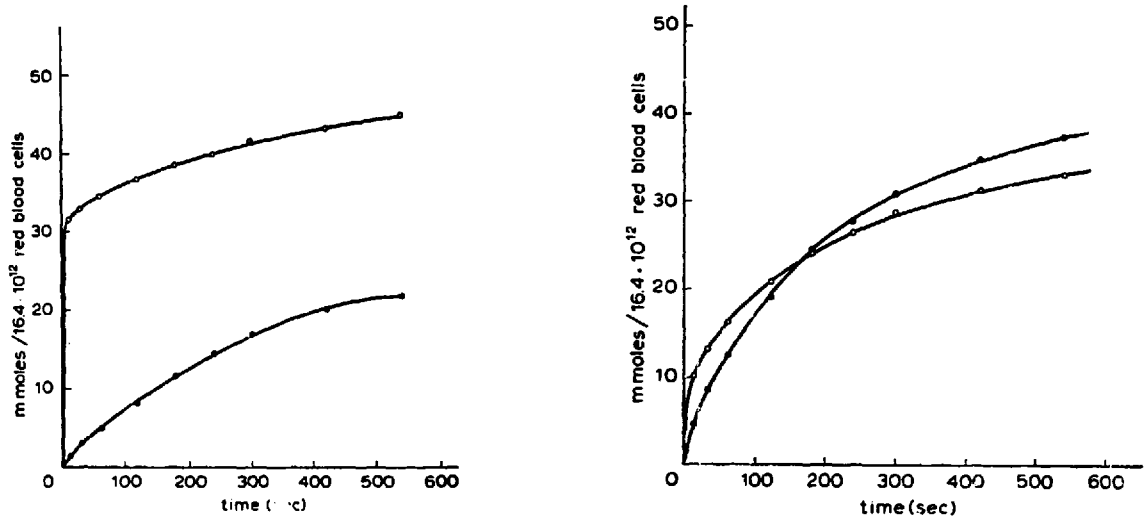


Fig. 1. Uptake of phosphate (●) and the loss of chloride (○) by pig red blood cells at pH 7.5 and 37 °C. To 48 ml buffered 300 mM sucrose (10 mM sodium phosphate) 12 ml erythrocyte suspension in unbuffered 300 mM sucrose were added. At various times 2-ml samples were added to 8 ml ice-cold 300 mM unbuffered sucrose and the red blood cells were spun down at once. The red blood cells were treated once more with ice-cold unbuffered sucrose and then lysed with water.

Fig. 2. Uptake of phosphate (●) and loss of chloride (○) by porcine erythrocytes at pH 5.5 and 37 °C. The same method was used as in the experiment of Fig. 1.

this short period, however, a nearly one to one exchange on molar basis takes place, suggesting a substitution of intracellular chloride by primary phosphate, rather than by secondary phosphate. The results obtained in a parallel experiment at pH 5.5 showed a less drastic decrease in intracellular chloride and an increased uptake of phosphate, as was also observed for human erythrocytes at low pH[26]. In this case a nearly one to one exchange of chloride and phosphate on molar basis was observed (Fig. 2).

It seems likely that at physiological pH and temperature another process, possibly an exchange of chloride and hydroxyl ions coupled to the buffering action of hemoglobin, is involved in the anomalous lysis behaviour of untreated red blood cells and red blood cells treated with buffered 150 mM NaCl. The existence of these processes in solutions of non-permeating non-electrolytes was suggested earlier[11,27,28]. In Fig. 3 a schematic representation of this exchange process in buffered sucrose

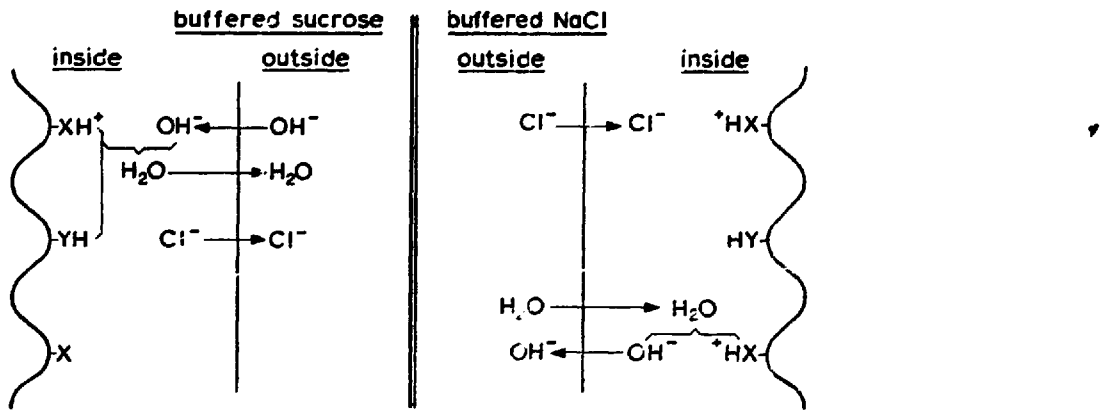


Fig. 3. Schematic representation of chloride–hydroxyl ion exchange, coupled to the buffering action of hemoglobin. X represents an imidazole or amino group and Y a carboxylic group.

and NaCl solutions is given. In buffered sucrose solutions (pH 7.5) an exchange of intracellular chloride against extracellular hydroxyl ions will increase the intracellular pH resulting in a concomitant liberation of protons from hemoglobin. The extracellular pH will decrease. These processes cause a decrease of the intracellular osmotic activity, since the inorganic anion concentration is lowered. Treatment with buffered NaCl solution (pH 7.5) causes an influx of chloride ions, an efflux of hydroxyl ions and a protonation of hemoglobin, together resulting in an increase of the intracellular osmotic value. It follows that the initial composition, the pH and the buffer capacity of the extracellular solution and to a lesser degree the duration of the treatment, will affect the following, closely connected parameters: (a) the direction and extent of chloride–hydroxyl exchange, (b) the extent of chloride–phosphate exchange in buffered non-electrolyte solution, (c) the intracellular and extracellular pH, (d) the net charge of hemoglobin, (e) the mean cellular volume.

We investigated the change in extracellular pH upon addition of erythrocytes, suspended in unbuffered sucrose, to buffered solutions (pH 7.5) containing isotonic sucrose or NaCl. In buffered 300 mM sucrose, a rapid and sharp decrease in extracellular pH to about 5.8, being almost complete in 30 s, was observed. Whereas the extracellular and intracellular hydroxyl ratio initially was about one, the intracellular and extracellular chloride ratio was near infinity, probably resulting in a

TABLE III

CORRELATION OF INTRACELLULAR pH, ANIONIC CHARGE, NET CHARGE OF HEMOGLOBIN AND CELL VOLUME OF PIG ERYTHROCYTES

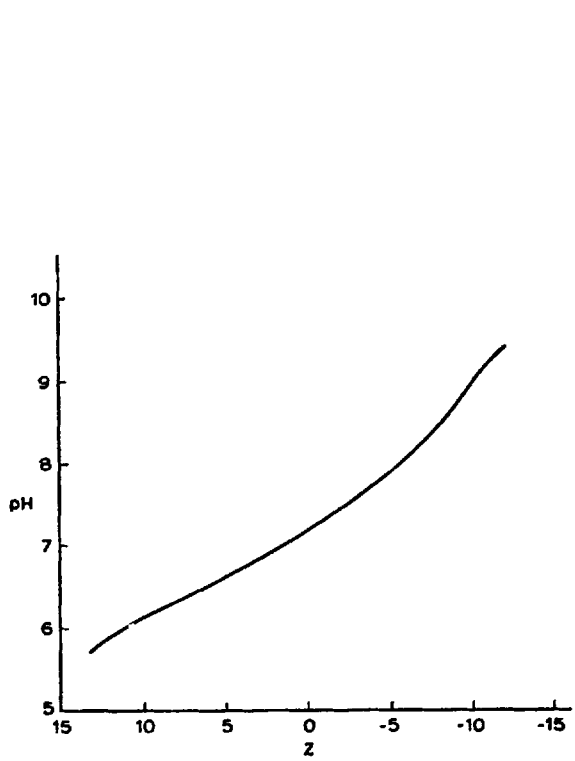
Samples containing approx. 2 ml erythrocytes were used. One group was washed twice with 8 ml 300 mM unbuffered sucrose only. A second group was washed four times with 8 ml 300 mM sucrose buffered with 10 mM sodium phosphate to different pH values, then two times with 300 mM unbuffered sucrose. A third group was washed four times with 8 ml 150 mM NaCl, buffered with 10 mM sodium phosphate to different pH values, then two times with 300 mM unbuffered sucrose. The total anionic charge was calculated from chloride and phosphate concentrations and the intracellular pH. The net charge of hemoglobin was calculated from hemoglobin content (36%) and intracellular pH by means of the titration curve of Fig. 4. Both charges are given in mequiv per $16.4 \cdot 10^{12}$ red blood cells. The cell volume was determined from [^{14}C]inulin dilution and compared to that found for red blood cells treated with unbuffered sucrose (100%). The values for chloride, phosphate and cell volume are means with S.E. for 6 determinations.

Treatment	*Extracellular pH*	*Intracellular pH*	*Chloride*	*Phosphate*	*Total anionic charge*	*Net charge of hemoglobin*	*Cellular volume in %*
Sucrose unbuffered	5.9	7.8	50.6 ± 0.4	6.7 ± 0.1	62.6	−25.0	100.0 ± 0.4
NaCl	8.5	7.8	56.4 ± 0.1	5.6 ± 0.1	66.5	−25.0	99.4 ± 0.4
NaCl	7.5	7.5	73.0 ± 0.1	5.6 ± 0.1	82.5	−14.1	103.4 ± 0.3
NaCl	6.5	6.6	129.8 ± 0.9	6.3 ± 0.1	137.4	+28.0	108.2 ± 0.4
NaCl	5.5	6.1	169.6 ± 0.7	6.3 ± 0.1	176.4	+64.9	114.2 ± 0.8
Sucrose	8.5	8.6	5.6 ± 0.3	15.3 ± 0.1	35.6	−47.5	91.9 ± 0.2
Sucrose	7.5	8.2	5.5 ± 0.1	22.0 ± 0.1	47.5	−37.7	93.0 ± 0.3
Sucrose	6.5	7.5	6.5 ± 0.2	42.6 ± 0.2	77.4	−13.3	94.0 ± 0.1
Sucrose	5.5	7.2	20.6 ± 0.3	48.3 ± 0.3	93.1	− 0.5	98.2 ± 0.6

considerable exchange of extracellular hydroxyl against intracellular chloride ions. After 30 s a slow increase in extracellular pH was seen, which might be due to a gradual loss of cations and anions[29] or to an exchange of intracellular hydroxyl against extracellular secondary phosphate ions. The latter pass the membrane much more slowly than chloride ions, as was shown in Fig. 1.

When erythrocytes suspended in buffered sucrose, were added to the buffered NaCl solution, a small and slow increase of the extracellular pH to about 7.7 was observed. The slow increase in extracellular pH might be caused by an exchange of intracellular hydroxyl against extracellular chloride ions since the extracellular/intracellular chloride ratio was nearly 1.9 at the start, assuming a chloride content of 52 mmoles/l cells (Table II) and a water content of 65 % (ref. 20). Since the intra- and extracellular pH were nearly equal, the initial ratio of extracellular and intracellular hydroxyl ion concentration was approximately one. Though the permeability to small anions like chloride[22] and hydroxyl[30] is high, equilibrium is reached only after a rather long period of time. Possibly, this is caused by the decreasing ratio of extracellular/intracellular chloride.

The exchange phenomena should also affect the intracellular pH, the anionic composition and charge, the net charge of hemoglobin and the cell volume. Since the extracellular pH and the composition of the solution of non-permeant will affect the parameters mentioned, we analysed pig red blood cells after treatment with sucrose and NaCl solutions at various pH values (Table III). The net charge of hemoglobin could be calculated from the titration curve of porcine oxyhemoglobin (Fig. 4),



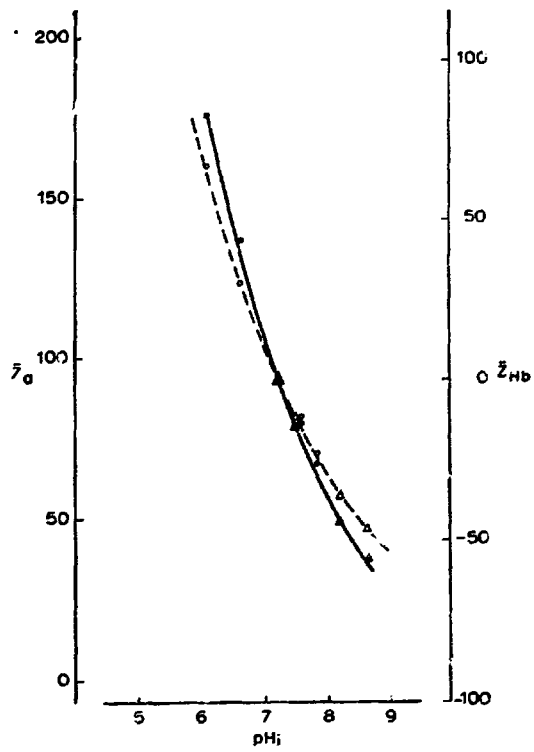


Fig. 4. Titration curve of pig oxyhemoglobin. Abscissa: pH. Ordinate: net charge of hemoglobin in equiv/mole. Protein concentration, 10 mg/ml. Ionic strength, 0.1. Temperature, 25 °C.

Fig. 5. Relation between intracellular pH (pH_i), net hemoglobin charge (Z_{Hb}; △, ○) and total anionic charge (Z_a; ▲, ●), both in mequiv per $16.4 \cdot 10^{12}$ red blood cells. Data were obtained from Table III. The points (△) and (▲) are derived from erythrocytes treated with buffered sucrose, the points (○) and (●) from erythrocytes treated with buffered NaCl.

assuming a hemoglobin content of pig red blood cells of 35 % w/v (ref. 20). The variations in anion composition, and thus in total anionic charge were considerable and appeared to be correlated with the changes in the net charge of hemoglobin (Fig. 5) as previously assumed by Jacobs[28]. The curves for anionic charge and net charge of hemoglobin have been vertically displaced with regard to each other so as to make them coincide at pH 7.2 where Z_{Hb} is zero and the anionic charge is 93.1 mequiv per $16.4 \cdot 10^{12}$ red blood cells. Changes in anion content and hemoglobin charge proved to be accompanied by changes in cellular volume (Table III). Since particularly the chloride content of erythrocytes varied with pH and the half time for chloride exchange is approximately 0.2 s (ref. 22), a rapid chloride–hydroxyl ion exchange, coupled to variations in mean erythrocyte volume, appeared to be likely. Furthermore, in buffered NaCl solutions only slight changes in phosphate content were observed, whereas the chloride content increased with decreasing pH values of the NaCl solutions. In buffered sucrose solutions, however, a more complex situation exists, since besides the decline of the chloride content at all pH values, an increased phosphate content was observed at decreasing pH values of the sucrose solutions.

The same experiment as reported in Table III was performed with human erythrocytes at 3 °C, since at room temperature considerable lysis takes place in isotonic sucrose solutions at neutral and alkaline pH values. The most pronounced changes in ion content in buffered sucrose and NaCl solutions, compared with unbuffered sucrose, are presented in Table IV. A substantial cation efflux was observed in buffered 300 mM sucrose at all pH values which phenomenon was also found by other investigators[6–10]. The changes in chloride content and pH were comparable with those observed in pig erythrocytes (Table III).

TABLE IV

CHANGES IN INTRACELLULAR pH AND IN CATION AND ANION COMPOSITION OF HUMAN RED BLOOD CELLS

Erythrocytes were treated at 3 °C with unbuffered sucrose, buffered sucrose (pH 8.5) and buffered NaCl (pH 5.5). Determinations were performed in duplicate. Other experimental details are the same as given in Table III.

Treatment	*Extracellular pH*	*Intracellular pH*	*Cation content*	*Chloride*	*Phosphate*
Sucrose unbuffered	5.8	7.5	121.7	63.6	3.2
NaCl	5.5	6.1	126.7	170.7	2.8
Sucrose	8.5	8.6	101.4	8.4	5.0

Proof that the chloride–hydroxyl ion exchange influences the osmotic resistance in buffered sucrose as well as the delay of lysis in buffered glycerol solutions, was obtained with porcine erythrocytes containing different chloride concentrations. Erythrocytes were used either untreated, or treated with 150 mM NaCl, buffered with 10 mM sodium phosphate at pH 6.0 and 7.5; they were then treated with unbuffered sucrose. Only the chloride content was changed in NaCl-treated erythrocytes It was increased by a factor of 1.8 at pH 7.5 and a factor of 3.4 at pH 6.0. The retardation of the lysis in buffered glycerol solutions (5 mM sodium phosphate, pH 7.5)

appeared to be larger at higher intracellular chloride content (Fig. 6). The osmotic resistance in hypotonic buffered sucrose (5 mM sodium phosphate, pH 7.5) was also influenced and appeared to be lower at higher intracellular chloride content. After treatment with NaCl at pH 6.0 the experimentally determined resistance in hypotonic

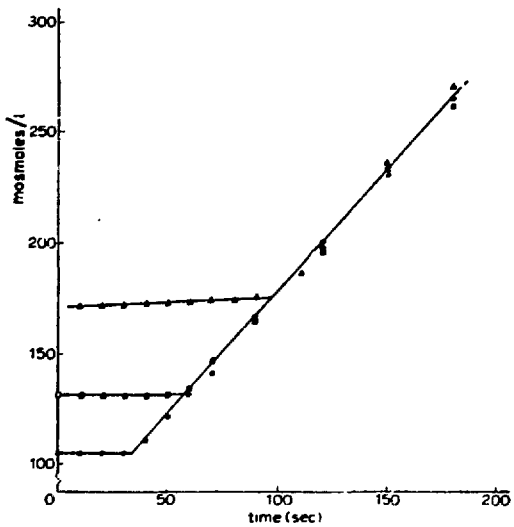


Fig. 6. 50% hemolysis values in mosmoles/l at 37 °C of pig red blood cells in buffered solutions of glycerol and sucrose (5 mM sodium phosphate, pH 7.5). Untreated erythrocytes in sucrose (○) and in glycerol (●). Erythrocytes treated with NaCl (pH 7.5) in sucrose (□) and in glycerol (■). Erythrocytes treated with NaCl (pH 6.0) in glycerol (▲).

buffered sucrose solutions appeared to be lower than the value obtained by extrapolation to $t = 0$ of the line which represents the delayed 50% hemolysis values in glycerol. The cause of this phenomenon is still unclear.

DISCUSSION

An explanation for the delay of lysis of NaCl-treated or untreated red blood cells in glycerol solutions can now be given. In strongly hypotonic glycerol solutions the erythrocytes lyse almost instantaneously due to water transport only. At sufficiently high concentrations of glycerol, however, two processes take place acting in opposite directions. Glycerol molecules pass the membrane leading to an increase of the mean cell volume. In the meantime a rather rapid chloride–hydroxyl ion exchange takes place, which is probably complete within seconds and leads to a decrease of the mean cell volume. It follows that when the buffer capacity of the glycerol solutions is sufficiently high and lysis does not take place at once, the erythrocyte will shrink rapidly to a certain minimum volume, almost independent of the initial intracellular chloride content. The buffer capacity of 1 mM sodium phosphate (pH 7.5) will generally be sufficient, since the ratio of the total red blood cell volume and the volume of the test solution is about 1:400. Moreover, the lysis rate of the erythrocytes will be equal after the disappearance of the differences in chloride content, which could be experimentally established (Fig. 6). Now we have shown that a delay of lysis may take place in solutions of permeating non-electrolytes, the question arises to what extent this phenomenon affects the time of lysis. In general the osmotic lysis method is performed at physiological pH value in isotonic

solutions of permeant with untreated or NaCl-treated red blood cells. Hence a normal or increased amount of intracellular chloride ions will be present. When the red blood cells are suspended in an excess of buffered, permeating non-electrolyte, a rapid chloride–hydroxyl ion exchange will take place resulting in a delay of lysis. Prevention of this delay would lead to a decrease of the lysis time. The lysis time determined in this way will represent the lysis rate due to permeant diffusion only. It can be concluded from Fig. 6, for instance, that whereas in 300 mM glycerol 50% lysis of untreated pig red blood cells would be found after approximately 240 s, the same 50% lysis value in 300 mM glycerol due to glycerol diffusion only would be found after approximately 200 s since a lysis delay of about 40 s was observed. The extent of the delay depends primarily on intracellular pH and chloride content, and also on extracellular pH and buffer capacity. Probably the intracellular pH of untreated erythrocytes of different mammalian species varies somewhat, whereas also differences in the intracellular chloride content exist[21]. Since, however, the chloride–hydroxyl ion exchange is complete in a short period of time, this phenomenon does not affect the permeability coefficient for glycerol when this is determined with the modified lysis method, presented in the preceding paper[1].

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