THE ELECTROPHORETIC MOBILITY OF RABBIT ERYTHROCYTES AND GHOSTS

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Between 1929 and 1934 Abramson (1929, 1934) carried out many measurements of the electrical mobilities of red cells and of the ghosts produced by various forms of hemolysis. He found that after hemolysis by water, the mobility is unchanged, as it also is if lysis is produced by small quantities of saponin, or by small amounts of complement; he therefore concluded that hemolysis can occur without necessitating a radical change in the chemical constitution of the surface, and put forward the idea that for hemolysis to occur it may be necessary to affect only certain "key spots," occupying only a very small portion of the cell surface.

The purpose of this paper is to extend the observations, and to bring the results into relation with those obtained by certain other methods.

Methods and Preparations

The electrophoresis measurements were made in the Abramson horizontal micro-electrophoresis cell, and the technique used was that developed by Abramson (1929, 1934) and more recently described by Moyer (1936). The electrophoretic mobilities were obtained from measurements at the stationary levels; as checks in the majority of determinations, however, measurements were also made at levels throughout the cell and parabolae plotted. The mobilities obtained by the integration of these parabolae always agreed within 5 per cent with the corresponding mobilities obtained from stationary level measurements. The measurements were all carried out at room temperature, and the mobilities converted to mobilities at 25°C. by allowing a 2 per cent increase per degree. Although the same electrophoresis cell was used for most of the measurements, a few of the determinations on unlysed red blood cells and watery ghosts were made in a second cell of the same type. The mobilities obtained in the two different cells checked exceedingly well with one another.

The solution in which the red blood cells and ghosts were suspended for

electrophoretic measurements was an isotonic buffer-glucose solution of pH 7.2. It was made by mixing 10 cc. of a M/15 phosphate solution (prepared by adding 70 cc. of M/15 Na_2HPO_4 to 28 cc. of M/15 KH_2PO_4) and 90 cc. of a 5.4 per cent glucose solution. The buffer-glucose solutions were all freshly prepared. In some determinations, as will be pointed out later, various concentrations of saponin were also included in the phosphate buffer-glucose solution. The unlysed erythrocytes, ghosts, oil droplets, and quartz particles on which mobility determinations were made were always first suspended in suitable concentration in 1 per cent NaCl before being added to the buffer-glucose solutions, 0.3 cc. of saline suspension being mixed with 40 cc. of buffer-glucose solution. The specific resistance of each suspension was determined at the same temperature at which the measurements were made.

The blood from which the cells were obtained was fresh, defibrinated rabbit blood. The cells were first washed three times with 1 per cent saline and finally suspended in 1 per cent saline.

"Watery ghosts," formed by lysis of erythrocytes with a hypotonic solution, were prepared as follows. The cells from 10 cc. of defibrinated blood, after three washings, were made up to the original volume by the addition of 1 per cent saline, and to the 10 cc. of cell suspension 60 cc. of water was added. After about 10 minutes, 10 cc. of 7 per cent saline was added, making the resulting 80 cc. of suspension fluid isotonic. The suspension was immediately centrifuged at high speed in celluloid cups for 15 minutes, the supernatant fluid removed, and the ghosts suspended in saline. No attempt was made to free them of the hemoglobin remaining in them.

"Freezing-and-thawing ghosts" were prepared as follows. A suspension of cells from 20 cc. of defibrinated blood, twice washed, was placed in the bottom of a 250 cc. beaker, and the beaker set upon a flat cake of dry ice. The suspension was allowed to freeze solidly, and then to thaw at room temperature. The resulting hemolyzed cells were then washed from one to three times, the number of washings being sometimes limited by the low yield of ghosts, and were finally suspended in 1 per cent saline.

"Chloroform ghosts," the result of hemolysis with chloroform, were prepared as follows. A suspension of red cells from 10 cc. of defibrinated blood, twice washed, was added to about 150 cc. of 1 per cent saline saturated with chloroform. After about 10 minutes had been allowed for hemolysis, the suspension was subjected to high speed centrifugation for about 15 minutes. The ghosts thrown down were washed and finally suspended in 1 per cent saline in the same manner as the freezing-and-thawing ghosts. Both freezing-and-thawing ghosts and chloroform ghosts, if washed three times, are quite white in color.

"Saponin ghosts" were made as follows. Washed cells were added to the already described phosphate buffer-glucose solution (0.3 cc. of cell suspension to 40 cc. of solution), containing in this case, however, also enough saponin (0.1 per cent or 0.01 per cent) to hemolyze them. Electrophoresis measurements were made directly on the saponin ghosts in the solutions in which they were prepared. In connection with saponin ghosts, mobility measurements were also made on

unlysed red blood cells in buffer-glucose solutions containing insufficient concentrations of saponin for lysis, namely 0.001, 0.0001, and 0.00001 per cent of saponin.

Mobility measurements were also made on microscopic quartz particles and paraffin oil droplets in buffer-glucose solutions containing various concentrations of saponin. The suspension of oil droplets was made by shaking the oil vigorously with 1 per cent saline for about half an hour. The droplets of which the mobilities were determined were about 1 μ in diameter.

RESULTS

In Table I are given the mobilities found for unhemolyzed rabbit erythrocytes and for ghosts of rabbit erythrocytes prepared by lysing

TABLE I

Condition of cells	Mobility	Average mobility
	μ/sec./volt/cm.	μ/sec./volt/cm.
Unlysed cells	1.01 (a) 1.03 (b) 1.02 (c) 1.01 (d) 1.08	1.03
Watery ghosts	1.11 1.00 1.01 (a) 1.02 (b)	1.03
Freezing-and-thawing ghosts	1.08 1.02 (d)	1.05
Chloroform ghosts	1.15 1.00 (c)	1.08

with water, by freezing-and-thawing, and with chloroform. The mobilities of all are within experimental error of one another. In several cases both the mobility of the unlysed cells and that of the ghosts in question was determined in the same suspension in the electrophoresis cell at the same time. The excellent agreement of such simultaneously determined mobilities, which are marked by similar letters in the second column of Table I, shows that there is no detectable difference between the mobility of unhemolyzed rabbit erythrocytes and the mobilities of their ghosts formed by lysing with water, by freezing-and-thawing, or with chloroform. Further evidence of the

agreement of these mobilities is presented in Fig. 1. In it electrophoretic mobilities of intact red cells, watery ghosts, freezing-and-thawing ghosts, and chloroform ghosts at various depths in the same electrophoresis cell (in separate suspensions in separate determinations, however) are plotted against the fraction of depth in the cell.

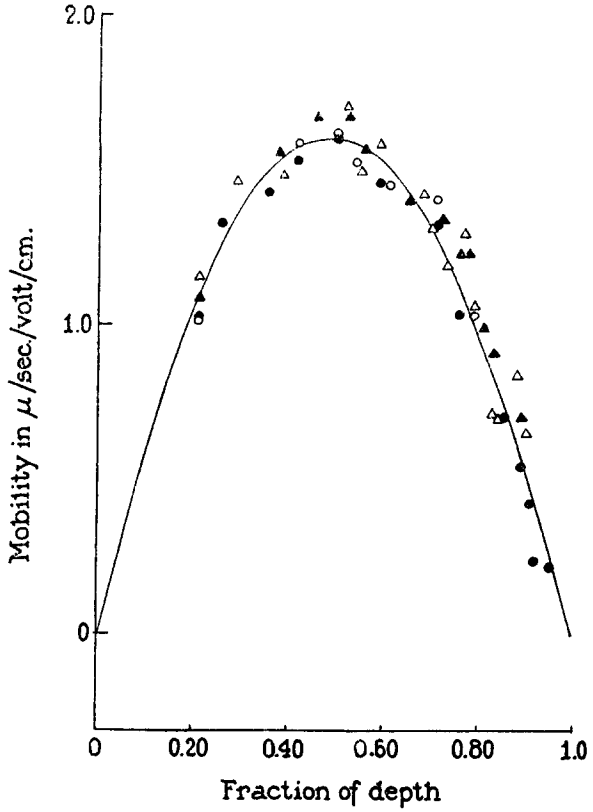

FIG. 1. Closed circle, unlysed cells; open circle, watery ghosts; closed triangle, freezing-and-thawing ghosts; open triangle, chloroform ghosts.
Theoretical parabola obtained from plotted points by method of least squares.

In Table II are presented the mobilities of unhemolyzed rabbit erythrocytes and saponin ghosts in phosphate buffer-glucose solutions containing various concentrations of saponin. As mentioned above, unlysed red cells were added directly to these solutions, and in the two solutions containing the higher concentrations of saponin the cells

were hemolyzed. The mobilities in Table II were determined from a series of measurements made in a single day, and all of the mobilities agree with themselves and with the mobilities in Table I within experimental error. The unlysed rabbit red cells in the presence of various non-lytic concentrations of saponin, and the saponin ghosts in the presence of lytic concentrations, have the same electrophoretic mobility, as far as our experimental results indicate, as unlysed rabbit red cells, watery ghosts, freezing-and-thawing ghosts, and chloroform ghosts in saponin-free solutions.

TABLE II

Saponin concentration	Mobility	Condition of cells
per cent	*μ/sec./volt/cm.*	
Zero	1.08	Unlysed
0.00001	1.09	"
0.0001	1.09	"
0.001	1.17	"
0.01	1.16	Ghosts
0.1	1.09	"

TABLE III

Saponin concentration	Quartz mobility	Oil mobility
per cent	*μ/sec./volt/cm.*	*μ/sec./volt/cm.*
Zero	3.47	3.81
0.0001	3.47	—
0.001	3.40	3.48
0.01	3.19	2.98
0.1	2.69	2.75

In view of the possibility that saponin might form an adsorbed film on the red cell surface, an attempt was made to determine the mobility of saponin-covered surfaces. Table III gives the mobilities of quartz particles and paraffin oil droplets in phosphate buffer-dextrose solutions containing various concentrations of saponin. The mobilities for quartz particles and oil droplets at the same intermediate saponin concentrations are not strictly comparable, for the quartz particles were in very much greater concentration (thus affording more surface) in suspension than were the oil droplets. However, in the suspensions

containing 0.1 per cent saponin, where there was surely enough saponin to cover all of the surface of the quartz particles and oil droplets, the mobilities are within experimental error of each other. It would appear from these results that saponin is adsorbed on both quartz particle and oil droplet, lowering their mobilities with increasing adsorption until they both have complete surface films of saponin and therefore the same mobilities. The electrophoretic mobility of saponin-covered surfaces therefore seems to be somewhat over 2.5 μ/sec./volt/cm., considerably higher than the mobility of about 1.07 μ/sec./volt/cm. of unlysed red cells and saponin ghosts in the same buffer-glucose solution containing various concentrations of saponin.

DISCUSSION

It is surprising that these different forms of lysis, which correspond to a variety of degrees of injury to the red cell, should be unaccompanied by any change in ζ-potential. The watery ghosts have a resistance and capacity virtually the same as that of the intact cell (Fricke and Curtis, 1935), although, over a period of minutes, they are permeable to cations (Davson and Ponder, 1938); on the other hand, they are impermeable to hemoglobin (Fricke, Parker, and Ponder, 1939), and exhibit the typical biconcave shape of the mammalian red cell. So far as it goes, the evidence is that this form of lysis is the result of the cell membrane being stretched beyond its normal area (Ponder, 1937; Castle and Daland, 1937), and that the permeability to pigment which results is followed by some sort of repair process. The resistance and capacity of the freezing-and-thawing ghosts is also like that of the intact cell, although there is a peculiar frequency dependence of the capacity (Fricke and Curtis, 1935); these ghosts are also slowly permeable to cations. But they are permeable to hemoglobin,[1] and the biconcave shape is lost. The action of the chloroform is presumably to dissolve out or otherwise disorient the lipoid components of the cell ultrastructure; the chloroform ghosts are permeable to cations and to pigment, and again the biconcave shape is lost. The

[1] At least in the direction from inside to outside, for they can be washed free of the residual hemoglobin which remains in watery ghosts. Watery ghosts are impermeable to hemoglobin placed in the fluid surrounding them, but we have no information about a similar impermeability of freezing-and-thawing ghosts.

action of saponin is probably the most drastic of all, for this lysin can react with both the protein and the lipoid components of the membrane ultrastructure (*cf.* Schulman and Rideal, 1937). The ghosts are roughly spherical, and are permeable to both cations and to pigment, and if sufficient saponin is used the resistance and capacity disappear entirely. Whether this is due to all the cells becoming more and more permeable as time goes on or as the concentration of saponin is increased, or to an increasing number of the cells becoming totally permeable in an all-or-none fashion, is not entirely clear.[2] At all events, increasing quantities of the lysin are taken up by the cells as time goes on (Ponder, 1935), and this presumably corresponds to increasing injury.

Nevertheless the electrical mobility is the same in all cases, and the same as for the intact rabbit red cell. It is difficult to account for this, but there are at least two lines of speculation open.

The first of these is Abramson's key spot hypothesis, which suggests that lysis results from certain key spots in the cell membrane breaking down, the greater part of the surface, however, remaining much as it was before. The results of "fading time" experiments tend to support this view in the case of lysis by hypotonic solutions (Davson and Ponder, 1938), Ca stearate monolayers (Ponder and Neurath, 1938), dilute saponin solutions (Ponder and Marsland, 1935), and possibly chloroform (Davson and Ponder, 1938); the fading times are such as would be expected if there were 10 to 50 holes of 250 A through which the pigment could escape, and their total area would be anything from 1/10,000th to 1/2,000th of the area of the cell surface.[3] The difficulty arises when we have to consider the case of concentrated

[2] Fricke and Curtis discuss the matter from the latter point of view. If the resistance and capacity of any cell are lost in an all-or-none fashion after saponin hemolysis, and if the loss is followed by disintegration (as Fricke and Curtis seem to think), it may be that the ghosts whose electrical mobility we have measured are structures which still retain their normal resistance and capacity. The alternative possibility, that a partial or complete loss of resistance and capacity is accompanied by no change in mobility, would be very interesting.

[3] In order to account for many immunological reactions, it is convenient to postulate the existence on the cell surface of key spots with specific reactivities, but the key spots referred to in this paper are not necessarily of this kind. In the meantime, it is better to regard them as merely the weakest spots in a membrane which, from its very nature, is unlikely to be altogether homogeneous.

saponin solutions (*e.g.* 1 in 1000), for, whether as judged by fading times or in any other way, such concentrated solutions, acting over considerable periods of time, are unlikely to have so restricted an action.

The second possibility is suggested by the particular form of the membrane ultrastructure (Schmitt, Bear, and Ponder, 1937 and 1938). As we now conceive it to be, the cell membrane has an outer, tangentially arranged, network or lattice of protein, probably the stromatin of Jorpes (1932) and Boehm (1935), and probably only a few molecules thick. Within this, there are radially arranged layers of lipoid, not more than two or four in number, and within this again is another layer of protein intimately connected with the gel of stromatin which Boehm believes, probably correctly, to fill the cell interior. It is possible that it is the outer protein layer, with its polar groups, which is responsible for the electrical mobility, while lysis results from the destruction or disorientation of the more deeply seated non-polar lipoid layers, which contribute little to the surface charge. All the lytic agents considered, in fact, may leave the layer responsible for the electrical mobility relatively untouched.[4]

The other point which emerges from this investigation is that the red cell ghost apparently does not adsorb a layer of saponin as do oil droplets and quartz particles. This is of interest in view of the fact that the red cell surface is the only surface hitherto investigated which does not adsorb gelatin; apparently it has quite special properties.

SUMMARY

Measurements of the electrical mobility of washed rabbit red cells and of ghosts produced by hypotonic solutions, freezing-and-thawing, chloroform, and saponin were made in the Abramson horizontal microelectrophoresis cell. These different forms of lysis, which corresponds to a variety of degrees of injury to the red cell, are unaccompanied by any change in electrical mobility. These observations are discussed from the standpoint of the possible structure of the cell membrane and the action of lysins upon it.

[4] The investigations of Monaghan and White (1936), and of Byler and Rozendaal (1938) show that the reactivity of the cell surface to gelatin and chicken serum is somewhat altered. These observers agree, however, that the electrical mobility of the hemolyzed watery ghost is the same as that of the normal cell.

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