THE ELECTROPHORETIC MOBILITY OF HUMAN ERYTHROCYTES—WHOLE CELLS, GHOSTS, AND FRAGMENTS

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The results of some preliminary experiments, involving comparison of the electrophoretic mobility of the ghosts (stromata) of human erythrocytes with whole cells, and of the fragments of cells with whole cells and ghosts, are reported in this paper. Abramson (1) found that ghosts have the same mobility as whole cells in buffered isotonic suspension. This observation has been confirmed. However, we have observed marked differences when a foreign serum is present. Fragments of ruptured cells differ in mobility from the original cells, even in the absence of foreign material. These results suggest a new approach to the study of the composition and structure of the red cell wall, and a possible new method for clinical study of blood in disease.

A modified Northrop-Kunitz or Abramson microelectrophoresis cell was used. The method has been adequately described by Moyer (2). Measurements on seven different suspensions of normal cells, in $M/15$ phosphate buffer at pH 7.4, made on 5 different days, gave values of mobility in $\mu/\text{sec.}/\text{volt}/\text{cm.}$ at 25°C. , ranging from 1.25 to 1.31, with a mean value of 1.29. This compares with the standard value of 1.31 given by Abramson (1) and Moyer (2). Each result reported is based on a number of individual observations. The standard deviation for normal whole cells varied between 2.4 and 5.9 per cent.

Ghosts and Whole Cells

Normal cells suspended in $M/15$ phosphate buffer at pH 7.4 were hemolyzed by two methods involving no change of chemical composition of the medium, ultraviolet irradiation and dilution. The latter

method was used in most cases. Some of the cells resist hemolysis for hours so that simultaneous measurements on whole cells and ghosts may be made. Within the limits of experimental error, no difference was ever found between the mobilities of ghosts and whole cells in pure salt suspension. Neither was any difference found when 4 per cent human serum was added to the buffer solution.

Upon adding chicken serum to a diluted suspension of human red cells, the mobility of the ghosts decreases while that of the whole cells remains unchanged. The mean mobility of the ghosts decreases with time, and, after a few hours, many of them exhibit irregularities on the surface. These may be fragments from ruptured cells clinging to the surface or cell material resulting from extrusion. Whatever

TABLE I
Effect of Chicken Serum on Human Red Cell Ghosts in $\mu/30$ Phosphate Buffer

Serum	η (μ /sec./volt/cm.)		Difference
	Whole cells	Ghosts	
<i>per cent</i>			<i>per cent</i>
2	1.57	1.47	6.4
6	1.57	1.33	15.3
8	1.47	1.10	25.2
10	1.51	1.00	33.8

the source, such irregular cells usually move more slowly than smooth cells. But, even before any rough cells appear, individual differences are much greater between ghosts than between whole cells. These facts place limitations on any effort to study quantitatively the variation of the effect with serum concentration. However, the nature of the mechanism is of such interest that such a study was undertaken.

A standard technique designed to avoid possible variations due to method of mixing, concentration of salt, and timing, was adopted. Whole cells were measured within 20 minutes after mixing, and ghosts between 30 and 90 minutes. The results are given in Table I. With this method, it was not possible to get significant results below 2 per cent serum, because of experimental error. The linear variation

with serum concentration appears to indicate a mechanism involving a chemical-type change. However, nothing is known about the concentration of active material, and equilibrium is not attained. Therefore, a simple mechanism involving adsorption of a substance giving a lower over-all charge density is not excluded.

Whole cells may be kept at room temperature in $M/15$ phosphate buffer for only a relatively few hours without change of mobility. By reducing the temperature to $6^{\circ}\text{C}.$, a suspension was kept for 7 days without change. With this method, the normal rate of change can be so reduced that any effect due to simple adsorption should be observable. But a 1 per cent foreign serum sample was kept for 11 days without appreciable decrease of mobility of whole cells and with no difference between ghosts and whole cells. It is, therefore, concluded that there is no direct effect on mobility of the cells from adsorption of foreign serum. At room temperature the active material in the foreign serum serves to catalyze a change which takes place even in its absence. Apparently, only ghosts are capable of reacting with the catalyst. Important information may be obtainable by testing known compounds for catalytic action.

Such a response to foreign serum suggests that abnormal substances resulting from disease may possibly be capable of catalyzing the reaction. A few samples of blood of patients were examined, and small effects were indicated in some cases, but the number of observations was not sufficient to allow definite conclusions.

Fragments and Whole Cells

Some cells break up in $M/30$ phosphate suspension to yield fragments of varying size. The behavior of fragments in pure salt suspension is similar to that of ghosts in foreign serum suspension—a marked decrease in mean mobility with time obtains and different fragments vary widely. The results from one experiment are summarized in Table II. It seems significant that the fragments have the same mobility as the ghosts immediately after breaking off. This indicates that the interior surfaces are similar in structure to the exterior, and perhaps identical. The relationship appears to be independent of hemolysis for fragments were produced mechanically

by grinding in a mortar in a M/15 suspension, with essentially the same result.

At the outset, it was thought that adsorption of serum proteins by the fresh surfaces could account for the decreasing mobility of fragments. Serum-coated quartz particles were found to have a mean mobility of 0.81 in M/30 phosphate + 4 per cent serum suspension. With this in mind, a series of experiments was run with the normal amount of serum, with all serum washed out, and with excess serum. But the results were always similar.

The mechanism of this change is probably similar to that which accounts for the behavior of ghosts in foreign serum and of whole

TABLE II
Change of Mean Mobility of Fragments with Time

	Time interval after diluting (min.)			
	0-20	20-30	30-90	135-150
Mean mobility (g).....	1.57		1.46	
(μ /sec./volt/cm. at 25°C.) (f).....	1.55	1.16	0.91	0.69

cells on long standing. A chemical change, with which the decreased mobility is associated, possibly simply a rearrangement, is normally proceeding slowly even in whole cells. Upon hemolysis, the cells are able to adsorb certain substances which catalyze the change. After disintegration of the cells, the change proceeds more rapidly.

SUMMARY

The electrophoretic mobility of human red cell ghosts decreases in the presence of chicken serum. The decrease is not directly due to the presence of adsorbed material but to a change which is catalyzed by the foreign substance. It is suggested that abnormal serum materials resulting from disease may serve as catalysts.

Fragments of broken cells have the same mobility as whole cells at first, then decrease even in pure salt suspension, while the whole cells remain essentially unchanged for hours.

The results suggest that the slow change of whole cells, the change

of ghosts in the presence of foreign serum, and the change of fragments are all manifestations of the same modification of structure or composition of the cell surface.

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