ELECTROKINETIC PHENOMENA. III

THE "ISOELECTRIC POINT" OF NORMAL AND SENSITIZED MAMMALIAN ERYTHROCYTES

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I

INTRODUCTION

Kozawa (1) observed in 1914 that the addition of sufficient acid to mammalian red blood cells brought them to an isoelectric point at a pH which was characteristic for the cells of each of the animals studied. The amount of acid needed, however, was not constant for any particular animal, but decreased with the time the cells were permitted to stand in contact with the acid. It is evident, therefore, from these early experiments that time as well as acidity was influential in producing a reversal of sign of charge. These facts have not hindered a more or less general acceptance of the isoelectric point of red blood cells as a physical constant, apparently in much the same sense that it exists for the proteins. The reversal by hydrogen ion of the sign of charge of surfaces coated with proteins like egg albumin, and of globulin particles, occurs independent of time, with equilibrium, as far as our present technic permits, occurring instantly and remaining constant. The isoelectric point of a system is not significant in a simple sense unless it remains constant with time. If it does not remain constant, the variation points to a change in the surface or substance investigated and the "isoelectric point" does not represent a physical constant characteristic of the system. If the point of equilibrium is unrelated to the original system, except in a secondary way, the isoelectric point determined may be the resultant of many forces whose resolution can hardly be determined by the simple determination of reversal of sign of charge.

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Coulter (2) (1920) studied the migration of sheep's red blood cells washed with saline solution and suspended in a saccharose solution made acid with HCl, acetate, or phosphate buffers. He used a macroscopic method which involved long experimental periods before readings of mobility could be made. Coulter concluded from his data:

". . . . that the direction and rate of movement in the electric field of both normal and sensitized red blood cells is a function of the hydrogen ion concentration. At concentrations less than pH 4.6 the charge carried is negative and increases in amount with the alkalinity; pH 4.6 represents the isoelectric point; at concentrations greater than pH 4.6 the charge carried is positive and increases in amount with the acidity."

"A comparison of the two curves[1] shows that on the alkaline side of the isoelectric point the charge of normal cells is greater and increases more rapidly with alkalinity than the charge of sensitized cells."

Shortly after the work of Coulter it was found by Eggerth (3) that the migration velocities of *B. coli* suspended in buffer mixtures commonly used for acid agglutination underwent a change in mobility, the organisms becoming less negative. He concluded that these changes were due to the extraction of a protein which was combined at the interface of organism and medium. In a second paper Eggerth (using a microscopic method to determine mobility) pointed out that similar changes occurred in erythrocyte suspensions.

Eggerth states, on the one hand:

"Human and sheep erythrocytes when placed in 0.01 N phthalate buffer solutions at reactions more acid than pH 5.2 undergo a progressive change in potential, becoming less negative or more electropositive. This change usually occurs within two hours at ordinary room temperatures. . . . This change is primarily due to the liberation of hemoglobin from the cells."

On the other hand, after thus showing that red cells were destroyed in acid solutions and that their mobilities varied with time, he states:

"The isoelectric point of erythrocytes in the absence of salt or in the presence of salts having both ions monovalent occurs at pH 4.7. This confirms the observations of Coulter."

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[1] Curves for normal and sensitized cells.

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Netter (4) has made a careful investigation of the electrophoretic mobilities of horse and ox cells in acid solutions to determine the isoelectric points. The isoelectric point of washed and unwashed horse red cells was at pH 4.7 in M/40 acetate. Washed ox cells were found to be isoelectric at pH 4.3 in M/40 acetate.

These data of Netter are concordant with those of Eggerth, not only in the existence of an isoelectric point for the red cells investigated but also in the fact that near the isoelectric point some time passed before "equilibrium" was obtained. He states:

"Das Potential stellt sich nach Uebertragen in die Loesung im allgemein schnell ein und bleibt constant, nur in der Gegend des isoelektrischen Punktes is das endgultiges Potential erst nach *2* Stunden spätestens erreicht; *alle Messungen wurden daher nicht vor 2 Stunden nach dem Ansetzen der Suspensionen begonnen.*"

Netter cites further the data of Eggerth, mentioning the effect of the products of red cell destruction.

Eagle (5) (1930) states:

"Similarly, sensitization changes the cataphoretic potential of red cells; however, according to Coulter and confirmed by us, the cataphoretic isoelectric point remains at its *normal* value (pH 4.7)"

(the italics are ours). Further on this author writes:

"Red cells[2] both normal and sensitized have a minimum velocity at about pH 4.7: in more acid reaction there is hemolysis, with currents making readings impossible."

Fig. 1 summarizes graphically some of the pertinent results of Coulter, Eggerth, Netter and Eagle. All of the data of these authors are early or late *equilibrium* values of the isoelectric point obtained in *acid* solutions incidental to the presence of the products of hemolysis.

The existence of an isoelectric point for the mammalian red cell will be here more closely scrutinized in the light of these previous investigations and of our own. It will be shown that it is, at present, more in keeping with experiment to believe that normal red cells have no known values of pH for their isoelectric points, when suspended in uni-univalent electrolytes. While reversal of sign of charge may occur,

[2] The animal from which the red cells were obtained is not given.

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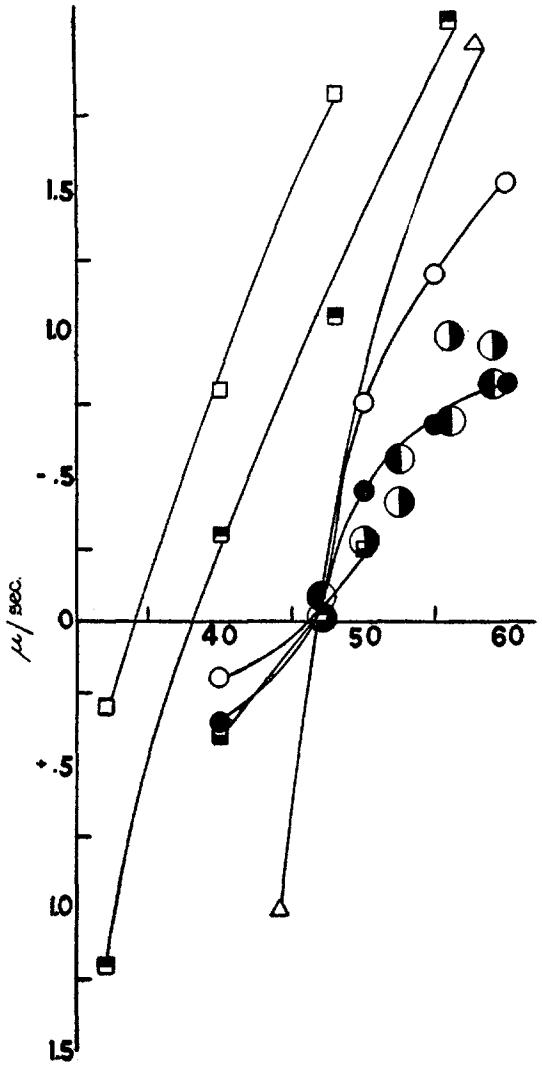

FIG. 1. The relationship between pH and electric mobility according to various authors:

○ Coulter; normal cells (relative units).
● Coulter; sensitized cells (relative units).
□ Eggerth; zero time
▣ Eggerth; 1.5 hours
▬ Eggerth; rabbit cells in 0.01 N NaCl.
} Human cells in saccharose buffered by 0.01 phthalate.
△ Netter; washed horse red cells in sugar buffered by N/40 acetate.
◑ Eagle; normal red cells in NaCl solution buffered by acetate (relative units).
◐ Eagle; sensitized red cells.
For further description, see text.

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it will be shown that its demonstration is most unlikely with intact, normal human and sheep red blood cells.[3]

II

Methods

The measurements were performed in the modification of the Northrop-Kunitz cell described previously (6). Measurements in acid solutions were usually made only at one level (0.2) in the cell, since destruction of cells occurred in acid solutions so rapidly that further measurements were inconvenient. At the level, 0.2, the speed of the particle relative to the liquid is measured.

All measurements were made within two minutes of the time that acid was added to the system.

The description of the experimental procedure is best given with each experiment, in the next section.

The red cell suspensions were buffered sufficiently to prevent the occurrence of significant changes in pH due to hemolysis. No experiments with saccharose or other sugar were performed, for the following reason. Change in ionic strength is known to influence greatly the solubility and state of proteins. More normal conditions may be obtained for proteins existing at a phase boundary by not varying ionic concentration or species unnecessarily. If the ionic strength is varied it is evident that a new variable is introduced.

Hydrogen ion activity was determined by the quinhydrone electrode. All measurements are referred to pH 4.62 as the pH of Michaelis acetate standard.

III

The Isoelectric Point of Normal and Sensitized Cells

Experiment I.—Unwashed Human Red Blood Cells. Human blood was drawn up into the stem of a white blood cell pipette from a pricked finger and mixed directly with 100 cc. of 0.85 per cent sodium chloride solution buffered by N/50 sodium acetate-acetic acid solutions. Measurements of mobility were made within 2 minutes of preparation of the suspension.

Note that reversal of sign of charge did not occur; nor was there an appreciable decrease in mobility with decrease of pH. The fact that the mobility at pH 3.9 was so close to that observed at pH 5.7, where practically no destruction of red cells occurs within the short interval,

[3] Preliminary data have also been obtained for rabbit red cells. The conclusions are similar.

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indicates that these mobilities are related more to the normal chemical constitution of the red cell than to complex phase boundary conditions occurring in acid media.

Experiment II.—Washed Human Cells. 5 cc. of human blood were poured into 25 cc. of 0.85 per cent NaCl and centrifuged. The cells were washed 6 times with 10 times their volume of 0.85 per cent NaCl containing N/100 sodium acetate. Measurements of mobility were made within 2 minutes after addition of acetic acid.

Table II confirms that data found in Table I.

TABLE I

pH	V μ/sec/volt/cm.
5.7	−1.15
4.6	−1.03
4.0	−0.97
3.9	−1.02

TABLE II

pH	V μ/sec/volt/cm.
5.6	−1.05
4.1	−1.10
3.6	−1.04
3.55	−0.92

Experiment III.—Washed Sheep Cells. Fresh defibrinated sheep cells which had been in the ice chest for 12 hours[4] were washed 8 times with about 10 times their volume of 0.85 per cent sodium chloride solution containing N/50 sodium acetate. The acetate was added to keep the suspension alkaline. Suspensions of red cells were made up as in Experiment I and measurements were made within 2 minutes.

Because destruction of cells occurs with such rapidity in acid solutions, the slight diminution in mobility described in Table III cannot be unequivocally correlated with decreasing pH. The possibility

[4] No appreciable change in mobility occurs if horse red cells are kept in serum in the ice chest as long as 3 days.

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remains that this slight but definite change may be due to adsorption of the products of erythrocyte destruction. This will be subsequently discussed in detail.

Experiment IV (A).—Sheep Cells in the Presence of Rabbit Serum. Sheep cells of Experiment III[5] were added to 50 cc. of 0.85 per cent sodium chloride containing, in addition, N/50 sodium acetate, and 1 cc. of washed[6] normal rabbit serum which had been heated to destroy complement. The sheep cells were allowed to

TABLE III

pH	V μ/sec/volt/cm.	Remarks
6.45	−1.16	In these acid solutions hemolysis was very rapid and at times complete within 2½ minutes
6.26	−1.03	
5.91	−1.12	
4.73	−1.01	
4.30	−0.98	
3.72	−0.97	
3.62	−0.94	
3.60	−0.88	

TABLE IV

pH	V μ/sec/volt/cm.	Remarks
6.18	−1.14	Control: No serum; pH 3.51, V = −.75 μ/sec/volt/cm.
4.25	−0.97	
3.90	−0.56	
3.67	±	

remain in contact with this solution for 15 minutes. Measurements of mobility were made within 2 minutes after addition of acid. See data in Table IV.

Table IV demonstrates that above pH 4.2 the presence of rabbit serum protein does not appreciably influence sheep red cell mobility. This is in harmony with our previous findings that human cells sus-

[5] Twelve hours later and washed twice with salt-acetate.

[6] This refers to a preliminary treatment of the "normal" rabbit serum with rabbit red cells to remove any amboceptor. This treatment does not remove an appreciable quantity of serum protein.

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pended in isotonic media containing 1.4 per cent of rabbit serum retain their surface integrity and migrate about twice as quickly as rabbit cells suspended in the same medium (7). The interpretation of the fact that red cells are isoelectric at about pH 3.6 is difficult. Since this change in mobility occurs in a region where red cell destruction is extremely rapid, it seems reasonable to conclude that the drop in

TABLE V

pH	V μ/sec/volt/cm.	Remarks	
		Controls pH	Without serum V μ/sec/volt/cm.
5.50	−1.00	5.55	−1.04
4.65	−1.00	4.65	−0.91
4.60	−0.95	3.54	−0.79
4.10	−0.53		
3.74	negative		
3.64	positive		

TABLE VI

pH	V μ/sec/volt/cm.	Remarks	
		Controls pH	No serum V μ/sec/volt/cm.
6.5	−0.58	4.72	−1.04
4.97	−0.60	3.5	−0.89
4.70	−0.45		
4.0	−0.43		
3.6	±		

mobility and attainment of an isoelectric state is determined by the presence of abnormal red cell surfaces as well as by the presence of serum protein. We believe, therefore, that the isoelectric point noted has no more significance than that which may be attached to those red cell systems described in the introduction.

Experiment IV (B).—Sheep Cells in Presence of Rabbit Serum. Experiment repeated in N/100 acetate buffer. See data in Table V.

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The discussion following Table IV applies here.

Experiment V (A).—Sensitized Sheep Cells. The sheep red cells used in IV (B) were kept in the ice chest for 6 days. At the end of this time they were washed 8 times with about 10 times their volume of 0.85 per cent sodium chloride containing N/50 sodium acetate. A very dilute suspension of the cells (about 1:2000) was permitted to remain about 15 minutes in contact with 24 cc. of physiological saline containing 1 cc. of an inactivated rabbit anti-sheep serum. At the end of this period a suitable mixture of electrolytes was added to make conditions identical with part (b) of this section. Measurements were completed within two minutes. See data in Table VI.

Experiment V (B).—Sensitized Sheep Cells. Experiment V (a) was repeated in N/100 acetate buffer. See data in Table VII.

TABLE VII

pH	V μ/sec/volt/cm.
5.5	−0.57
4.35	−0.60
4.1	−0.40
3.9	−0.28
3.8	negative
3.7	±
3.6	+0.24

The data of Tables VI and VII demonstrate the well known effect of specific immune serum on red cell mobility. In one sense they confirm Coulter's statement that the isoelectric point of sensitized cells is not perceptibly different from that of normal cells. In view, however, of the fact that an excess of serum protein is present, and furthermore that protein of the serum alone is capable of inducing an isoelectric state near the pH noted, we feel we must ascribe the similarity found here for the isoelectric point of normal and sensitized cells to the same effect, possibly an injurious one, that has been described as occurring in solutions as acid as these. Indeed, the wide differences in mobility between normal and sensitized cells in the zones where injury to the cell surfaces is not marked, make it more likely that if isoelectric points with regard to hydrogen ion activity could be determined without red cell destruction, these points would be different.

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What effect more powerful immune sera would have on the mobility is unknown for these conditions (See Fig. 2).

IV

The Adsorption of Gelatin by Red Cells

It has been previously remarked on several occasions that horse red cells in (alkaline) serum gelatin gels do not adsorb gelatin in the same

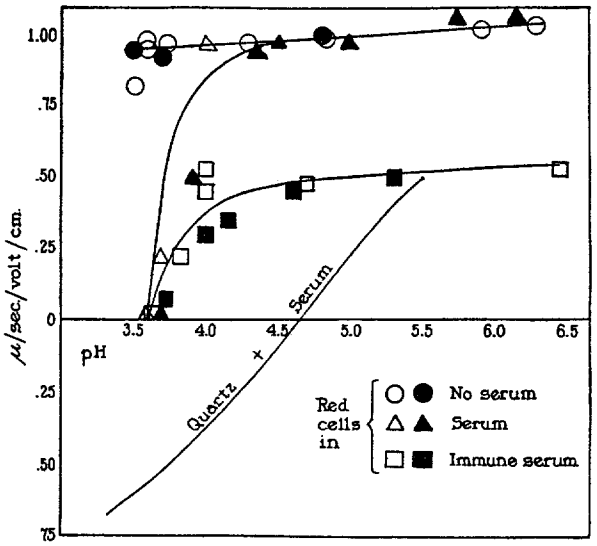

FIG. 2. A summary of the type of data obtained here for the electrophoretic mobilities of sheep red cells in 0.85 per cent NaCl buffered by acetate. Note that in the absence of serum under the experimental conditions noted in the text, the slope of the straight line describing the relationship between mobility and pH is very small. Serum has no demonstrable effect up to about pH 4.3. Immune serum changes the surface notably even above pH 4.3. Compare with Fig. 1. Quartz particles adsorb protein of the serum in characteristic fashion. (See: Abramson, H. A., *J. Gen. Physiol.*, 1929, **13**, 169.)

way that quartz particles do (8). That is, in just the same way that horse red cells preserve their surface integrity in the presence of serum, so do washed red cells keep their specific electrophoretic mobilities in the presence of gelatin in phosphate buffer at pH 7.35.

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We have seen in the preceding section that adsorption of some serum protein probably does occur in acid solutions. It was of interest therefore to observe the mobilities of washed red cells that had been in contact with gelatin, and studied in acid solutions in the presence of gelatin.

Experiment VI (A).—Washed Sheep Cells (Table VIII).

TABLE VIII

pH	V μ/sec/volt/cm.	Remarks
3.61	−0.87	Cells of Experiment II (b) in 0.2 per cent gelatin

Experiment VI (B).—Washed Human Cells (Table IX).

TABLE IX

pH	V μ/sec/volt/cm.	Remarks
5.9	−1.00	Cells of Experiment III kept 24 hours on ice, rewashed, and studied in 0.2 per cent gelatin
4.33	−1.06	
3.62	−0.92	
Control, quartz		
3.6	+0.40 approx.	
5.7	−0.25 approx.	

Experiment VI (C).—Washed Sheep Cells (Table X).

TABLE X

pH	V μ/sec/volt/cm.	Remarks
4.00	−0.93	Cells of Experiment IV (b) suspended in 0.2 per cent gelatin for 10 minutes and then examined
3.76	−0.75	
3.67	−0.65	

The data in Tables VIII, IX, and X indicate that even though red cells in acid solutions may react with certain components of a medium containing normal serum, a similar reaction does not occur with gela-

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tin. One is led to suspect that the reaction with the protein of the serum is not exactly as non-specific as the adsorption of serum protein by quartz. If so, the red cells should have adsorbed gelatin. Experiment VI (B) was made particularly striking by studying simultaneously quartz particles and red cells in the presence of gelatin. In these systems the red cells migrated to the anode and the quartz particles, because of their adsorbed protein film, migrated in the opposite direction.

It must not be thought that reversal of the sign of charge of red cells did not occur in the acid solutions. It did so repeatedly, but always after the 2-minute period of contact with the salt solution when the products of hemolysis were sufficiently concentrated to be an important factor. In *no case* was a positively charged red blood cell seen in acid solutions during the 2-minute period of observations.

It is of interest to describe how this reversal of sign of charge may occur. In Table X the cells suspended in the buffer at pH 3.67 were observed after the end of the 2-minute period during the very rapid hemolysis which occurs at this pH. Red cells which were seen migrating rapidly to the anode seemed to lose their hemoglobin suddenly and the pale "ghost" reverse its sign of charge migrating to the cathode.

V

The Reaction of Sheep Cells with Normal and Immune Sera in Phosphate Buffer[7]

An extensive series of measurements was made to investigate the effect of normal and immune rabbit sera on sheep cells We shall not give the data in detail, for these experiments in general merely confirm what has hitherto been known qualitatively. They are of interest because of the precision which has been obtained in 0.85 per cent NaCl buffered by M/150 phosphate of initial pH 7.35, the medium in which all observations of mobility have been made, and because of the associated studies of hemolysis made in conjunction with changes in mobility. In the experiments described in general in Fig. 3, five different immune sera and four normal sera were employed. Curve 1 in Fig. 3

[7] When studying the effect of sera on cells, at least 1 hour's time elapsed between preparation of suspension and measurement.

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was obtained by studying washed sheep cells in normal inactivated rabbit serum. It is evident that no change in mobility occurs up to about 1:50 of serum. Curve 2 has also been obtained with normal serum from another rabbit. At low serum dilution a definite lowering of electrophoretic mobility occurred. That this change was real, was evidenced by the fact that after addition of more sheep cells to the suspension, cells having two different mobilities were present. Curve 3 is a typical curve obtained with inactivated anti-sheep serum. Approximately the same value of mobility was obtained for all the

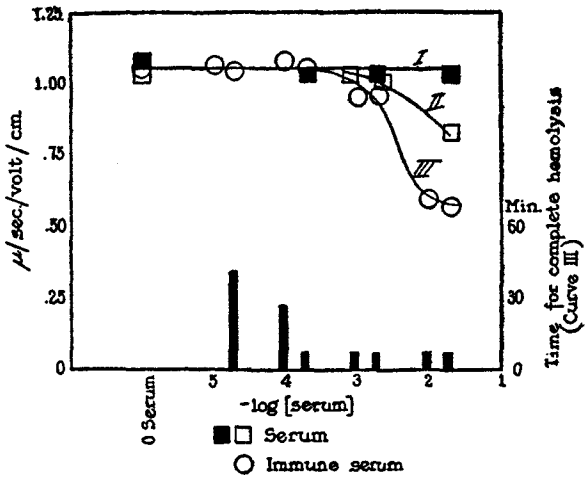

FIG. 3. See Part V of text. The upright black rectangles represent hemolysis times.

immune sera. Whether this is the lowest mobility obtainable cannot be decided upon from the shape of the curve, for the possibility exists that a more powerful anti-serum may be obtained than that employed.

The cells with which Curve 3 had been obtained were studied with a suitable dilution of complement to ascertain the relationship between the lowering of mobility and susceptibility to hemolysis by complement. It is to be noted that complete hemolysis on the addition of complement (0.03 cc. guinea pig serum to 1 cc. of a dilute red cell suspension) occurred in all except the highest dilution of serum, much before any perceptible change in mobility occurs. In other words,

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sensitization to hemolysis of the red cell surface occurs without any change in mobility. In fact, hemolysis was just as rapid in the 1:5000 dilution as in the lower ones. At this dilution, as is apparent from the figure, there is no change in mobility, but hemolysis is rapid and complete. Since the mobility of the cell is in general determined by its surface constitution, very few active groups of the cell surface are changed for preparation of the cell for hemolysis incidental to the presence of complement. The lowering of the electrophoretic mobility does indicate a notable change in the surface make-up of the cell. As far as hemolysis requirements are concerned this change is secondary and must be associated with other immunological properties of the cell surface acquired incidentally to sensitization by a large quantity of amboceptor.

VI

SUMMARY

A survey of the published electrophoretic mobilities of certain mammalian red cells reveals that the isoelectric points accorded to these cells are the result of equilibria incidental to red cell destruction. The electrophoretic mobilities of normal washed sheep and human cells have now been studied in 0.85 per cent NaCl solutions from about pH 3.6 to 7.4. All measurements were made within 2 minutes of the preparation of the suspension of red cells. In no case was reversal of sign of charge observed under these conditions. Reversal of sign of charge occurred only after sufficient time had elapsed to permit sufficient adsorption of the products of red cell destruction. There is little change in mobility as the pH of the medium is decreased. Reversal of sign of charge does occur in the presence of normal and immune (anti-sheep) rabbit sera. The isoelectric point determined under these conditions does not appear to be connected specifically with the immune body but is perhaps associated with phenomena incidental to red cell destruction and the presence of serum. The characteristic lowering of mobility by amboceptor occurs, however, from pH 4.0 to pH 7.4. The curves of mobility plotted against pH for normal and for immune sera support the viewpoint that the identity of the isoelectric points for normal and sensitized sheep cells is not primarily concerned with the immune reaction. It is most unlikely that an "albu-

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min" or a "globulin" surface covers red cells with a complete protein film. Although serum protein reacts with red cells in acid solutions, this is not demonstrable for gelatin. The lowering of mobility usually ascribed to anti-sheep rabbit serum may also occur, but to a lesser degree, in normal rabbit serum. This diminution of mobility is not, in the first place, associated with sensitization to hemolysis induced by complement. This supports the view that only a very small part of the red cell surface need be changed in order to obtain complete hemolysis in the presence of complement.

I am indebted to Professor Wm. J. Crozier for reading the manuscript and making many acceptable suggestions.

Addendum.—The viewpoint of McCutcheon, Mudd, Strumia, and Lucké (*J. Gen. Physiol.*, 1930, **13,** 669) in regard to the isoelectric point of cells has recently appeared.

BIBLIOGRAPHY

1. Kozawa, S., Biochem. Z., 1914, **60,** 146.
2. Coulter, C. B., *J. Gen. Physiol.*, 1920, **3,** 309, 513.
3. Eggerth, A. H., *J. Gen. Physiol.*, 1924, **6,** 63, 587.
4. Netter, H., *Arch. ges. Physiol.*, 1925, **208,** 16.
5. Eagle, H., *J. Immunol.*, 1930, **18,** 393.
6. Abramson, H. A., *J. Gen. Physiol.*, 1929, **12,** 469.
7. Abramson, H. A., *J. Gen. Physiol.*, 1929, **12,** 711.
8. Abramson, H. A., *J. Gen. Physiol.*, 1928, **11,** 743.

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