JCI The Journal of Clinical Investigation

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S. William Ross, Richard V. Ebert

J Clin Invest. 1959;38(1):155-160. https://doi.org/10.1172/JCI103785.

Research Article

MICROELECTROPHORESIS OF BLOOD PLATELETS AND THE EFFECTS OF DEXTRAN * †

By S. WILLIAM ROSS and RICHARD V. EBERT

(From the Department of Medicine, University of Arkansas Medical Center, Little Rock, Ark.)

(Submitted for publication April 28, 1958; accepted September 25, 1958)

The intravenous administration of dextran has previously been shown to produce a bleeding tendency in laboratory animals and in humans. Extensive studies concerning the hemostatic defect have yielded much useful information. Carbone, Furth, Scott and Crosby (1) found that the major abnormality consisted of a prolongation of the bleeding time. In seven of 11 patients given 1,500 to 6,500 ml. of dextran intravenously over a five day period the bleeding time was prolonged over 30 minutes. The concentration of platelets, clotting time, fibrinogen level, clot retraction time and prothrombin consumption test were not significantly affected by dextran. The prothrombin time was slightly abnormal. It appears unlikely that this finding explains the hemostatic defect because of the inconstancy and mild degree of the change. Adelson, Crosby and Roeder (2) found that smaller amounts of dextran were required to produce a prolonged bleeding time in dogs made thrombocytopenic by irradiation than in normal dogs. Comparable volumes of gelatin in the thrombocytopenic dogs did not prolong the bleeding time. Hemodilution, therefore, appears to be eliminated as a cause of the defect. These results have suggested that the platelet, the vessel wall, or both may be affected by dextran.

Since there is ample indication that the platelet is concerned with changes in the bleeding time, it seemed pertinent to procure more evidence concerning the effect of dextran on the platelet. Rothman, Adelson, Schwebel and Langdell (3) have shown that C^{14} labeled dextran is taken up by the platelet. In substantiation of this effect Ponder noted that the electrophoretic mobility of the platelet in saline appeared to be modified by dextran (4). He pointed out, however, that the electrophoretic mobility may be influenced by factors that are difficult to control or appraise. In the determination of the isoelectric point these uncontrolled factors may be eliminated or reduced significantly in their importance.

Microelectrophoresis has been used rather extensively to study the red cell and those changes in its environment that affect its surface charge (5, 6). Recently Creger, Tulley and Hansen (7) utilized this method to determine the effect of hydrocortisone on red cell-antibody union. Relatively few studies of the platelet have been carried out by microelectrophoresis. Abramson (8) found that the horse platelet migrated at a rate of 0.45 μ per second per volt per cm. in oxalated plasma. The pH was not recorded. The true mobility was determined by correcting for the electroosmotic effect at the center of the electrophoresis cell. This determination of the true mobility is accomplished more simply by observing the mobility at one of the stationary levels of the cell. Our studies include observations on the electrophoretic mobility and isoelectric point of platelets suspended in various buffer and protein solutions and the effect of dextran on these measurements.

METHOD

Blood was obtained from patients without hematologic disease and from normal humans. Multiple donors were used for the determination of each isoelectric point. Blood was drawn using nonwettable equipment. Platelet suspensions were prepared in the following manner: *1)* A silicone-coated needle was inserted into an antecubital vein after a tourniquet had been applied for no more than one minute. The first few drops of blood were discarded. *2)* Fifteen ml. of blood was then permitted to flow down the side of a silicone-coated tube containing 0.5 ml. of 5 per cent sequestrene. *3)* Thirty ml. of blood was spun at 5 to 10° C. in a refrigerated (International

* These studies were supported by a contract (DA 49-007-MD-736) with the Department of the Army, Office of Surgeon General, Research Development Division, and by a grant from the Leo N. Levi Memorial Hospital, Hot Springs, Ark.

† Presented in part before the Southern Section of the American Federation for Clinical Research at New Orleans, Jan. 20, 1957.

PR-2) centrifuge at 1,000 rpm for 25 minutes. *4*) The supernatant was aspirated with a plastic dropper and placed in a silicone-coated tube. *5*) This was then re-centrifuged at 2,000 rpm for 25 minutes at 5 to 10° C. *6*) The plasma was aspirated and the button of platelets was resuspended in 0.912 per cent saline. *7*) The latter was centrifuged at 2,000 rpm and the supernatant aspirated and discarded. *8*) The button of platelets was washed with 2 to 3 ml. of suspending solution (chilled) and resuspended in 20 ml. of suspending solution. The suspending solution was saline, or a mixture of saline and protein, or saline and dextran. The final ionic strength was 0.156. It was assumed that the proteins in the various solutions did not contribute significantly to the ionic strength. The pH of the saline or saline dextran solution was adjusted with citrate and phosphate buffers prepared according to Clark (9). The pH values of the protein solutions were adjusted by titration with 0.01 N HCl. Hemoglobin solutions were prepared by hemolyzing the blood with distilled water and removing the stroma with toluene. Human albumin and gamma globulin solutions were supplied by courtesy of the American Red Cross.

Specific conductivity measurements were made using General Radio Company Impedance Bridge, Type No. 650A, a CDA and a CDB Cornell-Dubilier Decade Capacitor, and a Leeds & Northrup Jones Conductivity Cell, Catalog No. 4914. Hopps and Engles (10) have discussed problems related to these measurements. Viscosity measurements were made using Ostwald viscosimeters. A horizontal type Abramson microelectrophoresis apparatus[1] was used (6). This cell was set in a plastic base which in turn was fixed to the stage of a phase microscope so that the center of the cell lay under the objective lens. One of the oculars contained a grid which was calibrated and served as the distance marker. The oculars were 20× and the objective was a 20× long working distance phase lens. The cell was filled with platelet suspension by turning the stopcocks to communicate with the funnel and waste outlet. All visible air bubbles were removed. The stopcocks were turned to close the cell and circuit and the current turned on. A two way switch allowed the current to be reversed manually and automatically reversed the current every one to three minutes. The cell was calibrated to make certain it was of proper shape and dimensions. This was accomplished by focusing at the top of the cell and timing the movement of platelets in first one and then the opposite direction over the measured distance. Usually 10 to 20 readings yielded a reliable average figure. These readings were taken at several levels from the top to the bottom of the cell.

The rate of migration was then plotted against cell depth. If the cell was made of uniform dimensions, a parabolic curve formed. According to the established theories of Smoluchowski (11) and Komagata (12), if the ratio of width to depth of the cell is adequate, the mean height of this curve will intersect the parabolic curve at 20.2 per cent to 21.1 per cent of the depth from the top as well as from the bottom of the cell. These levels then represent the stationary layers where mobility measurements unaffected by electro-endosmosis may be made. All measurements were made at one of these levels. The corrected velocity in μ per second per volt per cm. is equal to

$$\frac{V_{obs}\, K_s\, q\, \eta_r}{I}$$

where V_{obs} is the observed velocity in μ per second; K_s, the specific conductivity; q, the cross-sectional area of the microelectrophoresis cell in cm.^{2}; I, the current in amperes; and η_r, the relative viscosity. A discussion of some of the technical problems associated with this technique is given by Ponder and Ponder (13).

RESULTS

Mobility measurements of platelets were made in various citrate and phosphate buffers ranging in pH from 7.5 to 3.2. The corrected velocities were then plotted against pH and connected by a solid black line as shown in Figure 1. The isoelectric point was assumed to be the intersection of the zero mobility and the curve. The isoelectric point of platelets in buffered saline solution was 3.98 (Table I).

By this technique the curve and isoelectric point of saline washed platelets were then determined in serum, 1 per cent gamma globulin, and 4 per cent gamma globulin with pH adjusted by 0.01 N HCl. The isoelectric points ranged from 4.41 to 4.59 (Table I). These isoelectric points were significantly different ($p < 0.001$) from that of platelets in citrate buffer.

The isoelectric point of platelets in 3 per cent albumin was found to be 4.18. The difference between the isoelectric points of platelets in citrate buffer and those in 3 per cent albumin was significant ($p < 0.001$).

The effect of dextran[2] was studied by allowing washed platelets to remain in 3 per cent or 6 per cent dextran for 30 minutes. These platelets were then resuspended in a solution having a final concentration of 3 per cent dextran and an identical citrate concentration and ionic strength as in the previously described experiments. The isoelectric points of platelets exposed to 3 per cent and 6 per cent dextran for 30 minutes were

[1] Built by Scientific Glass Apparatus Co., Inc., Bloomfield, N. J.

[2] The dextran solution was supplied by courtesy of the R. K. Laros Co. It contained 6 per cent hydrolyzed fractionated dextran in 0.9 per cent sodium chloride solution for clinical use.

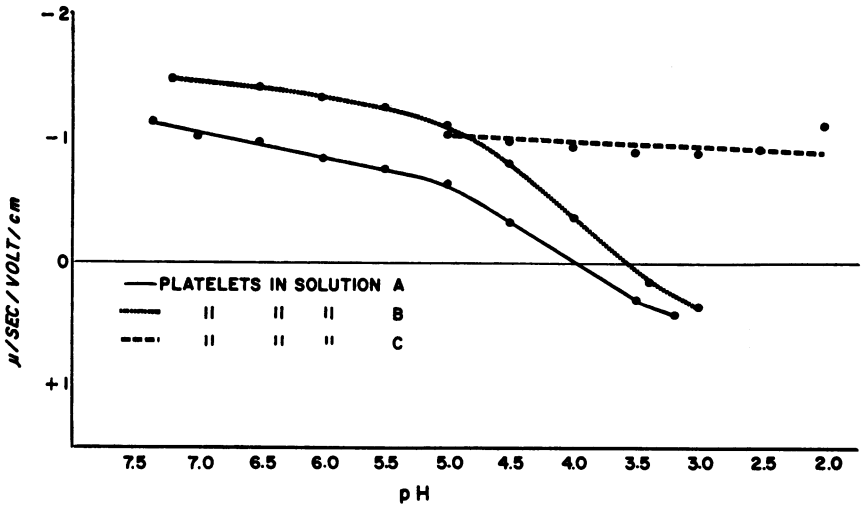

FIG. 1. CORRECTED VELOCITY CURVE OF PLATELETS IN VARIOUS SOLUTIONS AT DIFFERENT pH LEVELS

Solution A is made up of one part citrate or phosphate buffer and nine parts of saline. Solution B is made up of 6 per cent dextran in one part citrate or phosphate buffer and nine parts of saline. Solution C is made up of 0.5 mg. per cent heparin in one part citrate or phosphate buffer and nine parts of saline.

3.83 and 3.63, respectively. These differ significantly ($p < 0.001$) from the isoelectric point of platelets unexposed to dextran (Table I).

Platelets were exposed to 6 per cent dextran for 30 minutes. Serum and saline solution was added so that the final concentration of dextran was 3 per cent and of serum protein approximately 1 per cent. The isoelectric point was found to be 4.49 and was not significantly different from that obtained with platelets unexposed to dextran but suspended in serum. This clearly demonstrates that dextran does not prevent coating of the platelets by serum proteins.

Since most of the isoelectric points were found to be in a fairly narrow range it seemed desirable to explore the possibility of producing more marked changes in the isoelectric point of the platelets. The isoelectric point of platelets suspended in a 3 per cent hemoglobin solution was determined in the previously described manner and was found to be 5.27 (Table I). The difference between this and the isoelectric

TABLE I

Isoelectric points of saline washed human platelets suspended in various solutions*

Pretreatment	Suspending solution	I.E.P.†	S.D.‡	Range
	0.5 mg. % Heparin in P-C-S§	<2.18		
Exposed to 6% dextran, 30 min.	3% Dextran in P-C-S	3.63	0.10	3.48–3.80
Exposed to 3% dextran, 30 min.	3% Dextran in P-C-S	3.83	0.09	3.72–3.92
	P-C-S	3.98	0.08	3.83–4.10
3 saline washes	P-C-S	3.99	0.06	3.91–4.05
	3% Albumin in saline	4.18	0.08	4.08–4.27
	Human serum	4.41	0.16	4.13–4.58
	1% Gamma globulin in saline	4.49	0.13	4.34–4.68
Exposed to 6% dextran, 30 min.	Serum protein 1%, dextran 3%	4.49	0.08	4.38–4.62
	4% Gamma globulin in saline	4.59	0.04	4.54–4.63
	3% Hemoglobin	5.27	0.30	4.93–5.78

* Unless otherwise stated, platelet suspensions were washed once with saline.
† Isoelectric point.
‡ Standard deviation.
§ One part of citrate or phosphate buffer and nine parts of saline solution.

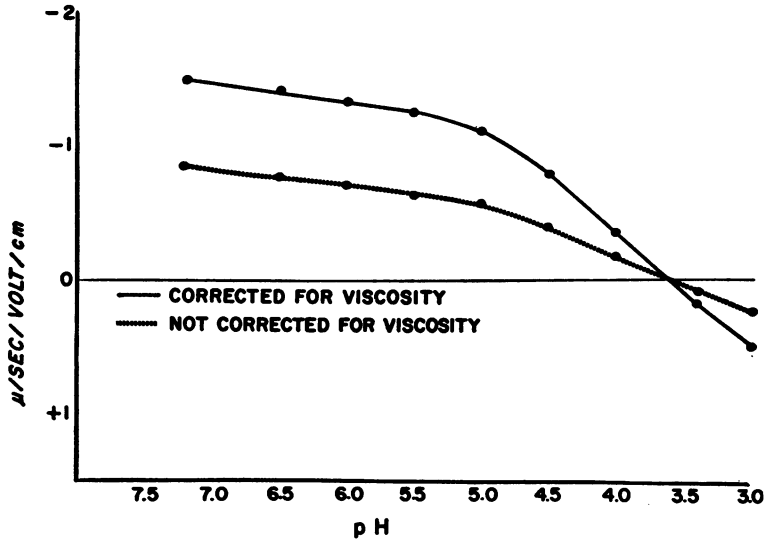

FIG. 2. THE EFFECT OF VISCOSITY ON THE MOBILITY CURVE OF PLATELETS SUSPENDED IN A SOLUTION CONTAINING 6 PER CENT DEXTRAN AND ONE PART CITRATE OR PHOSPHATE BUFFER IN NINE PARTS OF SALINE

point of platelets in 4 per cent gamma globulin was significant ($p < 0.001$). At the other end of the scale platelets suspended in 0.5 mg. per cent heparin and citrate buffer were negatively charged at a pH as low as 2.18 (Figure 1). The isoelectric point was not determined because of the instability of the velocity at these low pH values. Readings had to be made in less than two minutes or sudden changes in direction of migration would occur. Heparin was added to serum and solutions of albumin and gamma globulin. Platelets suspended in these solutions were found to have the same isoelectric point as platelets in similar protein solutions without heparin. This was true with concentrations of 2.0 mg. per cent heparin in the final solution.

It is evident from these experiments that the isoelectric point of platelets is modified by the suspending medium. The isoelectric points of platelets in the protein solutions are in the same relative order as the isoelectric points of those proteins. In no case, however, is the isoelectric point of the platelets the same as that of the protein. This may represent a partial coating of the particle which would fit Abramson's key spot hypothesis (6). It is also interesting to note that Abramson (14) found that quartz particles suspended in serum had an isoelectric point of 4.7 which is close to the isoelectric point of platelets in serum.

DISCUSSION

The theory of Helmholtz and Lamb indicates that electrophoretic mobility should be proportional to the zeta potential and inversely proportional to the viscosity of the medium, provided that the thickness of the double layer, the facility of slip and the dielectric constant remain unchanged. Ponder (4) used this relation in the form

$$\frac{U}{U_0} = \frac{v\,\eta}{v_0\,\eta_0}$$

where U was the zeta potential, v the mobility, η the viscosity, and the subscript zero referred to saline without dextran. His values of U/U_0 for platelets in 1 per cent dextran at pH 7.0 averaged 1.27, while our figure for platelets in 3 per cent dextran at pH 7.0 is 1.40.

Ponder questioned the validity of treating platelets as molecular particles with regard to the effect of viscosity on flow. The determination of the isoelectric point eliminates the problem of how to treat the effect of viscosity. In our experiments the isoelectric point was observed to be essentially unchanged whether or not the points of the curve were plotted with a consideration for the effect of viscosity (Figure 2).

The platelet suspended in a protein solution migrates in an electrical field in a fashion comparable to leucocytes and inert particles. The

evidence gained from electrophoresis suggests that the platelet is partially coated by the protein in which it bathes. This is in sharp contrast to the evidence that erythrocytes suspended in a protein solution are not coated by this protein (15, 16). It appears that there may be a variance in the type or degree of such coating between leucocytes and platelets but there is still an inadequate amount of information about the former to warrant any final conclusion.

Abramson, Gorin and Ponder (17) concluded that platelets suspended in serum are coated by serum albumin A. They arrived at this conclusion on the basis of similar mobilities among quartz particles, leucocytes and platelets in serum and the evidence by Moyer and Moyer (18) that quartz particles are coated by serum albumin A. In view of the isoelectric point differences between platelets in albumin and in serum it appears that platelets suspended in serum must be at least partially coated by globulin.

The effect of dextran on platelet mobility is interesting in that dextran is essentially nonionic. A possible reason for the effect of dextran on platelet mobility is that the dextran might conjugate with some of the proteins on the cell surface and by so doing may change the orientation of the proteins. Support for this view is found in the observation of Rozenfel'd and Plyshevskaya (19) that dextran does conjugate with protein.

The fact that dextran does not influence the isoelectric point of platelets suspended in serum does not militate against a significant influence of dextran on the surface of the platelet. It should be noted that heparin had a pronounced effect on the platelet mobility when the platelets were suspended in saline but that even in extremely high concentrations it did not affect the mobility of platelets in protein solutions. These latter results are in the face of Zucker's (20) observation that high doses of heparin increase the mesenteric bleeding time of rats and prevent the formation of an adequate platelet thrombus. Abramson's "key spot" hypothesis again may come into play as an explanation of the apparent dichotomy of results. He showed that sheep erythrocytes when sensitized by the addition of small amounts of rabbit amboceptor did not have their electrophoretic mobility altered and still could be hemolyzed by the addition of complement.

SUMMARY AND CONCLUSIONS

1. The isoelectric point of saline washed human platelets suspended in citrate buffer is 4.0.

2. When platelets are suspended in a solution containing human albumin, serum, gamma globulin or hemoglobin, the platelets appear to be partially coated by the protein in the surrounding medium, as evidenced by a change in the isoelectric point toward that of the protein.

3. Platelets suspended in serum have an isoelectric point significantly different from platelets suspended in albumin solutions alone but not significantly different from platelets suspended in a solution containing gamma globulin.

4. Heparin produces a marked increase in the negative charge of platelets in citrate buffer.

5. Dextran causes an alteration in the mobility and a significant lowering of the isoelectric point of platelets in citrate buffer.

6. Our observations support the view that dextran affects the platelet surface. It is possible but unproven that this change prevents the platelets from forming a platelet thrombus in dextran treated animals.

ACKNOWLEDGMENT

The authors appreciate the technical assistance of Mrs. Doris Ann Warren.

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