The Effect of Dextrans on Zeta Potential* (34050)

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The effect of dextran fragments on suspension stability of erythrocytes has been the subject of many previous studies. Measurements of red cell electrophoretic mobilities in dextran solutions were interpreted as showing an increased negative surface charge (1) but in those experiments some of the mobilities were run on heparinized blood—although heparin uniformly increases the erythrocyte negative surface charge. In addition, concentrations of dextran were used far in excess of those obtainable clinically and solutions of buffered dextrose, rather than plasma, were investigated in some of the experiments (2). *In vitro* observations of cell aggregation could not be correlated with the electrophoretic results since 70,000 ml wt dextran produced cellular aggregation in spite of an "increased negative charge."

Erythrocyte disaggregation, loss of suspension stability, or reversal of sedimentation rate has been uniformly produced by dextran fragments of <60,000 mol wt (3–5) and aggregation by higher molecular weight fragments (5).

In nonbiologic systems the suspension stability of colloids is clearly related to the zeta potential of the particle rather than net surface charge (6). The zeta potential is a reflection of surface charge (as measured by electrophoretic mobility) as well as the viscosity, dielectric, and ionic composition of the suspending solution. Values of 0 to —5 mV are associated with profound agglomeration and clumping; —5 to —15 mV with moderate agglomeration and > —20 mV with dispersal of the colloid (7). We have shown in other studies that divalent ions, such as calcium (8) and proteins (albumin) (9), can profoundly effect zeta potential. There has been additional work demonstrating a reaction between dextrans and proteins (10, 11) which may be important in understanding the effects of dextrans on erythrocytes.

This paper will report a series of experiments designed to determine the effects of dextrans of approximately 40,000 mol wt (LMD) and 70,000 mol wt[2] (clinical dextran-CD) on the zeta potential of erythrocytes in buffered saline and diluted albumin solutions. Using a standard particle (minusil) as a substitute for the erythrocyte the effect of LMD and CD in solutions containing physiologic concentrations of albumin will be reported. The results clearly demonstrate that LMD minimally increases negative erythrocyte potential in physiological solutions while CD markedly reduces potential in the presence of physiologic concentrations of albumin.

Methods. A commercially available cylindrical electrophoresis cell was used. This apparatus has been described in a previous publication (9). In addition to erythrocytes obtained by venipuncture without anticoagulants, a standard test colloid (minusil), of average diameter 1.1 μ, was used (9).

The zeta potential was calculated using the standard Helmholtz-Smoluchowski equation $\zeta = 4\pi\eta u/D$ where ζ = zeta potential; η = viscosity; u = electrophoretic mobility; D = dielectric constant. In order to express the results in millivolts the formula was converted to the following: $\zeta = 113{,}000\eta u/D$

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TABLE I. The Zeta Potential of Human Erythrocytes in Albumin Solutions.

Solution	Albumin (g/100 ml)	Specific conductance (μmhos)	Electrophoretic mobility ± SE ($-\mu$/sec/V/cm)	Zeta potential (mV)
Saline–sucrose	0	5400	1.06 ± .02	—15.5
Saline–sucrose	1.25	3200	0.80 ± .02	—10.9
Saline–sucrose	2.5	5800	0.69 ± .02	—10.8

where u is expressed in μ/sec/V/cm and η in poise. D of water (78.54) was used throughout the calculations. All runs were made at 25° and viscosity of each solution was measured in an Ostwald viscometer at 25°.

Results. The effect of small amounts of albumin on the zeta potential of human erythrocytes is shown in Table I. It can be seen that the zeta potential is lowered to values compatible with agglomeration by as little as 1.25 g/100 ml albumin. This effect is similarly noted in Table II when dextran-containing solutions were tested. Note the marked drop in zeta potential produced by albumin even with increasing amounts of dextran—in excess of concentrations obtainable physiologically. It should also be noted that both 40,000 and 70,000 mol wt dextran increase the negative zeta potential in saline—producing a marked dispersing effect.

The most striking effects of dextran are demonstrated in the experiments using minusil as shown in Fig. 1 and 2. Note the sharp increase in negative potential with the addition of dextrans of either weight. Albumin alone produces a fall in potential to the range —10 to —12 mV but 70,000 mol wt dextran decreases the zeta potential to —4 mV and then to zero at physiologic concentrations of albumin.

This effect of LMD on zeta potential is less striking than that of heparin. In other similar experiments we found that the zeta potential of erythrocytes increased from —21 to —33 mV when heparin (3 drops of commercial heparin to 25 cc of solution) was added.

Discussion. There is concern among clinicians with the suspension stability of erythrocytes particularly in the microcirculation. Agglomeration of cells in these small vessels could lead to significant sludging and decreased perfusion. LMD has been proposed as a substance which may have significant antisludging effects. Some investigators have suggested that dextran of 70,000 mol wt may have similar effects. The addition of a significant number of osmotically active particles alone may produce an antisludging effect by decreasing the viscosity of the solution (through the transfer of water). It is not possible to draw conclusions about the antisludging effects of the dextrans from our experiments. However, many observed phenomena are related purely to the effects of the

TABLE II. The Zeta Potential of Human Erythrocytes in Albumin and Dextran Solutions.

Solution	Albumin (g/100 ml)	Specific conductance (μmhos)	Electrophoretic mobility ± SE ($-\mu$/sec/V/cm)	Zeta potential (mV)
Saline–sucrose				
Dextran (40,000 mol wt)				
2.5 g/100 ml	0	3400	1.06 ± .02	—25.9
5 g/100 ml	1.25	2200	0.87 ± .03	—17.4
10 g/100 ml	2.5	4500	0.57 ± .01	—14.8
Dextran (70,000 mol wt)				
1.5 g/100 ml	0	3400	1.06 ± .03	—23.2
5 g/100 ml	1.25	2200	0.79 ± .02	—16.9
10 g/100 ml	2.5	4700	0.42 ± .01	—12.9

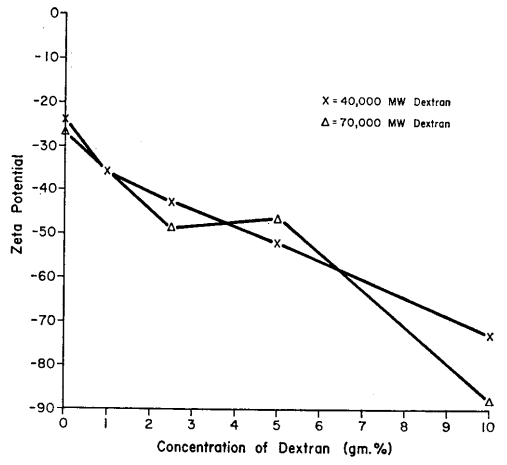

FIG. 1. The zeta potential of minusil in dextran solutions.

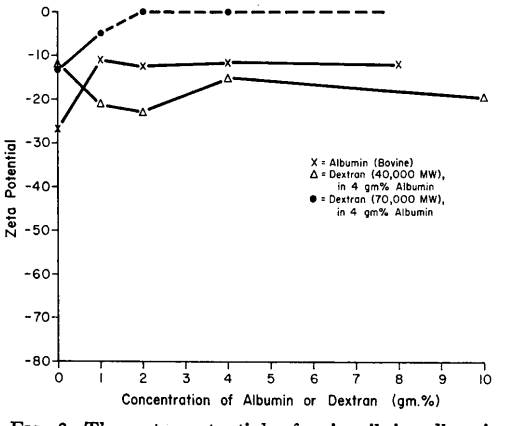

FIG. 2. The zeta potential of minusil in albumin and dextran.

dextrans on zeta potential. It has been shown that when zeta potential is greater than —20 mV there is strong suspension stability; at —5 to —15 mV there is a tendency toward agglomeration of particles and under —5 mV there is strong agglomeration (7). Our results would imply that albumin must be present to evaluate the effect of dextrans on zeta potential and these results correlate well with most of the observed phenomena. For example, the alteration of zeta potential from —10 to —20 mV by dextran of 40,000 mol wt would tend to increase the stability of erythrocytes and decrease their sedimentation rate as has been observed by others (3, 4). Dextran of 70,000 mol wt in the presence of albumin on the other hand lowers the zeta potential greatly which would lead to cellular agglomeration, increased sedimentation rate, and decreased erythrocyte suspension stability—all of which have been observed (4, 5). In fact, the use of higher molecular weight dextrans to precipitate proteins or other particles from solution could be predicted by these results (12).

It is possible that some of the hemorrhagic phenomena associated with infusion of large amounts of low molecular weight dextran are related to the increase in negative charge. In this sense dextran of 40,000 mol wt and heparin have similar effects since both increase the negative zeta potential of erythrocytes. If one were purely interested clinically in the erythrocyte-dispersing effect of dextrans he should use low molecular weight fragments to increase erythrocyte dispersal and higher weight (> 60,000 mol wt) fragments to decrease dispersal.

Summary. The effects of dextran solutions (40,000 and 70,000 mol wt) on the electrophoretic mobility of erythrocytes and a negatively charged colloidal particle—minusil—was calculated. Zeta potential was calculated from the standard Helmholtz-Smoluchowski equation. Results showed a decreased zeta potential of erythrocytes in albumin solutions and a plateau effect of both erythrocytes and minusil at a level of approximately —10 mV. Both dextrans uniformly increased zeta potential from —16 mV to an average of —24 mV in saline solutions. However, in physiologic concentrations of albumin, increasing concentrations of 40,000 mol wt dextran increased the negative zeta potential from —10 mV to —24 mV while 70,000 mol wt dextran decreased the zeta potential from —10 mV to —4 mV and then to zero.

The results imply that evaluations of the effects of dextrans on the zeta potential of erythrocytes must take into consideration interaction with albumin. Clinically, from these data 40,000 mol wt dextran would be expected to maintain erythrocyte stability in solution while 70,000 mol wt dextran would be expected to enhance agglomeration.

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