Role of Surface Electric Charge in Red Blood Cell Interactions

KUNG-MING JAN and SHU CHIEN

From the Department of Physiology, Laboratory of Hemorheology, College of Physicians and Surgeons, Columbia University, New York 10032

ABSTRACT The role of the surface charge of human red blood cells (RBC's) in affecting RBC aggregation by macromolecules was studied by comparing the behavior of normal RBC's with that of RBC's treated with neuraminidase, which removes the sialic acids from the cell membrane and reduces the zeta potential. RBC aggregation in dextrans with different molecular weights (Dx 20, Dx 40, and Dx 80) was quantified by microscopic observation, measurement of erythrocyte sedimentation rate, and determination of low-shear viscosity. Dx 20 did not cause aggregation of normal RBC's, but caused considerable aggregation of neuraminidase-treated RBC's. Neuraminidase-treated RBC's also showed stronger aggregation than normal RBC's in Dx 40 and 80. Together with the electron microscopic findings that the intercellular distance in the RBC rouleaux varies with the molecular size of dextrans used, the present study indicates that the surface charge of RBC's inhibits their aggregation by dextrans and that the electrostatic repulsive force between cell surfaces may operate over a distance of 20 nm.

INTRODUCTION

It has long been recognized that vertebrate red blood cells (RBC's) are negatively charged and that they move to the anode in an applied electric field. By the use of the microelectrophoresis apparatus, the electrophoretic behavior of human RBC's has been studied in various ionic and macromolecular environments (1-4).

Recent studies on RBC rouleau formation have led to the postulation of a mechanism involving the bridging of adjacent cell surfaces by a monolayer of macromolecules (5, 6). According to this concept of RBC aggregation, the adsorption force between the macromolecule and the cell surfaces must exceed the electrostatic repulsive force resulting from cell surface charge. The present experiments were designed to study the role of RBC surface charge in affecting the effectiveness of dextrans (neutral polysaccharides with various molecular weights) in inducing RBC aggregation. The enzyme neuraminidase was used to reduce the surface charge by removing the
N-acetyleneuraminic acid (or sialic acid) from the RBC membrane. The results of this investigation, together with the accompanying study on the effect of the ionic environment on RBC aggregation (7), have served to establish the role of surface charge in cellular interactions and to elucidate the electrochemical basis of rouleau formation.

MATERIALS AND METHODS

1. Preparation of Suspensions of Normal Red Blood Cells

Fresh blood from healthy human subjects was drawn into heparinized syringes. After centrifugation and removal of the plasma and the buffy coat, the RBC's were washed three times with a saline solution. The saline solution is composed of 0.15 M NaCl and contains 0.5 g/100 ml human serum albumin to prevent cell crenation and hemolysis (8, 9). The pH was adjusted to 7.4 with 0.3 M tris(hydroxymethyl)aminomethane. The washed RBC's were suspended either in the saline or solutions of dextrans in saline. The weight-average molecular weights and the molecular lengths of the dextran fractions (Pharmacia Labs, Uppsala, Sweden) are listed in Table I. Electrophoresis of dextran fractions on cellulose acetate membrane indicated that they are not charged. Conductivity and osmolality measurements indicate no significant contamination of electrolytes in the dextran fractions: less than 1 meq/liter for 1% dextran solutions in distilled water.

2. Removal of Sialic Acid from Red Blood Cells

To suspensions of washed RBC's in saline (hematocrit = 1%), neuraminidase prepared from Clostridium perfringens (Sigma Chemical Co., St. Louis, Mo.) was added to obtain a concentration of 15 μg/ml of suspension. The suspension was incubated at 37°C under constant shaking for 60 min. This incubation duration and the neuraminidase concentration of 15 μg/ml were chosen after preliminary experiments had shown that maximum removal of surface charge was attained under these conditions (see Results). The neuraminidase-treated RBC's were washed with the saline or dextran solutions and then suspended in the same.

| TABLE I |
| MOLECULAR WEIGHTS AND DIMENSIONS OF DEXTRAN FRACTIONS |
| Dextran fractions | Molecular weight* | Molecular length† |
|------------------|-------------------|------------------|
| Dextran 20       | 20,000            | 25               |
| Dextran 40       | 42,000            | 32               |
| Dextran 80       | 74,500            | 55               |

*Weight-average molecular weight determined by light scattering. Data supplied by Pharmacia Labs, Uppsala, Sweden.
† Values obtained from Ingelman and Halling (10), and Thorsén and Hint (11).
3. Electrophoretic Measurement and Calculation of Zeta Potential

The electrophoretic mobility of RBC's was measured in a cylindrical microelectrophoresis apparatus (Grant Instruments, Barrington, Cambridge, England) at a temperature of 25.0 ± 0.5°C as described by Bangham et al. (12). Suspensions of normal or neuraminidase-treated RBC's in saline or dextran solutions at a hematocrit of 0.1% were used. 20 measurements were made in alternate directions by means of a polarity changing switch.

The zeta potential, \( \zeta' \), which is the potential at the shear plane (13, 14), can be calculated with the use of the Helmholtz-Smoluchowski equation:

\[
\zeta' = 4\pi \eta' u' / D, \tag{1}
\]

where \( \eta' \) is the fluid viscosity, \( u' \) is the electrophoretic mobility of the RBC's, and \( D \) is the dielectric constant of the fluid medium. In cgs units, \( \zeta' \) is in statvolt, \( \eta' \) is in poise, and \( u' \) is in \( \text{cm s}^{-1}/\text{statvolt cm}^{-1} \). Since each statvolt is equal to 300 V, Eq. 1 can be written as:

\[
\zeta = 360 \pi \eta u / D, \tag{1a}
\]

where \( \zeta \) is in millivolts, \( \eta \) is in centipoise (cP), and \( u \) is in \( \mu \text{m s}^{-1}/\text{V cm}^{-1} \). For the saline solution used in the present experiment, \( \eta \) was determined as 0.91 cP at 25°C. From the dielectric constant of water (78.54 at 25°C) and the dielectric increment of -11 mol\(^{-1}\) for NaCl (15), the value \( D \) is calculated to be 76.9. The use of these \( \eta \) and \( D \) values in Eq. 1a yields a factor of 13.38 for converting \( u \) into \( \zeta \) in the saline medium.

The zeta potential calculated from cell electrophoretic measurements in saline or dextran solution containing 0.5 g/100 ml human serum albumin showed no detectable difference from the value obtained from measurements in the same solution without albumin.

4. Determination of the Dielectric Constant of the Media

The dielectric constant was measured by the alternating current bridge method (16) in an instrument system described by Pollack et al. (17). The dextran fractions were dissolved in distilled water and dialyzed against distilled water overnight before dielectric determination. The dielectric constant was nearly independent of the frequency, showing only 2% increase when the frequency was reduced from 100 to 10 kcycle/s. The dielectric constant of a sample was obtained by linear extrapolation to zero frequency.

5. Viscometry

A coaxial cylinder viscometer was used for measuring the viscosities of the suspending media and RBC suspensions. The operational principle of this viscometer has been described elsewhere (18). The viscosity of each suspending medium (\( \eta_\text{s} \)) was measured at temperatures of 25°C and 37°C. The viscosity of RBC suspensions (\( \eta_c \)) was determined at 37°C over a shear rate range of 400 to 0.01 s\(^{-1}\). The RBC concentration
in the suspensions was adjusted to 45.0 ± 0.5%, which was determined by the micro-

6. Erythrocyte Sedimentation Rate (ESR)

Red cell suspensions containing 45.0 ± 0.5% RBC's were used for the determination
of ESR in Wintrobe tubes (120 mm long, ID = 3.0 mm) at 37°C.

7. Microscopic Aggregation Index (MAI)

To avoid overcrowding, RBC's were diluted to a hematocrit of 1.0% in saline or
dextran solutions for microscopic quantitation of aggregation at 37°C (6). The MAI,
which gives the average number of RBC's in each aggregation unit, was calculated
from RBC counting on photomicrographs (6).

8. Electron Microscopy

The procedures for examining the thin sections of RBC rouleaux in dextrans under
electron microscopy has been described elsewhere (6). The RBC aggregates were
fixed by slow and gentle addition of chrome-osmium tetroxide fixative (21) or 1%
osmium tetroxide in 0.1 M phosphate buffer at pH 7.35 (22). After step dehydration
in ethanol and propylene oxide, embedding in Araldite (Ciba Products Co., Summit,
N. J.) and polymerization at 60°C, thin sections were cut and poststained with lead
citrate for electron microscopic examination. Only the cells with sharply outlined
membranes were taken for study.

RESULTS

1. Electrophoretic Mobility of Red Blood Cells

The electrophoretic mobility of normal RBC's in saline averaged −1.12
μm·s⁻¹/V·cm⁻¹ (SD ± 0.08), which gives a zeta potential of −15.0 mV. After
neuraminidase treatment, RBC's showed a marked reduction of electrophoretic mobility in saline (Fig. 1). Maximum decrease in the electrophoretic mobility to −0.1 μm·s⁻¹/V·cm⁻¹ was obtained with the use of 15 μg of
neuraminidase per ml of 1% RBC suspension, incubated for 60 min at 37°C.
Dextran reduced the electrophoretic mobility of normal RBC's in saline
(Fig. 2 A). In order to calculate the zeta potential from mobility data, it was
necessary to consider the effects of dextran on fluid viscosity and dielectric
constant (Eq. 1). The viscosities of the various dextran preparations increased
with the molecular size and the dextran concentration (Fig. 2 B). In order
to ascertain that the macroscopic viscosity determined for the dextran solu-
tions is the correct ηw value to insert in Eq. 1 a, experiments were performed to
study the sedimentation rate of RBC's (hematocrit = 5%) in saline and dex-
tration solutions. In the absence of an electrostatic field, the sedimentation rate \( (v) \) of RBC's due to gravity \( (g) \) was linearly proportional to the parameter \( (\rho_c - \rho_o)/\eta_o \), where \( \rho_c \) and \( \rho_o \) are the density values of the RBC's and the suspending medium, respectively. Calculations of the Stokes radius \( (r) \) from the equation:

\[
r = \sqrt{\frac{9 \eta v}{2(\rho_c - \rho_o) g}}
\]

showed that \( r \) was essentially constant at 2.5 \( \mu \text{m} \) for RBC's in saline and dextran solutions with \( \eta_o \) ranging from 1 to 12 cP. These results indicate that RBC's behave as other hydrodynamic particles and support the validity of the use of the macroscopic viscosity of dextran solutions in Eq. 1 a. Dielectric measurements on water solutions of all dextrans over a concentration range of 2.5-10 g/100 ml showed the same dielectric constant as that of water. The finding that dextrans did not alter the dielectric constant of water is in agreement with the results of Allgén and Roswall (23) but not those of Pollack and his colleagues (17). With the instrument used in the present experiment, it is difficult to perform dielectric constant measurements in saline solutions because of the high conductivity. It seems reasonable, however, to assume that dextrans also do not affect the dielectric constant in saline solutions. With the use of Eq. 1 a, dextrans were found to increase the zeta potential of normal RBC's in saline, especially the higher molecular weight fractions (Fig. 2 C).

The slow electrophoretic mobility of the neuraminidase-treated (N-treated) RBC's was further reduced by dextrans as the \( \eta_o \) was increased. Since the decrease in \( \nu \) and the increase in \( \eta_o \) were nearly proportional to each other,
the zeta potential of N-treated RBC's appeared not to be affected by dextrans. It was difficult, however, to make a quantitative assessment of the effect of dextrans on the zeta potential of N-treated RBC's because of the low values of mobility.

2. **Effect of Neuraminidase on RBC Aggregation and Disaggregation by Dextran**

The degree of aggregation of normal and N-treated RBC's in various dextran solutions is shown by the photomicrographs of Fig. 3 and the measured MAI plotted in Fig. 4. Normal RBC's were monodispersed in saline and in Dextran 20 at all concentrations. A minimal degree of aggregation of normal
RBC's was observed in Dextran 40 (Fig. 3), but only at concentrations of 3-4 g/100 ml (Fig. 4 B). With Dextran 80, normal RBC's showed increasing degrees of aggregation as the dextran concentration was raised to 4 g/100 ml (Fig. 4 C). Further increases of the concentration of this high molecular

Normal RBC  |  N-treated RBC

|                |                |
|----------------|----------------|
| **Saline**     |                |
| **D × 20 (4 g/100 ml)** |                |
| **D × 40 (4 g/100 ml)** |                |
| **D × 80 (4 g/100 ml)** |                |
| **D × 80 (8 g/100 ml)** |                |

Figure 3. Photomicrographs of 1% normal RBC's (left) and neuraminidase-treated RBC's (right) in saline plus dextrans indicated.
weight dextran, however, caused disaggregation of normal RBC's, which became essentially monodispersed at a Dextran 80 concentration of 8 g/100 ml (Figs. 3 and 4 C).

N-treated RBC's were also monodispersed in saline (Fig. 3). A significant degree of aggregation, however, was observed when Dextran 20 with concentrations above 3 g/100 ml was used (Figs. 3 and 4 A). N-treated RBC's showed stronger aggregation than normal RBC's in Dextran 40 (Fig. 4 B) and Dextran 80 (Fig. 4 C). Furthermore, the N-treated RBC's did not show disaggregation at high concentrations of Dextran 80 (Fig. 4 C).

The increase in dextran-induced aggregation of RBC's after N-treatment was also corroborated by two other methods of quantitating RBC aggregation. Thus, N-treated RBC's showed faster sedimentation rates in various dextrans than normal RBC's (Fig. 5). The viscosities of normal and N-treated RBC's were essentially the same in saline (Fig. 6 A, 0% Dx 20). When suspended in various dextran solutions, however, the viscosity of suspensions of
N-treated RBC's was higher than that of suspensions of normal RBC's at the same cell concentration, and the difference was especially pronounced at low shear rates (Fig. 6). The relative viscosity of the RBC suspensions at a shear rate of 0.1 s⁻¹ is shown in Fig. 7. Both the ESR and viscometry data, as the MAI studies, indicate that the disaggregation of normal RBC's at high dextran concentrations was not observed in N-treated RBC's (Figs. 5 and 7).

3. Electron Microscopic Studies

Electron microscopic examination of thin sections of rouleaux formed by both the normal and N-treated RBC's showed parallel surfaces of adjacent RBC's with a rather uniform intercellular distance (Fig. 8). The intercellular distance in Dextran 40 and longer dextrans was not significantly different between the normal RBC's and the N-treated RBC's. Dextran 20 did not cause aggregation of normal RBC's, but it resulted in rouleau formation of N-treated RBC's with a mean intercellular distance of 16 nm (SD 1.5, n = 30).

DISCUSSION

The negative surface charge of human RBC's results primarily from the presence of ionogenic carboxyl groups of sialic acids on the cell surface (24, 25). Neuraminidase, which cleaves sialic acids from sialoprotein (26), reduces markedly the electrophoretic mobility of RBC's (Fig. 1), and the residual mobility may be ascribed primarily to the other carboxyl groups of the surface (25, 27). The contribution of cationic (e.g. amino) groups of surface
proteins to the zeta potential is probably not significant since most aldehydes, which tend to bind the cationic groups of proteins (28), do not cause any detectable change in the electrophoretic behavior of the normal or N-treated RBC's (25, 29). Glutaraldehyde treatment causes a slight increase in electrophoretic mobility of RBC's at pH values above 6.5, but this probably

![Graph showing viscosity-shear rate relations between normal RBC's (dotted lines) and neuraminidase-treated RBC's (solid lines) suspended in saline plus dextrans at 45% hematocrit. A, B, and C show results in Dextran 20, 40, and 80, respectively.]

Figure 6. Comparison of viscosity-shear rate relations between normal RBC's (dotted lines) and neuraminidase-treated RBC's (solid lines) suspended in saline plus dextrans at 45% hematocrit. A, B, and C show results in Dextran 20, 40, and 80, respectively.
results from the appearance of additional ionogenic groups in the peripheral zone of the glutaraldehyde-treated RBC's (30).

The aggregation of normal RBC's by plasma proteins and dextrans depends on the macromolecular size (5, 6, 20). The critical molecular size of dextran appears to be that of Dextran 40, which causes a minimal degree of aggregation of normal RBC's (6). Dextran 20 does not cause the aggregation of normally charged RBC's. After the removal of surface charge by N-treatment, however, Dextran 20 was effective in causing RBC aggregation, with an intercellular distance of 16 nm. These results suggest that the electrostatic repulsive force extends from the surfaces of normal RBC's for at least 16 nm, preventing the aggregation of normally charged cells by the short Dextran 20. Since RBC aggregations induced by Dextrans 40 and 80, with intercellular distances of 19 and 22 nm, respectively (6), were also enhanced after charge removal (Fig. 4), it appears that the electrostatic repulsive force is

![Diagram of Figure 7](image-url)
Figure 8. Electron micrograph of RBC rouleaux in 3% Dextran 80. (A), Normal RBC's; (B), Neuraminidase-treated RBC's. Note the similarity in the intercellular relations in (A) and (B).
operative over a distance of more than 20 nm. Indirect evidence has also indicated the existence of such long range electrostatic forces in the equilibrium gels of tobacco mosaic virus (31) and the myosin filaments of the striated muscle (32).

According to the theory of electric double layer (13), the negatively charged cell surface is surrounded by an ionic atmosphere of electric double layer composed of counter-ions and co-ions in the suspending medium. Electron microscopic pictures of the intercellular relation in the rouleaux (Fig. 8) show that the RBC surface may be assumed to be an infinitely large plane. For this configuration, the potential distribution in the diffuse double layer ($\psi$) is a function of the surface potential ($\Psi$), the distance normal to the surface ($x$), and the ionic composition of the solution. For a solution consisting of a $z$-$z$ valent electrolyte, the potential profile in the diffuse double layer has the form (33):

$$\psi = \frac{4kT}{\varepsilon} \tanh^{-1}[e^{-\varepsilon x} \tanh(\varepsilon \psi_s/4kT)],$$  \hspace{1cm} (3)

where $k$ is the Boltzmann constant, $T$ is the absolute temperature, $\varepsilon$ is the electronic charge, and $\kappa$ is the Debye-Hückel function. When $\psi_s$ is less than 40 mV, Eq. 3 can be approximated as:

$$\psi = \psi_s e^{-\kappa x}.$$  \hspace{1cm} (4)

Thus the potential decreases almost exponentially from the surface with a slope $\kappa$, which is a function of the ionic composition (33):

$$\kappa = \left(\frac{8\pi^2 N e^2 z^2}{1000 D k T}\right)^{1/2},$$  \hspace{1cm} (5)

where $N$ is the Avogadro's number and $e$ is the molar concentration of the electrolyte. The reciprocal of $\kappa$ is customarily called the "thickness" of the diffuse double layer (13).

The repulsive force between the surfaces of two adjacent RBC's in a rouleau can be calculated from the potential distributions of the diffuse double layers. If it is assumed that two diffuse double layers overlap in their "tails" without affecting significantly the $\psi_s$ at the surface, the repulsive force per unit area between two surfaces can be calculated as (33):

$$F = \left[64 \pi e RT \tanh^3 (\varepsilon \psi_s/4kT)\right]e^{-\kappa d},$$  \hspace{1cm} (6)

where $R$ is the gas constant and $d$ is the distance between the surfaces. From this equation, for two surfaces with $\psi_s$ of $-15$ mV and separated by 150 mM NaCl solution, the relation between the repulsive force ($F$) and the distance ($d$) can be obtained (Fig. 9). At an intercellular distance of 20 nm, the repul-
sive force between two normally charged RBC's is of the order of $10^{-1}$ dynes/cm$^2$. It is pertinent to note that a shear stress of $10^{-1}$ dynes/cm$^2$ (or a shear rate of the order of 1 s$^{-1}$) can cause a considerable dispersion of RBC aggregates (34–36). Thus, the seemingly small electric repulsive force of $10^{-1}$ dynes/cm$^2$ may be expected to play a significant role in affecting RBC aggregation. The results on the shear stress required to cause mechanical disaggregation indicate that the force causing RBC aggregation must also be of a similar order of magnitude. According to the model of RBC aggregation by macromolecular bridging (5, 6), the aggregating force is postulated to be the attraction force existing between the red cell surface and the adsorbed macromolecules. Direct evidence of dextran adsorption on RBC surface has become available between the submission and the editorial revision of this paper. With the use of $^3$H-labeled Dextran 80, Brooks (37) has made the important observation that the number of dextran molecules adsorbed on human RBC surface increases nearly linearly with dextran concentration (up to 7 g/100 ml). The adsorption isotherm indicates a lower affinity of the dextran molecule than the positively charged macromolecule polylysine (38).
for the RBC surface. These results and the relatively low shearing force required to cause disaggregation suggest that the adsorption force for dextran on RBC surface is weaker than that due to electrostatic attraction or covalent bonding. Hence the surface adsorption force is probably van der Waals or hydrogen bonding in nature. Thus far, the adsorption isotherm of dextran has only been determined for normal RBC's (37). It would be interesting to know whether dextran adsorption is altered after neuraminidase treatment of the RBC's.

According to the LaMer-Healy model (39), the bell-shaped relation between the degree of aggregation and polymer concentration may result from a surface coverage during polymer adsorption. That is, at high concentrations of polymers, the free surface on the particle available for polymer bridging is reduced, and hence particle aggregation is inhibited. Brooks and Seaman (40), however, have proposed that disaggregation of RBC's at high dextran concentrations is due to an increase in the surface electrostatic repulsion. These two possibilities, i.e. surface saturation vs. enhanced repulsion, can be tested by the present experiments on N-treated RBC's. Since N-treated RBC's continue to form aggregates in concentrations of dextrans which cause disaggregation of normal RBC's at high dextran concentrations is due to an increase in the surface electrostatic repulsion. These two possibilities, i.e. surface saturation vs. enhanced repulsion, can be tested by the present experiments on N-treated RBC's. Since N-treated RBC's continue to form aggregates in concentrations of dextrans which cause disaggregation of normal RBC's (Fig. 4), it appears reasonable to conclude that disaggregation is related to the surface electrostatic repulsion of the normal RBC's. Electrophoretic studies indeed indicate that the addition of dextrans to saline causes an increase in the surface potential of normal RBC's and that this effect is greater with rising dextran molecular size and concentration (Fig. 2 C). Thus, the resulting increase in electrostatic repulsion between cells may explain the disaggregation of normal RBC's at high concentrations of dextrans. The effect of dextrans on electrostatic repulsive force between RBC's will be further discussed in the accompanying paper (7). The absence of disaggregation in N-treated RBC's indicates that surface saturation has not been reached with the dextran concentrations used. It is possible that surface saturation may occur at very high concentrations of dextrans, but such high concentrations cannot be attained because of the limitation of dextran solubility. The absence of surface saturation with dextran concentrations up to 15 g/100 ml has been shown by the recent study of Brooks on the adsorption isotherm of Dextran 80 on normal RBC's (37).

This investigation is part of a Dissertation submitted by Dr. Jan in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University. The authors wish to acknowledge Dr. Erich Schnagl of Pharmacia Fine Chemicals, Inc., Iscayaway, N. J., for his generous supply of the dextran fractions, Dr. Orlando Miller for the use of his microelectrophoresis apparatus, Dr. Rudolph Reckel of the Ortho Pharmaceutical Corp., Raritan, N. J., for kindly allowing us to use his apparatus for the determination of dielectric constant, the valuable technical assistance of Ignacio Alvarez de la Cumpa, Dagmar Igals, Orlando M. Leyva, and Juan Rodriguez, and the excellent secretarial help of Miss Ethel M. Goodrich.

This investigation was supported by Research Grant HL-06139 from the National Heart and Lung
Institute, the U. S. Army Medical Research and Development Command Contract DADA-17-72-C-2115, and by generous gifts from The Scaife Family Charitable Trusts of Pittsburgh, Pa., and Mrs. George W. Perkins.

Dr. Kung-ming Jan is a Postdoctoral Fellow of the New York Heart Association.

Received for publication 10 July 1972.

REFERENCES

1. FURCHGOTT, R. F., and E. PONDER. 1941. Electrophoretic studies on human red blood cells. J. Gen. Physiol. 24:447.

2. ABRAMSON, H. A., L. S. MOYER, and M. H. GORRIN. 1942. Electrophoresis of Proteins and the Chemistry of Cell Surfaces. Van Nostrand Rheinhold Company, New York.

3. HEARD, D. H., and G. V. F. SEAMAN. 1960. The influence of pH and ionic strength on the electrokinetic stability of the human erythrocyte membrane. J. Gen. Physiol. 43:635.

4. CASTAÑEDA, A. R., E. BERNSTEIN, F. BANGSTADT, and R. L. VARCO. 1964. The effect of polyvinylpyrrolidone, mannitol, dextrose and various dextrans on red blood cell charge. Bibl. Anat. 7:262.

5. CHIEN, S., S. USAMI, K.-M. JAN, S. H. MILLER, and H. FREMOUNT. 1971. Effects of macromolecules on the rheology and ultrastructure of red cell suspensions. Proceedings 6th European Conference on Microcirculation Karger, Basel. 29.

6. CHIEN, S., and K.-M. JAN. 1973. Ultrastructural basis of the mechanism of rouleaux formation. Microvasc. Res. 5:155.

7. JAN, K.-M. and S. CHIEN. 1973. Influence of the ionic composition of fluid medium on red cell aggregation. J. Gen. Physiol. 61:655.

8. PONDER, E. 1948. Hemolysis and Related Phenomena. Grune & Stratton, Inc., New York.

9. CHIEN, S., S. USAMI, R. J. Dellenback, M. I. GREGERSEN, L. B. NANNINGA, and M. M. GUEST. 1967. Blood viscosity: influence of erythrocyte aggregation. Science (Wash. D. C.). 157:282.

10. ISSELBACHER, B., and M. HALLING. 1949. Some physicochemical experiments on fractions of dextran. Ark. Kem. 1:161.

11. THORSEN, G., and H. HINT. 1950. Aggregation, sedimentation and intravascular sludging of erythrocytes. Acta Chir. Scand. Suppl. 134:1.

12. BANGHAM, A. D., R. FLEMS, D. H. HEARD, and G. V. F. SEAMAN. 1958. An apparatus for microelectrophoresis of small particles. Nature (Lond.). 182:642.

13. OVERBEKE, H. TH. G. 1952. Electrochemistry of the double layer. Electrokinetic phenomena. In Colloid Science. H. R. Kruyt, editor. Elsevier, Amsterdam.

14. WEISS, L., and R. F. WOODBRIDGE. 1967. Some biophysical aspects of cell contacts. Fed. Proc. 26:38.

15. KORTUM, G. 1965. Treatise on electrochemistry. Elsevier, Amsterdam.

16. POWLES, J. G., and C. P. SMYTH. 1960. Measurement of dielectric constant and loss. In Physical Methods of Organic Chemistry. A. Weisberger, editor. Interscience Publishers, Inc., New York.

17. POLLACK, W., H. J. HAGER, R. RECKEL, T. A. TOREN, and H. O. SINGHER. 1965. A study of the forces involved in the second stage of hemagglutination. Transfusion (Phila.) 5:136.

18. CHIEN, S., S. USAMI, R. J. Dellenback, and C. A. BRYANT. 1971. Comparative hemorheology—hematological implications of species differences in blood viscosity. Bio-rheology. 8:35.

19. CHIEN, S., R. J. Dellenback, S. USAMI, and M. I. GREGERSEN. 1965. Plasma trapping in hematocrit determination: differences among animal species. Proc. Soc. Exp. Biol. Med. 119:1155.

20. CHIEN, S., S. USAMI, R. J. Dellenback, and M. I. GREGERSEN. 1970. Shear-dependent interaction of plasma proteins with erythrocytes in blood rheology. Am. J. Physiol. 219:143.
21. DALTON, A. J. 1955. A chrome-osmium fixative for electron microscopy. *Anat. Rec.* 121:281.
22. MILLONIG, G. 1961. Advantages of a phosphate buffer for OsO₄ solutions in fixation. *J. Appl. Physiol.* 32:1637.
23. ALLEGÈN, L.-G., and S. ROSWALL. 1954. A dielectric study of a carboxymethylcellulose in aqueous solution. *J. Polym. Sci.* 12:48.
24. EVLAR, E. H., M. A. MADOFF, O. V. BRODY, and J. L. ONGLEY. 1962. The contribution of sialic acid to the surface charge of the erythrocyte. *J. Biol. Chem.* 237:1992.
25. COOK, G. M. W., D. H. HEARD, and G. V. F. SEAMAN. 1961. Sialic acids and the electrophoretic shift of the human erythrocyte. *Nature (Lond.)* 191:44.
26. GOTTSCHALK, A. 1960. The Chemistry and Biology of Sialic Acids and Related Substances. Cambridge University Press, London.
27. EDsALL, J. T., and J. WYMAN. 1958. Biophysical Chemistry. Academic Press, Inc., New York.
28. FRENCH, D., and J. T. EDsALL. 1945. The reactions of formaldehyde with amino acids and proteins. *Adv. Protein Chem.* 2:277.
29. HEARD, D. H., and G. V. F. SEAMAN. 1961. The action of lower aldehydes on the human erythrocyte. *Biochim. Biophys. Acta.* 53:366.
30. VASSAR, P. S., J. M. HARDS, D. E. BROOKS, B. HAGENBERGER, and G. V. F. SEAMAN. 1972. Physicochemical effects of aldehydes on the human erythrocyte. *J. Cell Biol.* 53:809.
31. BERNAL, J. D., and I. FANKUCHEN. 1941. X-ray and crystallographic studies. *J. Gen. Physiol.* 25:111.
32. ROME, E. 1968. X-ray diffraction studies of the filament lattice of striated muscle in various bathing media. *J. Mol. Biol.* 37:331.
33. SHELUdKO, A. 1966. Colloid Chemistry. Elsevier, Amsterdam.
34. CHIEN, S., S. USAMI, H. M. TAYLOR, J. L. LUNDGREN, and M. I. GREGERSEN. 1966. Effects of hematocrit and plasma proteins on human blood rheology at low shear rates. *J. Appl. Physiol.* 21:81.
35. SCHMID-SCHÖNBEIN, H., P. GAERTGENS, and H. HIRSCH. 1968. On the shear rate dependence of red cell aggregation in vitro. *J. Clin. Invest.* 47:1447.
36. BROOKS, D. E., J. W. GOODWIN, and G. V. F. SEAMAN. 1970. Interactions among erythrocytes under shear. *J. Appl. Physiol.* 28:172.
37. BROOKS, D. E. 1973. Effects of neutral polymers on the surface potential of cells and other charged particles. III. Experimental studies on the dextran/erythrocyte system. *J. Colloid Interface Sci.* In press.
38. NEvo, A., A. DE VRIJS, and A. KATCHALSKY. 1955. Interaction of basic amino acids with the red blood cell. I. Combination of polylysine with single cells. *Biochim. Biophys. Acta.* 17:536.
39. LAMER, V. K., and T. W. HEALY. 1963. Adsorption-flocculation reaction of macromolecules at the solid-liquid interface. *Rev. Pure Appl. Chem.* 13:112.
40. BROOKS, D. E., and G. V. F. SEAMAN. 1970. Dextran mediated interactions in the rheology of erythrocyte suspension. Abstracts of VI. Conference on Microcirculation, European Society for Microcirculation, Aalborg, Denmark. 138.