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# NOTES AND NEWS

## Report on Hemorheological Presentations at the Seventh Conference of the European Society for Microcirculation, Aberdeen, Scotland, 28 August–1 September 1972

THE VII Conference on Microcirculation of the European Society for Microcirculation was held in Aberdeen/Scotland from 28 August to 1 September under the presidency of Prof. A. L. STALKER. Some 205 communications were presented, not counting the motion picture demonstrations and exhibits. The increase of this number in comparison to previous meetings of the Society (124 in Aalborg/Denmark 1970, 167 in Gothenburg/Sweden 1968, 113 in Cambridge/England 1966) demonstrates the rising interest in the general field of microcirculation. Thus, it is increasingly difficult to give a complete summary of all of the material presented. This report will be focused on those papers dealing with blood rheology in general and with the red cell in particular; these papers were combined in the two "Sessions on Rheology". In addition, a review of the Invited Symposium "Ultrastructural and Electrochemical Aspects of Red Cell Aggregation" and of a pertinent paper delivered as part of the Invited Symposium "Endothelial Interactions with the Circulating Blood Elements" will be presented. This selection was partially determined by the aims and goals of this journal; however, it also reflects the personal preferences and limitations of the reporters, who were unable to be at more than one place at the same time.

Data obtained by filtration of blood through 8 $\mu$m bore filters were presented by A. M. EHRLY (Frankfurt/Main, Germany). This technique, used by several laboratories for the determination of RBC deformability, appears to have proved useful in the hands of the skillful investigator although considerable discussion was evident on the relative advantages and disadvantages of the different types of filters available. The uniformity of pore size as well as the shape and branching of the channels in the particular filter type used is extremely important for the data obtained and their interpretation. In addition to data on filterability of blood from various clinical patients, the author presented evidence that differences in deformability between different ABO groups cannot be observed.

Another approach to the investigation of the mechanical properties of individual RBC was taken by N. MOHANDAS, R. M. HOCHMUTH and J. R. WILLIAMSON (St. Louis, U.S.A.). Red blood cells were allowed to settle and adhere to the glass surface of a flow channel; the channel was then perfused at various flow rates. The deformation of the cells brought about by the shear stresses of the flow was studied microphotographically. A motion picture presented by the authors showed very long and thin tethers being pulled from the cellular membranes. These tethers were up to 100 $\mu$m long at a fluid shear stress of 2 dyn/cm$^2$ and were dependent upon the number of points of attachment (usually one or two). From these measurements, moduli of elasticity of the membrane on the order of $10^4$ dyn/cm$^2$ were calculated.

Red cell aggregation (RCA) was studied by various authors, and some new techniques have been developed, thus allowing a determination of the extent as well as the kinetics of this phenomenon under a variety of conditions. Among these techniques, optical methods play a significant role. Changes of light transmission occurring when a flowing layer (thickness 100 $\mu$m) of blood in a cuvette was brought to a stop were reported by H. J. BERMAN and R. L. FUHRO (Boston, U.S.A.). These changes were interpreted to reflect the process of RCA. The authors studied the effects of various Dextran fractions (MW $4 \times 10^4$–$2 \times 10^6$) on the aggregation of human and hamster blood. In agreement with the results obtained previously by other investigators, RCA was found to increase with the MW of the Dextran employed; Dextran 40 was claimed to possess a disaggregating effect. This disaggregation effect is somewhat surprizing, since most investigators appear to have agreed that no such effect exists *in vitro*. Human blood was more responsive to Dextrans than hamster blood, even if the human cells were suspended in hamster plasma. pH (range 6·7–7·7) was found to have no effect, whereas RCA increased with temperature (range 15–42°C).

Light reflectometry of human blood under controlled shear conditions in viscometric flow was performed by S. USAMI and S. CHIEN (New York, U.S.A.); they reported on the effect of fibrinogen concentration on RCA. The reflectometric output was linearly related to fibrinogen concentrations if the data were obtained at low shear rates (1 sec$^{-1}$). The authors conclude that the reflectometric technique provides a sensitive method for quantifying RCA under controlled flow conditions.

The data presented by E. VOLGER, H. SCHMID-SCHÖNBEIN and H. J. KLOSE (Munich, Germany) were obtained in a similar way, except that light transmission, rather than reflection, was recorded. In addition to steady state values at defined shear flow and at stasis, the rate of transmission changes due to onset of shear was measured and the extent of RCA as well as the time dependence of this process were estimated. The effect of increasing concentrations of various colloids (Dextrans, fibrinogen) was studied. The authors reported that the shear rates necessary to disperse RCA first rise and then fall with increasing colloid concentration due to the increase of shear stresses with suspending medium viscosity. At low shear flow, RCA is most affected by high colloid con-

centration and the aggregates formed continue to grow in size even at constant shear. This effect was called "flow induced secondary aggregation" by the authors and was found to increase with dextran concentrations up to 2·5 g per cent but decreased at higher concentrations regardless of the MW used.

In addition to the various colloidal materials tested by the various authors, a surface active substance (Pluronic F68) has been found to reduce physiological as well as Dextran-enhanced RCA. Although a somewhat more conventional technique (sedimentation studies) was used by K. U. BENNER, P. GAEHTGENS and K. E. FREDE (Köln, Germany) in their investigations, the authors believe that the results obtained are valid if the necessary corrections (suggested by HARDWICKE and SQUIRE 1953) are applied. Pluronic (MW 8300) appears to be an interesting agent since it shows a strong disaggregating effect without altering RBC shape and without causing hemolysis.

A functionally important aspect of RCA and red cell deformability was studied by R. ZANDER and H. SCHMID-SCHÖNBEIN (Mainz and Munich, Germany). The rate of oxygen release from RBC suspensions flowing in a cone-plate-viscometer under standardized flow conditions was investigated. The authors suggested that cellular deformation during flow results in a significant intracellular convection of oxygen and Oxy–Hb, which in rapid flow is quantitatively more important than intracellular diffusion. They conclude that the rate and extent of oxygen delivery in the microcirculation is, to a great extent, dependent on the ability of the RBC to be deformed during their passage through the capillary network.

The material presented by A. A. PALMER (Sydney, Australia) represented a continuation of his earlier studies on plasma skimming and axial drift of RBC in flow. The effect of gravity was studied *in vivo* (rat omentum) and *in vitro* (branching rectangular slits). The experiments demonstrated that, under normal conditions, the sedimentation process of the flowing cells is overruled by the axial drift, both *in vitro* and *in vivo*. If, however, the aggregation tendency is strongly enhanced (high molecular Dextran), an asymmetry of the cellular distribution and pronounced plasma skimming may occur under the influence of gravitational force, particularly at slow flow.

*In vivo* measurements of relative viscosity of blood in sequential vascular segments of the canine forelimb were reported by P. GAEHTGENS and U. UEKERMANN (Köln, Germany). It was found that alteration of the hematocrit in the perfused blood had significantly smaller effects on the resistance in the microvascular segment than in the larger arterial or venous vascular segments. The relative viscosity of the perfused blood was calculated to be approximately 27 per cent lower in the microvessels in comparison to the arterial and venous section. The results were interpreted as supporting evidence for the significance of the FÅHRAEUS-LINDQUIST effect in determining effective blood viscosity *in vivo*.

Two reports described attempts to replace the blood of dogs with a hemoglobin solution thereby rendering the animals virtually free of RBC. D. BRAASCH (Marburg, Germany) obtained excellent results with the use of a pig Hb solution prepared by a new precipitation technique. According to this author, dogs could be maintained at a hematocrit of less than 1 vol per cent with no apparent circulatory disturbances. Plasma–Hb concentrations of 3–4 g per cent provided sufficient oxygen transport capacity as judged by the author from the constant lactate levels. Data on specific circulatory parameters, such as cardiac output, systemic arterial and central venous pressure, blood volume and acid-base status, were unfortunately not given, although the author mentioned that the animals had to be hypervolemic. Thus, a comparison with the data reported by L. SUNDER-PLASSMANN, F. JESCH, J. SEIFERT, W. GROHMANN and K. MESSMER (Munich, Germany) appears difficult. These authors found that, in contrast to their own previous experiments with hemodilution by conventional colloidal solutions, the studies with hemoglobin solutions showed a significant decrease of blood volume and cardiac output leading to the development of severe hypoxia as judged from measurements of central venous $pO_2$ and arterial pH. The authors attribute the inferior volume effect of their Hb-preparations to a dissociation of the Hb molecule allowing the dimeric split product to leak into the interstitial space. Quite obviously the current state of hemodilution by Hb-solutions is far from being suitable for clinical purposes. It may, however, become an interesting tool for the evaluation of blood rheology in the living system.

The Invited Symposium "Ultrastructural and Electrochemical Aspects of Red Cell Aggregation", chaired by S. CHIEN (New York, U.S.A.), was organized to discuss the often elusive factors involved in erythrocyte aggregation.

The major theme of this session and, in fact, the general consensus of the various participants, was that the process of RBC aggregation involves a balance between attractive (aggregating) and repulsive (disaggregating) forces. The manner in which the equilibrium state, and thus the degree or intensity of cellular aggregation, is related to these forces was the basis for the several papers which were presented. Differing experimental techniques were employed in order to vary or quantify either or both of these forces; the following represents a brief description of the approaches and results.

By way of introduction, S. CHIEN (New York, U.S.A.) presented an overview of the historical aspects of RBC aggregation as well as a brief summary of the present state of the ongoing research activities in this field. This introduction thus served to highlight several key points which were detailed later by the other speakers.

Experimental data relating to the surface chemistry of the erythrocyte membrane were presented by G. V. F. SEAMAN (Portland, Oregon, U.S.A.) and D. E. BROOKS (Rehovot, Israel). Following a brief review of the molecular structure of the peripheral region of the red cell, the electrokinetic consequences of this region were discussed in some detail. In particular, the zeta potential of the cell was related to its electrokinetic behavior and the appropriate relations and precautions for calculating charge from mobility data were explored. Alterations of the surface structure, via a variety of specific and non-specific agents, was shown to alter the charge on the cell. Examples

presented included enzymatic procedures (neurominidase, proteolytic enzymes, nucleases), blocking of functional groups such as amino, carboxyl, etc. and the use of cross-linking and oxidizing agents.

The resulting data were discussed in terms of the molecular structure of the cell surface and the relations between cell charge and the adsorption of ions or molecules onto the cell surface. Both the cell charge itself and altered macromolecular adsorption were suggested as playing important roles in the overall process of cellular aggregation.

As a companion presentation to the general topic of erythrocyte surface chemistry, D. E. BROOKS (Rehovot, Israel) and G. V. F. SEAMAN (Portland, Oregon, U.S.A.) presented specific information on the effects of dextran on human erythrocyte interactions. Dextran, a neutral macromolecule, has received considerable attention in the field of RBC interactions for several years owing to the extensive use of some molecular weight fractions as blood plasma substitutes. Previous literature reports have not always been in agreement; erythrocyte aggregation and/or disaggregation have been claimed, with strong advocates on both sides of the argument. In the opinion of these reviewers, this presentation represents a significant step toward the resolution of this controversy.

Using adsorption, electrokinetic, low shear viscometric and microscopic techniques, several aspects of the interaction of dextran with erythrocytes in simple salt solutions were investigated. Dextran was shown to adsorb onto the RBC surface and to increase the electrokinetic surface potential (zeta potential) of cells as a result of this adsorption. Under appropriate conditions of concentration and molecular weight, dextran can adsorb to adjacent cell surfaces, thus causing aggregation. Brooks and Seaman indicate that with increased dextran concentration, and thus increased adsorption, electrokinetic repulsion rises faster than does the effective attraction due to intracellular bridging. Thus, above a certain critical zeta potential, the cells disaggregate owing to this repulsive force. This critical zeta potential was shown to increase with increasing dextran molecular weight and to be a function of the ionic strength of the suspension. This disaggregation appears to be unrelated to *in vivo* reports of reduced cell interaction, since it occurs only at elevated dextran concentrations. For example, complete disaggregation (for a $\overline{M}n = 77{,}600$ fraction) was observed at and above a 6–7 g per cent dextran concentration—elevated aggregation occurs below this concentration.

The importance of RBC surface charge and the balance between attractive and repulsive forces was also discussed by K. J. JAN and S. CHIEN (New York, U.S.A.) and was presented by S. CHIEN. In addition to the techniques employed by BROOKS and SEAMAN, these authors used sedimentation rates as an additional index of RBC aggregation. Measurements of intercellular separations in RBC aggregates were obtained through transmission electron microscopy.

Using various dextran fractions, aggregation followed by disaggregation was observed with increasing dextran concentration for fractions above a weight average molecular weight of 20,000. The surface potential of the red cell was found to be reduced by increasing the cationic concentration of the suspending media owing to cationic adsorption; dextran induced RBC aggregation was reduced under these conditions. General agreement with the results of BROOKS and SEAMAN was indicated, and a balance between aggregating forces (dextran bridging) and disaggregating forces (electrostatic repulsion) was proposed. Intercellular separation was shown to be a function of dextran molecular weight, with spacings being on the order of one-half the extended molecular length. The exact nature of the cell-cell bridges formed by dextran is, however, not yet clear. Single-molecule bridges between two cells were postulated by JAN and CHIEN; interaction of loops of molecules, with each molecule adsorbed to a separate cell, also appears to be possible. Concern was expressed by some participants regarding potential artifacts arising in the preparation of RBC aggregates for electron microscopic observation, inasmuch as the necessary fixing and embedding procedures may alter intercellular distances.

A general review of several aspects of red blood cell agglutination by polyelectrolytes and antibodies was given by D. DANON (Rehovot, Israel); the role of cell charge and thus attractive and repulsive forces was a major theme of the presentation. Cell separation via negative charge repulsion normally prevents agglutination; the higher the charge density, the more difficult it is for cells to approach each other. Agglutination can be induced with positively charged macromolecules, since these macromolecules form intercellular bridges. If, however, the surface charge density falls below a minimal level, agglutination no longer occurs because of insufficient attachment sites. Agglutination can also be induced via antibody attachment to antigenic sites.

Light and electron microscopy were used to explore the interactions of the polyelectrolyte poly-L-lysine (pLys) with RBC. Data relating polymer length (i.e. molecular weight) and agglutination were presented and the intercellular separation was shown to be less than the calculated length of the pLys molecule. N-acetyl neuraminic acid was found to be the molecule by which pLys is attached to the red blood cell.

The role of cell charge in the rate of agglutination was studied using young, old and neuraminidase treated cells; rate data were obtained using a Fragiligraph and cationized ferritin labelled antibodies allowed electron microscopy. Cell charge was observed to be important only for single stage agglutination. Two stage agglutination (Coombs), which occurs at a greater distance between red cells, showed less effect of cell surface charge. Three stage agglutination showed minimal differences between young and old cells. It was concluded that differences in cell charge have no effect on agglutination rates when agglutination takes place at distances greater than the zone of mutual cellular repulsion.

Red cell aggregation produced by proteins other than fibrinogen was the subject of the final paper of this sym-

posium; this paper was authored by H. SCHMID-SCHÖNBEIN, E. VOLGER, G. GALLASCH and J. N. MEHRISHI (Munich, Germany).

Sera from normals and from myeloma patients were fractionated by gel and glass chromatography, isoelectric focusing and free flow electrophoresis; protein fractions were identified by immune electrophoresis. RBC aggregation, using washed autologous erythrocytes, was observed microscopically and quantitated via light transmission in a cone-plate viscometer.

Aggregation was found to be produced only by those proteins contained in the first peak eluted from the chromatograph (containing alpha 2-macroglobulin, IgA, IgM and part of IgG). In normals, the magnitude of RBC aggregation was correlated with the alpha 2-macroglobulin concentration; sera from myeloma patients varied in aggregating ability as a function of IgA and IgM content. Red cell aggregation could be produced by all sera fractions, including pure albumin, by reducing the ionic strength of the suspending media below 5 mmho/cm. This ability of pure albumin to induce RBC aggregation at low ionic strengths appears to be related to association of albumin molecules into larger units (i.e. dimers, trimers, etc.).

Based on these results, the authors propose that RBC aggregation by serum proteins is a colligative rather than a specific property of the proteins. Thus, such factors as molecular weight, charge and molecular configuration, rather than a binding process of distinct proteins as found in agglutination, should be considered. They therefore strongly suggest that RBC aggregation be clearly distinguished from the apparently much more specific process of RBC agglutination.

The Invited Symposium "Endothelial Interactions with the Circulating Blood Elements", chaired by A. S. TODD (Dundee, Scotland), provided a multidisciplinary discussion of the biochemical, biophysical and hemorheological aspects of endothelium–blood interactions. Of immediate relevance to this report was the presentation by A. L. COPLEY (New York, U.S.A.), which dealt with the macro- and microrheological facets of the endothelium–plasma interface. The author discussed the well known but, as yet, not completely understood concept of a marginal or plasmatic zone occurring during *in vivo* blood flow and indicated that a portion of this zone may be immobile; this immobile portion was related to his concept of an endo-endothelial fibrin layer (EEF). The site for the homeostatic aspect of fibrin formation and fibrinolysis was proposed as being in this relatively immobile portion of the plasmatic zone.

The significance of an EEF layer was explored by the author and was related to several of his experimental observations. The macroscopic flow of blood *in vitro* was indicated to be facilitated by a fibrin layer on the walls of a glass capillary viscometer; the presence of an EEF layer *in vivo* was suggested as having physiological value, since circulation would be aided by the decreased resistance to blood flow. Rotational viscometric data, obtained in a system designed to provide an air–protein solution interface, were also related to events occurring at the endothelium–plasma interface. From this data, the author proposed that, at the endothelium, aggregation of proteins occurs by a two-stage process: (1) adsorption of proteins onto the surface of the EEF; (2) continued growth of this layer by additional adsorption. Continuation of this process was indicated as leading to partial or complete obstruction of microvessels; aggregation of platelets and/or fibrin gelation were viewed as secondary phenomena in the overall process of thrombosis.

PETER GAEHTGENS
HERBERT J. MEISELMAN

Institut für Normale und
Pathologische Physiologie
der Universität zu Köln,
Köln, Germany
and
University of Southern California,
School of Medicine,
Los Angeles, California