CLINICAL HEMORHEOLOGY, Vol. 7, pp. 137-140, 1987
0271-5198/87 $3.00 + .00 Printed in the USA.

SUMMARY AND CONCLUSIONS

Shu Chien

Division of Circulatory Physiology and Biophysics,
Department of Physiology and Cellular Biophysics,
Columbia University College of Physicians and Surgeons,
New York, NY 10032

In the Introduction written by Dr. A. L. Copley entitled "On Erythrocyte Aggregation and Desaggregation", he gave a historical background of this subject and referred to red cell aggregation ex vivo and in vivo in relation to rheological measurements. He also discussed the various factors which play a role in these processes. The organizer Dr. Stoltz pointed out that the aims of this Symposium are to define the state of the art of investigations on red cell aggregation, to understand the importance of this rheological phenomena in physiological and pathophysiological conditions, and to determine to what extent hemorheologically directed treatment modalities have a role in various clinical disorders.

I. Summary of Individual Presentations

In the first two papers, Drs. Lelievre and Lacombe and Drs. Lucius and Stoltz discussed the use of time-dependent measurements to study red cell aggregation and red cell deformability. They have studied the stress-strain relationship in blood and red cell suspensions by using both step shear and time-varient input such as sinusoidal shear. The rheological behavior of the system is partially attributable to red cell aggregation, including the time-dependent processes of thixotropy hysteresis, etc. With the use of their apparatus, they were able to demonstrate changes in red cell aggregation under a variety of experimental conditions, e.g. reduction in red cell surface charge with neuraminidase treament or elevation of fibrinogen concentration. These instruments have also been used to detect alterations in blood rheological behavior which reflect changes in red cell aggregation, when studies were performed on blood samples obtained from patients with disease states, such as diabetes, myocardial infarction, Raynaud's disease, scleroderma, etc.

Dr. Meiselman presented a paper for Dr. Nash and himself on the rheological parameters of red cell deformability in relation to aggregation. They have employed a modified Myrenne aggregometer to derive time-dependent parameters which describe the two-phase behavior of red cell aggregation, and used these parameters to characterize the blood samples obtained under a variety of conditions. Particularly of interest is their study on fractions of red cells separated by density. They found that the bottom, dense fraction, which are primarily composed of the older cells, is three times as prone to aggregation in dextran as the top fraction, i.e. the younger cells. This

is noteworthy because it is known that the older cells are less deformable and that cells with a reduced deformability are generally less aggregable. By using a number of experimental approaches, they concluded that there are at least two factors reponsible for the greater tendency for the older cells to aggregate, viz. the smaller mean corpuscular volume and the greater degree of dextran binding for the older cells as compared to the younger cells.

Dr. Boivin summarized the biochemical parameters of red cell deformability. He outlined how the membrane lipid molecules and protein molecules are arranged and interact with each other. With respect to the lipids he stressed their asymmetry in the membrane and the role of phosphoinositol. In regard to the proteins, he discussed the interactions of membrane proteins such as glycophorin C and 4.1 and the roles of cytoskeletal network proteins such as spectrin, actin, band 4.1 and band 3, particularly the influences of phosphorylation, Ca^{2+} and ATP on these membranes proteins and on red cell deformability.

In Drs. Donner and Stoltz's paper on the molecular rheology of red blood cells, they discused the electrical aspects of the behavior of blood cells, including the double layer surrounding the surface charge, and they focussed on the fluidity of cell membrane. With the use of a variety of techniques, including NMR, elctron spin resonance, and fluorescence techniques, one can study the movements of lipid and protein molecules in the membrane. With the use of the fluorescence technique, they demonstrated a decrease in the fluidity of the red cell membrane in several disease states such as acanthocytosis, liver disease, sickle cells diasease, and diabetes.

The morning session closed with a paper by Drs. Snabre and Mills on the electrostatic interaction in red blood cell aggregation. They formulated a new model in which the dextran molecule interacts with and penetrates into the glycocalyx on the red cell surface. As a result, the dextran molecules can alter the microarchitecture of the glycocalyx molecules on the red cell membrane, causing them to be extended. This leads to alterations in the charge field around each red cell and in the electrostatic interaction between adjacent cells when aggregated by dextran. They were able to use this model to deduce the aggregation behavior of red cells in dextran in the presence of various levels of ionic strength, and the results show excellent agreement with existing data in the literature. They also modeled the aggregation of liposome vesicles and the interaction between red cells and liposomes mediated by dextran.

The afternoon began with my discussion on the physicochemical basis and clinical implications of red cell aggregation. The physicochemical factors governing the energy balance on the red cell surface were discussed. For red cell aggregation to occur, the aggregation energy provided by macromolecular bridging must overcome the electrostatic repulsive energy and the work done by shearing forces, if they are present. The balance of energy can be probed by a variety of techniques, and the results indicate that the net aggregation energy depends on the type of molecules used to induce aggregation. The net aggregation energy is partially stored in the cell membrane as a change in membrane strain energy, thus leading to an alteration of cell shape; the degree of cell shape change would depend on the membrane properties of the cell as well as the net aggregation energy. The implications of red cell aggregation in physiological and pathophysiological states include their potential effects on blood viscosity and blood flow, transcapillary transfer, oxygen transport, as well as red cell distribution in the microcirculation.

Drs. Stoltz, Paulus and Donner presented a paper on the techniques for assessing red cell aggregation. They reviewed the various mechanical and optical methods for

determining red cell aggregation, including microscopic techniques, viscometric methods, and measurements of conductivity, ultrasound, light transmission, and light reflection. They presented a method recently developed for the dynamic measurements of red cell aggregation. By video digitization of microscopic pictures of red cell aggregation, they determined the ratio between the surface area and the perimeter of each unit of aggregates and deduced a parameter to express red cell aggregation, which is shown to correlate with variations in fibronogen concentration. They have applied this technique to a variety of clinical conditions, including acute myocardial infarction, diabetes, and macroglobulinemia before and after plasmapheresis; the results showed that red cell aggregation was enhanced in these disorders and that the abnormal red cell aggregation in macroglobulinemia recovered after plasmapheresis.

The last three papers in this Symposium are concerned with the clinical aspects of red cell aggregation. First, Mr. Dormandy discussed the importance of red cell aggregation in venous pathology. In thromboembolic disorders, he studied the alterations in rheological properties of blood and showed that the changes in blood rheology can be correlated with the venous thromboembolic events He formulated the hypothesis that rheological abnormalities can initiate a vicious cycle to cause progressive worsening of the thromboembolic events. Correction of the rheological abnormalities by using techniques such as defibrinogenation leads to an improvement of blood flow through the part of the body where the thrmoboembolism is occurring. His study showed that rheological factors, particularly red cell aggregation, plays an important role in venous pathological conditions.

Dr. Larcan presented data on arterial and venous thromboembolic diseases, showing changes in rheological properties of the blood, including an elevation of blood viscosity at low shear rates and an increase in red cell aggregation. These rheological abnormalities are correlated with an increase in α–2 globulin and a decrease in albumin, and with derangements in blood flow. In agreement with Dr. Dormandy, he pointed out that the rheological alterations may initiate a vicious cycle and that appropriate treatment of the rheological disorders can alleviate the pathological situations.

Dr. Stoltz reviewed the therapeutical aspects of red cell aggregation. He discussed several techniques, including plasmapharesis, hemodilution, a decrease of fibrinogen concentration with Ancrod, and the use of substances with specific actions on red cell aggregation. Several of these substances with actions on red cell aggregation were discussed: some with primary actions, others with secondary actions. In some situations he suggested the use of metabolic treatment. For example, long–term treatment with artificial pancreas in diabetes, by correcting the basic methobolic defects, may reverse the abnormal blood rheology, including red cell aggregation. He concluded that therapeutical approaches to reduce red cell aggregation can exert a beneficial effect on various circulatory disorders. He particularly pointed out the need for the development of substances with specific actions on red cell aggregation.

II. General Discussions, Overall Summary and Future Directions

The formal presentations in the Symposium were followed by a general discussion on the significance of red cell aggregation in physiological and pathophysiological conditions, i.e. whether red cell aggregation is beneficial or detrimental to blood flow and the delivery of nutrients to tissues. It was generally agreed that, even though red cell aggregation may have beneficial effect on blood flow under very special conditions, the overall effect of red cell aggregation probably is detrimental to circulatory transport, particularly under pathological conditions.

The following is a general summary of the Symposium. We first discussed the principles underlining red cell aggregation. We have a fairly clear notion of the physicochemical basis of this phenomenon, although there are many details that still need further investigations.

Many methods for the qualification of red cell aggregation were discussed, particularly dynamic methods which allow the study of the time-dependent behavior of red cell aggregation. How these methods can be transferred to clinical application needs be worked out, including how to choose the proper technique and how to standardize the methodology so that the results obtained from different laboratories can be compared. It is important for us to realize the limitation of each one of the methods in terms of their effectiveness in measuring red cell aggregation in different conditions. It is only with the realization of these limitations that we can apply the methods and intrepret the results appropriately.

There is a considerable number of studies involving experimental manipultions on red cell aggregation. As far as the suspending medium is concern, the macromolecules that cause cell bridging and the ionic conditions are important factors. The other component of the aggregation system is the red cells; cell geometry and cell deformability also play a role in determining the propansity of red cell aggregation.

There are many studies on clinical aspects of red cell aggregation. Besides the classical abnormalities in red cell aggregation in hematological disorders such as macroglobulinemia, conditions such as diabetes, myocardial infarction, Raynuad's disease, etc., also are clearly associated with an enhanced red cell aggregation, which presumably contribute to the pathophysiological changes in terms of blood flow and nutrient delivery. Therefore, the role of red cell aggregation in these clinical conditions should be considered.

They are a number of correlative studies between the in vitro rheology and the in vivo hemodynamics. Most of these studies show a positive correlation between blood viscosity and flow resistance. The reduction of low-shear blood viscosity or the lessening of red cell aggregation tends to improve the flow. The available data allow us to make the preliminary conclusions that excessive red cell aggregation in disease states is detremental and that an effort should be made to correct these abnormalities. Before we can finalize this conclusion, however, more studies need be done both in experimental animals and clinical patients. We should aim to relate the information on red cell aggregation in vitro to the in vivo physiological or pathophysiolgical conditions, i.e. how does red cell aggregation affect blood flow and oxygen delivery in vivo. Thus, this Symposium has motivated us to pursue further studies to establish the physiological and pathophysiological roles of red cell aggregation and also to investigate the possibility of devising specific drugs which would improve the flow properties of blood by minimizing red cell aggregation.

In closing, I would like to thank all the speakers, the discussants and the audience for their contributions to the Symposium. Especially I would like to express, both for myself and for all participants, our sincere gratitude for the warm hospitality and the excellent arrangements by Société de Biorhéologie de Langue Française and the NEGMA Laboratories, which have made the Symposium such a success and our stays here such an enjoyable experience.