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SUMMING UP AND CONCLUDING REMARKS: INTERNATIONAL SYMPOSIUM ON NEW METHODS IN BIORHEOLOGY

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This Symposium on New Methods in Biorheology was organized by Dr. Jean-Francois Stoltz and held in Nancy on August 18 and 19, 1983 as a satellite meeting preceding the Fifth International Congress on Biorheology. The Symposium was attended by approximately one hundred participants and nearly 60 papers were presented, either orally or in poster session. Many new methods, approaches and concepts were presented. The excellent organization, the pleasant surroundings, the quality of the presentations, and the optimal size of the group facilitated the exchange of information and ideas. This Symposium has been most successful in achieving its goals, as expressed by the President Dr. Stoltz in his inaugural address (1a) and the Honorary President Dr. Alfred L. Copley in his Introduction (1b), i.e. to present and discuss the state of the art of methodology in experimental and clinical biorheology.

In attempting to summarize this Symposium, I shall group the presentations under several headings; viz., methods for measuring the bulk rheological properties of blood (including coagulation) and other biological systems; studies on the components of blood rheology, including the use of multiple methods; methods using membrane and molecular approaches; and in vivo methodology.

I. Bulk Rheology of Blood and Blood Coagulation

Six papers were presented on the instrumentation for measuring the bulk viscosity and viscoelasticity of blood sample. With the exception of the paper by Doffin et al. (18), who utilize a falling ball method, the other five presentations were on rotational devices. All of these rotational

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viscometers have been used to determine the viscoelastic properties of the blood either under oscillatory shear (3,4,5) or in the transient period of step shear (15,16b), in addition to the measurement of blood viscosity at steady shear.

In all of the rotational instruments, as well as the falling ball viscometer, these are on-line computational facilities and/or automated devices. The inclusions of dynamic measurements of viscoelasticity and computation-automation attachments are the common features of the new developments of instrumentation for bulk rheological measurements.

The modified Weissenberg Rheogoniometer developed by King et al. (3) can be used to measure normal stress of blood and surface rheology of plasma and protein solution in addition to viscosity (shear rates 10^{-2} to 10^{3} sec^{-1}) and viscoelasticity (frequencies 10^{-3} to 40 Hz). The OP-rheometer reported by Isogai et al. (4) has a shear rate range of 10^{-1} to 40 sec^{-1} and a frequency range of 10^{-1} to 30 Hz. In the instrument designed by Ravey et al. (15), the viscosity-shear rate relationship can be derived from a single measurement. Brancher et al. (16b) used a ferromagnetic fluid and a magnetic field to control the rotation of the instrument.

All rheological methods for measuring the viscoelasticity of blood can also be used for determining that of blood coagulation. Since the viscous and elastic moduli of the clot are higher than those of the flowing blood, the shear stress levels encountered at at given flow condition are considerably higher in coagulation studies. Hartert, who introduced the thrombo-elastograph for measuring shear elasticity of blood clots, has developed a resonance thrombograph (2). By measuring the elasticity of fibrin through its resonance effect, this instrument can furnish new information on the coagulation structure and distinguish fibrinogen efficiency from platelet activity. Fukada, Kaibara and their colleagues (1,5) have developed a vertically oscillating parallel plate viscoelastorecorder to measure the viscous and elastic moduli of blood, plasma and fibrinogen-thrombin solution and test the antithrombogenicity of materials. The falling ball viscometer mentioned above (18) has also been used to determine clotting time.

In addition to reports on instrumentation, there were several papers on the methods of analysis and the theoretical aspects of blood rheology. Gupta and Garg (8) derived empirical expressions for calculating the hematocrit and viscosity in narrow capillary tubes from values of feed hematocrit and tube diameter. Maurrice (20) presented several mathematical and numerical methods for the nonlinear modeling of blood viscosity. Whittington and Harkness (14) analyzed the rheological behavior of placental blood with a two-parameter model and compared the results with those on the adult blood. McMillan (16) characterized the thixtropy of blood and correlated the stress relaxation of blood with the changing orientation of erythocytes. Braasch (27) analyzed the capillary flow resistance with a two-phase model (axial core of cells and marginal layer of plasma). Matrai et al. (21) pointed out the importance of mixing the blood sample for reproducible measurement of viscosity at low shear rates.

II. Rheology of Other Biological Systems

Three papers were presented on the rheology of mucus. Duvivier et al. (11) developed a new viscoelastometer for measuring the viscosity and elastic modulus (stress relaxation) of bronchial mucus. This is a Couette instrument

which rotates at a constant rate (shear rate = 0.45 sec^{-1}) and requires 1 ml sample. Arnould et al. (12) reported a new apparatus for measuring the thread forming properties (spinability) of biological fluids. Measurements have been made on 20 μl of cervical and bronchial mucus. Litt and Steiner (42) determined the molecular weights of tracheal mucin glycoproteins by using the technique of quasielastic light scattering.

Lorente et al. (32) compared several methods for evaluating the left ventricular wall motion from cineangiograms in terms of sensitivity and specificity. Bauer (30) used a photoelectric technique to record the pulsatile change in diameter of arteries in vivo. By using the finite deformation theory, he derived the elastic, viscous and inertial behaviors of the artery from the in vivo recording of the natural pressure and diameter. Pestin et al. (31) measured respiratory transfer impedances by determing air flow at the mouth in response to varying pressure around the chest. The results were analyzed by using a parameter estimation technique to derive the material properties of the airway and tissues.

Geiger et al. (36) designed two instruments for performing harmonic and relaxation tests on the rheological properties of ligaments and other soft tissues. Katz and Johnsonn (35) measured ultrasonic wave propagation and mechanical damping in wet bone and analyzed the fluid flow and relaxation of solid matrix.

Plouvier and Huong (44) identified by laser microRaman spectroscopy a microbial chromophore material in blood samples obtained from patients with infections, cancer and leukemia. Janot et al. (65) developed a new Laser-Doppler electrophoresis system which allows the measurement of the electrophoretic mobility of a large number of small particles in 1 minute. This instrument has been used to determine the effect of antibiotics on bacteria mobility.

III. Components of Blood Rheology

The major determinants of the rheological behavior of blood are hematocrit, plasma viscosity, cell aggregation and cell deformation. Papers were presented on the measurements of each of these components, as well as in combination.

A. Hemotocrit

Kiesewetter et al. (29) devised an instrument which determines the hematocrit of blood samples by measuring the ohmic resistance. This instrument has another compartment which allows the ohmic determination of the electrolyte concentration of plasma.

B. Plasma viscosity

Jung et al. (22) designed a capillary viscometer for the automated determination of plasma viscosity, with the use of disposable polyurethane capillary tubing. Matrai et al. (23) measured the surface rheological properties of plasma at the air-plasma interface.

C. Red cell aggregation

Several methods have been devised for the quantitation of red cell aggregation. Radtke et al. (13) used a branched channel to determine the yield shear stress required to initiate blood flow in pathological samples (erythrocyte-stasis meter). Kiesewetter et al. (47) measured red cell aggregation by monitoring the light transmission of red cell suspensions under controlled shear; the shear rate for minimum light transmission (erythrocyte disaggregation) was determined. This instrument also allows the simultaneous determination of hematocrit by ohmic measurements. Dintenfass et al. (49) designed a parallel-plate slit-capillary photoviscometer for studying the kinetics and morphology of red cell aggregation, with the aid of stereological method.

Gaillard et al. (48) compared red cell aggregation as determined in a transmission aggregometer, a laser reflectometer and the rheoscope. Braasch (9) compared the rheological effects of red cell aggregation in capillary and rotational viscometers. Ernst et al. (46) determined the degree of red cell aggregation by using the area under the blood viscosity vs. time curve.

D. Cell deformability

Several investigators presented methods for studying the deformation and/or orientation of red cells in suspension under shear flow. Nossal (59) reported on the ektacytometer which has been used to determine the elongation index of red cells as a function of shear stress and suspending medium osmolality. Ravey and Mazeron (60) compared the small angle light scattering pattern of red cell suspensions with that of prolate spheroids. Fuller and Frattini (41) measured the orientation of red cells in Couette flow by phase modulated dichroism. Berga et al. (63) studied red cell fragmentation by subjecting packed cell suspensions to high shear stresses in a cone-plate viscometer.

There were nine papers which dealt with the determination of blood cell deformability by filtration; most of them used polycarbonate sieves with 5 μm pores. Thao Chen et al. (52) employed the constant flow filtration technique to determine red cell deformability and the role of leukocytes in pore plugging. Malher et al. (52b) described an apparatus for the automated measurements of red cell filterability and plasma viscosity by the constant flow technique. This instrument was used by Gueguen et al. (53) to test the effects of hematocrit, buffer composition and blood storage on red cell filterability. Stuart et al. (55) used the hemorheometer developed by Hanss to study the influences of anticoagulants and temperature on whole blood and red cell filterability determined from the initial flow rate at a constant pressure. Comparing several methods for the removal of leukocytes prior to studying red cell filterability, Stuart et al. (57) concluded that prefiltration through Imugard IG 500 cotton wool was the method of choice. Sowenimo-Coker et al. (56) determined filtration resistance from the flow rate measured by weighing the outflow during filtration under gravitational force. Teitel (62) developed a computer-assisted polymicroviscometer to determine the filterability of concentrated red cell suspension from the pressure-flow relationship in which both parameters are varying with time. In all the above filtration studies, the pressure-flow relationship reflects the composite behavior of many cells through many pores in the filter. Roggenkamp et al. (50) and Seiffge (51) reported the use of a single-erythrocyte rigidometer (SER) to determine the passage time of individual red

cells through a polycarbonate filter with a single 5 μm pore. The passage time was calculated from the change in electrical resistance across the filter as single red cells tranverse the pore. With computer assistance, the distribution of passage time and its mean value can be obtained.

E. Simultaneous determination of various components of blood viscosity.

Several of the investigators presented results on the parallel measurements of several components of blood rheology. Dintenfass (6) measured blood viscosity in rotational and capillary viscometers, flow instability in a rotational viscometer, erythrocyte sedimentation rate, red cell aggregation in a parallel-plate photoviscometer (49) and rheology of thrombus in a thrombo-viscometer. Kiesewetter et al. (7) determined yield shear stress in the erythrocyte-stasis meter (13), hematocrit and red cell aggregation in the hematocrit-erythrocyte disaggregation apparatus (47), red cell filterability in single erythrocyte rigidometer (50,51), and plasma viscosity. Potron et al. (19) measured blood viscosity, viscoelasticity and thixotropy in a rotational viscometer, red cell deformation in the ektacytometer (59), and red cell filterability through polycarbonate sieves. Schmid-Schoenbein (61) reported on the uses of the rheoscope, red cell aggregometer, single erythrocyte rigidometer, red cell filterability and rheoscope-diffratometer to quantify red cell aggregation and red cell deformation. The simultaneous application of different procedures to study various components of blood viscosity promises to yield a more complete rheological profile, especially in disease states.

IV. Membrane and Molecular Approaches

There are many exciting, new developments on the application of physical techniques to study blood cell membranes and their molecular organizations. Leterrier et al. (38) used electron spin resonance spectroscopy to measure the internal viscosity of erythrocytes, the molecular organization of red cell membrane, and the orientation of red cells in shear flow. Koyama et al. (39) developed a nanosecond fluorometer to follow dynamically the fluorescence decay curve of fluorophores incorporated in the red cell membrane; the results have helped to elucidate the dynamic structure of the membrane. Andre et al. (40) used pulsed excitation in studies on fluorescence polarization and intramolecular interactions; this technique has allowed the study of membrane molecular dynamics in platelets, lymphocytes, spleen cells and tumor cells. Viriots et al. (45) have applied fluorescence spectroscopy to study intramolecular excimer formation of dipyrenylpropane in platelets.

V. Methodology for *in vivo* Investigations

In vivo investigations are necessary for assessing the relevance of *in vitro* rheological studies. Slaaf et al. (10) studied the rheological behavior of platelets in the living microcirculation. By using acridine-red labeled platelets, they were able to determine the concentration profile and cell orientation of platelets in the microvessels. Koyama et al. (25) constructed a grating laser microscope for the measuremens of red cell velocity in microvessels; this technique has been applied to arterioles in frog's foot web. Usami et al. (28) developed a microscope with extra long working distance which can be used for microcirculatory studies in deep structures, e.g. the pituitary stalk. Ehrly (33) reported on a tissue pO_2 electrode he

developed for the determination of muscle O_2 tension in man; this has been applied to studies on patients with intermittent clandication. The study by Bauer (30) on arteries in vivo has been mentioned above.

VI. Summary and Conclusions

In this 1 1/2 day Symposium the participants have actively engaged in discussions on the state of the art of methods in biorheology. The papers have covered all level of investigations: from molecules to membranes, cells, blood and other tissues, and finally to organs and systems, including in vivo as well as in vitro methods.

It seems to be the consensus of the participants that this has been a fruitful and valuable meeting. I am very impressed by the extent and the level of coverage. I would like to mention particularly the following features of the presentations at this Symposium.

1. Correlation of experimental methods with theoretical analysis.

2. Utilization of physicochemical principles and methodology (e.g., laser polarization, magnetism, fluorescence techniques, etc.).

3. Adoption of technological developments (e.g. computational facilities and automated operation).

4. Introduction of molecular approaches.

5. Application to life sciences in health and disease.

On the last point, I would like to recall the statement by Dr. Copley (1b) that biorheology is the missing link in life science. The recent developments in methodology in biorheology, as summarized at this Symposium, will eventually provide the necessary knowledge to close this missing link, so that biorheology will be applied to improve the welfare of mankind in health and disease. This meeting has given us an excellent start for future progresses in methods in biorheology. I am certain that discussions on this topic will continue at the 5th International Congress of Biorheology and the 3rd European Conference on Clinical Haemorheology in Baden-Baden. In the end, we shall have "Data in, concept out" instead of "Garbage in, Rubbish out" (1a).

VII. Acknowledgments

On behalf of all the participants, I would like to thank Dr. Stoltz and the Organization Committee of this Satellite Symposium for their wonderful organization and gracious hospitality, which make this meeting in the lovely city of Nancy a most valuable and memorable event.

References correspond to the abstracts number of the symposium