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FIRST INTERNATIONAL CONFERENCE ON HEMORHEOLOGY

UNIVERSITY OF ICELAND, REYKJAVIK, JULY, 1966

Appraisals of papers

DURING the Closing Session of the Conference, appraisals were given of the progress made at the Conference in the eight principal branches of the subject. These appraisals, in some cases reviewed after the Conference, are reprinted here. The Proceedings of the Conference as a whole will be published in book form; but it was felt that readers of BIORHEOLOGY would find it of interest to see this summary of the Conference in advance.

Hemorheological Theory

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WHAT form of hemorheological theory has emerged out of this conference? In what way, in other words, are we now better able to cast the rheological behavior of blood into mathematical form, i.e. into expressions whose parameters are material constants clearly related to the structural elements. Formulated in this way, however, the problem invites some cautionary remarks:

Firstly, is this objective fundamentally realistic, i.e. is blood a system sufficiently inert that the biological processes which run in it can be ignored in rheological experimentation? The present thesis seems to be that, with the exception only of some regimes, there exists a wide range of practical conditions over which the rheological representations, founded mainly on the basis of *extra vivum* studies, are indeed significant. Probably the best way to allay doubts and settle the issue is through the use of procedures which combine a rheologically well defined flow system with an immediate return of the blood to the living animal. By such artifices it should be possible to decide whether, where and over what range *extra vivum* results are relevant.

But even assuming that this aspect is settled and that we are dealing with the rheological behavior of blood in a regime where we are entitled to consider it as a physical system, we must still make proper and detailed allowance for its discontinuous two phase nature. In consequence, our experimentation depends on the dimensions of the apparatus, and the rheological behavior, in terms of a continuum model, cannot be a pure function of material constants. Whether this additional parametric dependence is due to a plasmolytic zone, in the wider vessels, or to bolus or group flow, in the narrower vessels, is not the relevant issue at this level of discussion. What is important and should be remembered is that no equivalent continuum representation, as for example, the Casson equation, may ever be extended beyond the range over which its applicability has been tested. In particular, since such representations involve implicitly the geometry of the flow system, the danger of extrapolation beyond the tested range refers not only to flow rates, but also to apparatus or vessel dimensions. On the other hand, even in the most discontinuous flow regimes we may, with justice but with caution, employ equations which are averages over the many situations which can arise simultaneously or in rapid sequence.

In this spirit, the connection between fundamental rheology and hemorheology was discussed by Joly, while Rivlin, in a review of non-Newtonian fluid behavior, particularly in non-circular tubes, pointed out the necessity of considering normal stresses in the flow of blood. Comparing rheological equations relating shear stress to rate of shear, Scott-Blair clearly favored the log-log Herschel–Bulkley equation over the square root formalism of the Casson plot, except for purely empirical purposes.

Despite their usefulness, however, we seem to be getting a little disenchanted with overall representations and the largest number of contributions by far dealt with models and theories which consider the two phase nature of blood, explicitly, i.e. the rheology of each of its components and the overall rheological effects resulting from changes in aggregation and other physico-chemical variations in the structure of the system.

Rheology appears in these studies in two guises, (i) as a framework in which to present the results and (ii) as a technique by which the sought-for structural information is to be established.

(i) The tendency to-day to discuss the rheological characteristics of each phase separately and to look for equations which will synthesize the separate rheological elements into the behavior of the overall system under various conditions of flow, is hampered, unfortunately, by the specific effects of some blood components and the high volume concentration of the particulate phase. It is clear that no simple superposition is involved.

Much very good work has been done, however, with model systems or semi-model systems, i.e. red cell suspensions in other than full plasma. In fact much of the best quantitative hemorheological information which we possess refers to semi-model systems of this kind. While it is not clear, of course, whether whole blood is adequately represented in this way, such measurements are of enormous importance for comparison with each other and against data to be obtained on whole blood.

(ii) Though the synthesis of the rheological description of the overall system from its parts is one aspect of the work, an equally, if not more, significant one is the insight which rheological methods are now beginning to provide into the structural and molecular aspects of blood; in close analogy to the use of the rheological approach made by polymer physicists over the years.

These new thoughts are also reflected in the development of measurement techniques. For *extra vivum* viscosity measurements an instrument involving a very narrow (5–60μ) gap was described by Dintenfass while Zimmer has developed a technique where samples are interposed into a continuous stream of saline passing through the apparatus. As the sample appears between the walls of the instrument, the changed mechanical response is recorded. The importance of meniscal resistance in tube viscometers was stressed by Jacobs.

Very interesting in particular were some *in vivo* techniques. Frasher reported on his success in introducing an arterial-venal shunt into dogs such that tube viscometers, or other devices, can be perfused with blood which derives and returns to the intact animal, which can be maintained alive and healthy for periods of up to 6 months. A similar idea underlies the use of a cannulated artery viscometer described by Charm while Wayland gave details of techniques which were developed to interpret flow in the trans-illuminated living vessels directly.

With the two phase nature of blood uppermost in mind, great emphasis was placed, in a large number of contributions, on the red blood cell, both as a unit and as an assembly of particles in suspension.

The most remarkable rheological property of the red cell is probably the flexibility of its wall. It seems that cells can be drawn into threads, during which process, despite hemolysis, their surface area, as Kochen has shown, remains constant and their ability to form an intact ghost cell, is retained. While normal cells easily pass through 5μ wide channels acetaldehyde hardened cells do not (Gregersen). The puzzle of the restoration of the bi-concave shape after enormous deformations was discussed by Burton, but if the latest observations of Stewart are confirmed the membrane may not be as structureless as most of the previous evidence seems to suggest. The likelihood that membrane flexibility varies considerably from sample to sample was pointed out by Thomas. Great differences in fragility and reversible deformability with cell age were indeed established quantitatively in the Fragiligraph by Danon. The correlation between flexibility and respiration, on the one hand, and pH and other environmental factors, on the other, were discussed by Sirs.

Mushroom-like deformation of single cells, completely analogous to that observed in the microcirculation, was demonstrated by Goldsmith using 10μ diameter glass tubes, and a mathematical model and a device to determine flow conditions using large scale models of variously shaped red cells in a tow tank were described by Bugliarello. The forces giving rise to these shapes should thus become known in due course. It is indeed not unlikely that the shape-change resisting forces of erythrocytes are an important rheological element in the flow of high hematocrit samples as Dintenfass points out. Changes in temperature alter surface area, but not volume, while changes in pH affect area, volume and visco-elastic properties. This was shown by Murphy while Seaman could prove that reducing the electric charge of the cell (by neuraminidase) left the overall blood viscosity unaffected.

Treating more concentrated cell suspensions, Goldsmith demonstrated the presence of the same rouleaux which are seen in *in vivo* systems. The stability of such rouleaux to flow can now be measured. It is to be noted in this connection that Seaman finds that a force of about 10^{-7} dynes is needed to break up normal red cell aggregates at an overall shear rate of 50 sec^{-1}. An interesting observation is due to Ehrly. Na oleate addition, to a total of 20–40 mg/100 ml fatty acid, reduces aggregation in case of pathologically aggregated blood, but leaves normal blood practically unaffected. The effect of various lipid meals on cell stickiness was discussed by Swank who pointed out that only saturated fatty acids seem to combine well with red cells. A method for determining aggregate shape from fluorescence depolarization was described by Pfeiffer.

The flow of cell suspensions through vessels only slightly larger than the cells was discussed by Whitmore, whose treatment explains why, under these circumstances, cells flow in stacked groups and why the overall viscosity is only a weak function of hematocrit. The flow of blood, or model suspensions, through wider tubes was the subject of a number of papers which were all concerned, in some way or another, with the problem of axial streaming and the presence of a plasmolytic zone. There is little doubt but that such a zone arises, at least in *extra vivum* experiments, even though only in a statistical sense. This was demonstrated in a plasma skimming apparatus by Palmer and in concentrated particle suspension experiments by Goldsmith and Mason and by Sacks. Charm basing himself on the Casson equation predicts a correlation of plasmolytic layer thickness and flow rates when Casson equation yield stress and velocity are large enough. A difference in transit time of cells and albumin through an isolated vascular bed in cat muscle was also demonstrated by Groom.

In wide blood vessels, however, the hematocrit seems to be uniform across the tube section and a plasmolytic layer, if it exists, is negligible. This has now been demonstrated by both Wiederhielm and Phibbs using quick freeze techniques. Both find preferred parallel orientation of the red cells to the vessel wall, no concentration changes across a diameter and no strong aggregation. Wiederhielm, moreover, has found the explanation why in tubes ten to fifteen times the red cell diameter maximum light absorption is apparently concentrated in two bands running parallel to and on each side of the tube axis. It has in the past been suggested that the enhanced transmission of light through the center is due to an axial outward migration of particles from the core. It now appears that the absorption there is normal, but that much of the incident light is specularly reflected in the region of the in-between band from red cells orientated parallel to the wall but at 45° to the incident beam. Wiederhielm could confirm this explanation by showing that the dark bands shifted as the inclination of the light source was changed.

Rheologically, plasma is not merely an inert suspending medium, and fibrinogen has been known for some time to be the rheologically active plasma component. Without fibrinogen, i.e. in serum, red cells do not form rouleaux. Copley has now shown that when fibrin-fibrinogen complexes are formed, their presence considerably augments aggregation. Furthermore, as Lee has demonstrated, blood viscosity is

considerably affected by any abnormal plasma protein structure or composition despite only very minor variations in hematocrit. Similarly, it is reported by Rees that increased Casson equation yield shear stresses result when fibrinogen and macroglobulins combine in what seems to be a network interaction with red blood cells. The role of beta-2-fibrinogen D in platelet aggregation was discussed by Barnhart.

Much of the discussion of plasma was devoted to the function of dextrans as plasma expanders and blood conditioners. There seems to be general agreement on the effects of high molecular weight dextrans. These increase cell aggregation and blood viscosity and both Gelin and Meiselman independently reconfirm this result. On the other hand, while Gelin and Groth believe that the decreased viscosity when low molecular weight dextrans are administered is accompanied by deaggregation, Wells, Meiselman and Bernstein, in separate investigations, attribute the reduced overall viscosity to hemodilution alone and find no change in aggregation even in cases where aggregation was first artifically increased.

To this discussion of the cell and plasma phase of blood and the rheological interplay between them, a new element was added which has till now been largely ignored. In a series of contributions the importance of surface effects on the rheology of blood was stressed. The discussion involved not only the interfaces cell-plasma and plasma-vessel wall, but also the interfaces in the micropores which are believed to exist in the membranes of the red blood cell and the vessel wall. Such pores, as Palade has pointed out, are the presumed vehicles of exchange flow between cell and plasma and the carriers of the extra-vascular circulation.

Basing himself on the properties of adsorbed polymer layers, Silberberg suggested that since the thickness of such films is able to vary considerably they could act as self-regulatory pressure or composition sensitive devices controlling the instantaneous flow of red cells in narrow vessels and the flow of components through the pores of the membranes. Lee did, indeed, point out that many serum proteins, but mainly α and β globulins, interact with the surface and undergo partial denaturation. Eirich, as well, referred to the profound surface effects of adsorbed macromolecules particularly polyelectrolytes and the many types of interaction which may be expected. The surface effects of macromolecules were also considered by Burton, who pointed out that rouleaux were almost certainly formed by long macromolecules linking cell to cell. In addition, he attributes the biconcave structure of the red cell to an internal membrane to membrane interaction involving macromolecules.

The presence of adsorbed macromolecules on the vessel wall is implied by the coatings which Frasher found were developed in his artificial shunt capillaries in dogs and by the thickening of the vessel wall at arterial forks observed by Stehbens. Unless these coats are very thick indeed no direct effects are to be expected in wide vessels, but Oka has suggested that indirect effects such as a wall slip could account for the reduced viscosity observed in model systems with fibrin coated walls. Tamamushi, as well, discusses slip and the effect of surface forces in the transmission of flow shear stresses.

The irreversible thermodynamics of membrane transport as linked to macromolecular transformation were discussed by Katchalsky while Seno proposed that a structural rearrangement in the membrane is involved in the transfer of macrolmoecular components across the cell or vessel wall.

The importance of including the vessel wall into the rheological picture is also well illustrated by the great progress which has been scored in hemodynamics where the analysis of pulsatile flow has been pushed very far. The overriding contribution of the visco-elasticity of the arterial wall in determining the nature of the system response was pointed out by Taylor, while the contributions of McDonald and of Seymour emphasize, in addition, the control asserted by the muscle component of the arterial wall. Overall mathematical models for the circulation were presented by Rubinow, and Attinger and discussed by Rouse, and the usefulness of high speed computing methods in their solution was acknowledged. It is to be noted that in these calculations the detailed rheological properties of the blood seem to play a minor role as useful, overall representations can be found with blood replaced by a Newtonian liquid.

Hemorheology is bound to remain a rather complex field for some time. Though the present working model, a two phase system, i.e. a suspension, in which surface interactions may be highly important, seems to be adequate for most purposes and is now generally accepted, it can be applied only qualitatively as the rheological information which would make the model quantitative is not nearly available. This is particularly true for *in vivo* situations where the complexities introduced by the vascular bed, which were described and discussed by Berman and by Joly, still have to be taken into account.

Since it appears that the rheological situations which arise in the circulation, are already sufficiently involved, the characterization of an all embracing model, valid also outside physiological flow regimes, seems at first glance to be unnecessary and ambitious. It turns out, however, that flow under non-physiological conditions may display differences and changes in the state of the blood which are indicative of a pathological variation not otherwise rheologically detectable. Such information is thus of diagnostic, clinical value. For example, in a contribution by Fukada, the possibility of disease linked rheological changes, using both a capillary viscometer and a double cylinder viscoelastometer, has been examined; similarly Casson equation yield stress was looked at by Rees and overall blood viscosity by Merlen and by Lee.

Hemorheological Techniques

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IT IS always difficult to apprise exactly the real achievement of an international conference immediately at the end. It is generally much easier to give a general survey of the fruitfulness of a conference a few months later, when everybody has had time enough to think about the various results which have been discussed. This difficulty is still greater when the conference is on a subject such as hemorheology which includes so many topics, extending from pure mathematical rheology to almost pure medicine.

Nevertheless some conclusions can be drawn forthwith concerning particular sessions of the present meeting. That is why I should like to make a few comments on the technical aspects of hemorheology.

In all the sciences, the techniques play an important part. In several sciences the techniques have reached a very high level of complexity and adequacy. In the case of hemorheology such a development is not yet attained. There is still much work to do in the technical field. That is the reason why the present conference has been very important and very useful in the domain of hemorheological techniques.

The nature of blood, the complexity of the macro- and microcirculation, and the intricate structure of blood vessels have led to the development, on a theoretical basis, of a rheology very different from the classical rheology. The scale of the constitutive elements of blood compared with the size of blood vessels, the deformability of these bodies and their variations in space and time have sometimes induced us to call in question again the assumptions used in the ordinary methods of mathematical rheology. Therefore, it has been necessary to attempt to establish the theoretical basis of a rheology valid on a microscopic scale, for heterogeneous media, the components of which vary and show physical properties depending on the state of motion.

Such a theoretical attempt can be fruitful only if the experimental data are adequately obtained. It is necessary that the experimentalist should provide the theoretician with an exhaustive set of well defined results clearly describing the various aspects of blood flow.The hemorheological techniques must be much more elaborate than in the case of classical rheology, in order to obtain a good selection of the parameters and to satisfy the exact condition required for an accurate description of the whole rheological behaviour of very complicated systems. For these experimental data, all the parameters must be completely characterized and show the properties of steady variables as required in physics.

In some cases it is difficult to approximate to such a situation. Therefore, the techniques used in practical hemorheology may sometimes appear as rather surprising methods to a physicist not familiar with the problems of hemorheology. Indeed very often a sample of blood is put into a more or less complicated apparatus, a button is pushed, the apparatus gives a signal, figures are read or a curve is recorded and a conclusion is derived. But, what, if anything, has been measured?

The signal given by an apparatus is generally not a measurement, in the physical sense of the word. A set of very severe conditions must be satisfied by a quantity for it to be a physical measurable quantity. The limiting conditions, the presence of a stationary state with regard to the environment, of reproducibility, and so on, must be strictly verified. The separation of the various parameters is rigorously necessary if one wants to know exactly what is measured.

Unfortunately such conditions, which are trivial in ordinary physics, are not always very easy to be satisfied in rheology as well as in biophysics, and are always very difficult to attain in hemorheology because of the extreme complexity of blood, blood vessels and, more generally, of living systems. This is the reason why the First International Conference on Hemorheology has been extremely useful and fruitful in order to promote, on a strong scientific basis, a valid development of hemorheology. Indeed, each speaker, in the various fields of hemorheology, has made a real effort to state precisely the exact conditions of his experiments.

In the session devoted to the technical aspects of hemorheology, very interesting papers have been presented, in which various aspects of the techniques used have been described. In each of these papers it has been pointed out that particular conditions must be satisfied for the validity of the measurements.

W. G. Frasher, H. Wayland and S. S. Sobin, for instance, have shown that sampling of blood in its native condition is necessary for the physiological significance of the measurements; and they have proposed an appropriate device in the form of a chronic external arterio-venous shunt. This shunt carries between 15 and 25 per cent of the cardiac output and functions free from clotting for prolonged periods without flushing or the use of anticoagulants. It allows one to perform outflow viscometry in artificial tubes cast in silicon resin.

The importance of direct observation *in vivo*, for instance by high speed cinephotography has been emphasized in the paper by E. H. Bloch. The flow is recorded at frame rates from 540 to 8000/sec, and the magnification permits an adequate resolution of all the elements of the blood. With this technique it is possible to establish the cellular flow pattern and to determine the boundary conditions at the vessel wall

in living animals. A similar method has been developed by G. P. Fulton, H. J. Berman, R. F. Slechta and A. M. Brooks in order to study the rheological disturbances introduced by various pathological changes.

H. Wayland, P. C. Johnson and W. B. Frasher have developed a very fine technique for the precise measurement of flow rates and shearing forces in living blood vessels. In capillary beds, vessel diameter is measured either with a flying spot microscope or with an optical scanning device. The velocity of blood cells is measured by a double-slit photometric method. For the determination of the pressure gradient two types of micropressure transducers have been used: the Wiederhielm active gauge and a miniature passive gauge using a low compliance pressure diaphragm fitted with solid state strain-gauge elements. They have emphasized the importance of the exact determination of the real tridimensional geometry in the studies of *in vivo* hemorheology. They have observed in the capillaries of the isolated mesentary of cats two different types of flow: a steady flow, the velocity of which is linear with pressure difference, and an oscillatory flow with a period of 6–10 sec related to the activity of precapillary sphincters. As pointed out by the authors this type of flow and the fluid transport across capillary walls make it difficult to isolate the flow characteristics which can be attributed to the rheological properties of blood.

In his paper, H. R. Jacobs has shown the importance of the perturbations introduced by the meniscal resistance to the flow in glass capillary tubes. He emphasizes the corresponding necessity of building viscometers minimizing the meniscal resistance in order to obtain significant values of blood viscosity.

The advantages of a microcapillary viscometer made of parallel plates of glass polished to an optical tolerance of few wave-lengths has been demonstrated in the paper by L. Dintenfass. In the series of microcapillary viscometers so constructed, the gaps between plates vary from 5 to 60μ, the length of the slit-capillary varies from 5 to 95 mm and the width of the slits varies from 40 to 100 mm.

S. G. Mason and H. L. Goldsmith have shown very elaborated apparatus enabling precise measurements of the motion of particles in various types of flow. The rotation of linear and flexible chains of spheres and discs have been studied in Couette flow and compared with that of rouleaux of human red cells of various lengths. These experiments confirm the necessity of very accurate measurements, as in physics, if one wishes to test the validity of theoretical deductions, which is extremely important for hemorheology as well as for fundamental rheology.

Unfortunately the paper by K. Weissenberg could not be read. In this paper some uses of the rheogoniometer were described. The advantage of such an apparatus is the following: one knows exactly what is measured, which is not always the case with other devices.

A new kind of viscometer has been proposed by J. Zimmer. This consists of a modified Couette viscometer in the gap of which the liquid is continuously renewed. The variations of the torque as a function of time are recorded. By alternating the circulation of a reference liquid and of the medium to be tested, one can obtain with this apparatus rapid information on the influence of various drugs on the serum viscosity.

Many other technical data were given in the various sessions not especially devoted to the technique. Important applications of the thrombodynamography were presented. This very clever technique bears on several rheological problems. On the other hand its physical, biophysical and also biochemical significance will certainly be studied thoroughly in the near future.

It would be useful to develop the theory of a number of other techniques. For instance, the flow through discs of sintered glass or other types of filters, as described in the communication of F. R. Eirich; and systematically studied in the paper by M. I. Gregerson and C. A. Bryant, seems to be, as a first approximation, a good simulation method of study of the flow in capillary beds, and a tentative technique for the determination of the force require for the deformation of the cells entering and traversing the pores.

This rapid survey of recent progress in hemorheological techniques shows that the aim of very accurate and significative experimental data in hemorheology is not too unrealistic. The realisation of this aim requires the collaboration of hemorheologists, biochemists, pure rheologists and biophysicists. Such a cooperation will be extremely useful for the rapid development of hemorheology as well as of basic rheology.

Model Studies and Phase Separation

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THE session on Model Studies comprised six papers given by Silberberg, Rouse, Goldsmith, Attinger, Sacks, and Bugliarello, and the session on Phase Separation had two papers, presented by Palmer and Charm.

Clearly, the first difficulty with regard to models is to define what a model really is—a difficulty that certainly cannot be resolved here. It should be pointed out, however, that there were papers in other sessions that by all rights would have fitted the session on models, notably Professor Burton's paper. Also, of course, mathematical and physical models have played an unconscious role in the mind of many contributors to this Conference.

Be this as it may, a striking characteristic of the session on models was its very broad scope, ranging from Silberberg's model of physico-chemical boundary processes at the molecular scale to Attinger's model of the peripheral circulation. Breadth of scope was associated with diversity of methods of approach: hydrodynamic modelling, mathematical modelling, visual observations, electrical analog modelling. Such breadth, both in scope and in approach, represents a quantum advance with respect to the previous symposia. This is most encouraging, since, unquestionably, the development of both the qualitative and the quantitative understanding of hemorheological phenomena in the context of the circulation can be seen only through constant interplay between prototype and model in exactly the same way and with the same conceptual difficulties that, as Rouse pointed out, obtain in the hydrodynamics of rivers. The link between model and prototype is, of course, given by *extra vivum* experiments such as those by Palmer and Charm.

What have been the specific accomplishments of the sessions on models and phase separation? It is always very difficult, as explained by Professor Joly in his remarks, to evaluate accomplishments so close to an event. Perspective can be gained only with time, and only time will tell what, in the model sessions, has been really relevant to the development of knowledge in hemodynamics and what instead has contributed to the development of physical science. The present commentary will be confined to a listing of some of the more significant points that have emerged both from the papers and the discussions.

1. We continue systematically to expand our knowledge of the behavior of model suspensions. (Sacks, Goldsmith and Mason, Bugliarello).
2. In several cases, model and *extra vivum* observations have given striking qualitative predictions of what is observed *in vivo* (as remarked, e.g., in the discussion of the papers by Phibbs and Wiederhielm).
3. We are acquiring the ability to model accurately by electrical analogy entire blocks of the circulatory system (Attinger). These models require an understanding of the role that the rheological characteristics of blood in the vessel well may or may not play in the circulation.
4. We have been alerted to the possibility that under certain conditions adsorbed macromolecules on the vessel wall may play a very important role in regulating the flow in very small vessels and in influencing the resistance in other situations (Silberberg).
5. We are achieving a greater quantitative insight into the transport processes taking place in the axial plasmatic gaps of true capillaries (Bugliarello).
6. We are becoming intently aware of the importance of bifurcations as controls for the hematocrit in downstream vessels (Palmer).
7. We have obtained further information on the peripheral plasma layer in small tubes (Charm) and hopefully settled the argument as to the meaning of data for the layer deduced from pressure flow relationships (Thomas, Bugliarello and Hershey's discussion of Charm's paper).

Cellular and Fibrin Clotting

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THE session on "Cellular and Fibrin Clotting" not only was the main topic of this week's program, but also seemed to be the central theme of the entire conference. This is understandable when we consider that clotting processes influence most the rheology of blood. There are principally two points which may be important with regard to the process of clotting. They are (1) the aggregation of platelets and (2) the production of fibrin. Both processes may influence each other in many different ways. Thus, the rigidity of the fibrin clot and its ability to retract is brought about by the platelets, which can be shown by thromboelastographic methods, as demonstrated by Leroux. By means of the retractograph, likewise developed by Hartert, it was found that retraction begins at the same time as clotting does, and is not merely a secondary process. The molecular mechanism of retraction, which is linked to an undisturbed energy metabolism of the platelets and determined by at least one plasma cofactor, is still, for the most part, unknown. The transformation of chemical into mechanical energy, which is well documented in the retractograph, appears to resemble that taking place in muscle contraction.

The formation of a special intermediate product during fibrin formation, during which only the first step in the clotting reactions (namely, the splitting off of fibrinopeptide A) takes place, was intensively examined by Copley. These fibrin monomers can be formed in the presence of very weak thrombin activity. They complex with fibrinogen, not resulting in a solid clot, but increase rouleau formation of the erythrocytes and, in this way, are of utmost importance in hemorheology.

In this respect, fibrinolysis is another important process. It brings about a resolution of clots and can be nicely viewed in the thromboelastograph, as also demonstrated by Leroux. Fibrinolysis is likewise related to other blood clotting events. It is inhibited in the presence of platelets. On the other hand, a high

molecular product of fibrinogen is able to aggregate platelets, as was shown by Marion Barnhart. This aggregation is reversible. It resembles the aggregation caused by ADP, and occurs with the production of pseudopodia when platelets come into contact with a glass surface.

The building of platelet aggregates is important for hemostasis and for thrombosis. However, it does not seem to be related to the vascular changes encountered in the early stages of arteriosclerosis, i.e. to the intimal plaques occurring preferentially at vascular forks, as shown by Stehbens in vital microscopic and electron microscopic studies. Stehbens described hemodynamic disturbances occurring at these forks, and succeeded in demonstrating a tendency of the platelets to adhere to leucocytes, but not to preferential locations in the vessel.

The paper delivered by Scott-Blair stood amidst these experimental studies on coagulation. Scott-Blair presented a number of different equations describing the flow of blood through artificial capillaries, and for the processes of fibrin polymerization and softening. With regard to clotting, the equations were derived from thromboelastographic curves and from torsiometric values obtained from Scott-Blair's own apparatus. With all respect for the mathematical and physical sophistication of this work, this appears to me as a first brave attempt at describing the whole of physiology and pathology of clotting from a general standpoint. I believe, however, we must admire with still greater respect the competence of nature, in her ability to balance the forces of cellular and fibrin clotting in such a way that the flow of blood is insured and the loss of blood is prevented.

Plasma Expanders

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THE four papers devoted to plasma expanders ranged from detailed *extra vivum* rheological studies to *in vivo* experiments on dogs and rabbits.

Taking first the *extra vivum* work, general conclusions were that all dextrans of nominal molecular weight in excess of about 20,000 are likely to cause some degree of aggregation of human red cells, and that the viscosities of solutions of dextrans at isotonic concentrations are appreciably greater than those of plasma. The yield shear stress and the sedimentation rate of suspensions of human red cells increase with increasing molecular weight of dextran, although a reversal in sedimentation rate may occur if the concentration of dextran raises the viscosity of the suspending fluid to a sufficiently high value. These results support the conclusions of many earlier workers in this field. It was also shown that additions of dextrans of 40,000 molecular weight to suspensions of human red cells which had been strongly aggregated by the presence of fibrinogen or high molecular weight dextran lead to no relative improvement in flow behaviour compared with similar suspensions which were diluted with albumin or saline solutions.

The *in vivo* experiments emphasised the many additional factors which have been extensively reported elsewhere and which must be taken into consideration when plasma expanders are used in living systems. High molecular weight dextran infusions in healthy dogs led to a fall in haematocrit, the appearance of aggregation and a dramatic decline in cardiac output. A corresponding fall in oxygen consumption was also reported and is probably attributable to the blockage by aggregates of some of the vascular beds. The addition of low molecular weight dextran was followed by a further fall in haematocrit and a fall in the blood viscosity to a value comparable with the original. There followed a recovery of cardiac output and oxygen consumption. In the work on rabbits suffering from hypovolaemia the recovery of oxygen tension in muscle was much better after infusion of albumin or dextran of 40,000 molecular weight than after infusions of whole blood of high molecular weight dextran.

A suspension exhibits a yield shear stress when a complete structure which can transmit the stress is present. An increase in the strength of the structure raises the yield stress. In the case of blood, therefore, some relationship between the degree of aggregation and the yield shear stress might reasonably be expected. The addition of a dextran solution to an aggregated suspension of red cells raises the viscosity of the suspending fluid but reduces the haematocrit. The lowered haematocrit leads automatically to a fall in the viscosity of the total suspension, relative to that of the suspending fluid, and reduces the yield stress. The influence of yield stress is most marked at low flow rates so that the reduction in haematocrit of human blood from 45 to 40 per cent lowers the relative viscosity at high rates of shear by 10 or 12 per cent, but reduces it at low rates of shear by some 40 per cent.

The *extra vivum* experiments described in the papers indicate that the viscosity changes in blood resulting from additions of dextran of 40,000 molecular weight are very similar to those following additions of saline or albumin. From this it might be deduced that the additives all had a similar effect on the degree of aggregation of the red cells. On the other hand, visual evidence from *extra vivum* experiments not reported at the Conference[1] is that dextran solutions of 40,000 molecular weight do give better dispersion

of human red cells than saline if present in concentrations exceeding about 2 g/100 ml. The discrepancy between the conclusion reached in the Conference papers that dextran of 40,000 molecular weight alters the flow properties of blood to the same extent as saline solution, and the visual observation that the same dextran fraction disperses the red cells better than saline solution, can only be resolved by assuming that the degree of dispersion of the cells is not related in a simple, direct manner to the viscous properties (particularly the yield stress) of the suspension.

The fall in haematocrit following *in vivo* infusion of dextran is accentuated by the osmotic diffusion of tissue fluids into the circulatory system. The improved dispersion of the red cells which is generally observed following infusion of dextran of 40,000 molecular weight can be attributed to an increase in the disruptive forces to which the aggregates are exposed (which results from the increased flow rate brought about by the reduced haematocrit and modified plasma viscosity) and possibly to a reduction in the internal strength of the aggregates. Unfortunately the strength of the aggregates, and the forces between them must be deduced from yield stress measurements made *extra vivum* and, as mentioned above, the correlation with aggregation is apparently not a simple one.

Further experimental work is clearly required to confirm the *extra vivum* conclusions before the relevance of significance of viscometric measurements to the behaviour of dextran *in vivo* can definitely be established.

[1] ENGLSEF, J., STALKER, A. L. and MATHESON, N. A. *Lancet* 1124, May, 1966.

In vivo *Hemorheology*

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IT IS difficult to summarize such a conference like this except perhaps in the broadest historical terms. The thing that occurs to me is the relation between Poiseuille's work and what came before and after. Before his time, the formula for the flow of blood or the flow of liquid rather, through a tube, was not known. The relation between rate of flow, pressure gradient and volume flow was very uncertain. This was, of course, because people were using large tubes and as soon as the flow rate got high enough, they got turbulent flow and the predictions became very uncertain. And they simply did not know what the relationship was. Poiseuille, interested in the flow of blood, studied the flow of water (thank goodness, it was water and not blood) in small tubes. We are very fortunate that it was water he studied, not blood, because he would have come against the anomalous properties of blood and we would have never learnt how even water went through anything. Now this represented a considerable advance and, I think, we are in the process now of seeing a similar advance between *in vivo* hemorheology and hemorheology *in vitro*.

Now just as before Poiseuille's time no one really knew how water flowed through small tubes, nowadays we have not yet a quantitative knowledge of how blood flows through small tubes in the body. Enormous amounts of data are available on the flow of blood in various artificial systems. The thing, I think, can be divided in the body into large vessel flow and small vessels flow where these anatomy effects become apparent. In the large vessel flow, I think as has been said by previous summarizers the situation is fairly clear and, in fact, the important rheological problem there is in the structure of the wall rather than the structure of the blood; and we are now fortunately in the position of gradually accumulating more and more evidence of the measurements, direct measurements made of the viscous properties of arteries in the animal. Large arteries with various fine calibers applied to them and smaller vessels perhaps will be accessible by microscopic technique, such as Dr. Wiederhielm has been describing. So we might be able to continue the rheological investigation of the arterial wall right down to arterioles and capillaries, and so on, and more attention is being paid to that now, which is a very good thing. As far as the small vessels are concerned for the flow problems here, at last, the quantitation is catching up with the enormous amount of qualitative observation which has been made in the past. People have been looking at and describing the flow of blood in small vessels for many years. An enormous amount of very accurate and interesting, descriptive material is available that it has only recently become possible to establish quantitative methods for this region and, I think, that just as though the big jump across the time of Poiseuille the measurements of the flow of fluids through anything were about to arrive at a similar situation for the flow of blood through small vessels. There is no doubt that the proper place to measure the viscosity of blood is in the arterioles and in the capillaries. The amount of information that can be got from model studies can only contribute to this and help with its interpretation. I cannot answer the whole question. And it is now, I think, largely a matter of instrumentation which seems to have been blossoming in the last few years that we are finally in the position to make accurate studies of what does go on in the small vessel circulation. I think this is a very hopeful future for this, indeed, extremely hopeful. And I am sure in the next meeting of this group there will be numerous papers on dynamic measurements of viscosity in small vessels by a

nnmber of techniques. The direct observation with and without various computer attachments to the machinery, other indirect ones, such as Groom has described, surely will be very useful in the differential passage of material through the circulation, can be subtly analyzed in this way. So in conclusion, I would say that as yet the new techniques are just flying their flags and, I think, with every promise of very great achievement in the next few years. The quantitative hemorheology of the microcirculation is just about to get launched and, I think, it is very exciting.

Blood Cellular Elements

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THERE were two sessions of the Conference specifically under this title, with twelve interesting papers. Of course, there were a great many other papers, under different general headings, that had reference to the form, elasticity, and interaction of the "particles" in blood. For many years there was lack of emphasis in rheology on the properties of the particulate matter, as largely determining the rheological properties of a suspension that is as closely packed with particles as is blood of normal hematocrit. Beautiful work has been done with ingenious experimental, microscopical methods and highly sophisticated hydrodynamic theory has been produced on the problem of *very dilute suspensions*. So far, no one could claim that we have mounted a real attack on the actual problem of flow of blood of 45–50 per cent hematocrit, where obviously the interaction (jostling, bumping, deformation) of the cells must be the dominant factor. For example, the contrast between what happens with relatively isolated cells in a shear gradient, e.g. from the beautiful work of Goldsmith and Mason, and what occurs in normal blood, e.g. from the remarkable pictures of Phibbs, is evident in the orientation of the cells. Isolated cells turn to the posture of discs with their plane at right angles to the axis of flow; whereas in blood of normal hematocrit most of the cells appear, in a cross-section of an artery, to be "edge-on".

While it may take many years to produce an adequate theoretical treatment of this dominant interaction of the cells in flow, we know now that the shape, deformability, and internal viscosity of the cell and of its membrane will be all important in that eventual formulation. Dr. Mason and his co-workers find marked differences, even in very dilute suspensions, in the behaviour of rigid vs. deformable particles. There is some rather neglected Japanese work[1] that shows that suspensions of rigid ("tanned") crenated cells have viscosities not five, but fifty times that of water! All this means that we must learn as much about the Physics of the red cell itself (and of the other cells of blood) as we can, before we can hope to formulate an adequate theory of the rheology of blood as it flows in the circulation in arteries and veins, as well as in capillaries, where obviously (in "bolus flow"), deformability is the important factor.

The Conference indicated how new methods of studies of the properties of the cell itself are being added to our armament. The tendency to form rouleaux, a property of the red cell membrane surface, has been studied quantitatively by rheological methods, e.g. the work of our Chairman, Professor Copley, at low shear rates. The fascinating microscopical observations of Goldsmith on the separation of rouleaux in a shear gradient offers a new quantitative approach to the wide-spread problem of cellular cohesion in Biology—important even as far afield as in cancer research. Micromanipulation, "poking and sucking" bits of red cells, is helping us understand what might happen to an erythrocyte in the circulation. The electron microscope is being used on the red cell membrane and its interior, with some results which we were shown that are difficult at present to interpret. I hope that the refined and sensitive methods of birefringence and dichroism, described by Professor Pfeiffer, will enable us to learn more about possible structure *inside* the normal red cell, for which our own analysis of the physical equilibrium of the membrane seems to call.

The method of the "Black Box", so popular with those of engineering training and with the Systems Analysts, though a powerful tool of theory, is, as it should be, most unsatisfying to the basic scientist, who must know what is inside the black box, and, if possible, watch the wheels go round there. The most encouraging aspect of this Conference to me has been the change in attitude of those who came to hemorheology through rheology, rather than through biology. Ten years ago I visited the laboratories of some of the Engineer pioneers of hemorheology. I was astonished to find that there was not even a simple microscope in their laboratories, and they actually had never seen for themselves the "particles" of the samples of blood on which they worked. Today, I am sure, this is not true in the laboratory of any hemorheologist. Even if only as best evidence that the erythrocytes in our suspensions are normal, microscopic examination of the fluid under study is essential. It has taken some time for hemorheologists to see how important is the study of the cellular elements, but the future of our subject surely depends upon this aspect of our research effort.

[1] WADANO, K. *Tokushima J. exp. Med.* **3**, 111, 1956.

Clinical Hemorheology

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THIS Conference has, indeed, been a great contribution for the clinician, in order to get acquainted with the basic sciences underlying various disturbances in flow as we can observe in pathology. It is not very long ago since the only measurement almost regarding shock treatment, was recording of blood pressure. Today, and as is evident in this Conference, this is no more a way of looking upon shock where the proper flow is the goal in treatment. It is, however, to the clinician, quite evident that we have difficulties in understanding the different languages talked by different scientists. One of the main goals of this society, I think, should be to work out clear definitions in some of the aspects of hemorheology. This will concern viscosity and different terms of viscosity. The equipment has no doubt been improved during the last years. More accurate measurements can be done on the shearing forces contributing to viscosity of blood. There is, however, still a little bit of a disagreement on what is an adequate viscosity of both plasma and whole blood viscosity and I think this depends on the equipment used for the determination. As earlier emphasized, there is quite a difference between the measurements made *in vivo*, *extra vivum*, and from *in vitro* models. This has also been clarified in experiments while *extra vivum* equipments had been used in providing energy for circulation *in vivo*. For us it has also been quite evident in the clinical papers presented that two hemorheological disturbances must be clearly separated from changes in vascular wall and vascular environment, as for example, the discussions on diabetes gave evidence of. There is no doubt a very great need today to differentiate between total flow, flow rate and the nutritional flow. Here again model studies have shown to be very significant in order to try to understand the forces which will separate off different cell particles to different branches and also, it has been stressed that the flow rate will be different in on-flow and off-flow types of the capillary tube. One of the main topics has been the clotting phenomena related to clinical situations and again, the primary thing has been the platelet aggregation and the events secondary to this aggregation have been all earlier emphasized and the methods applied here have shown specifically the effect of lysis on the clots. The effects of split products have been emphasized and apparently they will be able to accelerate the clotting process and alter viscous properties of blood. It has been fairly touched only on some new aspects on what fat means for the circulation. Here in a field which is so important in clinical rheology must be one of the main interests for the coming conferences. As a general field, hemorheology has come into the clinical situation in order to get a better understanding of the dynamics in pathology. This better understanding will open possibilities to work out new agents which will be able to act on different parts of shearing forces in the control of the flow of blood and in distribution of the different cell elements for different tissues. The clinical significance of this new discipline is without doubt and I think the conference here has initiated an interest among different disciplines, but it will come to the benefit of the patient, and that is what we are so happy about.