Symposium on Microvascular Methodology

held during the Third European Microcirculation Conference in Jerusalem, March 15th-19th, 1964

Edited by P.-I. BRÅNEMARK, Gothenburg

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Introduction

During recent years microvascular form and function has steadily grown to become a major topic in biologic research. This development also includes the rapid progress in biorheology as a connecting link between biology and physics. This state of affairs is, from the microcirculatory point of view, very gratifying. Moreover, it also implies that a field of research, which initially was rather restricted by the limited number of techniques available, in a very short period of time has acquired an extensive amount of varied methods.

However, the applicability of these methods for a specific microvascular approach may be difficult to evaluate. This was the reason for including a small and informal symposium within the Third European Conference on Microcirculation in Jerusalem, 1964. The symposium dealt with various techniques which are being used, or which could be used, for microcirculatory studies on structure and function, experimental as well as clinical. The intention was to provide an evaluation of the most important methods, recent developments and future prospects. Because of the limited time available detailed information on single methods could as a rule not be included, nor could all methods be discussed or even mentioned. Although discussed in the symposium the important contribution of electron microscopy could unfortunately not be included in this volume. The heterogenous topics dealt with clearly illustrate the wide spectrum of the microvascular field.

In the printed form of the symposium a general review of hemorheology has been added, since the application of methods in this specific microvascular field has been subject to much controversy during years, and it was considered of importance to clear basic things up in this area.

Göteborg, May, 1964

P.-I. Brånemark

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From the California Institute of Technology, Division of Engineering
Pasadena, Calif. (USA)

Rheology and Microcirculation[1]

By HAROLD WAYLAND

I. Introduction

The dominant impression which I have carried away from looking at some of the excellent movie films which have been produced of living microcirculatory beds has been of the flow of particles or clumps of particles through interconnecting channels of great complexity. It is fascinating, for example, to watch a stream of erythrocytes passing a small, branching channel, and try to guess which ones will go off down the narrower path. Superficial observation of such films raises many questions concerning geometry, flexibility of walls, existence or non-existence of valving mechanisms, why red cells aggregate under certain circumstances, and so on: a list better made out by a physiologist. Through all of these questions one basic observation remains, however: the flow in the microcirculation is that of a heterogeneous, multicomponent system. We cannot hope to understand blood flow in the microcirculation without understanding the way in which a concentrated suspension of erythrocytes in plasma behaves even if, for a first approximation, we can neglect the effect of the platelets and the white cells. We are thus led, *a fortiori*, to considering the particulate nature of blood.

In reading the literature concerning the flow of blood in the vascular system I have often been confused by the terminology. I expected to have to learn many terms from physology, but many of

[1] The research program which has laid the background for this paper is being supported jointly by the Los Angeles County Heart Association, the U.S. Public Health Service under Grant No. HE 07902 and the California Institute of Technology.

the terms I knew from fluid mechanics and rheology appeared in ways I did not always understand. Such terms as "laminar flow", "turbulent flow", "viscosity", "streamline flow", were being used without precise definition. The definitions which could be unscrambled from context did not seem to form a consistent pattern even among physiologists and biophysicists.

Since one of the primary problems in interdisciplinary research is that of communication, I shall risk redundancy and discuss the definitions of certain basic terms as I feel they should be used, particularly with respect to the microcirculation.

II. Some Basic Concepts and Definitions

In recent years the terms rheology, biorheology and hemorheology have become increasingly current. The term "rheology" was coined by ***Bingham*** in 1929 as the "science of deformation and flow". This definition is extremely broad, but traditionally fluid mechanics of inviscid and Newtonian fluids and the mechanics of elastic solids have been excluded from rheology. Since the fluids involved in blood flow are non-Newtonian and the solids viscoelastic we are on firm ground in accepting rheology as the all inclusive discipline for a description of the ***mechanical*** behavior of the fluids, the vessels and the tissues involved in any part of the circulatory system. ***Copley and Scott Blair*** [1962] and ***Whitmore*** [1963] establish a distinction between "hemorheology" and "hemodynamics". To me this is an unnecessary complication. Hemorheology is broad enough to encompass all of the mechanical aspects of the problem. Rheology certainly includes the dynamics of deformation and flow and, perforce, the dynamic description of a system flowing in non-rigid tubes must take into consideration the shape and mechanical properties of the tubes in which the flow takes place. Of course, if we wish a more inclusive term we might consider "hemangiorheodynamics". I will stick with "hemorheology".

One of the criteria which I mentioned as being an accepted distinguishing feature between ordinary fluid mechanics and the rheology of liquids is whether or not the fluid is "Newtonian". To understanding the meaning of this statement we must first define viscosity—a concept about which I have found a great deal of confusion.

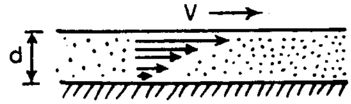

Fig. 1

Consider an infinitely large plate moving parallel to a fixed plate with a velocity V, and imagine that the liquid of interest is contained between the two plates. If the velocity field in the fluid varies linearly from zero at the fixed plate to V at the moving plate (i.e., at $^1/_3$ of the distance the velocity is V/3, etc.), we can define a definite *velocity gradient* in the fluid: $\Gamma = (V/d)$ (fig. 1). In the metric system Γ is expressed in centimeters per second per centimeter. The length dimension is often "cancelled"

$$\frac{\text{centimeters}}{\text{second}} \; \frac{1}{\text{centimeters}} = \frac{1}{\text{second}}$$

and we express the velocity gradient (also called the *shear strain rate*, or *shear rate*) in *inverse seconds:* compact, but sometimes misleading. If we could now measure the shear stress on the moving plate, i.e., the force per unit area required to keep that plate in uniform motion, we can express the relationship between the shear rate and the shear stress in the form

$$\tau = \eta \Gamma \tag{1}$$

If the proportionality factor η is a constant for all values of the shear rate Γ below some critical value ($0 < \Gamma < \Gamma_c$) and, further, if $\tau = 0$, when $\Gamma = 0$, we speak of the fluid as being *Newtonian*. For a Newtonian fluid a plot of τ against Γ must give a straight line passing

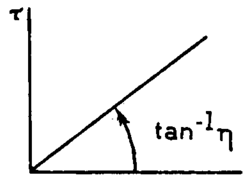

Fig. 2

through the origin. The slope of the line is the viscosity coefficient.[1] There is much confusion in the literature concerning the definition of a Newtonian flow regime: for example, one often reads of a substance "becoming Newtonian at high shear rates". This would be a correct statement for the case shown in curve A of figure 3: although the shear stress is not linear with strain rate for small values of Γ, it becomes asymptotic to a straight line through the origin for large Γ. It is not correct to speak of Newtonian behavior for large shear rate if the asymptote does not pass through the origin, as in case B, figure 3. The very question of a satisfactory definition of viscosity when the τ-Γ curve is not a straight line through the origin needs exploration. Taking the case in which we get a curve which passes through the origin (fig. 4) we can write

[1] The reciprocal of the viscosity coefficient is called the "fluidity". For non-Newtonian fluids the use of fluidity frequently gives simpler mathematical expressions than does viscosity, but since this falls outside the province of this paper fluidity will not be discussed.

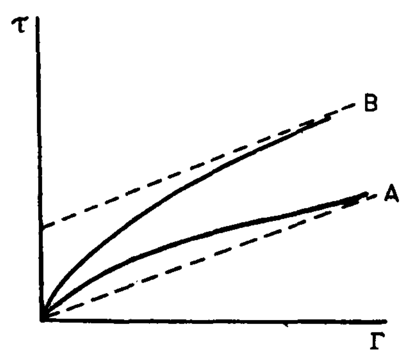

Fig. 3

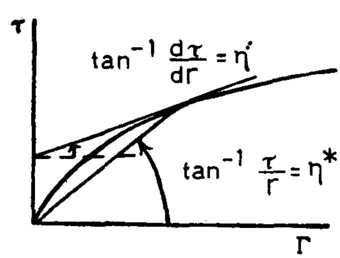

Fig. 4

$$\tau = \eta^* (\Gamma)\, \Gamma, \lim_{\Gamma \to 0} \eta^* (\Gamma)\, \Gamma = 0 \tag{2}$$

where η^* might be called the "secant viscosity"[1] to emphasize its relation to the stress-strain rate curve, since

$$\eta^* (\Gamma) = \frac{\tau}{\Gamma} \tag{3}$$

i. e., it represents the slope of the line drawn from the origin to the point at which τ and Γ are measured. An equally useful definition would be to define the viscosity corresponding to a given shear rate by the slope of the tangent to the τ-Γ curve. We would thus have a differential formulation

$$\eta' (\Gamma) = \mathrm{d}\tau/\mathrm{d}\Gamma \tag{4}$$

or, in the integral form

$$\tau = \int_0^\Gamma \eta' (\Gamma)\, \mathrm{d}\, \Gamma \tag{5}$$

where η' can conveniently be called the "differential viscosity". Each of these definitions is useful. Unfortunately some authors do not give the definition being used. Sometimes this can be ferreted out of the context of the paper. Sometimes it is impossible to tell.

When dealing with non-Newtonian viscosity the simplest way to avoid ambiguity is to plot the shear stress-shear rate curves at a prescribed temperature. Unless a substance is Newtonian, it is meaningless to give a value for the viscosity without specifying the conditions under which it was measured and the definition used for calculating it, nor is it of any basic scientific value to give either the slope of the secant or that of the tangent at a single point of an otherwise undefined curve. On the other hand, comparative results for several samples taken under identical conditions may be useful as a non-specific clinical test. Such an application has been made by *Harkness* and his coworkers [1963] in measuring the time required for a prescribed volume of blood plasma to flow tl :ough a given glass capillary under carefully controlled pressure difference and temperature. They have found a sufficiently good correlation between the flow-through time of plasma in his apparatus and certain pathological

[1] It has been conventional to call the quantity η^* the viscosity even for non-Newtonian systems (*Peterlin* [1953]). *Haynes* [1962] suggests "generalized viscosity" for this quantity, but this does not readily indicate how it is related to the stress-strain rate curve.

conditions of the patient from which the sample was taken to be clinically useful to them. As a rheologist I do not want to belittle the possibility of such single-point measurements in clinical applications—after all, sedimentation rate can be useful. We must realize, however, that such measurements will give too little information to apply the data to any possible analysis of plasma flow in any other situation. It would probably be best not even to apply the term "viscosity" to the results of such measurements. The ideal viscometer used as the basis of the definition of viscosity does not exist. The best approximation, and an excellent one when the instrument is carefully built and used, is the Couette or rotating cylinder viscometer. In this instrument the liquid is placed between two concentric cylinders. One is driven at a constant rotative speed and the torque required to hold the other stationary is measured. With a sufficiently small gap compared to the diameter and length of the cylinders; with careful attention to end effects; and maintaining the rotative speed low enough to avoid hydrodynamic instability of any sort, it is possible to make measurements which give essentially a true shear stress-shear rate curve. In the case of heterogenous mixtures special precautions are necessary even in a Couette viscometer. One of the postulates in the measurement of viscosity in such an instrument is that the velocity gradient is constant across the gap, and can be calculated from the velocities of the walls and the gap separation. In a slurry of particulate matter, it is impossible for the center of a solid particle to be coincident with a wall, and hence no particle will have precisely the velocity of the wall. *Cokelet* and *Merrill* [1963]

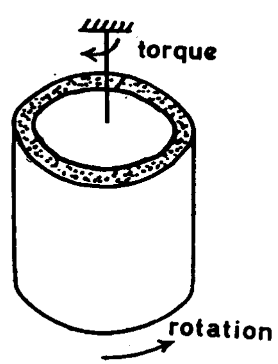

Fig. 5

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ingeniously surmounted this difficulty in measuring the bulk viscosity of erythrocyte suspensions by grooving the walls so that at the effective hydrodynamic boundary all components of the suspension had the same velocity.

Good instruments of the Couette type are expensive to build and require great care in their use, so that viscometric measurements depending on the rate of flow through a capillary tube are more often made. Here the interpretation of the results is somewhat more difficult if the viscosity depends on the shear rate. It is a well established principle of hydrodynamics that for most liquids we can assume that the velocity of the liquid is zero at a fixed wall. In a circular tube the velocity profile must be such that the velocity increases from zero at the wall to a maximum at the center. The velocity gradient, however, is a maximum at the wall and a minimum at the center, so if the viscosity depends on the velocity gradient, the profile will reflect this dependence. If the viscosity is constant the profile is parabolic (fig. 6 [a]). If the viscosity decreases for increasing shear rate the profile will be blunted toward the center (fig. 6 [b]), and if it increases

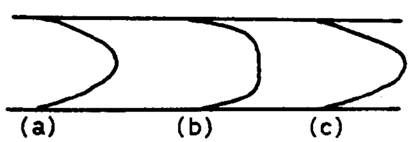

Fig. 6

with shear rate, the profile will be sharpened toward the center (fig. 6 [c]). The much used Poiseuille formula is based on the assumption that the viscosity is constant. Under this hypothesis we have, for a rigid tube of circular cross section, the rate of volume outflow given by the following expression

$$Q = \frac{\pi r^4 \Delta P}{8 \eta l} \tag{6}$$

where

r = radius of tube;
ΔP = pressure difference across length of tube;
η = viscosity coefficient (a constant)
l = length of tube.

This Poiseuille formula is often used without correction even when it is known that the viscosity varies with the shear rate. If we solve Eq. (6) for η we obtain

$$\eta = \frac{\pi r^4 \Delta P}{8 l Q} \tag{7}$$

which, for a given geometry and pressure difference will permit us to calculate an "apparent viscosity" from measurements of Q. It has sometimes been contended that we should talk about "flow resistance" instead of viscosity for microcirculatory systems. For a given geometry we would write

$$Q = R \Delta P \tag{8}$$

where we could call R the ***flow resistance.*** Actually, there is only a semantic distinction in calculating flow resistance instead of effective viscosity when the geometry is known; if the geometry is not known, the use of flow resistance permits us to lump our ignorance in a single coefficient instead of dividing it among r, l and η, which may be a laudable thing to do.

For many systems it is possible to calculate the shear stress-shear rate relation from a series of measurements made in a capillary viscometer at a series of pressure differences even though the dependence of viscosity on shear rate is not known (*Peterlin* [1953]). The shear rate-shear stress plot reconstructed from such data will coincide with one obtained with a Couette viscometer ***if the fluid can properly be treated as a homogenous substance.*** For a suspension in which the particle diameter is at most a few times smaller than the tube diameter, we can no longer expect to use the bulk properties (i. e., those properties for which the material can be treated as a homogenous substance) of the fluid to describe the flow behavior in these small tubes. Such effects are well known in the physiological literature; e. g., the Farhaeus-Lindquist effect in which the effective viscosity of erythrocyte suspensions appeared to decrease with tube diameter for tubes below about 100 microns in diameter is the classical example. The fact that the particles are large with respect to the diameter of the tubes should lead us to expect anomolous flow behavior in this case without having to call on such phenomena as axial streaming of the erythrocytes. For a clearer understanding of physiological processes we need a more precise description of events, taking into consideration the particulate nature of blood.

It is probably fair to say that, for blood, the shear rate-stress curve as normally treated by rheologists cannot be measured in a capillary viscometer. Since, physiologically, blood does flow in tubes (even if they are not rigid circular cylinders), this raises a significant question as to the usefulness of the usual physical measurements. I will return to this question in section IV.

Two other terms from hydrodynamics—*laminar flow* and *turbulent flow*—must be reexplored in the context of the microcirculation. Phenomenologically we can speak of laminar flow when there is a correlation between the velocity at one point in the flow with that at every other point in the flow. Such flows do not have to be steady: the intermittent shedding of vortices in the Karman vortex street following a ship, or down stream from an arterial stenosis can be validly classified as laminar flows. It is difficult, if not impossible, to give a universally acceptable definition of turbulent flow. It is certainly characterised by the fact that there is only a statistical correlation between the velocities measured at two points in the flow sufficiently removed from each other. There may be a well defined mean velocity at all points in the flow, but there will be random fluctuations of the velocity about the mean at each point.

Even in the macrocirculation it seems unlikely that fully developed turbulence exists except possibly in very limited regions. The very fact that the flow is pulsatile in the portions of the circulation where the velocity is the highest means that we must deal with transient phenomena. The flow velocities may readily be high enough that instabilities in the flow will increase rather than being damped out; but in most, if not all, parts of the normal circulation this will not result in the degree of randomness which is called turbulence by the fluid mechanist. Furthermore, great care must be exercised in applying the usual criteria for onset of turbulence which are applicable in Newtonian fluids like water, flowing in rigid tubes. Viscoelastic walls can markedly delay the onset of turbulence, and small amounts of macromolecular or particulate additives may also introduce significant qualitative changes in the nature of the flow. For example, *Lindgren* [1959] found that as little as 0.1% of bentonite markedly influenced the velocity of propagation of turbulent flashes in tube flow; and *Toms* [1949] and, more recently, *Fabula* [1963] have shown that very small concentrations of certain polymers (of the order of a few parts per million) can have marked effects on flow properties. At this point I would like to emphasize the parallel between the interest in engineering applications and a scientific understanding in, say, fluid mechanics, and the interest in clinical applications and basic understanding in medicine. *Fabula's* interest in drag reduction in turbulent flow has led him to the study of the effect of a large number of macromolecules on flow resistance in pipes and rotating discs in the turbulent regime. His work permits the choice of several materials which, in very small quantities, will lead to drag reductions of a factor of two or better for high velocity flow. On the other hand this work has thrown no light on the mechanism by which this drag reduction is achieved. Such empirical studies can be of great practical value, and should be encouraged. We must, however, be clear as to our aims. I will leave it to the reader to make his own parallels in the medical field.

The problems of flow instability and onset and development of turbulence in non-Newtonian fluids and, particularly, in suspensions are very little understood. It is safe to say, however, that the usual hydraulic criteria based on the Reynolds number cannot be meaningfully applied. In fact, the use of Reynolds number in hemorheology deserves some exploration.

Osborne Reynolds found that a certain dimensionless parameter given by the ratio of viscous to inertial forces was useful in interpreting certain classes of model experiments in hydraulics. In particular, when viscous and inertial forces were dominant, two systems in which this number was the same would have dynamically similar behavior. This became particularly evident in connection with transition from laminar to turbulent flow of a Newtonian fluid in a long, circularly cylindrical pipe. This particular parameter, now known as Reynolds number, is given by

$$\mathrm{Re} = \frac{\varrho \mathrm{v} l}{\eta} = \frac{\mathrm{v} l}{\nu}$$

where η is the viscosity of the fluid, ϱ its density, v a characteristic velocity, and l a characteristic length. ($\nu = \eta/\varrho$ is called the "kinematic viscosity".) For a Newtonian fluid ν is well defined. In laminar pipe flow of such a fluid the velocity at any point in the cross section bears a simple relationship to the velocity at any other point (and hence to both the mean and maximum velocities), and the diameter of the pipe gives a convenient reference length. Accepting the mean velocity (which is readily measured as the ratio of the volume flow rate divided by the cross section: $\dot{Q}/\pi R^2$) for the reference velocity, either the radius or diameter of the pipe as the reference length, and with a single number to represent the kinematic viscosity of a Newtonian fluid at any given temperature, the Reynolds number is readily defined. It is found, for example, that turbulence will set in in pipe flow when the Reynolds number is greater than about 1000 (based on the pipe radius).

On the other hand, in discussing the stability of the flow around a sphere falling vertically down a tube only a little larger than the ball, we have an additional length to consider: the radius or diameter of the ball. In this case, if we wanted to change the scale of the experiment, we would want to maintain two dimensionless numbers fixed in order to have dynamically similar situations: the Reynolds number (which could be based on either the ball radius or the tube radius) and the ratio of the two radii. If one were to find, for example, that in a given geometry the flow around the ball became unstable for a given velocity of fall, if one considered Reynolds number alone (based on the tube radius) one might predict that if the tube diameter were doubled the velocity of the ball would have to be doubled to reach instability. But how about the ratio of the ball to tube radius? According to dimensional analysis, the ball would also have to be doubled in radius to have a dynamically similar situation. The use of Reynolds number alone, without considering the other parameters of importance can be most misleading.

When it comes to describing the flow of blood in the smaller vessels, the problem becomes even more complicated. A mean velocity of flow can be defined, but this is not very meaningful in terms of stability criteria when there is a pulsatile component to the flow. The tube radius has a definite meaning only for circularly cylindrical tubes of fixed lumen, a condition which may obtain in the capillaries but nowhere else in the microcirculation. There is a serious question as to what "viscosity" to take, since both the matrix fluid, the plasma, and the suspension show non-Newtonian flow behavior. Even assuming we can define the Reynolds number in a meaningful way, we must introduce the ratio of the erythrocyte diameter to the tube diameter. (If the erythrocytes do not all have the same shape, an additional shape factor would have to be introduced.) So, even neglecting such physical parameters as the wall elasticity, the deformability of the erythrocytes and all chemical interaction phenomena we see that the Reynolds number *by itself* is not a very useful or meaningful parameter. A careful treatment of the various dimensionless parameters which are important can be very illuminating in analyzing complicated flows. But to put the entire burden on the Reynolds number in a situation as complicated as blood flow in any part of the circulation is apt to be more misleading than illuminating.

Another hydrodynamic term which needs to be more clearly understood is that of "boundary layer". The concept of a boundary layer, as introduced by *Prandtl*, is primarily applicable to fluids of low viscosity to permit the maximum use of the fluid mechanics of inviscid fluids. For many important flow situations in hydrodynamics and aerodynamics, it is necessary to introduce viscosity only close to bounding surfaces where the rate of shear is high. As long as the "boundary layer" (in which viscosity is important) is thin, the Navier-Stokes equations can be linearized, which greatly simplifies their solution. Outside this boundary layer the shear rate is small enough that viscosity can be neglected for many practical purposes permitting the use of ideal fluid theory to describe this part of the flow. It seems unlikely that there is any part of the circulatory system in which we can assume that no energy is dissipated in all parts of the flow—i.e., where viscosity can be neglected. We may be interested in discussing a layer of fluid near some boundary surface, but let us not call it a boundary layer and expect to be understood by a fluid mechanist. It is certainly valid to define a term and then use it with the newly defined meaning, but it will not make intercommunication easier if a well-established term is so redefined.

III. Mathematical Models

The physical scientist eventually tries to describe physical phenomena by means of mathematical equations. We must keep in mind that a mathematical description of some phenomenon is merely a particular type of model, and bears the same kind of relationship to the original situation that a mechanical model might bear. The particular equations of motion of classical fluid mechanics—the Navier-Stokes equations for a viscous fluid—are *differential equations*, i.e., they relate the motion at a point to that at neighboring points, or, more precisely, the properties of a small volume element to those of neighboring volume elements. Mathematically we make the assumption that these volume elements can be made as small as we please. We know that physically if the element of volume is chosen to be sufficiently small we will eventually reach a size in which the mole-

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