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STANLEY MASON: HIS CONTRIBUTION TO THE SCIENCE OF BIORHEOLOGY

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It has been a privilege to assemble and edit this issue of Biorheology dedicated to the memory of a great scientist, Stanley George Mason. In an obituary (1), I have previously given a brief account of his life's work and his studies in physicochemical hydrodynamics. The present article is an account of the manner in which his work has led to the advance of the science of Biorheology, and in particular how it laid the basis of much of our present understanding of the flow properties of mammalian blood cells.

FROM PAPERMAKING FIBERS TO RED BLOOD CELLS

Stanley Mason was led into the realm of Biorheology through his interest in the flow behavior of suspensions of pulpwood fibers in the papermaking machine. In 1948, as head of the Division of Applied Chemistry in the Pulp and Paper Research Institute of Canada on the campus of McGill University, he initiated a series of investigations in which the complex behavior of pulpwood fibers was modeled by that of simpler rigid and deformable spheres and spheroids. It was typical of the man that he dissected out the complex problem and devised a set of relatively simple but definitive experiments capable of being analyzed by rigorous hydrodynamic theory.

(a) First Experimental Approach: Couette Flow

In all, 30 papers were published bearing the title "Particle motions in sheared suspensions", the first in 1950 (2), the last in 1981 (3). These investigations dealt with particle behavior and interactions in simple shear flows and were carried out in a series of Couette flow devices using techniques that became progressively more sophisticated. Particles were observed through a microscope in the interior of a suspension in the stationary layer between counter-rotating cylinders (1,4) or discs (5). In this way, the elements of fluid under investigation could be kept in the field of view as long as desired One has to remember that such experiments had never before been achieved. In the early days (1,6), the graduate student would sit for hours peering through an old microscope into the Couette apparatus containing the particles suspended in corn syrup ("If the experiment did not work you could always eat it", quipped Dr. Mason). The Cartesian and polar coordinates of a rotating sphere, rod or collision doublet of spheres were obtained by stopping the flow and using a measuring eyepiece and goniometer. Later, a Paillard Bolex 16 mm cine camera, special projection tables for frame by frame analysis of the films, and suspending fluids with a wide range of densities and viscosities made life easier for the students, and enabled much more sophisticated experiments to be attempted. Thus, particle motions could be observed and recorded on cine film or videotape simultaneously in directions along and normal to the vorticity axis (7). The effect of electric fields applied along these axes on the motion of a rigid spheroid (8) or on two-body interactions between rigid spheres or liquid drops (9) was also investigated.

At all stages of the work, the experiments were designed to be rigorously tested by hydrodynamic theory. Thus, for the first time, the theory of Jeffery (10) on the rotation of rigid ellipsoids in a simple shear flow at zero Reynolds number was tested using prolate (rigid rods, 11) and oblate spheroids (rigid discs, 12). The theories of G.I.Taylor on the deformation, internal circulation and break-up of fluid drops (13,14) in irrotational (hyperbolic) and rotational (Couette) flow were tested (15,16). The theory of Smoluchowski for the shear-induced two-body collisions of rigid spheres (17) was tested (6) and the experiments extended to fluid drops (18). Later on, equations giving the complete solution for the trajectories of two colliding rigid spheres were experimentally tested (19) and the theory extended to the case where interaction forces other than hydrodynamic operate as sphere surfaces approach to within 50 nm (20,21).

(b) Microrheology

Already in 1957, when Dr. Mason became interested in particle behavior in suspensions flowing through circular tubes and its relevance to the circulation, a substantial body of experimental and theoretical knowledge of particle behavior in Couette flow had been acquired. It might have been applied to the mammalian red cell, an oblate spheroid capable of deformation at sufficient shear stress, or to the unactivated platelet, a relatively rigid oblate spheroid, or its activated form approximating a spiny sphere (22). However, this development had to await new experiments in circular tubes. Before describing this work, it is appropriate to define Mason's methodology, that of microrheology. The term microrheology was first coined by Dr. Mason when he suggested the title "The microrheology of suspensions" for the author's Ph.D. thesis (23). As defined in the chapter on the microrheology of dispersions "the general problem of microrheology is the prediction of the macroscopic rheological properties of a material from a detailed description of the behavior of the elements of which it is composed" (24). In the work described above, the materials were suspensions of rigid and deformable spheres and spheroids, the elements were the elementary volumes of Newtonian fluids each containing a particle. The behavior of the elements as seen under the microscope, first in isolation from each other and then in interaction in dilute and (as described below) concentrated suspensions undergoing laminar shear flow was investigated.

It has been argued that since mammalian blood is such a concentrated suspension of red cells, the individual corpuscles are crowded together and are incapable of individual undisturbed translation and rotation. What then is the use of studying the motions and two body interactions of individual cells in very dilute suspensions? Professor Alan Burton made this point at the 1st International Conference on Hemorheology in Reykjavik while commenting on the question whether rouleaux of red cells exist in the normal circulation. Dr. Mason answered: "...we recognized this crowding problem in a completely different context a good many years ago, in studying the flow behavior of fiber suspensions. But just recently, we presented a paper which is now being published (25) and in which we have studied the mircrorheological behavior of concentrated suspensions of rigid spheres ......where there is continuous interaction between the particles. One can argue that it is unrealistic even to think of an isolated particle in such a system. But I can assure you that we have put a lot of order into the extremely complicated phenomena that occurred there, and we have been able to do this because of our earlier studies with the single particles, with triplets, quadruplets and thus synthesizing the system (26)."

MODEL EXPERIMENTS IN HEMODYNAMICS

Since 1957, Dr. Mason and his students had been working on the flow of large bubbles through small tubes (27), work which was continued by the author (28) and which clearly had relevance to the movement of red cells in the capillaries. Indeed, Prothero and Burton (29) had used the flow of a series of entrapped air and liquid bubbles through capillary tubes containing water as a model for such flow.

Also, in view of the observations of many physiologists going back to the time of Poiseuille, that there is migration of corpuscles across the planes of shear away from the vessel wall, Dr. Mason considered it of particular importance to establish whether such axial migration could occur with single particles at low Reynolds numbers and to establish whether it could lead to plug or partial plug flow in concentrated suspensions with the particles unable to undergo independent rotation. Plug flow without independent particle rotation and an accompanying axial concentration with the development of a thin annulus of peripheral pure suspending liquid was earlier demonstrated in pulp fiber suspensions (30). The effect was shown to be associated with the flexibility of the fibers and their capacity to form continuous networks. As the flow velocity increases the thickness of the peripheral layer, in which the entire velocity gradient is concentrated, increases until a critical point is reached at which turbulence develops in the layer.

Mason pointed out that blood represents in many ways a system analogous to fiber suspensions. Both cases involve the flow behavior of a crowded system of flexible particles in which free rotation of the particles is prevented by their interaction. He noted that measurements of the electrical resistance of blood flowing through glass tubes (31) have led to the conclusion that there was plug flow in an axial core with the required shear occurring predominantly in an annulus of plasma at the wall. The data further suggested that at very high flow rates turbulence developed in the plasma annulus before apparent breakdown of the plug took place. We know now, ofcourse, that this is not the case. It is only at the lowest flow rates that a plug consisting of a network of rouleaux surrounded by a peripheral cell-depleted layer forms (32,33). As the flow rate increases, the plug breaks up and the velocity gradient spreads across the entire vessel.

The following then, are the key discoveries obtained by Dr. Mason and his students in the tube flow of suspensions which have contributed to our understanding of the rheology of blood.

(a) The Red Blood Cell as a Deformable Droplet: Axial Migration

At the time the first definitive experiments on axial migration in Poiseuille flow were carried out in Dr. Mason's laboratory, long held but false theories of particle migration were still circulating among physiologists and taught to medical students. These theories invoke the Bernoulli theorem to prove that there is a radial pressure gradient across the tube which results in a force moving particles from wall to axis. Schlarewsky (34) had proposed a density-dependent, and Fåhraeus (35), a particle size-dependent hypothesis for inward migration of blood cells from the tube wall. The latter hypothesis was in support of the finding that, in 100 μm diameter tubes at a Reynolds number ~ 0.4, white cells, although less dense but larger than red cells, are more axially distributed than red cells (36). However, the more common expressions of Bernoulli's theorem do not account for viscous dissipation of energy, and therefore apply strictly only to an inviscid fluid and can at best only be used for real, viscous fluids at high tube Reynolds number when inertial forces dominate viscous forces. The more complete version of Bernoulli's theorem, as well as the application of the laws of conservation of momentum show that there is no radial pressure gradient in the tube. Rather, it is the wall together with the deformation of the suspended particle, or with inertia of the fluid that produces migration effects.

The experiments in Dr. Mason's laboratory showed that there is lateral migration of deformable drops and flexible fibers across the planes of shear away from the tube or Couette cylinder wall at very low Reynolds numbers (creeping flow) but that such migration is absent with rigid spheres and spheroids (37,38). Thus, at a Reynolds number ~ 10^{-3} in an 8 mm diameter tube, a water drop of 0.35 mm diameter suspended in a 50 Poise silicone oil situated 2.6 mm from the tube axis migrated inward at an initial rate of just under 1 $\mu m\ s^{-1}$ at , reaching a radial distance 1.5 mm from the axis after 30 minutes. In contrast, a rigid sphere of the same diameter initially situated with its center 0.5 mm from the wall, had moved less than 30 μm away from the wall after 24 hr of flow at the same Reynolds number.

At this time, Segré and Silberberg had published results which showed that a two-way lateral migration of rigid spheres to an eccentric radial position occurs in Poiseuille flow at higher

Reynolds numbers when inertia of the fluid is significant - the tubular pinch effect (39). Mason subsequently demonstrated that the effect also occurs with rigid rods and discs. Moreover, in contrast to results obtained in creeping flow, in which the rotational orbits of single spheroids remained constant with time, depending only on the initial conditions of release into the fluid, in the presence of inertial effects the orbits tended to limiting values corresponding to *maximum* energy dissipation in the flow (40). It is of interest to note that the same effect occurs with normal and hardened human red cells (41). As a result of Dr. Mason 's collaboration with Howard Brenner, and the presence of Raymond Cox in the Suspension Rheology Group at the Pulp and Paper Research Institute at McGill, fluid mechanical theories describing both migration due to particle deformation (42) and fluid inertia (43,44) were developed. Migration due to inertia of the fluid may be likened to that of the curving of a spinning tennis ball (Magnus effect), although it is important to note that non-rotating particles also migrate, and that the presence of the wall is a cardinal feature of the two way migration.

It was also shown that the above effects occur in pulsatile flow (45,46), and that here also, particle motions could be rigorously analyzed with available theory.

(b) Concentrated Suspensions: Particle Crowding

In order to study particle behavior at high concentrations, transparent suspensions were prepared by matching refractive indices of suspending and suspended phases, and then adding a small fraction of particles of identical size and nearly the same density to act as visible tracer particles (47). As in dilute suspensions, significant differences were found between the flow behavior of rigid and deformable particles at low Reynolds number at which effects due to inertia are negligibly small.

At volume concentrations < 20% and particle to tube diameter ratios < 0.04, particle and fluid velocities in the tube were found to be identical, given by the familiar parabolic distribution. In the case of rigid spheres, as the concentration and/or particle size increased, particle and fluid velocities continued to be equal but the velocity distribution became blunted in the center of the tube with a core of partial plug flow in which the velocities were maximum and constant, and lower than the centerline velocity in Poiseuille flow at the same volume flow rate. However, these concentrated suspensions were still quasi-Newtonian in that the velocity profile was independent of flow rate and the pressure drop per unit length of tube was directly proportional to volume flow rate. This result, which holds true providing the particle distribution is uniform across the tube, is an effect which becomes important when the mean interparticle distance is of the same order as that of the sphere or spheroid and when both are not too much smaller than the tube diameter. The particle paths, which exhibited erratic radial fluctuations in the peripheral regions of the tube due to the continuous interparticle collisions, were reversible with respect to translation and rotation when the direction of flow was reversed (47). Such a "time-reversed" system can be thought of as a cine film of the real system run backwards. With the neglect of inertia the Navier Stokes equations become linear and are still satisfied under time reversal. It is the demonstration of this effect that Professor Fung remembers in his article in this issue (48). Time reversal does not exist in concentrated emulsions or in blood due to the irreversible inward migration of the particles.

In the case of concentrated emulsions of deformable drops, blunting of the velocity profile was also observed (49,50), but here, the extent of partial plug flow at a given particle concentration and size was smaller than that in suspensions of rigid spheres. Moreover, the degree of blunting of the velocity distribution decreased with increasing flow rate until at a limiting value, the Poiseuille velocity distribution was restored. Due to the deformation and distortion of the droplets, caused not only by shear but also by particle crowding, the amplitude of the radial displacements of the tracer droplets in the transparent emulsions were observed to be smaller than those of the tracer particles in suspensions of rigid spheres of similar diameter. As was subsequently shown in transparent ghost cell suspensions containing tracer normal red cells (51), deformation due to cell crowding plays an important role in the rheology of this system.

(c) Concentrated Suspensions: Wall Migration

In the presence of inertial effects, concentrated suspensions of rigid spheres develop a particle-free zone near the wall, both in steady (40) and in pulsatile flow (52,53). As a consequence of such a two-phase flow there is an additional blunting of the velocity profile as well as a decrease in the average particle concentration in the tube (54); c.f. the Fåhraeus Effect (55). Because of the lubricating action of the peripheral "plasmatic layer", the pressure drop over a given length of tube decreases with time as the layer forms (40,56).

In concentrated emulsions, a two-phase flow develops even at very low Reynolds number due to inward migration of the deformed droplets (49). Moreover, at a given Reynolds number, particle:tube diameter ratio and volume concentration, the rate and extent of formation of the particle-free layer is appreciably greater than in the rigid sphere suspensions. It should be noted, however, that even without any inward migration, theory predicts that a hydrodynamic wall effect will result in a decreased measured apparent viscosity with decreasing tube diameter (57); c.f. the Fåhraeus-Lindqvist Effect (58). In blood, it is the inward migration of the red cells (though small at normal hematocrits), together with the hydrodynamic wall effect which is responsible for the significant decrease in the measured apparent viscosity with decreasing tube diameter.

COLLOIDAL SUSPENSIONS

In 1970, Dr. Mason embarked on a series of investigations of the microrheology of colloidal dispersions. In conjunction with hydrodynamic and colloid stability theory, this work led to a method for detecting attractive and repulsive interaction forces $< 10^{-12}$N (< 0.1 µdyne) between colliding charged latex spheres in Poiseuille flow (21). Clearly, this was of great interest for the study of interaction forces between biological cells. The investigations were made possible by miniaturizing the technique for observing particle motions and interactions in tube flow. Previously, suspensions of macroscopic model spheres and spheroids (0.2 - 2 mm diameter) had been observed in flow through fixed, vertically mounted tubes of 2 - 10 mm diameter using a low power traveling microscope (37). The task was now to track particles of the order of 1 µm diameter in tubes of 50 - 200 µm diameter while observing their Brownian- and shear-induced motions. The solution was to build a "traveling microtube" in which the flow tube, mounted on a microscope slide on a platform together with the infusion syringe, was hydraulically moved in a direction opposite to the flowing particles, while keeping the optical axis fixed (59).

From the point of view of the Biorheologist, one of the more interesting applications of the traveling microtube technique, was the study of the trajectories of colliding latex spheres in aqueous glycerol solutions (to dampen Brownian diffusion) containing a polyelectrolyte (60). Some of the collisions resulted in the formation of permanent doublets (as opposed to transient doublets in which the spheres separated after collision), in which the surfaces were cross-linked by the polymer. In other cases, there was a temporary retardation and even a reversal of the direction of rotation of the collision doublet suggesting that a cross-bridge had formed which later broke up again.

As a result of this work, it was suggested to the author that he attempt to measure the strength of a cross-bridge of antibody between two red blood cells. Accordingly, experiments were carried out using swollen, sphered and glutaraldehyde-fixed red cells of antigenic type B suspended in aqueous glycerol containing 0.15 M NaCl and known amounts of anti B-antibody (61). Doublets of cells formed by two-body collisions at very low shear stresses were observed as they entered the flow tube, and then tracked in the traveling microtube apparatus in a steadily accelerating Poiseuille flow until break-up. From an analysis of the videotape of the doublet motion and known expressions for the normal force acting along the axis of the doublet (62), it was possible to calculate the hydrodynamic force at break-up and to estimate the strength of a single antigen-antibody cross-bridge, $< 10^{-11}$N.

The above is but one example of the impetus and direction that Dr. Mason's ideas and his presence gave my own work. Considering the frequency with which Dr. Mason's publications are referenced in papers on the flow of blood or suspensions modeled on blood, it is clear that he has influenced the direction of the research of other Biorheologists. We are fortunate that the Suspensions Rheology Group at the Pulp and Paper Research Institute is now led by one his former graduate students, Dr. Theodore van de Ven, who continues the tradition of excellence, and who has contributed to the success of this issue (63). Whatever the direction of future research in Biorheology, there is no doubt that the body of knowledge, experimental and theoretical, acquired in the laboratory of Stanley Mason will stand the test of time and continue to form the basis for the understanding of the microscopic and macroscopic rheology of blood and other biological suspensions.

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