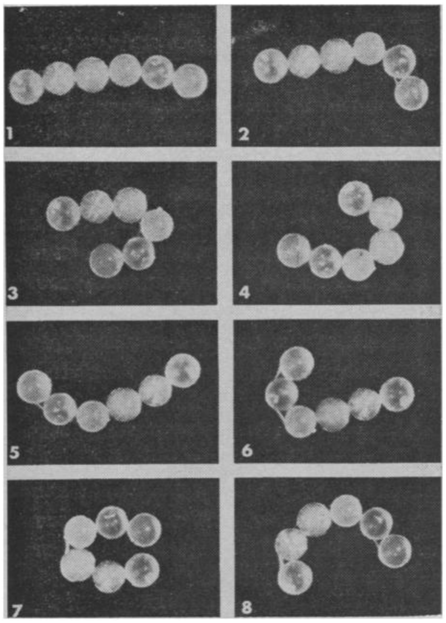

Fig. 2. A chain of polystyrene spheres, held together by the interfacial tension of a water meniscus bridging adjacent spheres, rotating and bending in shear flow in a periodic orbit similar to that of a flexible fiber or a rouleau of red blood cells.

ceeding quadrant when they are being pulled apart (see Fig. 1), the point of buckling and breaking generally occurring near the center of the chain.

When a liquid immiscible in the suspending medium such as water is introduced so that a meniscus bridges the gaps between the spheres, the chain can rotate and bend without breaking, since the interfacial tension provides tensile strength (Fig. 2). Such an aggregate undergoes a rotational orbit similar to that of a flexible thread or fiber in shear flow (*4*) with the mean curvature of the chain and the product TG, T being the period of rotation about the vorticity axis, increasing with G.

By direct manipulation (without the aid of an electric field) we have formed stacks of tiny polystyrene discs, and have found that they rotate like rigid rods and follow Jeffery's equations until reaching G's at which they start to bend and break apart, usually by sliding of the faces over one another. As with spheres, the addition of a second liquid phase can prevent breakup.

We have made two-dimensional (hexagonally packed) and three-dimensional (tetrahedrally packed) aggregates of spheres; when the aggregates are symmetrical they rotate about the vorticity axis at an angular velocity equal to $G/2$ as for a single rigid sphere (*3*).

The linear aggregates are interesting hydrodynamic models especially since they are amenable to theoretical treatment. We believe that they will prove useful in understanding the coiling and in some instances the breaking of fibers (*4*), macromolecules (*5*), and rouleaux of red blood cells (see *6*) in shear flows and other rheological aspects of such systems.

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Red Cells and Rouleaux in Shear Flow

Abstract. *The rotation and deformation of human red cells and linear aggregates (rouleaux) in dilute plasma suspension were observed in Poiseuille and Couette flow. Single undeformed erythrocytes and rouleaux rotated in orbits predicted by theory for rigid spheroids. Bending of rouleaux occurred at orientations at which compressive forces act on the particles and the degree of flexibility increased with the number of cells in linear array.*

I have undertaken an investigation into the microscopic flow properties of blood, that is, the motions and interactions of the constituent cells and plasma, by adapting techniques previously employed with model suspensions (*1, 2*). In the initial phase of the work reported here, the experiments were limited to a study of the behavior of single human erythrocytes and linear and branched aggregates of cells, such as may be observed when dilute red cell suspensions in heparinized plasma undergo laminar shear flow in vitro at velocity gradients below 20 sec^{-1}.

Two methods were used to follow and record, with the aid of a cine camera, the particle translational and rotational motions under the microscope. In both cases the vessel diameter was large compared to the suspended cells and aggregates.

Poiseuille flow. The suspensions flowed through polypropylene or glass tubes of 80 to 200 μ diameter embedded in chambers on a glass slide mounted on a vertical microscope stage which could be mechanically driven at variable speeds in a direction parallel to the tube. Matching the speed of travel of the stage to that of a particle in the tube enabled continuous viewing along an axis normal to the median plane of the tube over 2 to 3 cm.

At mean linear velocities $\bar{u}$ ranging from 1 to 4 tube diameters per second (that is, at rates of shear at the tube wall $G = 4\ \bar{u}/R_0$ from 8 to 32 sec^{-1}, R_0 being the tube radius) the rotations of single cells resembled those of rigid discs (*1, 2*), the angular velocity of the axis of revolution being a maximum when the cell face was at right angles to the flow and minimum when aligned with the flow (Fig. 1*a*).

Small linear aggregates or rouleaux of four cells measuring approximately 8 by 8.5 μ rotated with almost constant angular velocity. Larger aggregates, however, behaved as rod-like particles (*1, 2*) with the angular velocity a maximum when the long axis was at right angles to the flow and a minimum when aligned with the flow.

Moreover, rouleaux containing n cells in a linear array, of regular shape and not deformed by the flow, rotated in the spherical elliptical orbit predicted by Jeffery (*3*) for rigid oblate ($n = 1, 2, 3$) or prolate spheroids ($n > 5$). This is illustrated in Fig. 1 for the variation with time of the angle ϕ of the particle axis of revolution with an axis normal to that of the tube.

Most remarkable, however, was the ease of deformation of the rouleaux while rotating. Depending on the magnitude of the rate of shear and particle length, buckling set in in the second ($90 < \phi < 180$) and fourth quadrants ($270 < \phi < 360$) of the orbit (Fig. 1*b*) where compressive forces act on the cells to push them together. In the succeeding quadrants, where the forces become tensile, the rouleaux gradually straightened out. In some cases, as illustrated in Fig. 1*b* for a 15-cell rouleau of axis ratio 4, the variation of the angle ϕ with time still quite closely approximated the curve predicted from Jeffery's theory.

In this, the particle rotations resembled the "springy orbits" (Fig. 2*a*) of flexible threads of dacron, nylon, or rayon (*4*) and short wood pulp fibers (*5*) for which it was shown that the critical value of the product (suspending phase viscosity × shear rate) at which bending first sets in may be related to the particle axis ratio and bending modulus of the material of the rod (*2, 6*). My results show that at a given particle axis ratio, the onset of bending for a rouleau occurred at a value of G 10^7 times smaller than that for a dacron filament of the same diameter (*6*). At high magnification the deformation of individual cells in the rouleaux was visible. In view of the known ease of bending of the single red cell (*7*) and the measured membrane tension in vitro of only 2/100 dyne cm^{-1} (*8*), these observations are not surprising.

Also in common with flexible threads, wood pulp fibers, and chains of spheres in which a liquid phase meniscus bridges adjacent particles (*9*), very long linear aggregates of erythrocytes (> 20 cells) were observed to execute "snake" orbits (*4*) in which the two ends of the rouleau were capable of independent movement and in which (as illustrated in Fig. 2*b*) a bend which began at one end of the particle ran along the length of the rouleau which then straightened out again.

Couette flow. The suspension was contained in a gap of 100- to 500-μ width between two horizontal concentric glass discs mounted in place of the stage of a microscope and independently driven in opposite directions at continuously variable speeds. Here, the particles were viewed in a direction normal to the face of the discs and normal to the plane of shear in contrast to the tube where the motions were viewed along an axis parallel to the plane of shear.

The motions of the cells and rouleaux, seen in the median plane of the tube as complete rotations, were now observed as a rocking to-and-fro (*2, 10*) between equal angles on either side of an axis normal to the direction of flow and passing through the center of rotation of the discs. At the higher G which obtained in Couette flow (from 30 to 70 sec^{-1}) springy orbits were seen with rouleaux of only four cells and the flexibility of long rouleaux was most striking.

Also observed in Couette flow between counter-rotating discs was the growth of linear aggregates by collision between smaller rouleaux which were brought into proximity by the velocity gradient and combined to form longer particles. Conversely, at sufficiently high G, breakup of long linear and branched aggregates was evident. It is hoped from such experiments aided by theory (*6*) to measure the attractive forces between the cells of the rouleaux.

Finally, it is evident that many of the phenomena observed in blood flow at the microscopic level would not have been understood but for the existence of experimental and theoretical results on the microrheology of simpler model particle systems such as those described in the preceding report (*9*).

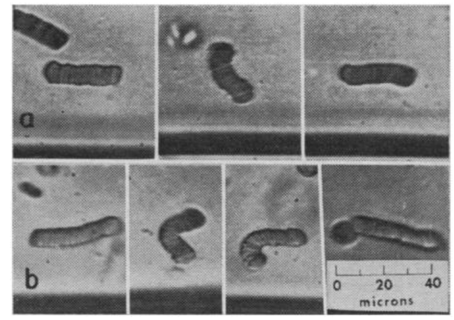

Fig. 2. Photomicrographs of the bending of rouleaux in flow (from left to right) through a tube of radius 60 μ, one wall of which is shown in the lower part of each photo. (*a*) Springy half orbit of a 16-cell rouleau. (*b*) Flexible or "snake" orbit of a 20-cell rouleau.

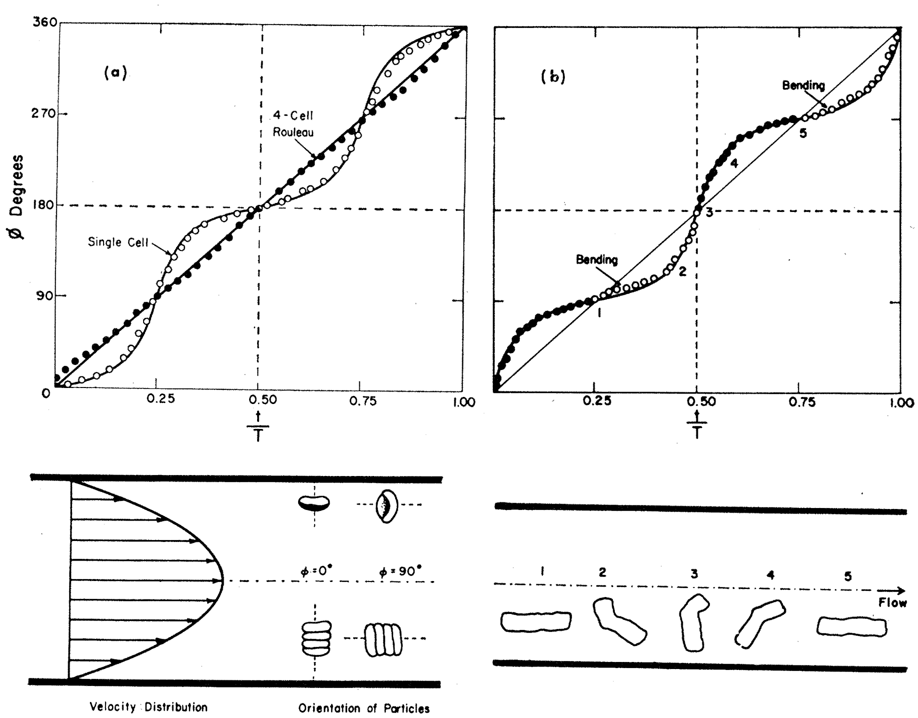

Fig. 1. The measured variation in the ϕ-orientation of a single erythrocyte and rouleaux during one complete orbit having the period T in Poiseuille flow. (*a*) Single cell (open circles) and a four-cell linear aggregate (filled circles). The lower portion shows the particle orientations at different angles ϕ. (*b*) A 15-cell rouleau which bent in the second and fourth quadrants (open circles) as shown by the tracings in the lower portion. The lines drawn were calculated from Jeffery's theory (*3*) for the rotation of a rigid spheroid.

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