

duced. Considering that about 13 and 34 percent of the triplets of methylated polyUG-1 and methylated polyUG-2, respectively, contain 7-methylguanine, many of the peptides might well be too short to be precipitated by the trichloroacetic acid and hence would not be counted.

The methylation of guanosine could affect the template activity by changing its charge since, at pH 7.2, where all the above experiments were run, only 50 percent of the 7-methylguanosine is in the protonated form. To evaluate the effect of charge, incorporation experiments were conducted at pH 6.0 where the nonprotonated form of 7-methylguanosine is reduced to 6 percent (as compared with 1 percent for guanosine at pH 7.2).

Figure 1 shows comparisons of the kinetics of incorporation for each polymer at pH 7.2 (open symbols) and pH 6.0 (filled symbols). The overall efficiency of incorporation is reduced to about 40 percent by the lowering of pH, as can be seen from the polyUG control (circles). The activity of both of the methylated polymers is also reduced by about the same amount (40 to 50 percent). The decrease in the ionized form of 7-methylguanine did not increase the template activity of the methylated polymers relative to the control polyUG, suggesting that it is not the ionization but probably the presence of a sterically important 7-methyl group which hinders the activity, and that base-pairing alone is not sufficient for codon recognition during peptide synthesis *in vitro*.

The methyl group might hinder the base-pairing process, binding of the template to ribosomes, or perhaps other enzymatic reactions related to codon recognition and peptide formation. The results in Table 3 suggest that the methylated polymers may not bind to ribosomes as well as polyUG does. Under conditions of limiting ribosomes, the addition of methylated polyUG-1 or methylated polyUG-2 before the addition of polyUG does not inhibit the overall template activity of polyUG. In other experiments the system could be incubated with methylated polyUG-2 up to 10 minutes without decreasing total disintegrations measured after the later addition of polyUG. Either the polyUG can displace a previously weakly bound methylated polymer or the methylated polymers bind very slowly. The decreased activity of the methylated polymers might then be related to this weak binding property.

In the foregoing experiments we have

shown the steric effect of the added methyl group of 7-methylguanine on the mechanism of translation *in vitro*. This result suggests further that the mutagenic effects of chemical methylation observed *in vivo* might be the result of altered base-pairing during polynucleotide transcription.

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Chains of Particles in Shear Flow

Abstract. *Interesting physical models of threads, rouleaux of red blood cells, and other flexible structures (including macromolecules) have been prepared with chains of spheres and discs. When suspended in a viscous liquid undergoing shear flow, the aggregates rotate as nearly rigid bodies, even though they have no tensile strength or stiffness until shear rates high enough to cause bending and then breaking are reached. By adding a second liquid phase which causes a meniscus to bridge adjacent particles, sufficient tensile strength can be provided to cause bending without breakage.*

Spheres suspended in a dielectric liquid attract one another in an electric field and eventually arrange themselves as linear chains aligned in the direction of the field (1). Since the spheres do not necessarily touch one another, the aggregates thus formed may be regarded as threadlike particles with neither tensile strength nor stiffness. Nevertheless, they rotate and remain straight in Couette (shear) flow after the electric field is removed (Fig. 1).

From a consideration of the lubrication equations for spheres in close proximity, we have demonstrated theoretically that when the spheres are in contact, the aggregate should rotate as a rigid body, without relative rotation of the spheres and without bending, in a spherical elliptical orbit similar to that predicted by Jeffery for a rigid prolate spheroid (2). However, when there are small gaps between the spheres, the chain length should vary periodically between a minimum when the chain is oriented at right angles to the direction of the shear flow and a maximum when parallel to it.

We have confirmed these predictions with tiny (0.05 cm in diameter) metal-coated polystyrene spheres suspended

in oils of the same density in which chains of as many as 20 spheres were formed by applying a 60-cy/sec alternating field of 2 kv/cm. Quantitative measurements were made with chains containing from 2 and 10 spheres at velocity gradients (G) up to 2 sec^{-1} established in a Couette apparatus consisting of two counter-rotating cylinders (3). At high G 's the chains buckle in the quadrant in which the spheres are being pushed together and generally break in two at the same position in the chain in the suc-

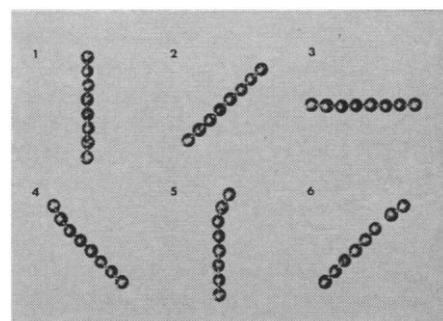


Fig. 1. A chain of eight spheres formed by applying an electric field in the vertical direction (1). With the electric field off, and the shear field on, the chain rotates clockwise (2, 3), buckles under axial compression (4, 5), and finally breaks under tension (6).

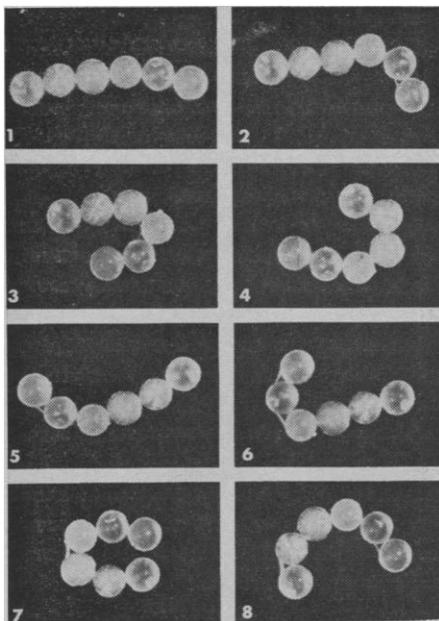


Fig. 2. A chain of polystyrene spheres, held together by the interfacial tension of a water meniscus bridging adjacent spheres, rotating and bending in shear flow in a periodic orbit similar to that of a flexible fiber or a rouleau of red blood cells.

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. 1a).

Small linear aggregates or rouleaux of four cells measuring approximately 8 by 8.5μ 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. 1b) 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 il-