Fabric Formation in Centrifugal Fields: Fibrin (35975)

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(Introduced by N. G. Anderson)

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Zonal rotors were developed to permit large-scale purification of particles by zone sedimentation in density gradients, since differential sedimentation (pelleting) does not yield a pure product (1). However, pelleting is an efficient way to concentrate rapidly sedimenting particles, and, paradoxically, zonal rotors provide a fast, large-volume means to do this (2).

Fabric formation is a corollary of the pelleting case. If particles sedimenting in a uniform solution undergo polymerization, they will sediment more rapidly into a region of higher hydrostatic pressure. If the polymerization reaction is accelerated by increased pressure (3), an accelerated accumulation of mass by the polymer will result, with attendant increase in sedimentation rate—this accelerative cycle to end when the polymer strikes the centrifugal wall of the rotor. It follows that polymer length will be determined in part by the polymerization rate and the centrifugal force. If the polymerization reaction includes cross-linking, then successively pelleted polymers will interact to form a continuous structure; otherwise, a felt-like fabric will result. If the rotor wall is a circumference around the axis of rotation, the uniformity of the centrifugal field will produce a sheet of constant thickness and properties. Specific conformations and thickness gradients may be produced in fabric sheets by appropriate machining of the rotor wall.

The evolution of these concepts was induced by the serendipitous appearance of fibrin sheets in a rotor filled with blood plasma, which, in turn, has led to the study of the phenomenon and the fabric.

Materials and Methods. Fresh-frozen, citrated human plasma was thawed at 4° and the cryoglobulins were removed by low-speed centrifugation. Thirty-four milliliters of 1 *M* $CaCl_2$ was added to 1125 ml of supernatant plasma while stirring at 4°, then loaded into a titanium B-XV (4) or Spinco Ti-15 (5) rotor at 3000 rpm with an overlay of isotonic saline. Centrifugation was for $\omega^2 t = 8.6 \times 10^{11}$ sec^{-1} at the maximum velocity of the rotor and 15°. We then opened the rotor, removed the fluid and septa, and peeled the fibrin sheet from the wall. The sheet was stored in isotonic saline or distilled water at 4°. The Spinco Ti-15 rotor was superior to the B-XV rotor for pelleting and fabric formation because of its unitary wall design (*i.e.*, the Ti-15 rotor can be opened without disturbing material sedimented on the centrifugal wall—material sedimented on the upper half of the B-XV rotor wall is scraped off by the septa when the rotor is opened).

Results. The temperature sequence is important in fibrin sheet formation. The clotting reaction rate is temperature dependent, and at 5° is of the order of 48 hr to completion. By loading the calcium-treated plasma at low temperature, then allowing the rotor to warm to 15 to 20° after maximum speed is attained, the polymerization reaction takes place in the maximum centrifugal field.

The size of the fibrin sheet is determined by the dimensions of the rotor wall and the volume of plasma. In the present case, a

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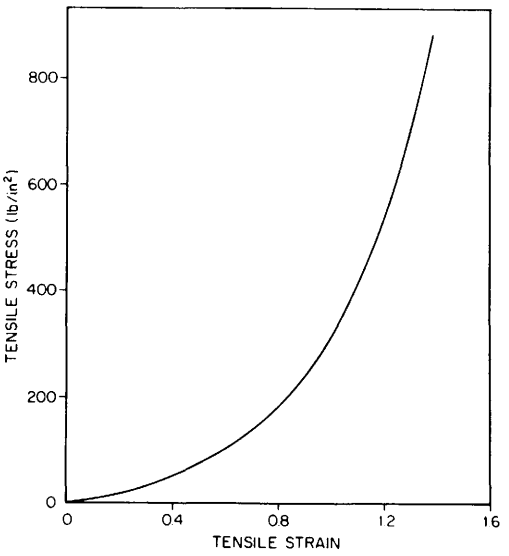

FIG. 1. Stress-strain properties of fibrin fabric stored in isotonic saline at 4° for 7 days after preparation: test strip dimensions (unstressed): 2 × 1.25 × 0.006 in. Sample was stretched at a rate of 0.2 in./min at 25° in air. Tensile strain is $\Delta l/l_o$.

cylindrical sheet 56 cm in circumference, 7.5 cm in height, and 0.016 cm thick (hydrated) was obtained. The thickness varies slightly at 90° (14-cm) intervals where the septa approach the rotor wall. When the fabric is removed from the rotor, it is thin and transparent, but swells and becomes progressively opaque in the suspending medium. This may be caused by hydration of the fabric following compressive dehydration by the centrifugal field; which agrees with the loose mesh structure seen in the electron microscope.

The tensile properties of the fibrin sheets were investigated with a tensile testing machine (6), and the ultimate stress was found to be 880 psi (Fig. 1). The stress-strain curve is that of an elastomer and reflects, in part, the porous structure of the fabric. Another sample was stretched to a tensile strain of 0.85 and held for 5 min, with a relaxation of only 11% of the load. The machine was then reversed and the strain was removed at the same rate. Zero load was obtained at a residual strain of 0.12. When the sample was again stressed, it fractured at a load 36% higher than found in the previous case, but with a strain of only 1.0. An accurate value of the stress was not obtained in this case due to difficulties in determining the cross-sectional area of the sample during its final loading. Samples of fibrin that had been held in distilled water for 7 days fractured at only 50% of the load withstood by those held in saline. Fibrin sheets were dried at room temperature, then rehydrated with apparent retention of elasticity and strength.

Electron microscopy of the unstressed fabric reveals a loose mesh of fibers occupying less than half the volume of the sheet (Fig. 2A). However, in a section taken from the fracture area of a stretched sample, the structure has been compressed with virtual disappearance of the void space (Fig. 2B). The surface of the fabric was found to be smooth and amorphous, both by scanning electron microscopy and light microscopy.

Discussion. Fibrin fabric is of interest due to the rare immunogenicity of human fibrinogen and native fibrin in man (7, 8). It is currently being tested as a burn dressing (9), and preparations are being made to investigate its suitability as a substrate for cell growth in surgical reconstruction. Conceivably, the loose mesh of the fibrin sheet would become permeated with fibroblasts and be replaced by a "scar-tissue" membrane. It may also find application in diffusion chamber fabrication, platelet function tests, and as a tissue culture surface. At the present time, the largest zonal rotor in operation, the K-VIII (10), is capable of producing sheets 76 × 42 cm. For applications requiring additional strength, the rotor can be fitted with a loosely woven fabric liner of other material (*e.g.*, Dacron, nylon, cellulose) and the fibrin sedimented into it. It follows that enforced tubular structures can be formed in long, fast rotors of small radius through which the polymerizing fibrin suspension may be passed in a continuous-flow-with-pelleting operation (1).

In the present preparation scheme, the serum globulins and albumin are sedimented into the fibrin fabric and must be washed out. In an alternate procedure, we have pre-

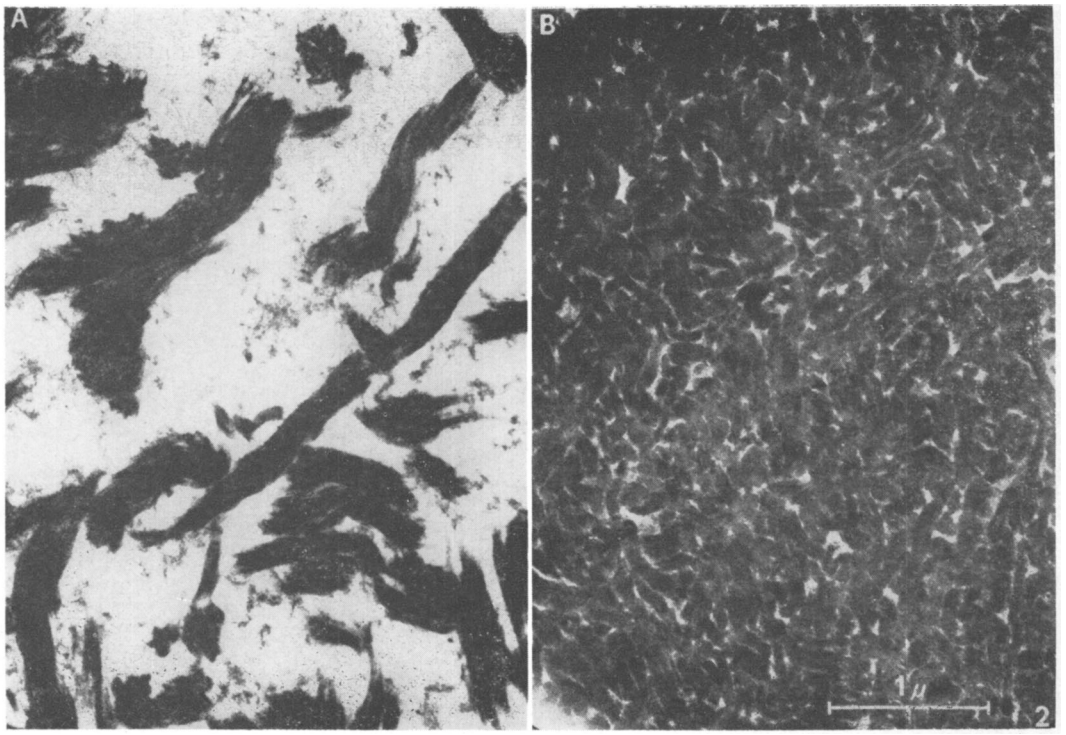

FIG. 2. Thin sections (0.006 μ) of fibrin fabric cut parallel to plane of the sheet: (A) unstressed; (B) stretched to fracture point. Fixed in glutaraldehyde and postfixed in Veronal-buffered osmium, pH 7.3; uranyl acetate and lead citrate stain.

pared sheets by precipitating fibrin through a zone of 20% sucrose sufficiently thick that nonpolymerized proteins did not reach the rotor edge. As an extension of this procedure, the polymerized fibrin could be specifically modified by passing through sequential zones of reagents (11). Fibrin sheets of maximum purity are obtained by reacting puried fibrinogen and thrombin in the rotor in place of plasma.

This concept of fibrin formation is applicable to any system in which polymerization can occur in a centrifugal field. It is particularly appropriate for biological substances because of the low shear forces involved. Production of fabric is batchwise with existing centrifuges, but continuous formation is technologically conceivable.

Summary. Human fibrin polymerizing in a centrifugal field will sediment to form a continuous fabric. This fabric is an elastomer with an ultimate stress of 880 psi.

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