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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION. XVI. FIBRIN CLOTS, FIBRIN FILMS, AND FIBRINOGEN PLASTICS

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Research Article

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XVI. FIBRIN CLOTS, FIBRIN FILMS, AND FIBRINOGEN PLASTICS[1,2]

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The first papers in this series (1 to 5) have described the properties of the constituents of plasma in solution. The physical chemical properties of sedimentation, electrophoretic mobility, osmotic pressure, viscosity, and double refraction of flow reflect the behavior of the protein molecules moving as individuals in the dissolved state. The physiological properties which are of importance in medicine, such as restoration of blood volume, immunization, and agglutinin reactions, are presumably related to individual molecular behavior.

Highly specific properties of certain molecules, such as fibrinogen, lead to their association to form solid structures. This introduces a new set of physical chemical properties, and a new set of physiological properties which apply to surgery rather than to medicine. The preceding paper has dealt with the physical chemistry of the normal clotting process (6). Now, it has been found that, when the human plasma proteins which are involved in the normal process are allowed to react *in vitro*, under controlled conditions, clots may be obtained with a wide variety of physical characteristics. We shall describe these clots, as well as two kinds of derived products—the fibrin films and fibrinogen plastics—which we have developed from the same proteins.

The clots, films, and plastics are structural materials whose value depends upon a combination of histological behavior following implantation in tissue with certain mechanical properties. The histological and clinical sequences are the subject of other communications in this series (7 to 11). The mechanical properties, which can be controlled to provide a wide variety of structures, are treated here.

The clots, films, and plastics are derived from Fraction I of human plasma (1), which consists largely of fibrinogen. In the *clots* and *films*, the fibrinogen has been converted to fibrin, by the action of thrombin (Fraction III-2). In the *plastics*, the fibrinogen has not been clotted, nor ordinarily separated from accompanying globulins, but the entire fraction has been subjected to an irreversible molding treatment.

FIBRIN CLOTS

The fibrin clots are comparatively tenuous structures, and only a small proportion of their volume—generally 2 per cent or less—is occupied by the fibrin which is responsible for their solidity. They are far less stable than the films and plastics, and are ordinarily prepared immediately before use, by mixing appropriate sterile solutions of human fibrinogen and thrombin. In clinical use, they may be formed *in situ*, the solution mixture being allowed to clot in a cavity or on a surface according to the nature of the treatment (8, 12).

The physical properties of the clots may, according to conditions of preparation, be graded continuously between two extremes, which for convenience are designated Type A and Type B. The former are transparent, gelatinous, and friable; have low tensile strength and maximum elongation (*i.e.*, cannot be stretched very far); adhere well to surfaces upon which they are formed; and do not readily synerize (*i.e.*, lose

[1] This work was originally supported by grants from the Rockefeller Foundation and from funds of Harvard University. Since March, 1942, it has been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] This paper is Number 22 in the series "Studies on Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

water with a contraction in volume). The latter (Type B) are opaque, doughy, and non-friable; have a high elongation, and plastic flow; do not adhere well to surfaces upon which they are formed; and synerize very readily.

The clotting times involved in the formation of the clots may be varied. Their order of magnitude may range from a few seconds to 15 minutes, for those of Type A; and from a minute to an hour, for those of Type B.

FIBRIN FILMS

The term fibrin film is applied both to a single lamina of plasticized fibrin and to modified sheets, consisting of fibrin backed by other layers (bandage, waterproofing, etc.). The plain fibrin films form the basic material for the other types. They can provide a variety of mechanical properties, ranging from a soft and resilient to a tough and rubbery structure. Products with different properties may be prepared to meet the mechanical and chemical specifications for different clinical needs.

Dimensions. Plain fibrin films have thus far been prepared in sheets, seamless tubes, and fibers. The sheet thickness is usually specified in terms of milligrams of fibrin per square centimeter, which may range from 1 to 50. These figures, multiplied by 0.03, give the approximate thicknesses in millimeters (Figure 1). The

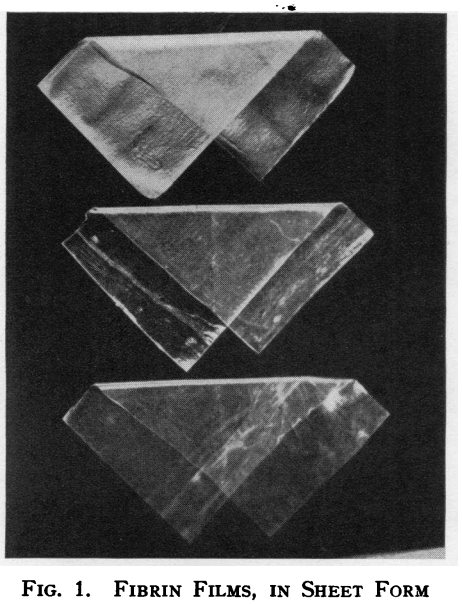

FIG. 1. FIBRIN FILMS, IN SHEET FORM

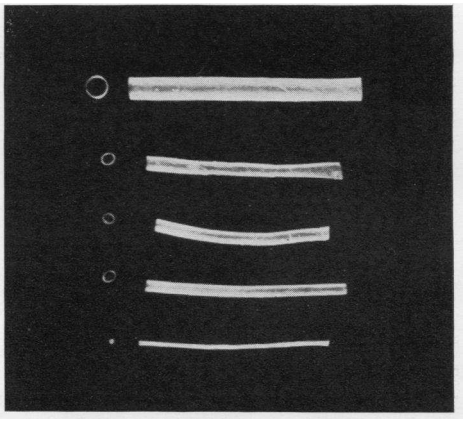

FIG. 2. FIBRIN FILMS, IN SEAMLESS TUBING FORM

tubes have been prepared with different diameters (*e.g.*, 2 to 20 mm.) and wall thicknesses (*e.g.*, 0.1 to 1.5 mm.). Several sizes are shown in Figure 2. The fibers have been prepared with diameters ranging from 0.2 to 1.0 mm. (Figure 3).

Composition. The films are composed of protein plus plasticizer. A "plasticizer," in technical terminology, is a substance of low molecular weight (usually a liquid) which is incorporated to make a plastic material soft and flexible rather than hard and brittle. In the fibrin films, the plasticizer may be simply water. If, for storage and distribution, other liquids are employed, due consideration has been given to their lack of toxicity, local and general. As used in surgery, the films are frequently immersed for some time in water or saline; this procedure

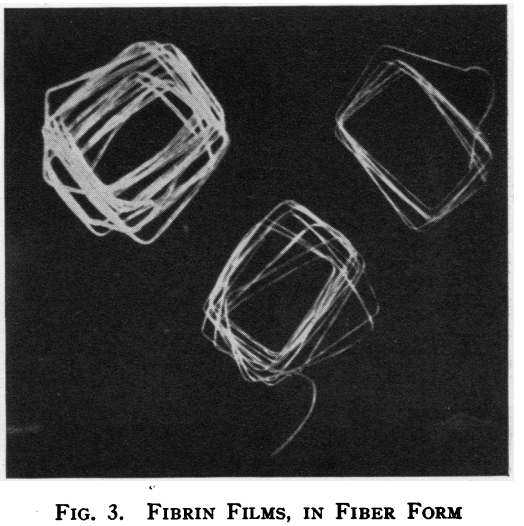

FIG. 3. FIBRIN FILMS, IN FIBER FORM

removes part or all of any other plasticizer and replaces it by water. The protein moiety is ordinarily at least 90 per cent fibrin, and the occluded human globulin may, as a first approximation, be neglected.

The proportion of fibrin may vary from 15 to 100 per cent by weight, the remainder being plasticizer. The mechanical properties depend to a very great extent upon these proportions, that is, upon the extent to which the fibrin units are diluted and forced apart by the liquid plasticizer.

Mechanical properties. The mechanical properties of the fibrin films can best be described by stress-strain curves, in which the stress (or load per *original unstretched* cross-section area) is plotted against percentage elongation (the increase in length expressed as percentage of the original length). Similar curves are commonly used to describe the behavior of rubber and synthetic plastics, as well as natural and synthetic fibers.

The shape of the stress-strain curve is primarily influenced by the proportion of fibrin in the film.

When the amount of fibrin is *small*, and the plasticizer occupies most of the volume, the stress increases linearly until an elongation of a little over 100 per cent is reached, and then goes up much more rapidly (Figure 4). Thus the film has an easy rubbery stretch to 100 per cent, and thereafter "firms up" so that further stretch is more difficult. This type of curve we have called *linear*. Sometimes films break before the firming-up stage is reached (Figure 4a) but this is attributed to flaws rather than to any essential difference in structure.

When the amount of fibrin is *large*, the stress-strain curve rises steeply at first, then at about 30 per cent elongation flattens off, and finally rises steeply at the end (Figure 5). Thus, the film, which may be qualitatively described as "tough" rather than "rubbery," requires a strong pull at the beginning of stretch, then deforms more easily as the stretching continues, and eventually firms up at 100 to 150 per cent elongation. This type of curve is described as *S-shaped.*

The difference between linear and S-shaped stress-strain curves may be interpreted in terms of the attractive forces between the units of the fibrin structure (whether these units are molecules, micelles, or other aggregates, or crystallites, remains to be determined). When the units are kept apart, initial stiffness is avoided; when the units are closer together, the attraction between them requires a high initial stress to start deformation.

It is of interest to compare the linear and S-shaped curves of Figures 4 and 5 with the stress-strain curves of certain natural biological

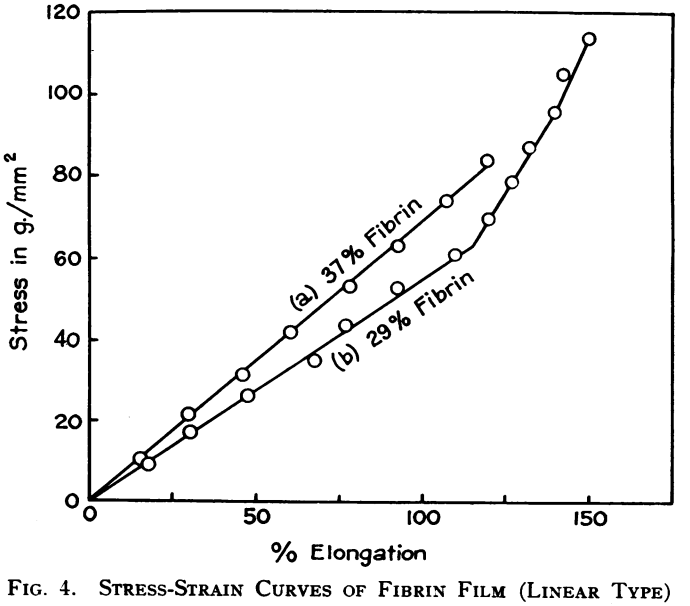

FIG. 4. STRESS-STRAIN CURVES OF FIBRIN FILM (LINEAR TYPE)

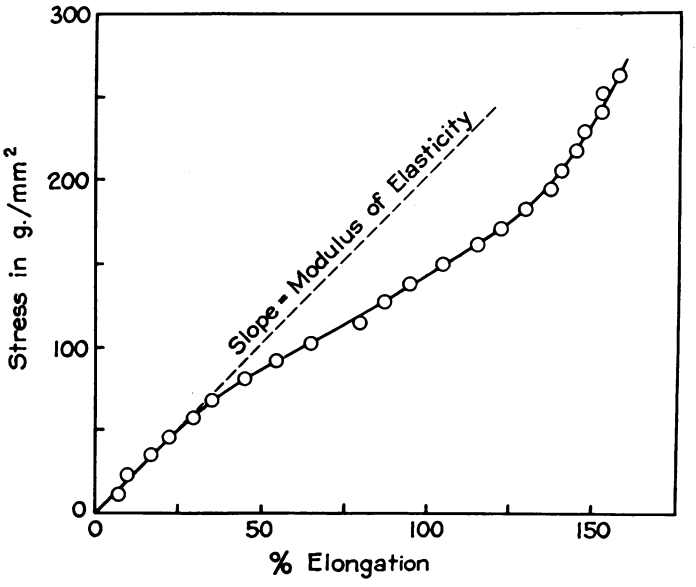

FIG. 5. STRESS-STRAIN CURVE OF FIBRIN FILM (S-SHAPED TYPE)

structures. The linear curve (Figure 4b) is very like that of the *ligamentum nuchae* (13) (Figure 6), whereas the S-shaped curve (Figure 5) is very like that of a wool fiber (14) (Figure 7) except for a difference in scale. The *ligamentum nuchae*, of course, normally contains a large amount of plasticizer (*i.e.*, tissue fluids); whereas wool has a rather low content of plasticizer (*i.e.*, moisture).

The mechanical behavior of either of these natural structures can thus be imitated by the fibrin film, and it may be possible to prepare types of film to simulate other anatomical structures if the mechanical specifications are provided.

From the stress-strain curve, three characteristic constants of a film can be evaluated: the modulus of elasticity, which is the initial slope, the tensile strength, or stress at break, and the maximum elongation at break. The last two are less reproducible than the modulus, since the point of break may depend upon the presence of a flaw rather than the yielding of a homogeneous protein structure.

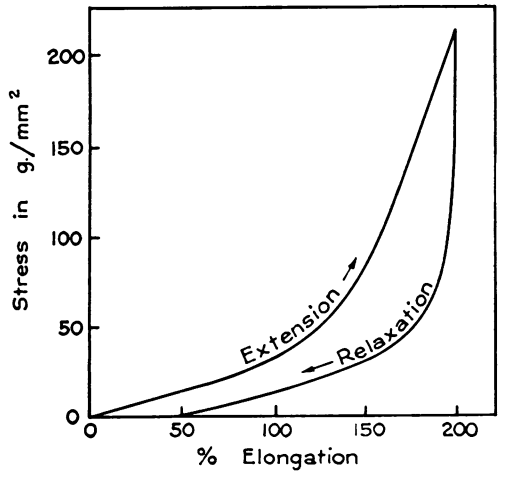

FIG. 6. STRESS-STRAIN CURVE OF *LIGAMENTUM NUCHAE* (BOVINE)

After Wöhlisch, du Mesnil de Rochemont, and Gerschler (13).

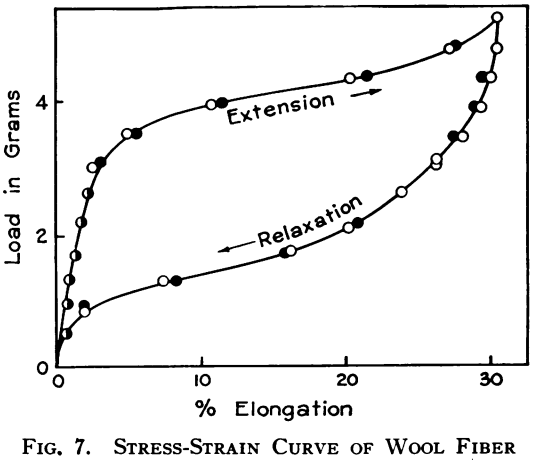

FIG. 7. STRESS-STRAIN CURVE OF WOOL FIBER

After Harris, Mizell, and Fourt (14).

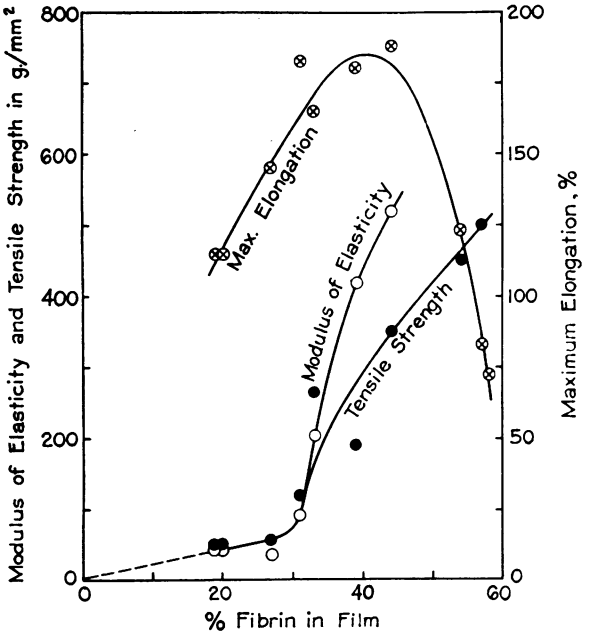

FIG. 8. MODULUS OF ELASTICITY, TENSILE STRENGTH, AND MAXIMUM ELONGATION OF FIBRIN FILM, PLOTTED AGAINST PERCENTAGE FIBRIN IN THE FILM

The three characteristic constants depend enormously upon the proportion of fibrin in the film, as shown in Figure 8. The modulus and the tensile strength increase slowly at first, and then rise very rapidly when a certain critical concentration of fibrin is exceeded. The maximum elongation goes through a maximum as the proportion of fibrin in the film is increased. This phenomenon is easily explained. When the fibrin concentration is small, the film is soft, and the coherence of the fibrin units is so weak that they pull apart before a high elongation is reached. At an intermediate concentration, the coherence is strong enough to allow a high elongation while the units still hang together, but not strong enough to interfere with the considerable internal rearrangement of molecules which must occur when a body is stretched to three times its original length. At a still higher fibrin concentration, the attractions are so strong that the internal rearrangements cannot take place; the film supports a high stress but snaps before it has stretched very far.

Chemical properties. The chemical properties of the fibrin films may be altered by various treatments. For example, the untreated film is readily digested by proteolytic enzymes, including trypsin, pepsin, the natural lysin which is concentrated in some of the fractions of blood plasma (1, 6), and the lytic enzyme of hemolytic streptococcus.[3] A small amount of the natural human plasma lysin, occurring largely as an impurity in the thrombin used to form the fibrin, is often retained in the film, so that the latter may lyse spontaneously when plasticized with water alone. Certain of the other plasticizers that have been employed inhibit the natural lysin and are therefore used in storage.

After certain treatments, the film becomes increasingly resistant toward proteolytic enzymes, and also toward absorption in living tissue.

Modified fibrin films. Besides the plain Type P fibrin film, modifications have been prepared as follows:

Type F film consists of plasticized fibrin with a backing of elastic bandage or elastic knitted cotton material for use in bandaging (Figure 9).

Type W film consists of plasticized fibrin with an elastic waterproof covering, made of non-toxic synthetic plastic, selected to have the same stress-strain characteristics, as far as possible, as the fibrin itself.

Type WF film consists of plasticized fibrin with a backing of elastic bandage or elastic cotton material, to the other side of which is applied an elastic waterproof coating.

The possible value of these fabric-backed and waterproof films is discussed in another paper of this series (9).

Sterilization. Fibrin films of certain of the types described above have been prepared asep-

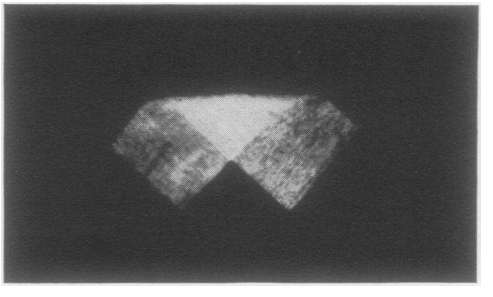

FIG. 9. TYPE F FIBRIN FILM (BACKED BY ELASTIC KNITTED COTTON)

[3] Prepared by Dr. E. A. Bering, Jr.

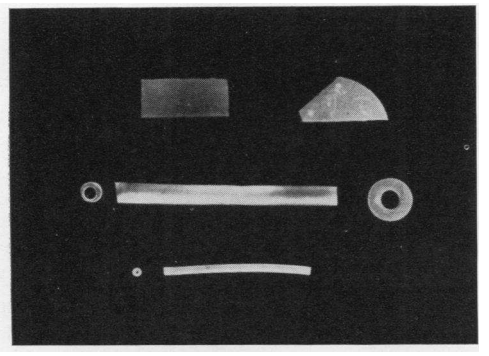

FIG. 10. FIBRINOGEN PLASTICS

tically. For large scale production, however, procedures for sterilization of the films after manufacture are desirable. Several methods have been attempted, some of which, successful on an experimental scale, have yielded the products used clinically (11). Investigations of the most satisfactory methods of sterilizing each type of film are still in progress.

FIBRINOGEN PLASTICS

The fibrinogen plastics are thermosetting, *i.e.*, they are set in molds under the action of heat, but cannot be softened or remolded subsequently.

Composition. The fibrinogen plastics, like the films, are composed of protein plus plasticizer. The proportion of protein may range from 25 to 75 per cent. The protein moiety is ordinarily Fraction I. Fibrinogen purified from Fraction I may also be employed. When plastics are used in surgery, the original plasticizer may be to a large extent replaced by water if the material is immersed for some time in water or saline before introduction into the body. After introduction into the body, the plasticizer will eventually be replaced by tissue fluids in any case.

The high permeability of fibrinogen plastics to water may be contrasted with the behavior of rubber and many synthetic plastics. Whereas the latter, generally speaking, are impermeable to water but permeable to oils and hydrocarbons, the fibrinogen plastics are permeable to water but impermeable to oils and hydrocarbons.

Optical properties. The plastics are translucent to light. Unlike the films, which are almost colorless, the plastics range in color from straw to dark brown. Like the films, they become doubly refracting under stress.

Mechanical properties. As in the case of films, the mechanical properties of the plastics depend largely upon the relative proportions of protein and plasticizer. As an approximate description, those containing 25 to 50 per cent protein are rubbery, those containing 50 to 75 per cent protein are leathery, and those containing more than 75 per cent protein are horny in character.

The stress-strain curves are similar to those of the films, except that the maximum elongation is much less, rarely exceeding 50 per cent.

SUMMARY

Fibrin films and fibrinogen plastics are structural materials derived from the proteins of human plasma involved in the natural coagulation process. They may be prepared in a wide variety of shapes, sizes, and mechanical properties. In the latter characteristics, which are dependent largely upon the proportion of protein present in the solid mass, the films and plastics may simulate different natural anatomical structures. It is possible to modify their susceptibility to attack by proteolytic enzymes, as well as their rates of absorption in living tissues.

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