CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

XX. THE DEVELOPMENT OF FIBRIN FOAM AS A HEMOSTATIC AGENT AND FOR USE IN CONJUNCTION WITH HUMAN THROMBIN [1,2,3]

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The control of unusually rapid capillary oozing or of free venous bleeding where the site of hemorrhage is unsuitable for hemostasis by ligature, clip, or electrocoagulation has long been a problem in all fields of surgery. Many methods have been suggested to control such bleeding, but all have disadvantages which make them unsatisfactory in certain situations. This has been especially true in the field of neurosurgery.

The oldest and still probably the most commonly used method of controlling such bleeding is pressure applied with gauze sponges or cotton patties soaked in warm saline. This method, though often highly successful, is time consuming, and as Harvey (1) pointed out in 1918, and Putnam (2) has recently reemphasized, a tampon of this sort which must be removed will often drag the clot away from the bleeding point with recurrence of the bleeding. This fact has led to a search for a substance which would effect hemostasis and could be left *in situ* without exciting injurious tissue reaction.

First, the body tissues, fat, fascia, and muscle, were tried. Today the use of muscle, first introduced by Cushing in 1911 (3), remains the most satisfactory method to obtain hemostasis in difficult situations. Subsequent to 1911, Grey and Harvey (4, 5), both associates of Cushing, tried for the first time tampons made of both animal and human fibrin. Histological studies of absorption and resolution of implants of these materials in rabbits, cats, and dogs revealed less reaction than that occasioned by muscle implants. Blocks of sterile fibrin paper were found to be admirable hemostatic agents when used either alone or in conjunction with certain fluid clotting agents available at the time.

In the history of surgery many such fluid clotting agents have been used, ranging from the "Koagulen" of Fonio (6), prevalent in the second decade of this century, to the highly purified thrombins of animal origin (7 to 14) which have been developed within the last 10 years.

Putnam (2) has reported highly successful results using pledgets of soluble cellulose (15, 16, 17) with animal thrombin and later with thrombin of human origin, which became available in sufficient quantities for widespread clinical use in the course of large scale plasma fractionation (18, 19).

The latter program has, for the first time, made possible the achievement of an absorbable hemostatic agent, combining the functions of tampon and thrombin, which contains as components only human proteins involved in the natural clotting mechanism.

This material, known as fibrin foam, can be made with a wide range of physical and biological properties. It has been made in two general types: *One*, known as fibrin foam, contains a minimum amount of thrombin, is for

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[2] This paper is Number 25 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.

[3] This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the United States Navy. The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

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use with separately packaged thrombin; *the other*, made with thrombin intrinsically combined in it, needs no extra thrombin for hemostatic use. Ease of manufacture and clinical preference have dictated the selection of the separately packaged fibrin foam and thrombin as a primary standard for production. Fibrin foam has been extensively studied from the point of view of histological reaction, and has been successfully used in the clinic. These studies are reported in another paper of this series (20).

THE PROPERTIES OF FIBRIN FOAMS

The name "foam" derives from the fact that in the dry state the structure comprises dense strands of fibrin fibers, between which there are air spaces of macroscopic and microscopic size (Figure 1). The resultant product is thus of low density; the multiple channels within it permit penetration of fluids and give rise to an absorbent action. When dry, the foam appears as a homogeneous, dull, rough, slightly brittle, porous mass. As the moisture content increases, the foam loses its brittleness, becomes compressible, rubbery, slightly resilient, and when entirely wet there is some spontaneous shrinkage. These properties may all be varied by suitable alterations in preparation.

Range of mechanical properties. The two essential proteins, fibrinogen and thrombin, are separated in Fraction I and Fraction III-2, respectively, of the plasma fractionation process (18, 19). By varying the proportions of these constituents and the physical and chemical conditions under which foams are made, a wide range of mechanical and biological properties may be attained. At one extreme of this range is a light, fluffy, highly compressible product which wets with great ease and which, when wet, loses approximately 90 per cent of its dry volume through spontaneous shrinkage. Near this end of the range are foams best suited for attaining hemostasis of small persistent bleeders, such as are found on the dura. At the other end is a dense, firm, relatively less compressible product which wets slowly, and when wet, shrinks but 50 per cent of its dry volume. These types are more suitable for packing large cavities such as tumor beds.

While the mechanical strength of various types of foam depends upon conditions of their preparation, any given foam will show a variation in mechanical strength dependent upon its moisture content. The differences in mechanical strength, though not striking when the foams are dry, become pronounced as the moisture content is increased.

Further modifications of the physical properties of foams by chemical and physical treatment may be attained, notably in the direction of increased flexibility and elasticity. Such foams tend to regain their shape if a deforming force be removed, and thus do not lend themselves readily to moulding.

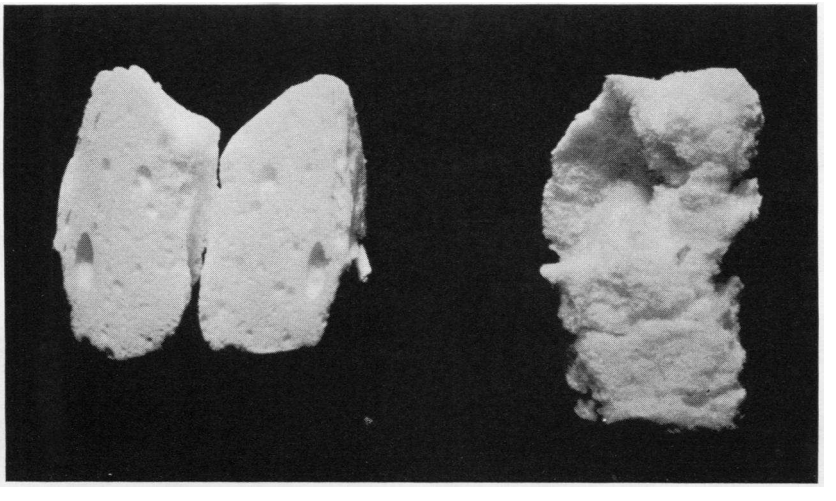

FIG. 1. FIBRIN FOAM IN THE DRY STATE

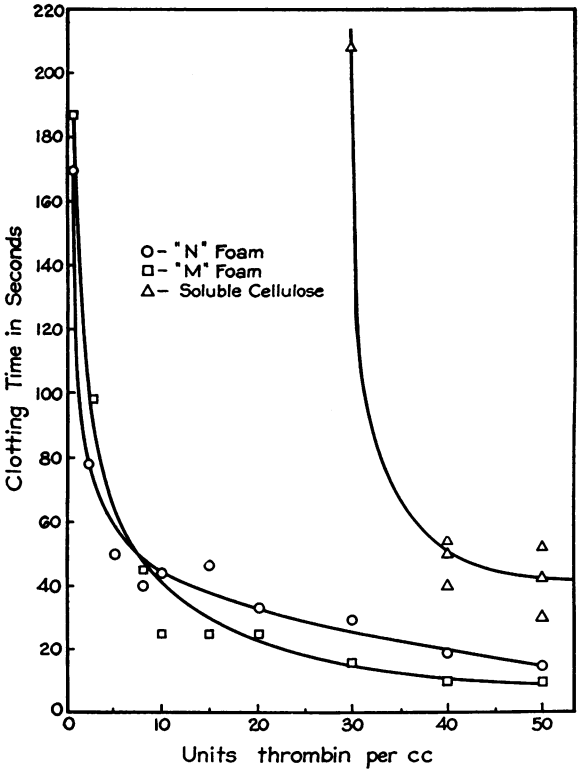

FIG. 2. *In Vitro* CLOTTING OF FIBRIN FOAM AND SOLUBLE CELLULOSE USED WITH THROMBIN SOLUTIONS OF VARYING STRENGTH

Thrombic activity. As in the instance of mechanical properties, a wide range of thrombic activity is attainable by varying the procedure of production. Fibrin foams of low thrombic activity, packaged as *fibrin foam*, are usually used with solutions prepared from separately packaged thrombin. *In vitro* or *in vivo*, the clotting time of a *fibrin foam* depends upon the strength of the thrombin solution used with it. This may be demonstrated by measuring the time required to clot 1 cc. of a standard fibrinogen solution with constant sized pieces of foam which are soaked in thrombin solutions of varying strength. Figure 2 shows this *in vitro* test applied to two kinds of fibrin foam, and soluble cellulose (supplied through courtesy of Dr. Tracy Putnam and Dr. Kenyon). Thus, foam would appear to be highly effective with weaker thrombin solutions than soluble cellulose.

If a foam is intended to be used alone as a hemostatic, the amount of thrombin incorporated in it will determine its *in vitro* and *in vivo* clotting times. With any given foam of this type, the *in vitro* and *in vivo* clotting times will vary with moisture content, the length of time it soaks in saline, and the volume of solution.

Curve 1, Figure 3, is the *in vitro* clotting time of constant sized pieces of foam when soaked in an infinite excess of saline, while curve 2 measures the effect of soaking the foam in just slightly more saline than the foam could absorb. In both cases, the clotting time reaches a minimum when the foam is first completely wet, but with an infinite amount of saline the thrombin continues to diffuse out and the clotting time rises, while with a small amount of saline the thrombin diffusion soon reaches equilibrium and the clotting time becomes constant.

Fibrinolysis. In that the source materials for production of foam, Fraction I and Fraction III-2, both contain fibrinolytic activity, the rate of lysis of the wet foam *in vitro* will be in the main a function, first, of the concentration of this activity, and secondly, of the susceptibility of the fibrin. Both of these factors may be altered by appropriate measures. Thus, it is possible to prepare a foam which when wet will lyse spontaneously with considerable speed *in vitro*, which is readily susceptible to bacterial fibrinolysin and which disappears relatively rapidly when implanted in tissue. On the other hand, it is possible to produce a foam, with the natural fibrinolysin inactivated, whose fibrin is only slightly susceptible to bacterial fibrinolysins.

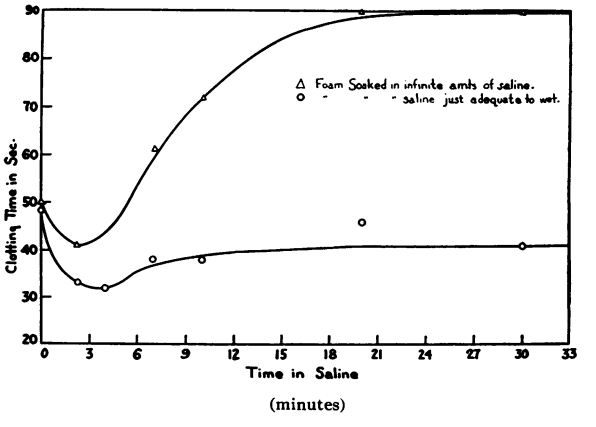

FIG. 3. EFFECT OF SOAKING FIBRIN FOAM THROMBIN IN VARYING AMOUNTS OF SALINE ON CLOTTING TIME

Curve 1 (△) Curve 2 (○).

In this case, both lysis *in vitro* and disappearance in tissue are slow.

Sterilization of fibrin foams. The fibrin foams released for clinical use are sterile. In that resterilization by boiling or by the autoclave seriously changes the mechanical properties, the thrombic activity, and the histological sequences following implantation, the unused material in an opened package should be discarded.

Incorporation of anti-bacterial agents. Antibacterial agents may be used with fibrin foam whenever they are indicated. These agents may be incorporated in the foam, put up with the thrombin, or mixed at the time of use.

COMMENT

Fibrin foam is one of a group of fibrous structures of potential hemostatic value which can be prepared from the products of human plasma fractionation. From the range of properties attainable in this group of structures, it becomes the province of the surgeon to chose that combination which is best suited to his particular problem.

The clinical and pathological studies reported in subsequent papers have been carried out on a group of foams which, in physical characteristics of porosity, compressibility, and strength, are near the light, fluffy end of the possible range. From the standpoint of bacterial fibrinolysis, the natural enzyme has been inactivated and the fibrin rendered resistant. Fibrin foam (low thrombin) has been used in conjunction with separately packaged thrombin.

Whereas this type of matrix would seem peculiarly adapted to and has, at present, been most used in neurosurgery, it may well emerge that an entirely different structure would be required should these products prove of value for other situations; for example, in the control of oozing as presented by chest wounds, injuries to the liver, and gynecological surgery.

SUMMARY

A fibrous protein matrix of a wide range of mechanical and biological properties has been prepared from the human plasma proteins involved in the natural coagulation mechanism. This matrix is designed to combine the function of an absorbable tampon with thrombin activity for use in those instances of hemorrhage where conventional surgical methods of hemostasis are not entirely satisfactory.

Fibrin foam for use with thrombin solution has been the first of these to be available for widespread clinical use. Its structure and characteristics are considered in detail.

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