CORNEAL WOUND HEALING II
VARIATIONS IN ADHESIVE POWER OF FIBRIN IN VITRO STUDIES

ALBERT L. BROWN, M.D.
Cincinnati, Ohio

AND

(By invitation)
FRANK A. NANTZ, M.D.
Cincinnati, Ohio

The adhesive power of fibrin found in the aqueous of rabbits and dogs following perforating corneal wounds has been previously reported. The remarkable tenacity with which corneal wounds are sealed in the rabbit suggested the employment of fibrin in the repair of corneal wounds in man because such natural formation in the aqueous of man is practically nonexistent. To this end the role of fibrin in the cycle of corneal healing in the rabbit and dog was studied in vivo. These induced studies showed that the remarkable tendency of corneal wounds and large corneal defects produced by the excision of corneal strips is due to the adhesive power of aqueous fibrin, which establishes primary union of the wound edges. The healing process is accomplished in three main stages: (1) Immediately after the corneal wound is produced fibrin forms and seals the wound. If the wound is large, fibrin fills the gap. So long as aqueous filters through the wound, fibrin is deposited; (2) in a few hours clot retraction begins. Excessive strands of fibrin tend to pull back in the conjunctival sac and the mass of fibrin in the wound contracts into a single rounded mass. The fibrin plug can be torn out but it is immediately replaced. The clot retraction continues pulling the wound edges together; (3) on the seventh or eighth day fibrinolysis destroys the fibrin mass which is replaced by normal fibrosis. The scar is firm and dense in fourteen days. Thereafter, the scar gradually becomes thinner and lighter, often clearing remarkably in from six to eight weeks.

Since fibrin is not found in human aqueous, comparative studies in vivo are of no significance so far as man is concerned. In vitro experiments were therefore contrived to note the adhesive power of fibrin on isolated corneal segments. Thus, the conditions in man could be more nearly simulated to note whether the fibrinogen-thrombin (fibrin) reaction alone was responsible for the adhesion between corneal edges in the absence of other normal tissue and fluid surroundings.

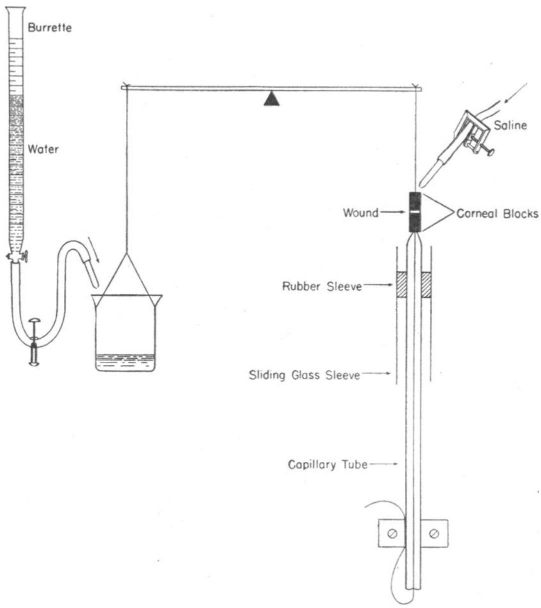
EXPERIMENTS ON THE ADHESIVE POWER OF FIBRIN IN VITRO

The method employed in studying the adhesive power of fibrin for isolated corneal blocks consisted in the preparation of a standard corneal wound which was then sealed with fibrin under controlled conditions. The sealing power of fibrin was measured by the tension (in grams) necessary to separate the wound.

Corneal strips were cut with a Castroviejo keratoplasty knife with blades set 2½ mm. apart so that all blocks were of constant width. Each strip was cut in half, thereby creating a standard corneal wound.

I. *The effect of various concentrations of fibrin on wound tension:* A knowledge of the adhesive power of various concentrations of fibrin was deemed of primary importance because the ease in handling the substance depends on its density and fluidity. Then, too, the absorbability from the inner eye varied with different concentrations.

The material used contained 60 per cent pure fibrinogen. Normal saline was added to the dried material and the concentrations computed on the basis of 60 per cent purity. A sample of the various concentrations was applied to the cut edges of the corneal blocks under the same external conditions. These conditions were as follows: temperature—37° C.; time—30 minutes; surrounding medium—air; hydration—14 drops of saline per minute.



Apparatus for testing adhesive power of fibrin between corneal blocks.

The tension exerted by the water in the balance arm measured the adhesion between the corneal blocks.

Mechanical adhesion increases progressively as a direct variant of the concentration, reaching a maximum at 20 per cent in normal saline. Since the material used contained 60 per cent pure fibrinogen, the maximum concentration of 20 per cent represented 12 per cent pure fibrinogen. This maximum concentration was prepared by adding 1.6 cc. normal saline to each ampule of material. At higher concentrations, the mixture was no longer fluid but became a sticky, thick mass, unsuited to corneal application.

The wound tensions produced by the workable concentrations were as follows:

Fibrinogen concentration in per cent	*Wound tension in gms.*
3	0.33
6	0.60
9	0.88
12	1.70

It is obvious that the adhesive power in low, workable concentrations for small corneal edges (2½ mm. in width) was very considerable.

II. *The effect of plasma and platelets on the adhesive power of fibrin:* The presence of blood in the human wound lends adhesive power to the wound edges. Herein, not only is fibrinogen activated by thrombin from the wound edges but other blood elements play a role as previously noted.

Plasma and platelets were added to fibrin to observe any enhancement of adhesive power. Corneal blocks were suspended in the right balance arm as before but their preparation differed. One group of blocks was placed in normal saline and another group was kept in plasma plus the buffy coat of clotted blood for one hour before the experiment. The results were as follows:

	Wound tension in gms.
Corneal blocks kept in normal saline	1.70
	1.60
	1.80
	Average 1.70

	Wound tension in gms.
Plasma plus buffy coat	2.80
	3.30
	2.70
	Average 2.90

Conditions of the experiments: temperature—37° C.; time—30 minutes; surrounding medium—air; hydration—14 drops of saline per minute.

The enhancement of the adhesive power by the addition of other blood elements, particularly plasma and platelets was as much as 60 per cent. The clinical significance of this may be important inasmuch as corneal wounds occur in avascular tissue and corneal sections are often made in front of the limbus without much bleeding.

III. *The effect of swelling on the adhesive power of fibrin:* Smooth, cleanly cut, dry corneal wound edges were found to adhere with fibrin readily. Swelling interfered markedly with the continued apposition of the corneal blocks. The tissues least capable of swelling adhered more firmly. This was demonstrated in a comparative study of various combinations of tissue approximations after placement in saline solution and in air.

Type of tissue	*Surrounding medium*	*Wound tension in gms.*
Cornea to cornea	normal saline	0.8
Cornea to cornea	air	1.7
Cornea to sclera	normal saline	2.0
Cornea to sclera	air	3.0
Sclera to sclera	normal saline	3.6
Sclera to sclera	air	4.6

Conditions of experiment: temperature—37° C.; time—30 minutes.

The edges of the corneal blocks swell slightly when they are cut and suspended even in dry air. The scleral blocks show no such immediate tendency and swell only slightly in saline solution. This was noted only grossly. The comparative water absorption was not measured by weight. In the first type of tissue approximation (cornea to cornea), suspension in saline solution produced maximal swelling, while in the last (sclera to sclera) it was minimal.

IV. *The effect of hydration on the adhesive power of fibrin:* The maximum adhesive power of fibrin for tissue edges was exhibited in an atmosphere of about 45 per cent at 37° C. Corneal blocks suspended in the chamber and sealed together with fibrin were subjected to various degrees of hydration. These variations were maintained by altering the number of drops of saline solution dropping on the wound edges per minute. Complete hydration was obtained by suspension in saline solution. Immediate adherence, as noted above, took place. At the end of 30 minutes the wound withstood a tension of 0.8 gms. After this time, the wound edges began to separate and were completely apart in 2 hours. There was no reunion after this time. The amount of hydration was reduced, causing a greater adherence of the corneal blocks. It was found that the amount of hydration produced by the application of 14 drops of saline solution per minute to the wound edges maintained an almost constant level of adherence. As this amount was reduced the level rose and became greatest when the application of solution was stopped and air became the sole medium.

Conditions of the experiment

Hydration	*Wound tension in gms.*	
	2 hrs.	*12 hrs.*
Normal saline bath	0—spontaneous separation	
14 drops of saline per minute	2.3	2.8
Air	8.2	206.0

The significance of these observations leads to those conclusions formerly reported in vivo.

Fibrin forms rapidly and in great quantity to close a corneal wound in the rabbit. If it is removed, greater quantities form, completely closing and covering the wound inside and out. If left in place, the fibrin forms a dense coat over the wound, thus waterproofing it against the surrounding fluid medium. The wound is allowed to heal, protected as well as sealed by the fibrin.

V. *Onset of fibrinolysis:* The adhesive power of fibrin was found to differ markedly in varying degrees of hydration. Corneal blocks sealed with fibrin separated in 2 hours in a completely fluid medium. Drying in air restored and enhanced the adherence enormously. However, the hydration produced by the application of 14 drops of saline solution per minute maintained the wound tension at a fairly constant level. The onset of fibrinolysis occurs when this curve reaches zero.

This was demonstrated to be approximately the case by suspending corneal blocks in the chamber and sealing their edges together with fibrin. The petcock was adjusted to deliver 14 drops of saline solution per minute to the edges. The wound tension attained a relatively high level in 2 hours, held fairly constant for four days and then slowly declined to zero on the eighth day.

Time in days	*Wound tension in gms.*
1	2.5
2	2.3
3	2.7
4	3.1
5	2.3
6	2.8
7	1.6
8	0.0

CONCLUSIONS

Although these in vitro experiments cannot exactly correspond to conditions in vivo, they are suggestive. In the living rabbit, fibrin seals a corneal wound mechanically until fibrosis can become established. It is difficult to determine exactly when fibrinolysis begins but the in vitro maintenance of a constant level with a gradual loss in adhesive power over eight days demonstrates the mechanical course without the body.

BIBLIOGRAPHY

Brown, A. L., and Nantz, F. A.: Am.J.Ophth., **27**:1220, 1944.

DISCUSSION

DR. THEODORE L. TERRY, Boston, Mass.: Dr. Brown was a pioneer in the field of fibrin used therapeutically. The present study of Brown and Nantz is of real value, different in manner from the many studies in the use of fibrin film and fibrin foam in general medicine.

The work with which I am familiar, relating largely to problems of war wounds, has not adapted itself to ocular injuries in practical ways. The fibrin film produced by Cohn and his associates is not easily stitched into place and the sutures pull out easily. Cohn's skin grafting unit, however, is of considerable interest. Solutions of fibrinogen and thrombin are readily made by adding saline to the dry preparations. The one is painted on the raw undersurface of the skin to be grafted and the other is painted on the bed for the graft. Fibrin is formed when the graft is placed in its bed, thus obviating the necessity of stitches. It has a use in plastic operations of the lids.

DR. ALBERT L. BROWN, closing: We felt when we started this that it had some practical application to war wounds during the war. We have not satisfied ourselves as to the exact use after the war, even now. We have tried to use this in a few eyes which were to be enucleated, having made corneal sections previously and allowed them to heal with the patient's permission. We are not ready to express ourselves on this yet. In fact, we are pretty much in the position that Columbus was in on his first voyage: When we started out we did not know where we were going, then when we got there we did not know where we were, and when we got back we did not know where we had been. We hope to find out in the next few years about the application of fibrin in various types of eye conditions.