in mouse strains which have been specially selected over many generations for low and high serum cholesterol levels, respectively.

The abundance of microperoxisomes in the retinal pigment epithelium, and the paucity in neural retina and choroid suggest that these organelles may have an important role in the eye. Considering their response to induced hypolipidemia and evidence implicating them with metabolic disorders of lipid metabolism,9, 10 it is tempting to speculate that microperoxisomes are associated in some way with the digestion or renewal of rod outer segment membranes. The fewer microperoxisomes observed in prenatal and neonatal animals compared to adults may reflect differences in renewal kinetics and not simply developmental changes. The persistence of microperoxisomes in the retinal pigment epithelium of hibernating frogs and also in mice with congenital retinal degeneration indicates that active turnover of rod outer segments is not required to maintain the capabilities provided by microperoxisomes. In any case, probably these organelles have various functions, some of which are unrelated to rod membrane turnover. Microperoxisomes are sensitive intracellular indicators of metabolic state, and they can be useful in distinguishing normal from pathologic conditions,9, 10 but their precise relation to lipid metabolism remains unknown.

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REFERENCES


Use of a platelet-fibrinogen-thrombin mixture as a corneal adhesive: experiments with sutureless lamellar keratoplasty in the rabbit.* A. RALPH ROSENTHAL, CHRISTINA HARBRURY, PETER R. ECBERT, and EDWARD RUBENSTEIN.

A platelet-fibrinogen-thrombin mixture utilizing autologous platelets was studied for its potential as a corneal adhesive. In the rabbit it demonstrated sufficient adhesive properties to allow 50 per cent of lamellar keratoplasties (autotransplants) to remain in place without the use of sutures. The mixture retains significant adhesive properties for four to six days. It is simple to prepare and apply. It also appears nonantigenic and nontoxic to the cornea; it does not incite inflammation, nor interfere with corneal clarity or the regrowth of corneal epithelium.

Thrombin1 and a mixture of plasma and thrombin2 have been used in the past as tissue adhesives in ophthalmic surgery. These agents did not gain acceptance, presumably because of unsatisfactory adhesive properties. Work at our institution has...
demonstrated effective tissue adhesive properties of a combination of platelets and fibrinogen catalyzed by thrombin in artery and vein anastomoses in rats. This paper describes initial experiments which have been undertaken to evaluate the efficacy of this physiologic material, i.e., a platelet-fibrinogen-thrombin (p-f-t) mixture, as a corneal adhesive. In order to best evaluate not only the adhesive properties but also the corneal toxicity of the p-f-t mixture, autotransplanted lamellar keratoplasties were chosen for the preliminary experiments. The experience with this mixture in sutureless lamellar keratoplasty as well as the histopathologic observations of the corneal healing in its presence will be described.

Method. Experiments were carried out on 10 male and female albino New Zealand white rabbits weighing from 2 to 4 kilograms. The animals were anesthetized with intravenous sodium pentobarbital (20 mg per cubic centimeter), the corneas were anesthetized with one drop of 0.5 per cent proparacaine hydrochloride, and 6 mm. half-thickness lamellar keratoplasties were performed bilaterally. The grafts were replaced to the eye from which they were removed. Platelets, fibrinogen, and thrombin were applied in sequence to the lamellar bed in one eye. The other eye was used as a control. No corneal sutures were placed in either side. The lids were closed with 4-0 silk sutures over rubber bolsters. The eyes were examined with the slit lamp one day, two days, four days, seven days, two weeks, and three weeks postoperatively. Two animals were observed for four to five weeks and one animal for eleven weeks.

Histopathology. Routine hematoxylin and eosin, periodic acid-Schiff, and Masson's trichrome stains were performed in eyes from all the above rabbits plus additional rabbits in which the p-f-t mixture was used to repair half-thickness lamellar keratoplasties. Thus, eyes were examined histopathologically one day, four days, seven days, two weeks, three weeks, four weeks, and eleven weeks postoperatively.

The components of corneal adhesive were: (1) Platelets: cardiac puncture was used to obtain 40 to 50 ml of the animal's own blood one to two hours prior to surgery. Acid-citrate-dextrose was used as an anticoagulant. The platelets were prepared by differential centrifugation and used immediately after preparation. (2) Fibrinogen: purified human fibrinogen was obtained from normal human donors. (3) Calcium: $1.7 \times 10^{-3}$ M solution of CaCl$_2$. (4) Thrombin: bovine thrombin, 1,000 units per milliliter. One or two drops of each component were applied in sequence to the lamellar bed and the graft immediately applied. The material was allowed to set for two to five minutes before the tarsorrhaphies were performed.

Results. The results of sutureless lamellar keratoplasty using the p-f-t mixture as compared to the controls are seen in Table I. In controls without the mixture only one graft remained in its bed for 48 hours and it subsequently fell off. In comparison, when the p-f-t mixture was used, all grafts were in place at 24 hours and nine out of the ten in place at 48 hours. However, by six days, three further grafts were dislodged. After this only one additional graft came loose from its bed; this occurred at one week and was associated with the development of a severe purulent conjunctivitis. Therefore, all grafts which survived the first week remained in place and were clear without vascularization for the remainder of the period of observation (range 20 to 77 days).

Histopathology. At one day the tissue adhesive, which is a homogeneous eosinophilic substance, was seen to form a thin layer between the corneal bed and the lamellar graft. The adhesive filled the fine interstices between the collagen lamellae at the cut edge but did not penetrate deeply into the corneal stroma (Fig. 1). After four days the adhesive was still present but no fibroblastic activity had yet commenced.

Seven days after surgery the thin layer of adhesive started to disappear at the periphery of the graft. Fibroblast proliferation was evident along the entire boundary of the adhesive (Fig. 2). The fibroblasts did not penetrate the adhesive but crossed the graft boundary only where the
Fig. 2. Interface between the lamellar graft and the corneal bed with a layer of adhesive (A) in between. Fibroblastic activity (F) is seen on either side of the adhesive with minimal tendency to invade the adhesive. Hematoxylin-eosin, ×300.

Fig. 3. The graft came loose from its bed leaving a layer of glue in the stromal defect. The epithelium (E) from the periphery of the cornea is seen sliding and growing around the edge of the adhesive (A). Periodic acid–Schiff, ×120.

A layer of adhesive had disappeared. At fourteen days the adhesive was no longer visible and healing had progressed. At three weeks the graft was well healed. Further remodeling of the scar was observed at four and eleven weeks.

In one eye in which the graft came loose from its bed two days postkeratoplasty, the adhesive provided a scaffold over which the epithelium grew (Fig. 3).

The adhesive incited little or no inflammatory reaction. At day one in two eyes there were a few polymorphonuclear leukocytes at the cut edges of the graft as well as around a globule of the adhesive at the periphery; however, in a control eye in which the operation was performed without the adhesive the same degree of acute inflammation was apparent. This minimal inflammatory response was, therefore, attributed to the surgical trauma and not to the presence of adhesive. At four days postoperatively there was no inflammation in any of the eyes. Furthermore, there was no visible macrophage response to the adhesive. The p-f-t mixture gradually disappeared and its degradation products presumably were absorbed into the surrounding cornea.

Discussion. In the vascular tree, a system which contains fluid under pressure, it is essential to be able to seal a leak promptly. This is accomplished by the prompt formation of a platelet-fibrin plug at any injury site. The platelets avidly adhere to the freshly exposed collagen and secondarily adhere to each other and to interlaced fibrin strands to form a fibrin plug.

This quality of platelets to adhere to collagen, each other, and fibrin is what suggested to us that this combination would be able to function as a physiologic adhesive in corneal surgery. Because the corneal stroma is largely composed of collagen, it provides the ideal milieu for platelet adherence and aggregation.

An advantage of a platelet-fibrin adhesive is that all components have existing biodegradative pathways in the body and that the platelets can be obtained from the patient, thus assuring immunologic compatibility. Also, the components can be prepared by sterile technique.

The success of achieving clear bonded lamellar keratoplasties using the p-f-t mixture (50 per cent after two weeks) compares quite favorably with the results of Cardarelli and Basu who were able to achieve only a qualified success in fixing lamellar corneal transplants with isobutyl cyanoacrylate.

In 10 cases out of 49 a permanent bond was achieved but the grafts became moderately opaque.

Those properties of the material which appear to make it particularly advantageous as a corneal adhesive are: (1) it is nontoxic to the cornea since it incites no corneal inflammation and, therefore, does not impair the ultimate corneal clarity. (2) After setting it is soft, rubbery, and pliable. Any excess of the surface of the wound is smooth and nonirritative to the lids and cornea. It can be molded and cut easily with a scissors. (3) It does not prevent epithelialization, but allows the corneal epithelium to slide over it. (4) It is slowly absorbed, probably under the action of the proteolytic enzymes of the cornea and does not act as a permanent barrier to healing. (5) The platelets are simple to prepare and apply and should be available at one to two hours notice. The thrombin and fibrinogen are commercially available.

The adhesion created by the p-f-t mixture appears to be developed immediately and to maintain its full power for four days after application. The subsequent loss of adhesive power correlates with the disintegration and death of the platelets (platelet half-life is four to five days).
corneal wound strength is not developed until three weeks. However, the current experiments suggest that sufficient corneal healing had taken place by four to seven days to allow continuation of the wound healing and subsequent adherence of the lamellar graft.

Though we did not attempt to do so in the current experiments, there appears to be no reason why the p-f-t mixture should not be reapplied at any time if segmental loosening is observed. Similarly, it appears that the material may be useful in the repair of corneal perforations and corneal wound leaks.

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REFERENCES