Physical properties of experimental vitreous membranes. I. Tensile strength. TETSUTO NUMATA, IAN J. CONSTABLE, AND DANIEL E. WHITNEY.

The tensile strength of vitreous membranes, induced by silk sutures imbedded in the vitreous body and removed one to two weeks later, was investigated in rabbits. Three types of membranes were distinguished by their appearance under an operating microscope: dense, opaque, cylindrical membranes, (Type 1), broken with 2 to 16 Gm. of force and elongated from 127 to 200 per cent before breaking; thin, cylindrical membranes, (Type 2), broken with 200 mg. to 2 Gm. of force and elongated from 51 to 152 per cent before breaking; and thin film-like membranes imbedded in formed vitreous gel, (Type 3), broken with 200 mg. to 5.5 Gm. of force and elongated from 53 to 186 per cent before breaking. Vitreous membranes formed two to eight weeks after surgery resisted forces greater than those required to detach rabbit retina. The results of the experiment are relevant in the design of vitrectomy instruments.

A satisfactory surgical instrument for vitreous membrane removal by the closed transcleral route must possess properties such as small size (maximum 2.0 mm. diameter tube), a separate infusion chamber to maintain pressure, and a suction line to remove cut membranes without pulling on the easily detachable retina. These requirements have been quite well recognized by others. However, knowledge of the mechanical properties of the materials being cut would be very helpful for the systematic design of specific details such as shape, material, and finish of the cutting edge, and speed and stroke of the cutting mechanism. The present experiment was carried out to investigate the tensile strength of vitreous membranes produced in rabbit eyes.

Materials and methods.
Production of vitreous membranes. Ten rabbits were used for this experiment weighing between 1.7 to 4.2 kilograms. Animals were anesthetized with intravenous pentobarbital sodium (25 mg. per kilogram) and pupils were dilated prior to surgery with phenylephrine 10 per cent and scopolamine 0.3 per cent. The eyes were propsected and held by forceps applied to the superior and inferior recti. Three to six sutures (4-0 silk) were passed through conjunctiva and sclera across the vitreous cavity at the level of the equator by means of a straight sewing needle. After one or two weeks, sutures were removed and, thereafter, membrane formation was observed by indirect ophthalmoscopy and by slit lamp biomicroscopy.

Membrane preparation for testing. Two rabbits were killed every week by an overdose of anesthetic and the eyes were enucleated and put in normal saline at 25° C. Each eye was sutured into a molded plastic hemisphere, then opened by an incision around the limbus. The anterior segment was lifted with forceps and the lens cut away from the vitreous face. The membranes were then dissected out from the eye under an operating microscope and transferred onto a flat, black plexiglass plate. Then, membranes were cut into test specimen sizes. The individual form clamps were made by cutting soft polyurethane form into 1.6 by 1.6 by 0.4 mm. blocks and glueing them onto strips of aluminum foil (Fig. 1). Membrane test specimens were transferred from the black plexiglass plate onto form clamps submerged in normal saline at 25° C, and the ends of each membrane were then pressed between two such forms (Fig. 1). Form clamps prevented membranes from slipping during pulling and from being pinched directly by the plexiglass clamps. Pinching might create a local weakness in membranes where they would break with very small forces. Each membrane specimen secured at both ends by form clamps was carefully transferred into a plexiglass testing bath filled with normal saline at 25° C, and secured tightly into two plexiglass clamps as shown in Fig. 1. The specimen was then pulled until broken by a trans-
Fig. 1. Apparatus for testing tensile strength. The transducer (above) moves up at constant speed, stretching the vitreous membranes which are held between two plexiglass clamps (lower left). The form clamps (lower right) prevent uneven force from being applied to the vitreous membrane.

ducer mounted on a microscope stand, which was driven up and down by a motor at $\frac{1}{2}$ rev. per hour (0.3 mm. per minute extension) and $\frac{1}{2}$ rev. per minute (9 mm. per minute extension). The transducer was calibrated using milligram weights and its outputs were recorded on a Sanborn 350 recording machine.

Results.

Observations made on the membranes formed. The number of membranes obtained from each eye varied between nine and twenty-two. Membranes formed were classifiable into three categories on the basis of appearance under the operating microscope (Fig. 2). Dense, opaque, white membranes approximately 1 mm. in diameter or less were found bridging two nearby suture perforation sites (Type 1). Incomplete and thin films of vitreous gel surrounded these membranes. This type of membrane tended to be thicker and wider near the perforation sites tapering down in width toward its middle. Fine, cylindrical membranes, approximately 0.05 mm. in diameter, wider near the perforation sites, tapering down (Type 2). Diameters, however, varied and were never constant throughout the length of the membranes. Therefore, small sections of each membrane specimen were selected for testing so that they were as regular as possible. Considerable amounts of formed gel surrounded this type of membrane, but most of it was broken down by the preparation for testing. These membranes were often transparent and hard to observe under the operating microscope. Thin, film-like mem-
branes imbedded in formed vitreous gel were found at random locations in the vitreous cavity (Type 3). This material was difficult to handle and trim into the small size necessary for testing. These membranes were often quite transparent, and could only be seen by means of oblique illumination.

Variable amounts of vitreous gel surrounded each type of membrane and could not be easily separated from them, but was continuous only in Type 2 and Type 3 membranes. Although, Type 1 and Type 2 membranes were found radiating out from suture tracks, Type 3 membranes bore no direct relationship to them, being present at random sites within formed vitreous gel. All membranes tended to increase in density up to approximately four weeks, then became thinner and more transparent. None of the membranes had caused actual retinal detachment by pulling on the retina at local attachment points within the period of observation.

**Tensile strength and membrane extension.** Membranes first elongated, then finally broke at a random site (Fig. 3). The tensile strength and the extent of elongation of membranes before breaking are depicted in Fig. 4 as a function of age. Two different extension speeds, 0.3 mm. per minute and 9.0 mm. per minute, did not reveal any strength differences. Type 1 membranes showed a breaking strength of 2 to 16 Cm. and a breaking elongation of 127 per cent to 200 per cent. Type 2 membranes showed a breaking strength of 200 mg. to 2 Cm. and breaking elongation of 51 per cent to 152 per cent. Type 3 membranes showed a breaking strength of 200 mg. to 5.5 Cm. and a breaking elongation of 53 per cent to 186 per cent. All membranes tested were stronger than the force required to detach the retina. No strength differences were observed between two-week-old and eight-week-old membranes. A clear association between membrane age, strength, and elongation could not be made.

**Discussion.** The method of producing experimental vitreous membranes by means of sutures was found to be reliable. This method has several
advantages over the commonly used method of injecting blood. The vitreous body remains clear, allowing detailed clinical examination during formation of membranes and during experimental surgery. A reproducible variety of membranes are formed and, most importantly, formed vitreous gel is not radically destroyed as occurs after blood injection in the rabbit. This last point is particularly relevant when assessing the efficiency of vitrectomy instruments.

Type I membranes were usually much stronger than either Type 2 or Type 3: Type I membranes represent ingrowth of scar tissue at perforation sites. Their greater strength was probably due principally to larger amounts of these materials rather than to actual structural differences. The fine strands bridging suture tracks (Type 2) were of similar strength to lace-like membranes embedded in formed gel (Type 3). It was difficult to be certain to what degree their tensile strength was dependent on the newly formed membranous tissue and to what degree it was dependent on the formed gel. Attempts to measure the tensile strength of normal formed vitreous gel were unsatisfactory for several reasons. Normal vitreous gel could not easily be cut into small regular sizes suitable for testing, repeated handling tended to break up the gel, and the clear gel was invisible when immersed in saline. However, handling indicated that the formed gel alone was not strong enough to contribute much of the strength of Type 2 and Type 3 membranes.

It was inevitable that a wide range of tensile strength and extension would be obtained since individual membranes varied in diameter along the segments being tested, and since only gross trimming of surrounding formed vitreous gel and membranes could invariably be broken if stretched a maximum of 200 per cent. These facts suggest cutting mechanism designs in which membranes are first clamped before cutting. With such a design, the dependence on perfectly fitting shearing surfaces would become less critical. Systematic determination of the clamping surface and clamping force necessary would require data on adhesive force and frictional force characteristics of vitreous membranes. Experiments to define these properties for rabbit vitreous membranes will be reported later.

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Key words: tensile strength, vitreous membranes, vitreous gel, vitrectomy.

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same pilocarpine dose in free fluid. Analysis of flux to be independent of the available dose revealed a whole order greater than that induced by the linearly with time. At 240 minutes total flux was continuous flow of a tear analog but excluded lenses containing equal doses was quantitated in a previous publication. All parameters reported, including flush and control runs in advance of the pilocarpine administration, were held constant for experiments now described. However, single fluid pilocarpine instillations were replaced by delivery of presoaked hydrogel polymer lens buttons. Each of these was trephined when fully hydrated with pilocarpine solution. The experiment was designed so that the total available pilocarpine dose in each lens button was equivalent to the "secondary" dose in the upper chamber in the previously described single-fluid dose experiments.

Lenses buttons were presoaked in 30 to 50 ml. 16.0 percent pilocarpine-HCl in saline for 48 hours with a change of solution at 24 hours. Each button was blotted in a standard manner, then eluted for a minimum of 1 week in 100 ml. of saline at 4° C. The buttons were then matched on the basis of pilocarpine capacity independent of weight or thickness. Matching buttons were accumulated until a "pool" of prematched buttons was available. Each lens button from this "pool" was used randomly for determination at various time intervals. Long and short intervals were run sequentially. From time to time, a group of lenses were taken from the "pool" and re-tested by full soaking and elution for constancy of pilocarpine-HCl space. Buttons were discarded if any evidence of fraying or mechanical damage was present.

Quantitation of pilocarpine flux enhancement across isolated rabbit cornea by hydrogel polymer lenses. David L. Krohn and Julianna M. Breitfellner.

The comparative effect on pilocarpine flux across rabbit cornea induced by two hydrogel polymer lenses containing equal doses was quantitated in a transport chamber. This closed system featured continuous flow of a tear analog but excluded variables of the internal eye influencing concentration. Flux induced by both lenses increased linearly with time. At 240 minutes total flux was a whole order greater than that induced by the same pilocarpine dose in free fluid. Analysis of pilocarpine in tear analog effluent showed the flux to be independent of the available dose retained in the hydrogel polymer lens, suggesting that corneal transport of pilocarpine to the aqueous may involve mediation by a carrier system.

Clinical studies\(^{1-3}\) and animal experiments\(^{4,5}\) have demonstrated enhanced transepithelial pilocarpine flux induced by presoaked hydrogel polymer lenses. The mechanism of this enhancement has been uncertain. In addition, exact measurements of the effect of these polymers relative to that of ordinary drop administration or constant flow delivery have been unavailable, since aqueous concentration in the living eye after topical administration of any type is variably influenced by tear elution and multiple intraocular events. The latter include chamber inflow and outflow, preferential uptake of the drug by tissue in the internal eye,\(^6\) isomerization and hydrolysis,\(^7,8\) and, in the case of radioisotope determinations, interchange of isotope with solvent.\(^9\)

This report describes quantitation of the effect on pilocarpine flux across isolated rabbit corneas of two well-known hydrogel polymer lenses. A transport chamber system was used which incorporates the effect of tear flow but excludes the complex variables of pilocarpine concentration in the living internal eye. It is a closed system in that all administered pilocarpine can be accounted for. The intention was to compare relative flux efficiency of the two polymer lenses at various points in time. It was anticipated that some inference as to mechanism of transport enhancement relative to equal dose "drop" administration might be available from this comparison.

The transport chamber and flux data for single drop administration of pilocarpine were described in a previous publication.\(^10\) All parameters reported, including flush and control runs in advance of the pilocarpine administration, were held constant for experiments now described. However, single fluid pilocarpine instillations were replaced by delivery of presoaked hydrogel polymer lens buttons. Each of these was trephined when fully hydrated with pilocarpine solution. The experiment was designed so that the total available pilocarpine dose in each lens button was equivalent to the "secondary" dose in the upper chamber in the previously described single-fluid dose experiments.\(^11\)

Lenses buttons were presoaked in 30 to 50 ml. 16.0 percent pilocarpine-HCl in saline for 48 hours with a change of solution at 24 hours. Each button was blotted in a standard manner, then eluted for a minimum of 1 week in 100 ml. of saline at 4° C. The buttons were then matched on the basis of pilocarpine capacity independent of weight or thickness. Matching buttons were accumulated until a "pool" of prematched buttons was available. Each lens button from this "pool" was used randomly for determination at various time intervals. Long and short intervals were run sequentially. From time to time, a group of lenses were taken from the "pool" and re-tested by full soaking and elution for constancy of pilocarpine-HCl space. Buttons were discarded if any evidence of fraying or mechanical damage was present.