the conversion of our results (as expressed in HEL) to HTL units. By dividing the mean value of lysozyme (6.1 mg. per milliliter HEL) into the conversion factor, a value of 1.5 to 2.0 mg per milliliter HTL was arrived at. This value correlates with the results obtained by the lysozyme method of Bonavida and Sapse. Our experiments indicate that when the tears are further diluted, the activity of the enzyme increases. This might be explained by the presence of inhibitors in tears whose activities are reduced by dilution. Another possibility might be that a dimer form of the enzyme with no activity was present to an appreciable extent initially and was changed to the active monomer form upon dilution.13, 14

The collection and dilution procedure used here does not affect the reading appreciably. This conclusion is supported by the fact that tear lysozyme readings from both eyes, varied to the same extent as repetitive readings of the same diluted sample. In other words, the variability of the method stems mainly from the assay procedure itself.

The spectrophotometric method can be used to describe the distribution of tear lysozyme level in a population as well as to compare normal subjects to a group of patients with a certain disease. For this purpose, one reading per person is sufficient. However, when the aim is to establish the individual tear lysozyme level for diagnostic purposes, one reading may not be sufficient, depending on the sensitivity of the method used.

The method was used to determine the normal level of tear lysozyme in both eyes of 60 healthy subjects. The group was analyzed as a whole, since previous reports had indicated that tear lysozyme level was not affected by sex and only very slightly by age. One of our findings is that lysozyme level is the same in both eyes. Accordingly, it is possible to use the mean lysozyme level of both eyes to obtain a better estimate of the level in each healthy person.

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A surgical approach to full-thickness eye wall biopsy is proposed as a method that may be applied to poorly understood diseases of the retina and choroid. An eye basket is sutured to the eye for stabilization, and two trephines mark an area to which diathermy is applied until penetration is achieved. The specimen is removed with fine
forceps and scissors. A scleral homograft closes the defect. In ten rabbits, no eyes were lost and all ten retinas were intact. All biopsy specimens were suitable for histologic study.

The study of diseases affecting the retina and choroid has been limited by the unavailability of tissue specimens, except when unfortunate circumstances, such as sympathetic ophthalmia, necessitate enucleation, or when postmortem specimens become available. Our tools have been limited to such methods as ophthalmoscopy, fluorescein angiography, electroretinography, and other nonhistologic methods of observing tissue responses to a disease process. These circumstances have led to an incomplete knowledge of the progress of diseases such as retinitis pigmentosa, and uveitis, and to the call for tissue biopsy so that such techniques as electron microscopy and molecular biology can be applied. In light of the recent success of full-thickness eye wall resection in both animal models and human eyes, we attempted to prove that full-thickness eye wall biopsy is a viable procedure that will facilitate the study of disease of the retina and uveal tract.

Materials and methods. Ten pigmented rabbits weighing 2 to 3 kilograms were anesthetized and prepared for surgery using the eye basket. Seven and four millimeter trephines cut through the sclera to the choroid (Fig. 1). Diathermy was applied between those markings until perforation was achieved (Fig. 2). The specimen was then removed with a fine forceps and corneal scissors (Fig. 3) and immediately fixed in a neutral buffered solution of 1 per cent formaldehyde-1 per cent glutaraldehyde.

Vitrectomy, when necessary, was performed with forceps and scissors. The eye was closed with an 8 mm. scleral graft from a donor eye using 7-0 chromic gut. The eye basket was then removed (Fig. 4). Intraocular pressure was restored with a solution of gentamicin sulfate and saline, equivalent to 8 µg per milliliter of gentamicin, injected through the pars plana via a 25-gauge needle. The conjunctiva and lids were closed with 5-0 chromic gut.

Topical Neosporin ointment was applied after indirect ophthalmoscopic examination. The rabbits were observed daily for one week, then weekly thereafter. Intraocular pressure was measured by applanation tonometry at varying intervals.

After overnight fixation, each biopsy specimen was washed, postfixed, again washed, dehydrated in ethanol and propylene oxide, and embedded in Araldite. Thick sections were stained with Mallory’s stain, thin sections were stained with uranyl acetate and lead citrate. All were examined by electron microscopy.

Three months postoperatively, one animal underwent electroretinography.

![Fig. 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933061/) Eye basket is sutured to the sclera and two trephine cuts through the sclera are shown.

![Fig. 2](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933061/) The area between the trephine cuts has been treated with diathermy.

Animals were killed at varying intervals and the eyes enucleated. All globes were immediately fixed in neutral buffered 1 per cent formaldehyde-1 per cent glutaraldehyde, dehydrated in ethanol and chloroform, and embedded in paraffin. Sections were stained with hematoxylin and eosin and examined by light microscopy.

Results. Immediately after surgery, indirect ophthalmoscopy revealed markedly edematous retina surrounding the biopsy site resulting from the diathermy. Two of the ten eyes exhibited traces of blood at the periphery of the graft. One eye bled at the pars plana during restoration of intraocular pressure. In all cases the blood reabsorbed without consequence.

The eyes were inflamed after surgery, exhibiting conjunctival hyperemia and edema, anterior
chamber exudates, and some vitreous haze. One eye developed a small segmental cataract and another a total nuclear cataract soon after surgery. In two eyes the anterior chamber exudates caused corneal endothelial damage resulting in a localized opacification. External and anterior segment inflammation cleared in one or two weeks. Vitreous haze persisted variably for up to four weeks postoperatively. By six weeks all eyes were quiet, and the biopsy site appeared as a white disk surrounded by a chorioretinal scar. The rest of the fundus appeared normal in each case. Two months after surgery intraocular pressure was within the normal range for all rabbits, although four eyes exhibited lower pressure in the surgically altered eye than in the contralateral eye by as much as 10 mm Hg. There was slight flattening of the scotopic electro-

retinogram B-wave in the biopsied eye when compared to the contralateral intact eye.

Light microscopy of enucleated eye revealed chorioretinal scars at the edge of the graft and normal retina elsewhere.

Histologic examination of the ten biopsy specimens (Fig. 5) revealed that six had fully attached retinas, two had small portions artifically detached, and two had fully artifactually detached retinas. The nerve fiber layer of seven of the ten retinas showed a uniform vacuolation, which occasionally extended into the ganglion cell layer. Normal retinal histology was found in all other layers.
Ultrastructural examination (Fig. 6) confirmed these findings, showing that all retinal layers were normal in the center of the biopsy specimens with the exception of a partial vacuolization of myelinated nerve fibers in the nerve fiber layer.

**Discussion.** Biopsy specimens taken with our technique show no significant structural and ultrastructural changes as a result of the procedure. Despite peripheral ultrastructural changes, and the artifactual detachments noted in four specimens, all ten specimens were histologically normal in central areas and adequate for both light and electron microscopic study.

Surgical and postoperative complications observed during the course of the experiment were few in number. All observable retinas remained attached and appeared normal by ophthalmoscopic and histologic observation. The corneal damage seen in two eyes was the result of the heavy anterior chamber exudates. This may be avoided by treatment with anti-inflammatory drugs. Furthermore, it has been consistently observed in this laboratory that the rabbit eye is more sensitive to surgical trauma than that of other experimental animals and that of man. The two cataracts, one only a small segmental one, might easily be avoided in a species with a smaller lens. The likely cause in this instance was postoperative hypotony and subsequent condensation of fibrin from secondary aqueous.

This procedure provides a method for studying unusual ophthalmologic diseases affecting the retina and choroid. The resulting tissue specimens will be available for histologic study with the electron and light microscopes, for biochemical and immunologic analysis, and for tissue culture. Repeated biopsies could be used to follow the progress of a disease such as animal models of retinitis pigmentosa and other retinal dystrophies.

With further refinement, this procedure could be used in the diagnosis and study of human chorioretinal disease. One indication for this procedure is the diagnosis of sympathetic ophthalmia, a condition that requires enucleation as a diagnostic and therapeutic procedure. When this diagnosis is suspected in cases of blind eyes this procedure allows a tissue diagnosis that could save eyes currently being enucleated. Diseases such as retinitis pigmentosa and uveitis, which have multiple etiologies and threaten permanent visual loss, could be biopsied and studied with emphasis on new therapeutic approaches that could save remaining vision. Informed consent and philosophical judgment weigh heavily on this aspect of the future use of this procedure.

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**REFERENCES**


**Excitation and emission spectra of fluorescein dye in the human ocular fundus.**

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The excitation and emission spectra of fluorescein dye were determined during angiography from different sites in the human ocular fundus. All spectra were markedly shifted toward longer wavelengths relative to the spectra of fluorescein in aqueous solution. This effect is most pronounced for the macular area; however, it decreases for the choroidal background and even more for the retinal vessels. The results are relevant to the