Full-thickness eye wall biopsy. II. In primates. GHOLAM A. PEYMAN, PAUL HOMER, RICHARD KASBEER, AND JOSEPH VLCHEK.

We devised a technique to perform an intact full-thickness eye wall biopsy in primates. An eye basket is sutured to the eye wall for stabilization. Trephines demarcate and incise a 4 to 7 mm. circumferential area of sclera; diathermy deepens the incision until perforation is achieved. The biopsy specimen is removed and immediately fixed for histologic evaluation, and the eye wall defect is covered by a 7 mm. scleral homograft. Our results showed that histologically excellent biopsies can be obtained with minimal damage to the eye.

Diseases such as retinitis pigmentosa and uveitis are visible and well described clinically but are poorly understood pathogenetically. We felt that chorioretinal pathology could be studied more precisely if there were a method to take a small biopsy specimen of the eye wall without disturbing ocular function. In light of recent successful whole wall resections in animals and humans and good preliminary eye wall biopsy results in rabbits, we attempted to do biopsies on species monkey eyes.

Materials and methods. Eight eyes from five specia monkeys were used in this study. The monkeys were sedated with intramuscular phenylcyclidine and anesthetized with intravenously administered thiamylal. Proparacaine hydrochloride was dropped onto the eye for anesthesia as needed. The pupil was dilated with 1 per cent cyclopentolate hydrochloride and 10 per cent phenylephrine hydrochloride. A large temporal canthotomy and peritomy were performed. The side arms of the eye basket were positioned periliminally beneath the superior and lateral rectus muscles and sutured to the sclera with 8-0 running black silk. A scleral incision was made to the choroid with a 7 mm. trephine. Another incision was made centrally within the first one with a 4 mm. trephine. Diathermy was applied between these two incisions until perforation was achieved. The specimen was excised with corneal scissors and immediately fixed in a neutral buffered solution of 1 per cent formaldehyde - 1 per cent glutaraldehyde. Small amounts of vitreous (0.2 to 3 ml.) protruding through the biopsy site were cut with scissors.

A scleral homograft (7 mm. in diameter) that had been presoaked in 8 µg per milliliter of gentamicin sulfate was sutured to the area of resection, using running 7-0 chromic gut. Intraocular pressure was restored with 8 µg per milliliter of gentamicin sulfate in normal saline. The conjunctiva and canthotomy were closed with 5-0 chromic gut.

The monkeys received systemic oxytetracycline (Terramycin) for five days. They were observed daily for the first week following the procedure, and weekly thereafter. Prior to death, electroretinograms were done and compared to those of unoperated contralateral eyes. They were killed at intervals from four to twelve weeks.

All eyes were enucleated and immediately fixed in a neutral buffered 1 per cent formaldehyde-1 per cent glutaraldehyde solution. Seven of the eight eyes from which biopsies were taken were dehydrated in ethanol and chloroform, then embedded in paraffin. Histologic sections were cut on a microtome. The sections were stained with hematoxylin and eosin and examined by light microscopy.

The eighth eye and all of the biopsy specimens were prepared for electron microscopy as previously described.1

Results. Seven of eight eyes tolerated the procedure well (Table 1) with minimal loss of vitreous and little or no hemorrhage at the site of the graft.

Eye No. 5, which received a friable scleral graft that detached while intraocular pressure was being restored, had large vitreal loss and the graft had to be resutured. It never regained its preoperative configuration.

The seven eyes were quiet, the fundi clear, and intraocular pressures normal at the time of death. They had well demarcated white scleral grafts surrounded by chorioretinal scar (Fig. 1) formation. Indirect ophthalmoscopic examination before and after the biopsies revealed no evidence of retinal detachment or degeneration.
Table I. Results of eye wall biopsy in eight monkey eyes

<table>
<thead>
<tr>
<th>Eye No.</th>
<th>Complications</th>
<th>ERG</th>
<th>Time of death (weeks)</th>
<th>Histology of operated eye</th>
<th>Retinal histology of specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>Good photoreceptor function</td>
<td>12</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>Good photoreceptor function</td>
<td>7</td>
<td>Normal</td>
<td>Fixation artifact</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>Good photoreceptor function</td>
<td>7</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>Good photoreceptor function</td>
<td>12</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>5</td>
<td>Friable scleral graft; postoperative phthisical eye</td>
<td>*</td>
<td>4</td>
<td>Phthisis bulbi</td>
<td>Normal</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
<td>*</td>
<td>4</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>7</td>
<td>None</td>
<td>Good photoreceptor function</td>
<td>6</td>
<td>Normal</td>
<td>Specimen lost</td>
</tr>
<tr>
<td>8</td>
<td>Postoperative purulent conjunctivitis†</td>
<td>*</td>
<td>4</td>
<td>Retina normal inflammatory infiltrate in conjunctiva†</td>
<td>Normal</td>
</tr>
</tbody>
</table>

*ERG's not performed on eyes enucleated before six weeks after operation.
†Probably not a result of the surgical procedure.

Fig. 1. Side view of sagittal section taken through center of the eye showing the graft-retina interface. L, indicates lens; C, cornea; G, graft.

Electroretinograms of five operated eyes, when compared to three unoperated contralateral control eyes, showed good retinal photoreceptor responses under scotopic and high-intensity conditions.

Histologic examination by light microscopy showed normal monkey eyes with scar formation at the graft edge and chorioretinal adhesions in the area immediately surrounding the graft. There was infiltration of inflammatory cells at the graft site (Fig. 2), but no evidence of inflammation in the vitreous. The eye prepared for electron microscopy with special attention to the macular area was interpreted as normal.

Histologic examination of the eight biopsy specimens revealed that four specimens had fully attached retinas. One specimen had partial and three specimens had complete artifactual detachment of the retina as a result of processing. The fully attached retinas and two of the artifactualy detached retinas showed normal retinal morphology on light microscopy. Of the two remaining biopsy specimens, one retina showed full-thickness vacuolization artifically induced by a delay before immersion in fixative and one retina was lost during fixation. The choroid and sclera were normal in all specimens.

Electron microscopic examination of the four normal biopsy specimens revealed normal fine structure in all layers (Fig. 3).

Discussion. An editorial in Investigative Ophthalmology as recently as 1974 lamented the fact that the means were not yet available to perform a biopsy of the retina in order to bring the powerful tools of cellular and molecular biology to bear on the diagnosis of retinal disease. In addition to the transscleral eye wall biopsy which we initially developed in rabbits, there has been a recently reported transvitreal biopsy technique.

We feel the technique that we have devised is a technical adjunct to electron microscopic and biochemical studies which may eventually help
elucidate the mechanisms involved in metabolic disorders such as retinitis pigmentosa. This procedure may be diagnostically useful in determining the cause of certain obscure cases of progressive uveitis. In addition, it may prevent the unnecessary enucleation of a blind eye by establishing the diagnosis of sympathetic ophthalmia.

For the most part, the monkeys tolerated the procedure well, and we are encouraged by the results. The graft in eye No. 5 probably detached because of friable sclera, which, together with vitreous loss during surgery, may account for the eye's becoming phthisical postoperatively. Thereafter, we were more careful in screening the scleral homograft material used, and this problem did not recur.

Good light and electron microscopic biopsy results were obtained by our technique. The histologic anomalies in the biopsy specimens were probably due to correctable causes. We were using dull trephines throughout the first seven trials. Noting that the retinas had detached artificially in four specimens, we obtained sharper trephines and excellent histologic results with no retinal detachment on the eighth procedure.

Another important factor that we feel affected the histologic quality of the biopsy was the speed at which the resected area was removed from the eye and immersed in fixative. Rapid immersion may improve histologic quality.

The few problems we encountered may be prevented in the future with meticulous adherence to good surgical technique, good scleral homograft material, and sharper trephines. Scrupulously performed procedures can render good histologic results without significant loss of ocular function.

Fig. 2. Transition zone between normal eye wall and scleral graft. Note normal retina adjacent to graft site (arrows). P, indicates pigmented proliferation secondary to diathermy; I, inflammatory cell response at edge of homograft (hematoxylin and eosin, x110).

Fig. 3. Pigment epithelial cell (P) and associated outer photoreceptor segments (OS). CC, indicates choriocapillaris, and IS, inner photoreceptor segments; x5,000.

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REFERENCES