

In this experiment, the electronic pachometer when used by an untrained operator was no more accurate than the standard corneal pachometer when used by someone familiar with its use. Although the electronic pachometer is able to provide corneal thickness measurements to four decimal places, the ultimate accuracy of the machine depends on the end point alignment of the corneal parallelapiped. This end point was difficult to repeat when multiple measurements were made on the same cornea, but the accuracy of the measurement was no different than that obtained with the standard pachometer.

The electronic pachometer is easy to use and gives repeatable results with a high degree of accuracy under conditions in which the observer has not been specifically trained in its use. One advantage of the electronic pachometer is the ability to measure different areas of the cornea in a repeatable fashion because of the 5° separation of the fixation lights. With the addition of a microcomputer (Diagnostic Concepts, Inc.) which can calculate the mean and standard deviation for a series of readings, the electronic pachometer would be very useful for evaluating corneal thickness under various experimental conditions such as the continuous wear of hydrophilic lenses.⁸ The rapidity with which these measurements can be made and recorded would be advantageous when large populations are to be studied. The standard pachometer in trained hands is an accurate instrument and compares favorably with the electronic pachometer for central corneal thickness readings, but it is more difficult to use when measuring peripheral corneal thicknesses.

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Basophils in vernal conjunctivitis in humans: an electron microscopic study.

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The histopathological changes of vernal conjunctivitis comprise stromal infiltration by lymphocytes, plasma cells, and neutrophilic, eosinophilic, and basophilic leukocytes and epithelial invasion by mast cells and eosinophils. Blood vessels show swelling and death of endothelial cells and increased permeability with associated extravasation of erythrocytes and fibrin. The presence of basophils in ocular tissue has not been reported previously, and their occurrence in conjunction with the other pathological changes enables vernal conjunctivitis to be compared with, and classified as a manifestation of, delayed-type hypersensitivity of the cutaneous basophil type. Thus the mechanism is probably a mixture of both delayed and immediate immunological responses.

The clinical characteristics of vernal conjunctivitis have been extensively described,¹⁻³ and when the patient presents with a typical picture, diagnosis is seldom difficult. However, although "the pathological changes in vernal conjunctivitis have been fully investigated,"¹ there have been only two electron microscopic studies of this condition.^{3, 4} In view of the absence of an adequate theory to fully describe the mechanism of vernal conjunctivitis, which is considered to be allergic,^{1, 3} atopic,^{2, 3} or probably a mixture of delayed and immediate immunological responses,² this investigation was begun.

Methods. Biopsy material consisted of conjunctival tissue from the upper tarsus of two patients and from the upper and lower tarsal regions of a third patient, all of whom had typical palpebral vernal conjunctivitis (Fig. 1), and from the upper tarsal region of one patient in which the conjunc-

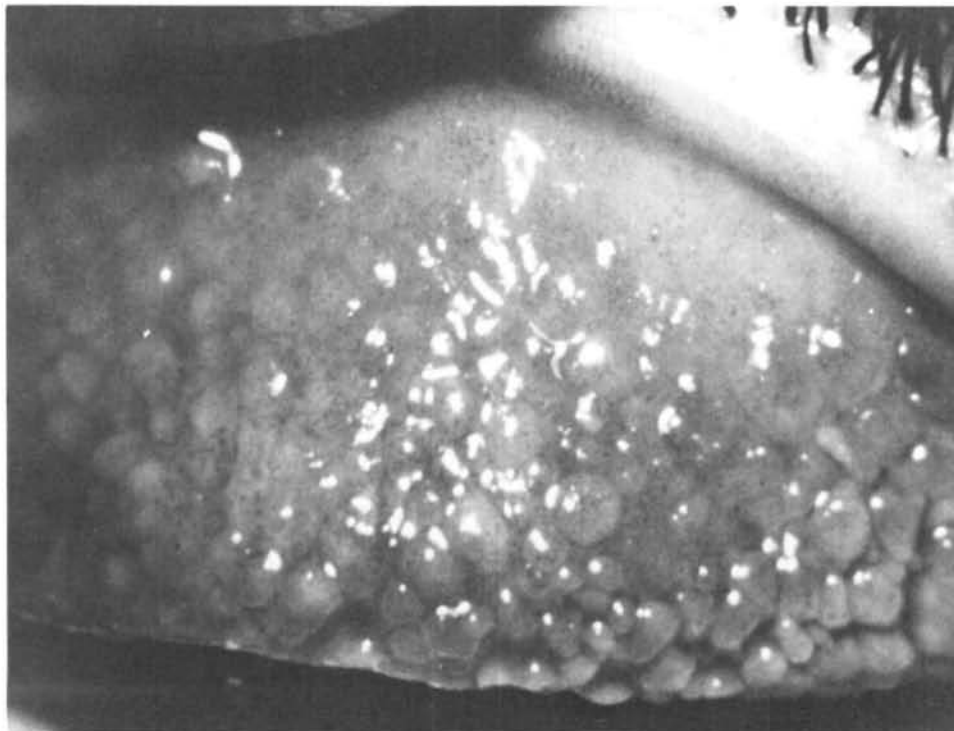


Fig. 1. Cobblestone excrescences on conjunctiva of upper eyelid of patient with vernal conjunctivitis.

tiva appeared normal. In addition to the clinical features, including the presence of cobblestone excrescences in the upper tarsal conjunctiva of both eyes, the diagnosis was confirmed by scrapings of the affected areas of epithelium, which revealed the presence of eosinophilic leukocytes in all patients except the control.

Immediately on removal, the conjunctival tissue was placed in buffered glutaraldehyde fixative (4% in 0.067M sodium cacodylate, pH 7.35) at room temperature. After 3 hr. the tissue was placed in buffer solution (0.067M sodium cacodylate) for a similar time and dissected into small pieces.

Thence, the tissue was processed according to one of the following schedules:

1. *Normal osmium.* Tissue was post fixed for one hour in 2% osmium tetroxide in 0.067M sodium cacodylate. Dehydration in acetone was followed by embedding in Araldite. Uranyl acetate (0.5% in 75% acetone) block stain was used, and sections were stained with lead citrate⁵ and uranyl acetate (1% aqueous).

2. *Osmium potassium ferrocyanide (OPF).* Post-fixation was with 2% osmium tetroxide and 1.5% potassium ferrocyanide in 0.067M sodium cacodylate for 1 hr. This is a slight modification of the OPF method of Dvorak et al.⁶ Tissue was again

dehydrated in acetone and embedded in Araldite. Sections were stained with lead citrate⁵ only.

Sections were cut on an LKB Ultratome 111 (LKB Instruments, Inc., Rockville, Md.) and examined on a Philips E.M. 200 electron microscope (Philips Electronic Instruments, Mt. Vernon, N. Y.).

Results. A full description of the clinical features of vernal conjunctivitis will not be included here, since these have been fully documented.¹⁻³

Light microscopy. In all patients with vernal conjunctivitis there was extensive thickening of both conjunctival stroma and epithelium, which showed new large downgrowths into the stroma. There was also a massive stromal cellular infiltration which consisted of lymphocytes, plasma cells, and polymorphonuclear leukocytes, eosinophils, and basophils. Lymphocytes, eosinophils, mast cells, and perhaps basophils, which were difficult to distinguish from mast cells, were also found in the epithelium.

Electron microscopy. All tissues studied in this investigation were fixed by the same standard method known to produce excellent preservation of ultrastructural details. However, since two quite distinct methods of postfixation were employed, the staining characteristics of the two groups of tissues differed. "Post-fixation with

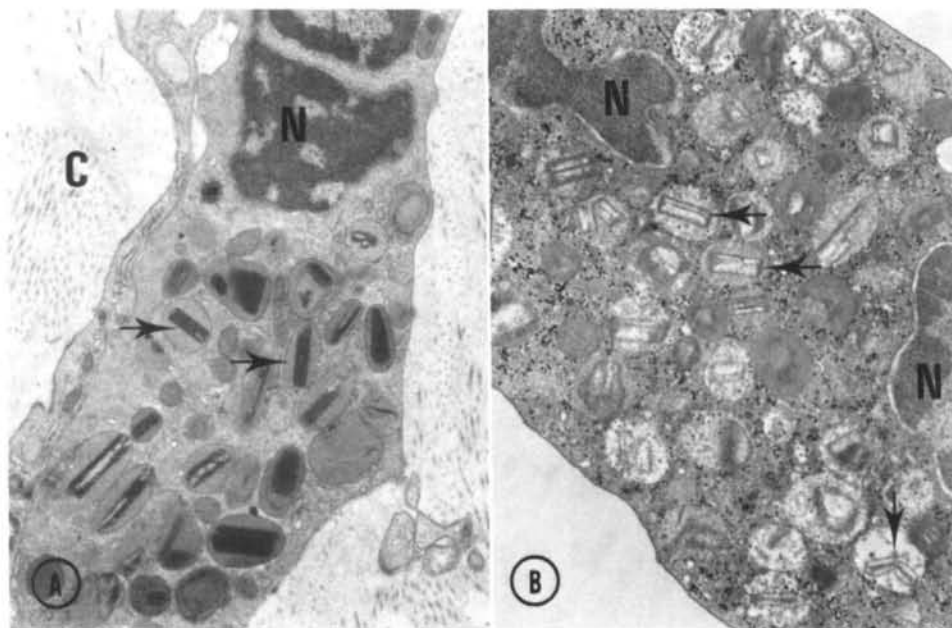


Fig. 2. A, Part of an eosinophilic leukocyte from the superior tarsal conjunctiva of a patient with vernal conjunctivitis. With normal osmium and uranium stains, the cytoplasmic granules are seen to contain crystals which are electron-opaque and appear dark (arrows). N, Nucleus; C, collagen. (Normal osmium; $\times 13,250$.) B, Part of an eosinophil in an edematous area of inferior tarsal conjunctiva from a patient with typical vernal conjunctivitis of the superior tarsal conjunctiva. OPF postfixation without uranium staining was used and the crystals (arrows) show dark outlines and pale centers against the background of the finely granular matrix of the granules. N, Nucleus. (OPF; $\times 15,500$.)



Fig. 3. Elongated basophil in the superior tarsus in a vernal conjunctivitis patient. There are six distinct nuclear lobes (N), and the cytoplasm contains irregular-shaped granules with fine particles (arrows). The small black particles within the cytoplasm are glycogen granules, and there are no pseudopodial extensions of the cell membrane. C, Collagen. (OPF; $\times 11,800$.)

osmium potassium ferrocyanide (OPF) method allowed good staining of all membranes and extracellular material and of cellular glycogen. Nuclear chromatin and ribosomes were less well stained.⁶ This was in contrast to the tissues treated with normal osmium tetroxide in which cytoplasmic details were well displayed.

The use of cacodylate buffer in the OPF method has not affected the results. A comparison of the electron micrographs of OPF postfixated material reported here with those in the literature⁶ in which OPF postfixation and sodium phosphate buffer were employed shows that there were no differences in either the preservation or staining of the tissues due to the use of sodium cacodylate buffer.

As observed with the light microscope, both epithelium and stroma from patients with vernal conjunctivitis were thickened. The increase in stroma was primarily due to very extensive masses of collagen fibers with a periodicity of approximately 600 Å. In some areas, particularly within the cobblestone papillae, the collagen fibers were packed so densely and with so regular an orientation that the appearance resembled that of cornea.

In all pathological specimens, eosinophilic leukocytes were present and were readily recognized by their multilobed nuclei and numerous characteristic cytoplasmic granules containing crystal structures. The appearance of these cells was different with each type of postfixation. When normal osmium was used, the crystals were very dark (electron-dense) with occasional light centers (Fig. 2, A). With OPF postfixation, the crystals stained lightly but were clearly outlined within the granule (Fig. 2, B). Glycogen granules were readily visible as small black particles in the cytoplasm of all eosinophils when the OPF method was employed (Fig. 2, B). Loose eosinophil granules were also found in the tissue of all vernal conjunctivitis patients.

Between the cells of the epithelium and its downgrowths were quite numerous eosinophils and also mast cells and a few basophils.

Plasma cells with well-developed and sometimes dilated rough endoplasmic reticulum and an extensive Golgi apparatus were very numerous in some areas of stromal tissue.

Although positive differentiation between mast cells and basophils was difficult in the conjunctival tissue from the one patient in which postfixation was with normal osmium, the OPF method enabled easy recognition of basophil features. Basophils were observed in all specimens taken from vernal conjunctivitis patients. They had multilobed nuclei with up to six lobes (Fig. 3) and characteristic cytoplasmic granules. These granules had a trilaminar membrane and contained fine particulate material, the density of which determined the

appearance of the granules. Some granules had tightly packed particles, whereas others were almost empty or contained glycogen granules or myelin-like figures (Figs. 3 and 4). The presence of these empty granules, which resembled vacuoles, indicated a state of partial degranulation or release of the granule contents of the basophils.

In contrast to the situation in mast cells, glycogen granules were plentiful in the cytoplasm of basophils, and the fingerlike pseudopodial projections of the mast cell were replaced by broad-based extensions.

In two of the four specimens, the tissue showed some edema and the lymphatic vessels were dilated. In the majority of blood vessels from two of the three vernal conjunctivitis patients, one or more of the endothelial cells was thickened and swollen, with loss of cytoplasmic organelles (Fig. 5). Sometimes the cell membrane was broken, and there was complete disruption of the cell. Extravascular erythrocytes and fibrin with a periodicity of approximately 200 Å were observed in the specimens from the upper tarsal conjunctiva of all three vernal conjunctivitis patients.

Examination of the conjunctival tissue from the control patient confirmed the light microscopic findings. Neither eosinophils nor basophils were observed, and mast cells were found only in the stromal tissue. Blood vessels were normal, and neither erythrocytes nor fibrin were observed in the extravascular tissues.

Discussion. The clinical features^{1, 2, 7} and the classic cellular and pathological characteristics of vernal conjunctivitis as observed in light microscopic studies¹⁻³ have been described frequently, and more recently the ultrastructural findings^{3, 4} have been reported. However, in none of these publications has the presence of basophils in the tissue been described, although Kimura and Thygeson⁸ have reported basophils in scrapings from patients with vernal conjunctivitis.

The use of the OPF method of Dvorak et al.⁶ has overcome the difficulties in distinguishing basophils from mast cells according to criteria which they have established.⁹

The presence of mast cell granules free in the tissues and the variations in the appearance of the cytoplasmic granules, some being wholly or partially empty of particles, represent the various stages of degranulation and release of substances from both mast cells and basophils.⁹

The majority of reports on vernal conjunctivitis^{1, 2, 4} make no mention of vascular changes such as those described here, i.e., enlarged, swollen endothelial cells with reduced or absent cell components and broken cell membranes. Only Morgan³ states that "endothelial swelling may be seen and the walls of the blood vessels may sometimes be hyalinized." These vascular changes

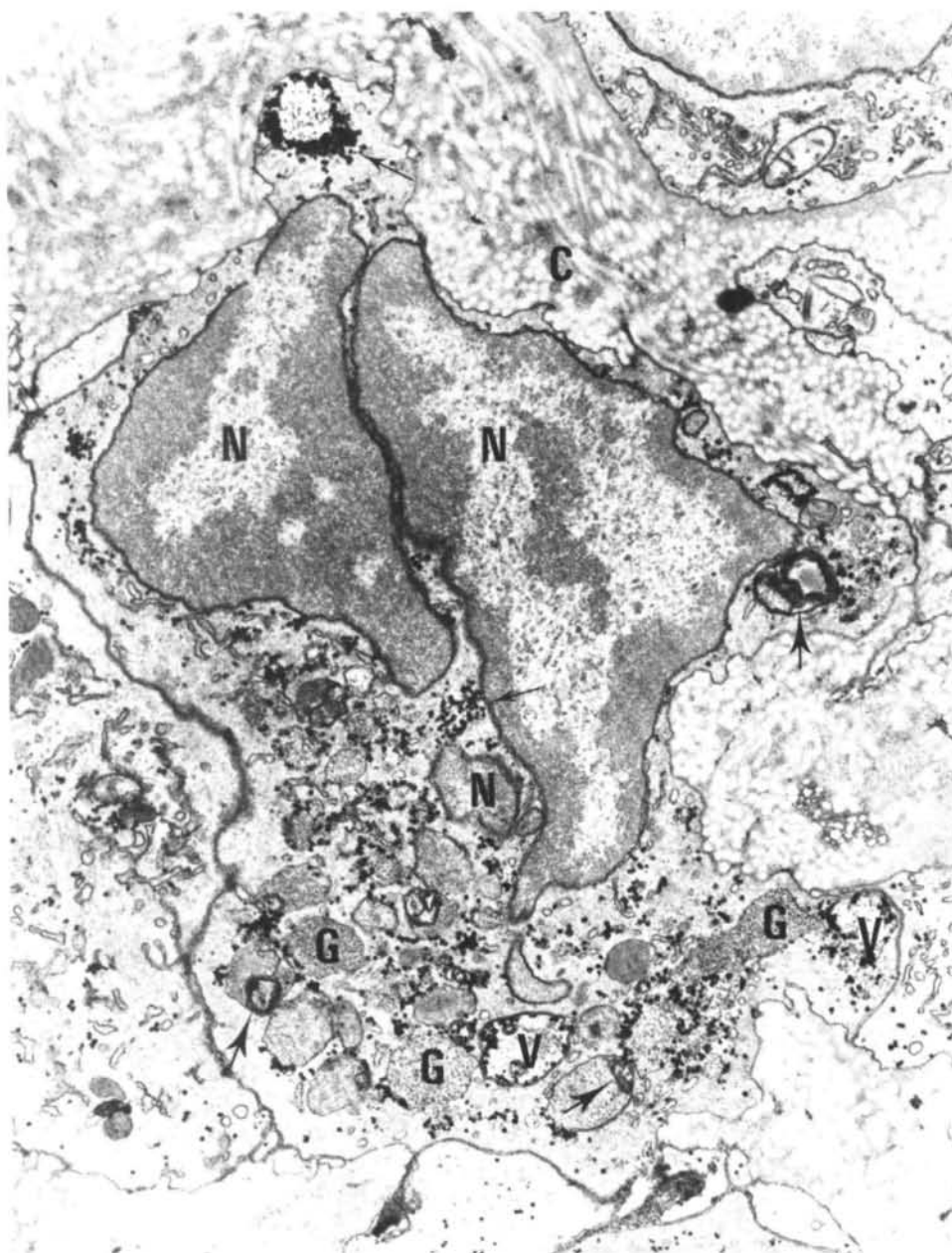


Fig. 4. Basophil in the superior tarsus in vernal conjunctivitis. There are three nuclear lobes (N) and numerous large granules filled with fine particulate material (G). Some granules are essentially empty (V), but others contain myelin-type membranous figures (large arrows). There are numerous black particles (small arrows) or glycogen granules. There are no pseudopodial projections from the cytoplasm. C, Collagen. (OPF; $\times 21,500$.)

appear to closely resemble the hypertrophy and hyperplasia of endothelial cells and individual endothelial cell necrosis observed in the blood vessels in delayed hypersensitivity in man.⁹

The present observation that fibrin is present in the stromal tissue of vernal conjunctivitis patients may help to clarify a controversy as to whether

the appearance of hyalinized connective tissue is related to the presence of fibrin in the tissues.¹ Takakusaki,⁴ using electron microscopy, also described a fine filamentous substance "which may correspond to the hyaline degeneration of the extra-cellular substance in the connective tissue characterising this disease." "It is probable that

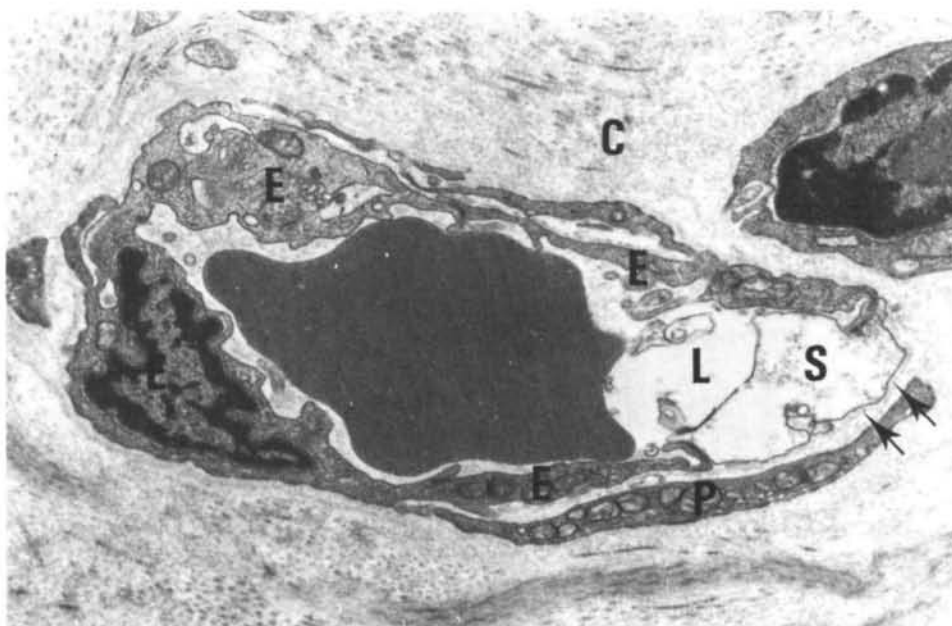


Fig. 5. Small blood vessel from the superior tarsal conjunctiva of a vernal conjunctivitis patient, showing several normal endothelial cells (*E*) and pericytes (*P*) surrounding an erythrocyte in the lumen (*L*). One endothelial cell (*S*) is pale, swollen, and devoid of organelles or other cell components. The basement membrane (arrows) is intact. *C*, Collagen. (Normal osmium; $\times 18,250$.)

this substance is derived from blood plasma and exudated through the capillary wall."⁴ Morgan³ also used the electron microscope but was unable to discern any deposition of fibrin in the stroma.

The coincidence of all of the pathological features described here and in particular the presence of basophils in the tissues indicate a marked similarity between vernal conjunctivitis and a type of delayed hypersensitivity found in animals and called cutaneous basophil hypersensitivity.¹⁰ Delayed-type hypersensitivity of the cutaneous basophil type in the human skin is characterized by the following features.⁹ In addition to the often described infiltration by lymphocytes, plasma cells, and macrophages, there are infiltration of the dermis and epidermis by basophils and to a lesser extent by eosinophils, increased vascular permeability with edema and erythrocyte extravasation, mast cell degranulation, microvascular alterations affecting endothelial cells, and alteration of the clotting system with deposition of fibrin.

Thus, since all of these are present, vernal conjunctivitis must be, at least in part, a manifestation of delayed-type basophil hypersensitivity.

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The ultrastructure of human limbal collagen. M. BRUCE SHIELDS, JOHN D. SHELBURN, AND SUSAN W. BELL.

In human limbal tissue from four glaucomatous and four nonglaucomatous eyes, the average collagen fibril diameter in the inner and outer layers was 100.86 and 115.33 nm., respectively. In both layers the fibrils occupied an average of two thirds of the total area. The possible implications of this observation in the trabeculectomy procedure are discussed.

Spitznas¹ studied the ultrastructure of human scleral collagen and reported that the diameter of the fibrils in the outer layers is significantly larger than that of the inner layers. Purnell and McPherson² suggested that the larger fibril diameter, which they extrapolated to looser fibril arrangement, might provide less resistance to aqueous flow through the intervening ground substance. They offered this in support of the concept that the route of external filtration by trabeculectomy is through the substance of the protective scleral flap. The study by Spitznas, however, was performed on sclera posterior to the ora serrata and in nonglaucomatous eyes.³ The purpose of this paper is to report observations on the ultrastructure of collagen in the region of the limbus, which lies directly over the usual trabeculectomy fistula, in glaucomatous and nonglaucomatous eyes.

Materials and methods. Limbal tissue was excised from four eyes with primary open-angle glaucoma at the time of posterior lip sclerectomy, by means of a Holth punch. Limbal tissue was also excised from four nonglaucomatous eyes at the time of enucleation for malignant melanoma. The tissue was excised immediately following enucleation, with the same technique as that for the posterior lip sclerectomy. Limbal tissue was

Table I. Ultrastructure of human limbal collagen

	Average fibril diameter (nm.)		Fibril concentration, avg. & (range) (percent of area)	
	Inner portion	Outer portion	Inner portion	Outer portion
Glaucoma cases:				
1	96.51	96.51	54 (55-71)	66 (61-73)
2	113.10	124.74	61 (58-66)	64 (57-71)
3	94.67	109.22	63 (52-67)	65 (62-64)
4	103.01	103.69	67 (59-77)	68 (65-70)
Average	101.82	108.50	64 (56-70)	66 (61-71)
Nonglaucoma cases:				
5	89.34	102.34	69 (63-74)	71 (65-76)
6	78.76	84.49	68 (67-69)	68 (65-73)
7	116.98	134.73	66 (62-71)	65 (61-73)
8	114.46	167.03	64 (61-67)	61 (59-62)
Average	99.89	122.15	67 (63-70)	66 (63-71)
Average of total	100.86	115.33	66 (60-70)	66 (62-71)

also removed from the melanoma eyes with scissors to be compared with the punch specimens for possible artifacts induced by the punch.

Each specimen was immediately fixed in 0.1M cacodylate-buffered 3 percent glutaraldehyde, post-fixed in 2 percent osmium tetroxide, and embedded in Epon. Thin sections were examined on a transmission electron microscope (Model 100 B; JEOL USA, Electron Optics, Medford, Mass.). An inner portion of collagen tissue adjacent to Schlemm's canal and an outer portion adjacent to episcleral tissue were studied in each specimen. Five to ten random areas, in which all collagen fibrils were seen on end with no interbundle spacing, were photographed at 40,000 $\times$ and enlarged to 110,000 $\times$ for each inner and outer portion.

Collagen fibril diameter was measured in millimeters on each photograph and converted to nanometers. The area occupied by the fibrils was also determined as a percentage of the total area by point counting with a modified Weibel grid.⁴

Results. The average diameter of collagen fibrils varied widely between different bundles of the same limbal specimen in the eight eyes studied. The over-all average diameters of the fibrils and the percentage of total area they occupied are shown in Table I. No difference was seen in the specimens excised by punch and by scissors. Fig. 1 shows an example of the appearance and relative size of the collagen fibrils in the limbus, and Fig. 2 shows the distribution of fibril diameters in the inner and outer layers of the same case.

Discussion. In a separate in vitro study, it was found that ferritin-labeled fluid can flow through