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Electron Microscopic Studies on the Trabecular Meshwork in Glaucoma simplex

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Summary. In 20 cases of chronic simple glaucoma with cataract a trabeculectomy was carried out and the bioptic specimens were studied by electron microscopy. For the purpose of comparison similar specimens obtained from 14 cases of secondary glaucoma and 7 control eyes were investigated by the same methods. In many cases of glaucoma simplex deposits of homogeneous osmiophilic material (plaques) were found between the cell layers of the cribriform area of the trabecular meshwork adjacent to the inner wall of Schlemm's canal. These were not present to such an extent within the control specimens. The plaques differ in structure and size, and appear first in foci. In advanced stages of the glaucomatous disease, the entire juxtacanalicular region is filled with osmiophilic plaques of this kind. The nature of these substances is unknown.

A further characteristic finding in the glaucomatous specimens is the extreme hyalinization of the trabecular lamellae, especially in chronic simple glaucoma. The process of hyalinization is described briefly.

It is assumed that the osmiophilic plaques at the filtering inner wall of the canal are of greater importance to the increase of outflow resistance observed in chronic simple glaucoma, than are the hyalinized trabecular lamellae.

Zusammenfassung. In 20 Fällen von Glaucoma chron. simplex mit Katarakt wurde eine Trabekulektomie ausgeführt und das entnommene Biopsiestückchen elektronenmikroskopisch untersucht. Zum Vergleich wurden Biopsien von Trabekulektomien verschiedener Sekundärglaukome (14 Fälle) und Kontrollaugen (7 Fälle) mit gleicher Methodik untersucht. In den Glaukomfällen fanden sich zwischen den Zellschichten des Trabeculum cribriforme, in der Nachbarschaft der Innenwand vom Schlemmschen Kanal auffallend viele homogene osmiophile Verdichtungen („Plaques") unterschiedlicher Struktur und Größe. Diese Plaques treten herdförmig auf. In fortgeschrittenen Fällen erscheint der ganze juxtacanaliculäre Bereich des Trabekelwerkes von derartigen osmiophilen Plaques durchsetzt. Die Natur dieses Materials ist nicht klar.

Eine weitere Besonderheit der Glaukomfälle stellt die Hyalinisierung der Trabekellamellen dar. Für die beim Simplexglaukom beobachtete Erhöhung des Abflußwiderstandes spielen die Plaques an der filtrierenden Innenwand vermutlich eine größere Rolle als die hyalinisierten Trabekel.

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Early fine structural changes on the tissues of the chamber angle region in chronic simple glaucoma have been inaccessible to study up to now. Since glaucomatous eyes are usually enucleated only in the advanced stages of the disease, morphological studies of such eyes provide no information concerning the primary events (cf. Elschnig, 1928; Hogan and Zimmermann, 1962). Early changes can be studied only on biopsies. Our previous light microscopic investigations were based on specimens of Elliots trephinations (Rohen and Unger, 1959; Unger and Rohen, 1960). This material, however, usually contained only fragments of the trabeculum; complete sections of Schlemms canal with intact inner wall were a rarity. The situation is different with respect to the trabeculectomy recently recommended by Cairns (1968) and Witmer (1971). This operation consists of the removal of the inner scleral lamella of the chamber angle, which includes a section through the entire canal with outer wall and the whole trabecular meshwork. Electron microscopic analyses of such biopsies provide more accurate information on the morphologic changes in glaucoma. Above all, they can be performed in early stages of the disease.

Material and Methods

1. Patients

The present study involves fine structural findings on specimens excised in trabeculectomy from a total of 41 eyes. In some cases, the surgically obtained specimens either contained no trabecular meshwork, or it was lost during preparation. For the purpose of comparison only those cases were examined, which contained enough of the trabecular meshwork to permit exact evaluation of pathological changes.

The material was divided into three groups. The *first group* included cases of chronic simple glaucoma with senile cataract (Table 1); intraocular tension was usually within normal limits under treatment with miotics. Surgery was indicated because of the decrease of visual acuity due to progressive lens opacity. The glaucoma was thus in a relatively early stage. A few patients of this group had a slightly more advanced chronic simple glaucoma without cataract; the intraocular tension was not compensated with miotics; therefore glaucoma surgery was indicated.

The *second group* included all other glaucomas, e.g., capsular glaucoma (pseudoexfoliation of the lens capsule), chronic simple glaucoma with high myopia, absolute hemorrhagic glaucoma and some other secondary glaucomas of various etiology (Table 2).

The *third group* was made up of control cases (Table 3), and consisted mostly of eyes which had been enucleated because of malignant tumors of the choroid or of the periorbital tissue. After enucleation these eyes underwent trabeculectomy in exactly the same way as described later. In three patients with senile cataract, but without glaucoma, the same combined trabeculectomy cataract-operation was performed. Finally, for purpose of comparison recently reported findings on age changes in the human trabecular meshwork are used (Rohen and Drecoll-Lütjen, 1971).

Table 1. Group 1. *Glaucoma simplex with senile cataract (20 cases)*

Case	Name	Sex	Eye	Age	Diagnosis	Cataract	Mean intraocular tension (mm Hg)	Tonography C-value (3–7 min)	Trabecular meshwork	
									Plaques	Hyalinization
205/69	F. R.	♂	l	69	Gl. chron. simpl.	+	33	0.06	++	+++
229-69	E. E.	♂	l	64	Gl. chron. simpl.	0	23	0.07–0.18	++++	+++
233-69	J. E.	♂	r	85	Gl. chron. simpl.	+	22	0.09	+++	+++
5-70	M. F.	♀	r	71	Gl. chron. simpl.	+	19	—	+	++
69-70	F. H.	♀	l	78	Gl. chron. simpl.	+	24–37	—	+++	+++
82-70	A. S.	♂	r	67	Gl. chron. simpl.	+	28	—	++	++
83-70	P. K.	♂	l	45	Gl. chron. simpl.	+	18–21	—	0/+	0/+
103-70	R. M.	♂	l	69	Gl. chron. simpl.	+	42	0.06	++	++
105-70	O. M.	♀	r	80	Gl. chron. simpl.	+	23	0.05	+++	+++
106-70	J. J.	♂	r	73	Gl. chron. simpl.	+	32	0.1	+++	+++
111-70	A. K.	♀	r	64	Gl. chron. simpl.	+	25	0.06	++++	++++
122-70	M. H.	♀	r	66	Gl. chron. simpl.	+	27	0.06	++	+++
123-70	M. Z.	♀	r	74	Gl. chron. simpl.	+	21	—	+++	+++
124-70	J. K.	♂	l	65	Gl. chron. simpl.	+	24	0.07	+++	+
163-70	R. K.	♀	l	82	Gl. chron. simpl.	+	22–24	0.07	+++	+++
11-71	R. K.	♀	l	71	Gl. chron. simpl.	+	21	0.25	+	++
44-71	F. K.	♂	l	81	Gl. chron. simpl.	+	20	—	++	+++
17-71	A. K.	♂	l	85	Gl. chron. simpl.	+	28	—[a]	+++	++
13-71	J. P.	♂	r	75	Gl. chron. simpl.	+	32	—[a]	+++	++
12-71	H. O.	♂	l	78	Gl. chron. simpl.	+	28	—	+++	+++

[a] Monoculus.

Table 2. Group 2. *Complicated and secondary glaucomas (14 cases)*

Number	Name	Sex	Eye	Age	Diagnosis	Intra-ocular tension	Tonography C-values (3–7 min)	Trabecular meshwork	
								Plaques within inner wall	Hyalinization
169-69	O. B.	♂	l	65	Capsular glaucoma	32	0.17	—	+
197-69	M. G.	♂	l	16	Juvenile glaucoma Turner syndrome	30	0.10	+	0
203-69	A. G.	♀	r	39	Sec. glaucoma after perfor. injury	44	0.05	+++	0/+
204-69	M. B.	♀	l	71	Narrow angle glauc.	20	0.10	0/+	+
226-69	A. I.	♀	r	78	Sec. glauc. after metaherpetic keratitis	11–35	—+	0	+
227-69	M. G.	♂	r	39	Juvenile glaucoma Turner syndrome	35	0.13	0	0/+
4-70	L. P. T.	♂	r	41	Sec. glauc., chron. iritis, high myopia	32	0.10–0.07	0/+	0
21-70	A. M.	♂	r	70	Capsular glaucoma	26	—[a]	+++	+
104-70	J. H.	♂	r	76	Capsular glaucoma	16	0.18	++	+
117-70	F. G.	♂	r	60	Glaucoma abs. (haemorrh.)	45	—	0/+	0/+
164-70	W. G.	♂	r	72	Capsular glaucoma	48	—	+++	++
21-71	Z. M.	♂	r	16	Congenit. glaucoma	54	—[b]	—	++++
43-71	B. B.	♀		71	Glaucoma acutum	58	—	0/+	++
53-71	W. G.	♀	l	50	Pigment glaucoma with cataract	50	0.09	+	0

[a] Monoculus. — [b] Blind.

Table 3. Group. 3. *Control group (7 cases)*

Number	Name	Sex	Eye	Age	Diagnosis	i.o. tension	Trabecular meshwork	
							Plaques	Hyalinization
51-70	A. L.	♀	l	61	Carcinoma in the orbite	normal	0	+
85-70	O. S.	♀	l	71	Melanoma of the choroid	normal	0/+	0
121-70	A. T.	♀	l	70	Melanoma of the choroid	normal	0/+	0
116-70	C. T.	♀	r	2	Glioblastoma of the retina	normal	0	0
45-71	E. B.	♀	l	69	Pseudoexfoliation of the lens capsule	normal	+++	0/+
46-71	A. K.	♂	l	78	Cataracta senilis	normal	0	+
47-71	J. M.	♀	r	75	Cataracta senilis	normal	+	+

2. *Surgical Technique*

a) *Trabeculectomy* alone (without simultaneous cataract operation) consisted in the excision of a piece of the internal scleral lamella in the chamber angle region. First the limbus was exposed by dissection of a limbus based conjunctival flap, then a scleral lamella was cut to half its thickness (about 4 mm wide). A 2×2 mm piece of the internal scleral lamella, including Schlemm's canal and trabecular meshwork, was then excised. At the same time a peripheral iridectomy was performed. The outer scleral flap was fixed with 30 µm Perlon sutures, and the conjunctival flap with 6–0 catgut.

b) *The combined glaucomatous cataract operation with trabeculectomy* was based on a modified four plane sclero-corneal incision; the internal scleral lamella, together with Schlemm's canal and trabecular meshwork, was excised at the 12 o'clock position. A large limbus based conjunctival flap was dissected and the limbus cleanly exposed from 9 to 3 o 'clock. Episcleral vessels were slightly cauterized. A first incision through half the thickness of the sclera (about 0.4 mm) was carried out from 9 to 3 o'clock, nasally and temporally at a maximum of 1 mm outside the limbus, but a 12 o'clock at least 3 mm away from it. The scleral lamella was then dissected down into the cornea. At the 12 o'clock position a 2×2 mm piece of the inner scleral lamella was excised, along with a peripheral iridectomy. The corneal incision was extended on either side with scissor, and the extraction of the lens, suturing of the corneoscleral incision, and closure of the conjunctival wound, were then performed.

3. *Morphological Methods*

Immediately after operation the biopsy specimens were immersed for 30 min in 2.5% glutaraldehyde, buffered to pH 7.3 with Sørensen buffer and kept at 4.0° C. The fixative was then washed out several times with Sørensen buffer. After that, the specimens were immersed in an isotonic saccharose solution and finally refixed for 3 hours in 1% osmium chromate (Dalton's solution) and embedded in Vestopal in the usual manner. Ultrathin sections were made with the LKB Ultratome IIIa or with the Om U2 Ultramicrotome (Reichert). For light microscopy semithin sections about 0.5–1.µm thick were stained by Richardson's method or with methylene blue. All specimens were stained en bloc prior to hydration with 0.5% uranyl acetate, in order to enhance contrast of the membranes. Ultrathin sections were studied with the Elmiscope IA electron microscope (Siemens).

Results

Most biopsies contained a more or less broad section of corneoscleral lamella, Schlemm's canal with its inner and outer walls, the main part of the trabecular meshwork, usually the scleral spur, occasionally a piece of iris and the tip of the ciliary muscle (Fig. 1a). In some cases, only the posterior two-thirds of the canal and the adjacent trabeculum were excised (Fig. 1b). Even in those cases in which Schlemm's canal and the trabeculum were only partially intact it was possible to examine the fine structure of the inner wall of the canal and the trabecular meshwork. In many cases, the trabecular lamellae and the inner wall appeared to be stretched out in their natural positions.

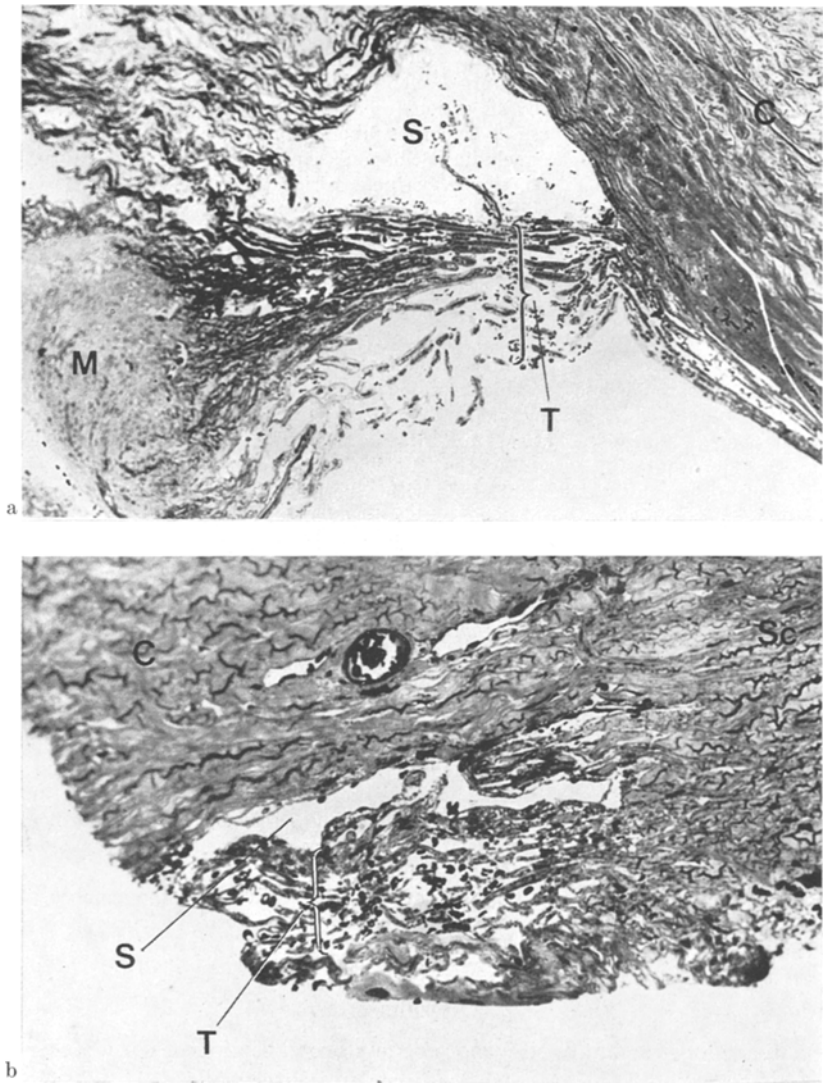


Fig. 1a and b. Semithin sections of two trabeculectomy specimens, stained with Richardson's stain. a Pat. Fr. R., R 205/69, magn. 40×, b Pat. A. G., R 203/69, magn. 64×. *C* cornea, *M* ciliary muscle, *S* lumen of Schlemm's canal, *T* trabecular meshwork

In general, the specimens of the *control material* (group 3) exhibited normal findings, like those reported in earlier studies (Rohen, 1964; Rohen and Lütjen-Drecoll, 1971). The trabecular meshwork consists of relatively thin lamellae with broad intertrabecular spaces. Its cribriform

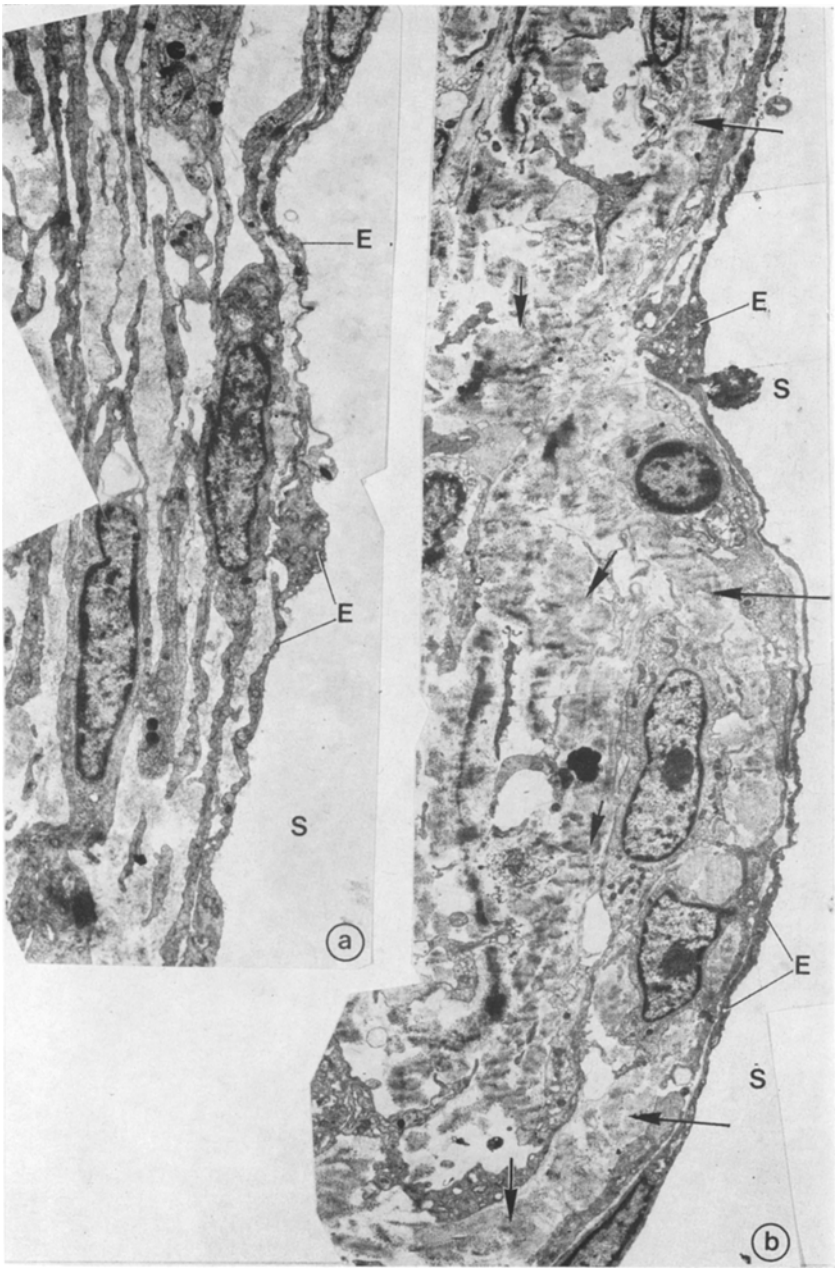
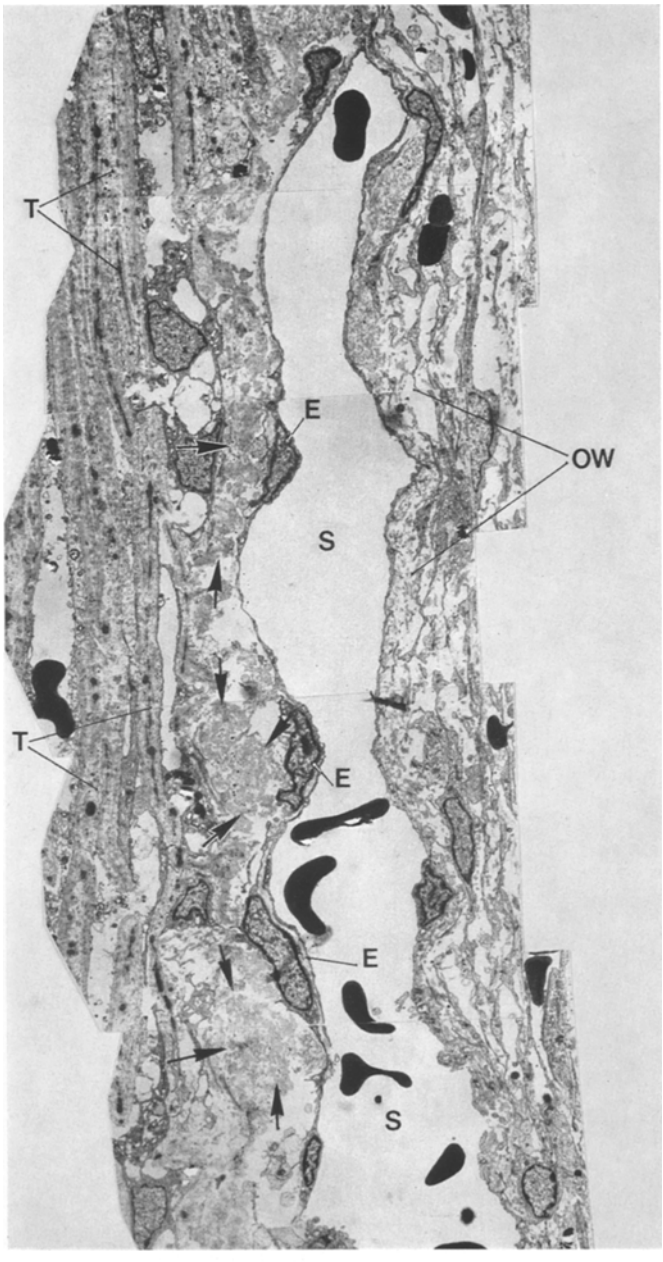
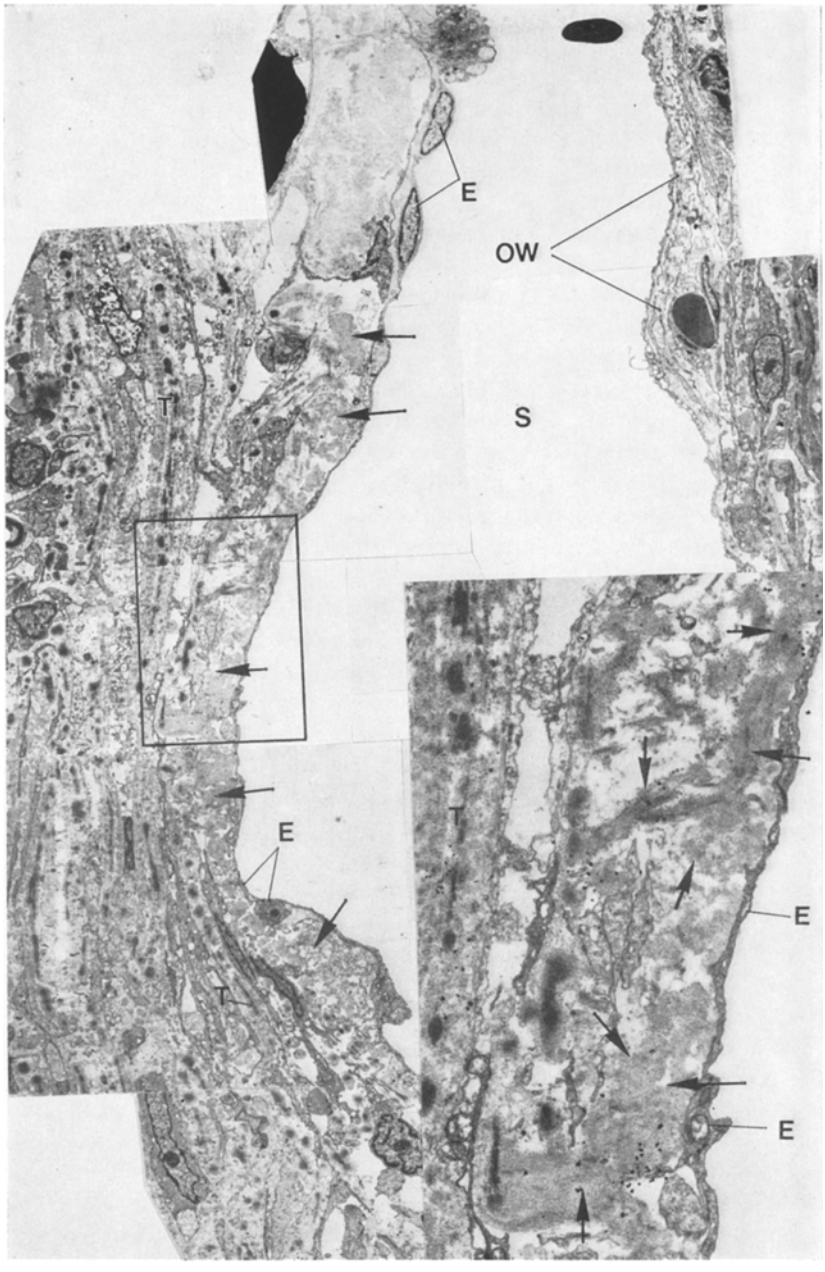


Fig. 2a and b. Electron micrographs of trabeculectomy specimens of one normal (a) and one glaucomatous, (b) eye. a Control material, Pat. A. L. 51/70, orig. magn. ×7000, b Pat. J. E., 233/69, magn. 5000× Chronic simple glaucoma without cataract. Notice densification of the juxtacanalicular zone be osmiophilic plaques (arrows) between the cellular layers of the trabeculum cribriforme in simple glaucoma (b), compared with tissue of the control case (a). *E* inner wall endothelium, *S* lumen of Schlemm's canal



a

Fig. 3a and b. Composite picture of electron micrographs of a sagittal section through the entire Schlemm's canal and adjacent tissue layers in a case of chronic simple glaucoma. Fig. b is a continuation of the lower part of Fig. a. (Trabeculectomy specimen, Pat. E. E., magn. ×2500; insertion in Fig. b: magnification of the indicated region of the inner wall ×7500). Notice numerous osmiophilic



b

plaques in the entire juxtacanalicular region of the inner wall (arrows). Similar densifications also occur occasionally in the outer wall (OW). *E* endothelial lining of the inner wall of Schlemm's canal, *S* lumen of Schlemm's canal, *T* trabecular lamellae, *Ow* outer wall of Schlemm's canal

portion appears thin and delicate. Homogeneous osmiophilic densifications in the juxtacanalicular region are found only to a slight extent (Fig. 2a). Case 45/71 provides an exception. It involved a cataract with pseudoexfoliation of the lens, but no increase in intraocular tension. Electron microscopy of the inner wall of Schlemm's canal in this case revealed extensive deposits of varying density, consisting of fine fibrous material, which separated the juxtacanalicular cell layers.

On the basis of the morphological findings (group 1), the cases of *chronic simple glaucoma* with and without cataract are being treated as one group. Two main features are already evident, even in *light microscopy*: first, many cases exhibit a marked thickening of the trabecular lamellae, usually due to extensive hyalinization of the subendothelial layers of the trabeculae. Secondly, the whole zone of the cribriform portion and the inner wall of Schlemm's canal appear broadened, due to deposits of homogeneous material under the endothelial cell layer.

Electron microscopy suggests that the thickening of the cribriform region of the meshwork in this group is caused mainly by small deposits of homogeneous, osmiophilic material scattered in varying frequency and density between the cell layers of the juxtacanalicular region (Fig. 2). In Table 1 the intensity of these plaques is indicated by the number of crosses. It is especially characteristic that these densifications appear in foci. Initially they are located outside the double endothelial layer which forms the inner wall of Schlemm's canal. More extensive aggregations of such structures form plaques which force apart the cells of the cribriform trabeculum and penetrate directly into the endothelium of the inner wall of the canal. In some cases (e.g., 229/69), the entire inner wall zone contains so many osmiophilic plaques that the whole filtration region is rendered homogeneous and hyalinized in appearance (Fig. 3).

At higher magnifications *three forms* of densifications can be differentiated. Most frequently they are homogeneous and of variable size (Fig. 4a), and they are less electron-dense than the elastic elements of the trabeculum, but easily distinguishable from deposits of precipitated protein or fibrin. A second form differs from the first only in density and degree of affinity for osmic acid. It too has a homogeneous structure, but is considerably more electron dense. After contrasting with lead citrate, this form of densification appears almost black (Fig. 4b), thus resembling somewhat the central core of the elastic fibers. Intermediate forms between 1 and 2 are common, and consist of gray, homogeneous plaques with a dense, osmiophilic core. A third form is characterized by a periodic arrangement (Fig. 4b). Within a homogeneous ground substance nodular densifications appear at regular intervals, connected by extremely delicate fibrillar structures. Occasionally such complexes

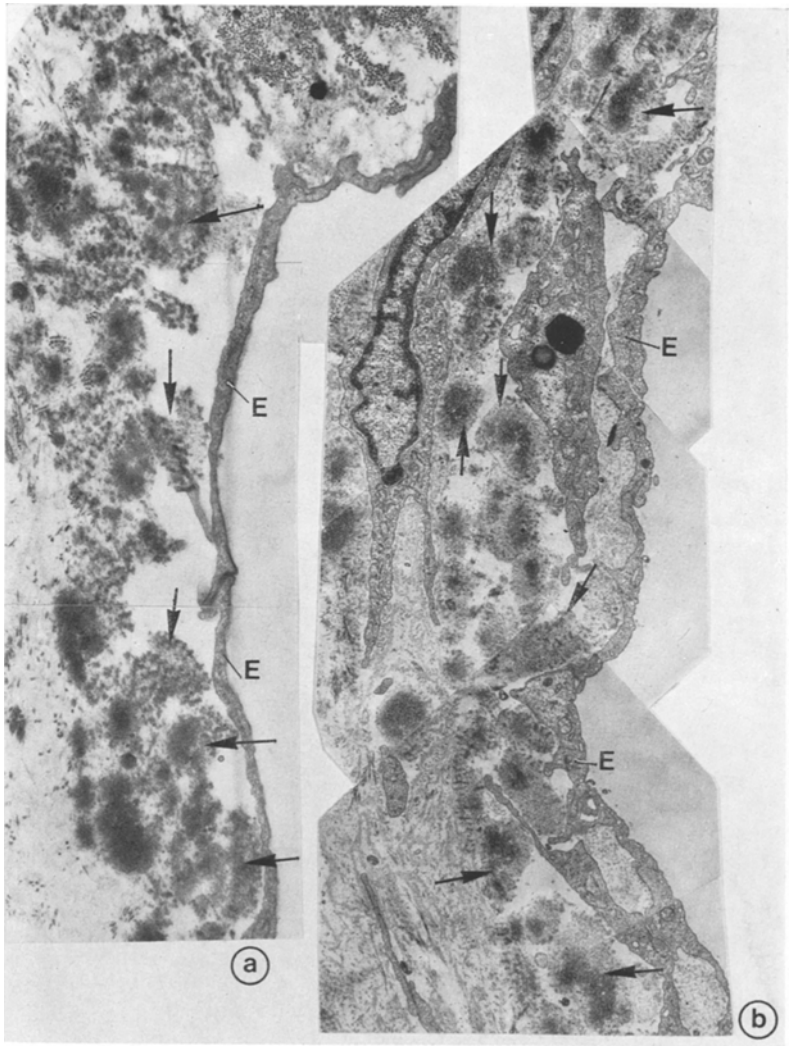


Fig. 4a and b. Electron micrographs of the juxtacanalicular zone of Schlemm's canal in two cases of chronic simple glaucoma, showing the various forms of osmiophilic plaques. (Trabeculectomy specimens, Fig. a, Pat. J. K., 124/70, ×1500; Fig. b, Pat. J J., 10000). In Fig. a, the plaques consist of partly periodic, nodular structures embedded in a strongly osmiophilic ground substance (arrows), and partly of fine fibrillar deposits as well. In Fig. b homogeneous osmiophilic plaques with central densifications are dominant (arrows). *E* endothelial lining of the canal

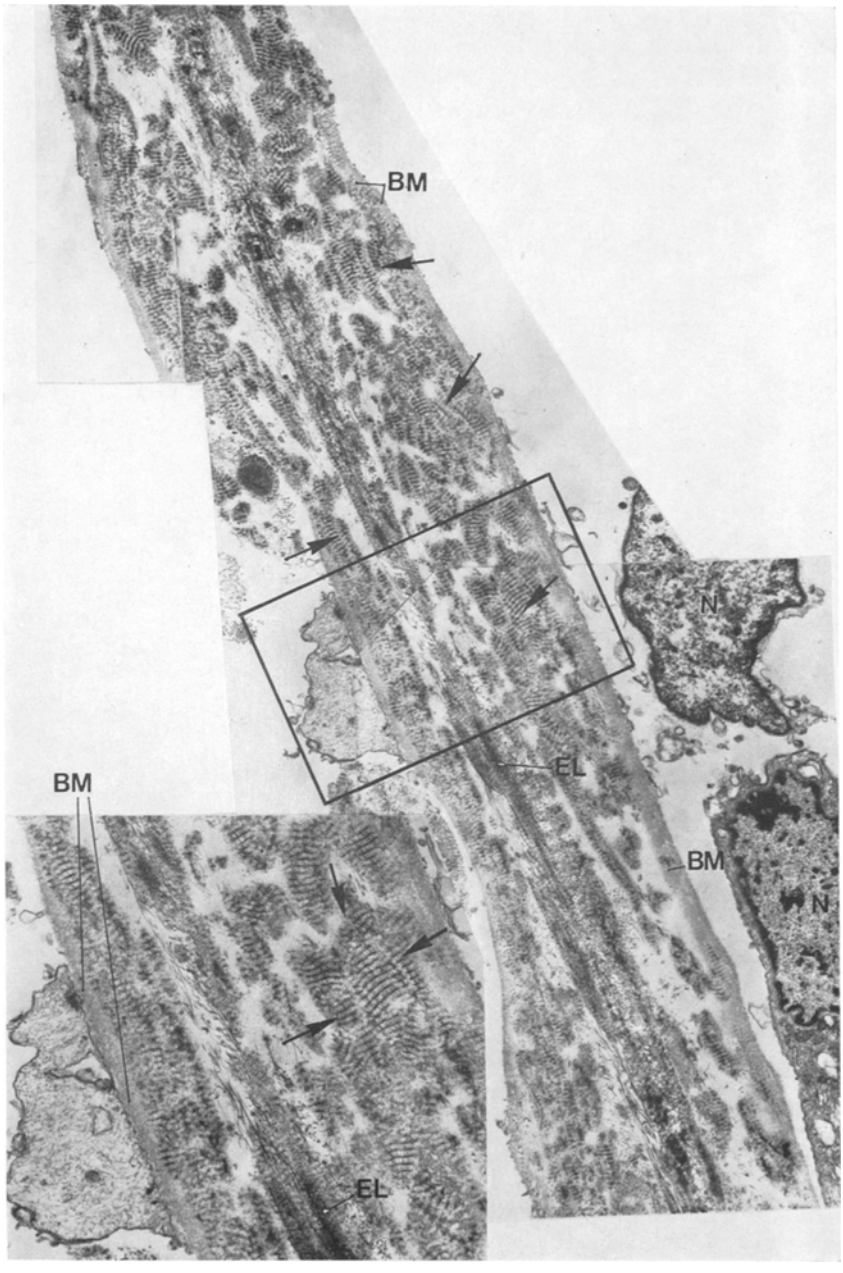


Fig. 5. Sagittal section through a strongly hyalinized trabecula in chronic simple glaucoma with cataract. (Trabeculectomy specimen, Pat. Fr. H., ×7500; inset: magnification ×15000). Notice the spreading of the subendothelial basement mem-

bear a resemblance to inclusions of lattice (curly) collagen in hyalinized membranes of the trabecular lamellae.

The characteristically homogeneous, osmiophilic deposits can also occur between the cellular elements of the *outer wall* of Schlemm's canal although the changes here are much less marked (Fig. 3).

The corneoscleral and uveal portions of the trabecular meshwork appear extensively hyalinized in most cases of the first group. Only case 124/70 showed a remarkably slight degree of hyalinization. Nevertheless, coarse, periodic densifications were present in the inner wall of the canal (Fig. 4b). Pigment glaucoma was suspected on the basis of clinical findings. This case could therefore also have been assigned to group 2.

Hyalinization begins with a thickening of the subendothelial basement membrane and deposits of lattice (curly) collagen in the marginal portions of the trabecula. Solid deposits of lattice collagen can form nodular swellings in the individual trabecular lamellae. In later stages of hyalinization the lattice collagen complexes aggregate to form a homogeneous mass resembling a basement membrane and extending to the central core of the trabeculum. Lattice collagen also occurs in the core of the lamellae, where it apparently is formed by parallel juxtaposition of collagenous fibers, together with homogeneous sheath material. The uveal trabeculae are usually more extensively hyalinized than the corneoscleral portion (Fig. 5).

It is difficult to present quantitative data concerning the degree of hyalinization, since it proceeds at different rates in different regions. We have attempted a qualitative estimation of the extent of hyalinization in various parts of the canal and filtering angle on the basis of composite electron micrographs showing sections through the entire trabecular meshwork. The results are indicated by the number of crosses in Table 1.

In the *second group* (glaucoma capsulare, secondary and narrow angle glaucoma, etc.), the morphologic picture with respect to the structures described above is quite heterogeneous. In four cases of capsular glaucoma with elevated intraocular pressure, trabecular hyalinization was relatively slight. No homogenous plaques such as described above were found in the region of the cribriform trabeculum or the inner wall. Instead, the juxtacanalicular area contained extensive aggregations of the finest fibrils, embedded in a very weakly osmiophilic ground substance. The fibril complexes were scattered at random between

branes, caused by massive deposits of lattice collagen (arrows). At some places the trabecular endothelium is artificially detached. *BM* basement membrane. *El* elastic fibers, *N* nuclei of trabecular cells

the cell layers of the meshwork of the cribriform trabeculum, and often on the endothelium of the inner wall.

Two cases of *acute glaucoma* (43/71 and 204/69) with tension maxima up to 60 mm Hg, showed almost total absence of alterations of the inner wall. Marked hyalinization of the trabeculae was found in 43/71, but to an extent normally found at this age (71 years). In addition, extreme hyalinization of the trabeculae was found in a case of *buphthalmus* (21/71). We have seen similarly exaggerated hyalinization in light microscopic studies of other cases of juvenile glaucoma. Unfortunately, the ultrastructure of the inner wall of the canal could not be evaluated in this case.

In secondary glaucoma the trabeculae usually appear normal and extensive hyalinization is rare. Homogeneous, osmiophilic plaques in the region of the inner wall have been found only in case 203/69, a secondary glaucoma after perforating trauma. In this case, the tonographically measured outflow facility was 0.05. Nonetheless the plaques in this case are less electron dense than in simple glaucoma, and no periodic elements are seen.

Discussion

In a previous light microscopic study of fifty cases of chronic simple glaucoma, we found that the majority showed pathological changes in the region of the inner wall of Schlemm's canal (Rohen and Unger, 1959; Unger and Rohen, 1960). These alterations had the appearance of coarsening and cell proliferation, but, occassionally merely resembled deposits of homogeneous material in the juxtacanalicular portion of the trabecular meshwork. Electron microscopic findings on the present material, however, indicate that these thickenings are due mainly to accumulation of homogeneous osmiophilic plaques deposited predominantly between the cell layers of the cribriform trabeculum. Evidence of genuine proliferative reactions was not seen in electron microscopy.

It is tempting to assume that these plaques are blocking the filtration pores of the inner wall, thus causing the increased outflow resistance observed in glaucoma. This hypothesis is difficult to prove on the basis of the present data. First of all, in trabeculectomy only a small section of the trabecular meshwork is excised; it may not reflect the functional condition of the entire circumference, especially if one considers the focal distribution of the plaques. Secondly, no definite correlation with tonographic C-values could be established (Table 1); this is, however, not necessarily of great significance because of the large range of error inherent in tonography and because of the fact, that tonography could not be performed in all cases prior to surgery.

Nevertheless it is important to note that the homogeneous plaques surrounding the inner wall occur predominantly in simple glaucoma and almost never in acute glaucoma of the controls. Our previous light microscopic studies failed to reveal changes in the inner wall in "congestive glaucoma". Changes observed in pseudoexfoliation of the lens capsule will be discussed elsewhere; they involve densifications in the cribriform trabeculum, but instead of homogeneous plaques as in simple glaucoma they consist of dense masses of fine fibrillar material, the ultrastructure of which resembles that described by Ringvold (1969, 1970) for lens and iris tissue. Case 45/71 showed exfoliation, but no glaucoma. It is conceivable that a glaucomatous increase in intraocular pressure would have developed later. At the time of surgery, however, most parts of the trabecular meshwork maintained adequate aqueous humor drainage. These findings in capsular glaucoma suggest that a thickening of the inner wall region, irrespective of its definite nature, may cause increased outflow resistance.

It is less probable that an increase in outflow resistance is due to the hyalinization of the trabecular lamellae (cf. Rohen, 1971). Since intertrabecular spaces are too large to be completely blocked by the above mentioned alterations unless the trabecula adhere to one another and to the inner wall, thus blocking the actual filtration area of the trabeculum cribriforme. In general, the process of hyalinization in simple glaucoma develops much like that described for secondary glaucoma (Rohen and Straub, 1968). The present study does not provide evidence of any correlation between the degree of hyalinization and duration of the disease.

On the whole, the changes that are described here, in the trabecular meshwork of eyes with chronic simple glaucoma are considerably more marked than those known to occur in normal eyes of persons of comparable age (Rohen and Lütjen-Drecoll, 1971). Nevertheless, it is of course difficult to provide quantitative data, and it should not be forgotten that simple glaucoma is a disease of elderly patients, in whom hyalinization and thickening of the same structures would have proceeded to an advanced stage even under normal conditions.

The critical question of whether the alterations in the inner wall described here are specific for the glaucoma and whether they represent the long sought early changes, still cannot be answered with certainty. Most of the excised material, however, came from patients with clinically proven glaucoma, although tension was usually maintained within normal limits with miotics. These eyes had therefore not yet reached the later stage of the disease. On the basis of the new findings of Bill one must assume that densifications in the vicinity of the inner wall may produce an increased resistance to outflow, if a sufficiently large

portion of the filtration surface of the canal endothelium is affected. If this is true, the increase of intraocular tension could have many conceivable causes. In the case of chronic simple glaucoma, we do not know as yet what factors contribute to the deposition of the homogeneous material in the subendothelial layers of the inner wall of the canal, nor do we know anything about the nature of this material. If is conceivable, however, that it is a protein and may be composed of antigen-antibody complexes. If this were so, glaucoma could be classified among the auto-immune diseases. This, however, remains purely hypothetical and further studies will have to be carried out along this line.

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