THE DEVELOPMENT OF THE TRABECULAR MESHWORK AND ITS ABNORMALITY IN PRIMARY INFANTILE GLAUCOMA*

BY *Douglas R. Anderson,* MD

INTRODUCTION

IN CONGENITAL GLAUCOMA, THE PERIPHERAL IRIS IS SEEN BY GONIOSCOPY TO BE inserted more anteriorly than normal, and the trabecular meshwork seems to have a shiny surface.[1-4] At the time of goniotomy, this surface tissue is incised, and the peripheral iris falls posteriorly as if the incised tissue had been holding the iris insertion forward. Since the operation usually relieves the elevated pressure,[4] it is natural to assume that the shiny surface is an imperforate membrane that had prevented aqueous humor outflow.

Histopathologic confirmation of this presumed membrane or other pathologic features typical of infantile glaucoma has been difficult to obtain. Specimens enucleated after long-standing severe glaucoma, perhaps after several surgical procedures, contain secondary changes that hide the nature of the initial abnormality. Other infantile glaucoma specimens were obtained at autopsy from infants who died of associated fatal congenital anomalies. The abnormalities in these eyes may not be the same as the abnormality that produces primary infantile glaucoma in otherwise normal infants. Furthermore, most investigators have not clarified which morphologic features of the angles of infantile glaucomatous eyes are characteristics of infant eyes (known to differ from adult eyes

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since the final stages of development of the angle are postnatal) and which are features that distinguish the glaucomatous infantile eye from the normal infantile eye.

However, these difficulties are not the only reasons for uncertainty. Even when paying attention only to appropriate specimens obtained early in the course of primary infantile glaucoma, several investigators[3,6-10] have not been able to agree whether or not there is an imperforate membrane covering the trabecular meshwork surface corresponding to the shiny surface seen clinically, or whether some other mechanism (such as abnormal insertion of the ciliary muscle into the meshwork) accounts for the pressure elevation and the response to goniotomy.

In an effort to learn more about the underlying abnormality, I studied undamaged trabecular tissue obtained at surgery from five eyes of three patients with primary infantile glaucoma early in the course of the disease. I also obtained the two eyes of an infant with glaucoma who died at the age of six months of an unrelated illness. These specimens were examined by scanning electron microscopy, as well as by transmission electron microscopy and phase-contrast light microscopy. For comparison, 26 normal eyes of 16 fetuses, premature infants, and term infants of various ages were studied by similar methods. In addition, previously obtained tissue specimens (14 normal and five glaucomatous) were studied in paraffin sections by light microscopy, even though phase-contrast and electron microscopy were not performed. Thus, a total of 10 eyes with infantile glaucoma and 40 normal eyes of fetuses and infants were studied.

MATERIALS AND METHODS

SPECIMENS OF INFANTILE GLAUCOMA

Satisfactory specimens of anterior chamber angle tissue were obtained by trabeculotomy-trabeculectomy from five eyes of three patients. Their case histories are given below. Five additional specimens had been obtained but were technically unsatisfactory and are not included in this report. The diagnosis of infantile glaucoma was confirmed in all cases and is documented in the accompanying case histories. All the children were less than six months of age, and none had previous surgery.

The tissue samples were obtained by the following surgical procedure: beneath a conjunctival flap a rectangular partial thickness scleral flap, hinged anteriorly at the limbus, was dissected. In the bed under the flap, two radial incisions were made into the outer wall of Schlemm's canal.

Harms' probes were used to perform a trabeculotomy temporally through the more temporal of the two radial incisions, and nasally through the more nasal of the two incisions. The undisturbed region between the two radial incisions was then excised by careful sharp dissection with a razor blade and Vannas scissors. As the tissue was removed a peripheral iridectomy was performed in a manner that left the iris stump (and a piece of the anterior ciliary body) attached to the block of trabecular tissue; the specimens described in this report were removed without apparent serious disruption of the tissue attachments.

After fixation in 2.5% glutaraldehyde in 0.1 M phosphate buffer, the tissue blocks were subsequently cut in two. One piece was processed for scanning electron microscopy through the freon critical point drying method. The other was post-fixed in 2% osmium tetroxide fixative (0.1 M phosphate buffer), embedded in epoxy resin, sectioned, and studied by phase-contrast microscopy. Sections two microns thick were stained with paraphenylenediamine and examined by phase-contrast microscopy. Thin sections were prepared from certain tissue blocks selected for transmission electron microscpy after staining with uranyl acetate and lead citrate.

An additional specimen of paraffin-embedded tissue was obtained from Dr Richard Green (Wilmer Ophthalmological Institute). This specimen was from Case 3 of the report by Maumenee.[9] Before preparation for scanning electron microscopy by freeze-drying, the paraffin-embedded tissue block was immersed in warm xylene, cleared of xylene in absolute ethanol, and hydrated through a series of decreasing concentrations of ethanol in water.

Standard light microscopy alone was used to study previously prepared paraffin sections of eyes from five additional patients with infantile glaucoma. These post-mortem specimens were obtained for study through the courtesy of Dr David Cogan and Dr Taylor Smith at the Massachusetts Eye and Ear Infirmary. One child was known to have congenital rubella and another child died of a congenital heart defect. One had a successful goniotomy and died a year later of pneumonia. Clinical details of the other two specimens are lacking.

NORMAL EYES

Anterior segment tissue or whole eyes from human fetuses, neonates, infants, and children were obtained after abortion, after death, or after enucleation of the eyes because of rhabdomyosarcoma or retinoblastoma. A total of 40 such specimens were collected, covering the range of age from five months of gestation to five years postnatal. Several were obtained from Dr Brooks Crawford of the University of California at San

Francisco, others from Dr Harry Quigley, others through the cooperation of the Pathology Department of the University of Miami School of Medicine and Jackson Memorial Hospital, and other from tissue caps in pathology laboratories of the Massachusetts Eye and Ear Infirmary. These specimens (26 eyes from 16 children), were studied by scanning electron microscopy either in the Howe Laboratory of Ophthalmology or in the Department of Anatomy at the University of Miami School of Medicine, by phase-contrast microscopy, or by both methods. The methods of tissue preparation were the same as for the infantile glaucoma specimens, except that freeze-drying was used instead of critical point drying for scanning electron microscopy. The other 14 specimens of normal eyes were previously prepared slides obtained for light microscopic examination from the files of the Massachusetts Eye and Ear Infirmary (Dr Taylor Smith) and from the pathology files of the Wilmer Ophthalmological Institute (Dr Don Nicholson and Dr Harry Quigley).

CASE REPORTS

CASE 1

This black boy was born in October, 1970, full-term, at the end of an uncomplicated pregnancy. At the age of two months the left cornea became cloudy and, very shortly thereafter, the right cornea as well. There was tearing of the eyes, and the child was fretful, but no photophobia was noted. Medical attention was sought promptly, and under anesthesia the Shiøtz tonometer gave a reading of 4 with a 10-gram weight in each eye (40 mm Hg). Both corneas were 13 mm in diameter and opaque. A trabeculotomy-trabeculectomy was performed on the left eye on January 6, 1971 in a manner that allowed removal of an untouched portion of the trabecular meshwork for microscopic examination. The right eye underwent a similar operation two weeks later at which time the pressure in the left eye under anesthesia was 20 mm Hg, but the right eye still had a pressure of 40 mm Hg. Hence, tissue was available from both eyes.

Over the next six years the corneal diameter remained at 13 mm. The intraocular pressure was typically in the low 20's, occasionally in the high teens, on multiple examinations. The disc in the left eye retained a larger cup with a paler rim compared to the right eye, but in both eyes there was a rim of tissue around the entire circumference. The stable cup-disc ratio is 0.7 in the left eye and 0.5 in the right. The visual field as examined with a tangent screen appeared normal in August, 1977. The visual acuity is 20/40 in both eyes.

CASE 2

This is the case of a black boy born at term in February of 1971. The mother had syphilis during the first trimester. The child subsequently was shown to have a positive serologic reaction, and he received appropriate penicillin treatment.

At the age of three months it was suspected that the child could not see very well. Under anesthesia the pressure was in the range of 25–30 mm Hg in both eyes. The corneal diameters were 12–13 mm. There were breaks in Descemet's membrane, and cupping of the disc was particularly apparent in the right eye. A trabeculotomy-trabeculectomy was performed on the right eye in June of 1971 in such a manner as to remove an untouched portion of the trabecular meshwork. A month later the Schiøtz tonometer gave a reading of 6 with a 5.5-gram weight for the right eye (14.6 mm Hg) but 3.5 with a 5.5-gram weight (22.4 mm Hg) or (10 with a 7.5-gram weight) for the left eye. Therefore, a similar procedure was done on the left eye. The tissue specimens from both eyes proved quite satisfactory for microscopic examination.

On subsequent examinations over the next several years, cupping of the right eye was always greater than the left. The pressure was typically in the mid-teens, but always higher in the right eye. In April, 1977, when the pressure under anesthesia was 21 mm Hg in the right eye, a second trabeculotomy was done, this time in the lower temporal quadrant. In 1977, when the child was six-years-old, the vision was 20/60 in both eyes, and the pressure was 16–18 mm Hg with the applanation tonometer. The larger cup in the right optic disc persists.

CASE 3

This girl of Brazilian parentage was adopted at the age of three days, and is said to have weighed four pounds at the time of birth. The eyes were "always somewhat large" and became cloudy when the child was three months old. When she was examined at the age of five months, the right cornea was 13 mm in diameter and the left cornea was 14 mm in diameter. With a 7.5-gram weight, the Schiøtz tonometer gave a reading of 4.5 for the right eye (28 mm Hg) and 4.0 for the left (30.4 mm Hg). These findings were confirmed by a subsequent examination under anesthesia, at which time a trabeculotomy-trabeculectomy was performed on the right eye, removing an untouched portion of tissue for microscopic examination. One month later, pressure in the right eye was approximately 20 mm Hg. The pressure was still elevated in the left eye and it underwent a trabeculotomy, this time without removal of a block of tissue. Two months later, the pressure was under 20 mm Hg in both eyes. The course of this patient was not followed subsequently at this institution.

RESULTS

NORMAL DEVELOPMENT

At 20 weeks of gestation (five months) the anterior iris surface and the edge of the corneal endothelium meet to form the peripheral confine of the anterior chamber. In the corneoscleral limbus, just posterior to the junction of the iris and corneal endothelium, is a nest of cells constituting the differentiating trabecular meshwork (Fig 1). In the uveal tissue at the

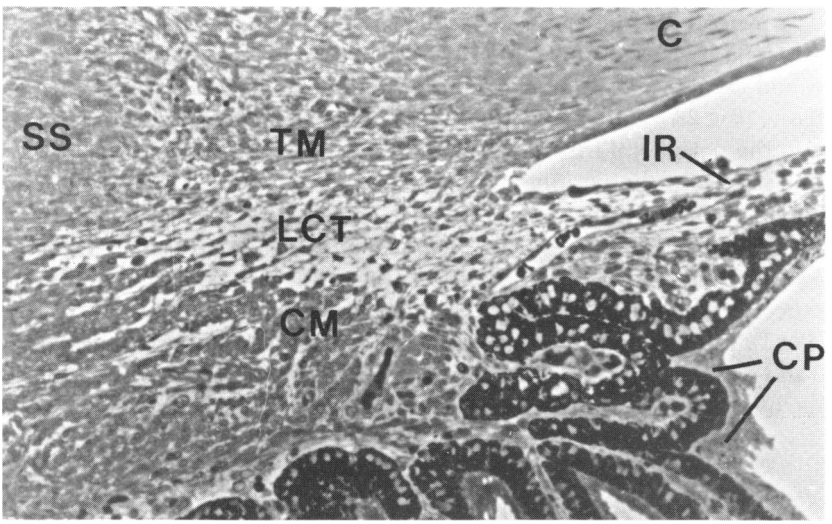


FIGURE 1
Angle of a 450-gram (20 week) human fetus. The trabecular meshwork is buried beneath uveal tissue, with the ciliary muscle and the ciliary processes overlapping the trabecular meshwork. Loose connective tissue (LCT) separates the ciliary body from the trabecular meshwork (paraphenylene diamine, ×200).

Abbreviations used in all Figures: AC = anterior chamber, C = cornea, CM = ciliary muscle, CP = ciliary processes, IR = iris, JCT = juxtacanalicular connective tissue, LCT = loose connective tissue, SC = Schlemm's canal, SS = scleral spur, TM = trabecular meshwork.

same level, facing the trabecular meshwork, is the ciliary body (ciliary muscles and ciliary processes) to which the root of the iris is attached. Between the trabecular meshwork and the ciliary muscle is a loose collection of cells similar to the iris stroma with which it is continuous. These are seemingly the ciliary body stromal cells that ultimately will line the recess of the anterior chamber angle.

Later in development, the peripheral margin of the anterior chamber moves posteriorly and the inner surface of the trabecular meshwork becomes exposed to the anterior chamber.[3,7-15] At one time it was supposed that the anterior chamber recess deepens by atrophy of the rarified tissue that in the earlier stage separated the trabecular meshwork and ciliary body. Later views[13] have emphasized the role of cleavage into this loose tissue, since there is no evidence of tissue loss (atrophy).

However, it is evident that the developmental process does not consist of simple cleavage or atrophy, for with either process the uveal tract would simply split away from the corneoscleral shell and the trabecular tissue (Fig 2A). The result would be that the ciliary muscle would extend into the peripheral iris, and the ciliary processes would be on the posterior surface of the peripheral iris.

The fact is, however, that the ciliary muscle and the ciliary processes remain attached to the corneoscleral envelope, but become recessed compared to their former position (Fig 2B). The ciliary muscle, and especially the ciliary processes, overlap the trabecular meshwork initially (Fig 1), but are later recessed to a position behind the scleral spur[15] (Figs 3 and 4). This repositioning can be explained only by a posterior sliding of the uveal tissues in relation to the cornea and sclera, presumably due to a

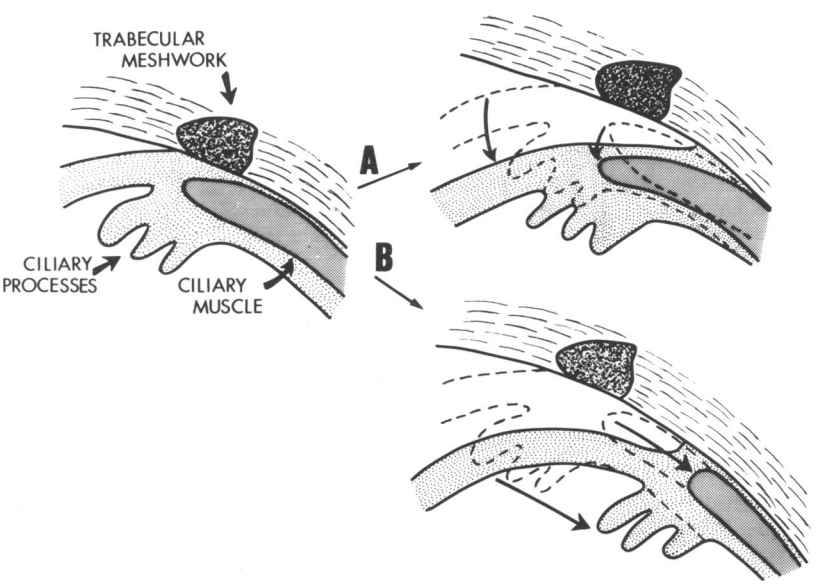


FIGURE 2

Process of exposing the trabecular meshwork to the anterior chamber during development. If the uveal tract simply splits away (A) by cleavage or by atrophy of tissue, the result would be an angle configuration in which the ciliary muscle extends into the iris and the ciliary processes are on the back of the iris. However, with slippage of the layers (B) due to differential growth rate, the ciliary muscle and the ciliary processes that initially overlapped the trabecular meshwork surface come to lie posteriorly.

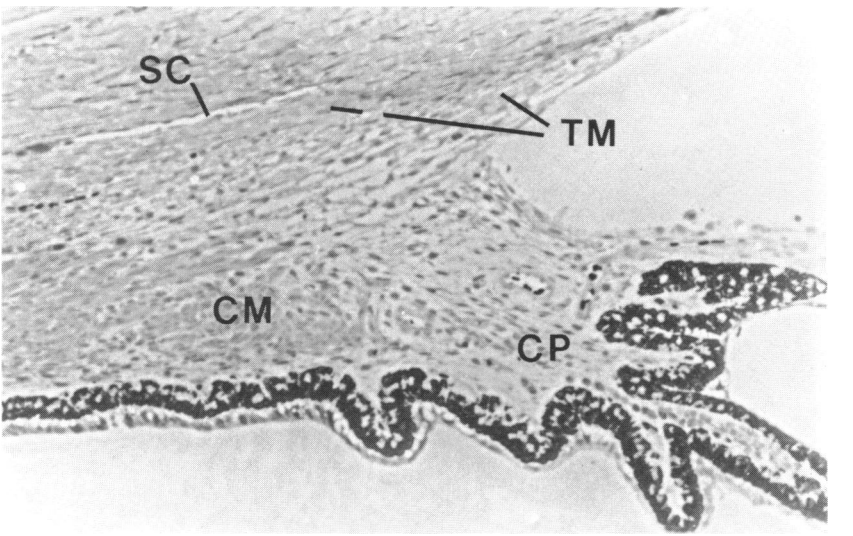


FIGURE 3
Anterior chamber angle of a 930-gram (27 week) human fetus. The uveal tract has receded so that the anterior portion of the trabecular meshwork is exposed to the anterior chamber. However, the ciliary muscle still overlaps the posterior portion of the trabecular meshwork, and the ciliary processes are even further forward, being anterior to Schlemm's canal (paraphenylene diamine, ×200).

differential growth rate of the various tissue elements. The repositioning process is not just a sliding of the uveal tract along the inner side of the sclera. There is also a repositioning of the various layers within the uveal tract in relation to one another: initially the innermost muscle fibers have a position relatively more anterior than the outermost fibers. Also, as can be seen in Figure 3, the ciliary processes are initially much further forward than the ciliary muscle. However, later, the ciliary muscle and the ciliary processes both recede to the same level and lie side-by-side, posterior to the trabecular meshwork and scleral spur.[15]

To allow slippage and progressive exposure of the meshwork to the anterior chamber, there must not be any restraining adhesions between the slipping layers, and particularly not between the uveal tract and the corneoscleral shell. Therefore the region of the recess of the future anterior chamber must naturally consist of a loose connective tissue that can yield to the slippage forces. This loose connective tissue may have potential spaces or clefts that suggest atrophy or impending cleavage when viewed in histologic section.

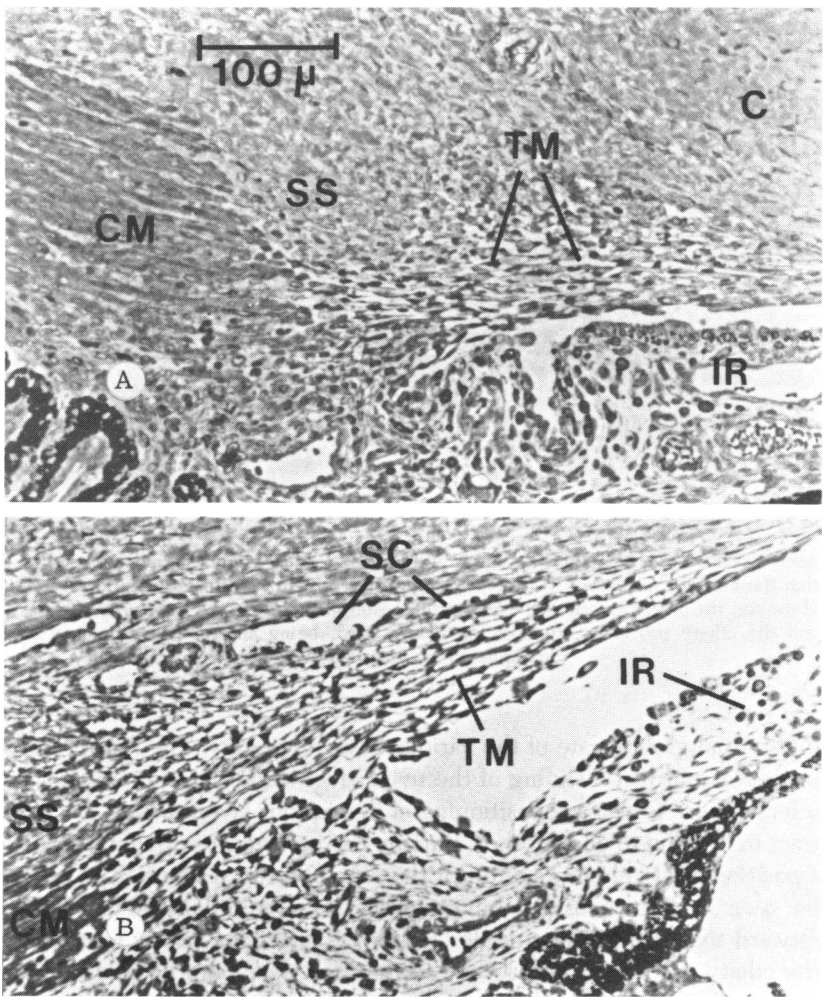


FIGURE 4

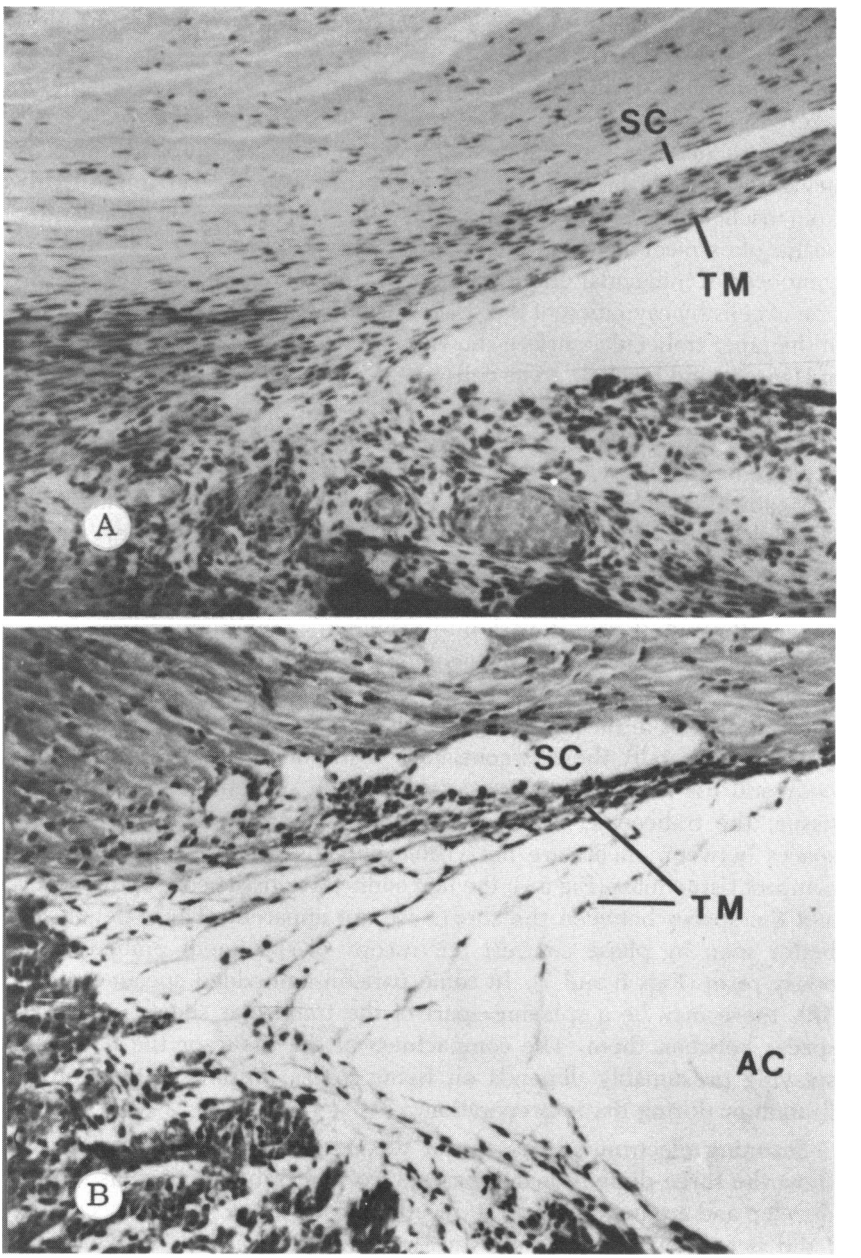
Two different areas of the anterior chamber of the same eye of a 1400-gram human fetus. A: The ciliary muscle and the ciliary processes are side by side at the level of the scleral spur, and the angle recess has receded to the point that most of the trabecular meshwork is exposed to the anterior chamber. Spaces are seen between the trabecular beams covered with trabecular meshwork endothelial cells. Schlemm's canal is not yet formed in this region. B: Vesicles can be seen on the inner wall of Schlemm's canal (paraphenylene diamine, ×200).

It is important to note that as the trabecular meshwork becomes uncovered, it does not seem to have a distinct endothelial covering, and thus differs from the adjacent cornea[14]: the surface layer of the meshwork is not an endothelial covering, but is simply the exposed surface of a multilayered mesenchymal mass that will become the multilayered trabecular meshwork endothelium. At about seven months, as the meshwork is being uncovered, the cells at all depths of the meshwork separate slightly from each other and the entire trabecular tissue cavitates.[16] Slender beams of extracellular matrix develop, forming trabecular sheets that are composed of trabecular endothelium and collagenous beams. The separation of cells by cavitation of the tissue would seem the same as the events at the inner trabecular surface shown in the scanning electron microscopy of Hansson and Jerndal.[17] The cells on the newly exposed surface separate from one another, just as the deeper cells are separating from one another. But this is not disintegration of an endothelial layer, because there is no endothelial layer—only the surface of a multilayered tissue mass.

Meanwhile, Schlemm's canal appeared as a distinct endothelial-lined channel on the outer face of the meshwork. Outflow facility develops,[18] and the endothelial cells on the inner wall of Schlemm's canal develop large vacuoles (Fig 4B), morphological evidence that aqueous humor is flowing through the meshwork into Schlemm's canal.

At term, the ciliary body attachment and the iris have receded just about to the level of the scleral spur. The trabecular beams are definite, and the vesicles in the inner wall of Schlemm's canal are more prominent (Fig 4). Postnatally there is continued posterior migration of the ciliary body and iris for about one year.[3] In paraffin sections of formalin-fixed tissue, the trabecular sheets are sometimes in apposition, so that the spaces between sheets are not visible and the meshwork appears as a compact tissue mass (Fig 5A); the fine connective tissue core of the beams and the spaces between the sheets are not apparent. These details are better seen by phase contrast microscopy of specimens embedded in epoxy resin (Figs 6 and 7). In some paraffin-embedded specimens (Fig 5B), there may be a splaying apart of the trabecular sheets with large spaces between them. The compactness of the tissue or the degree of splaying presumably depends on tissue forces present at the time of fixation or during tissue preparation.

Scanning electron microscopy of the normal specimens (Figs 8–10) show the three-dimensional appearance of the trabecular beams as they develop and are unveiled by the receding uveal tissue. In my specimens, I did not catch the stage, shown by others previously,[17] in which the trabecular cells first separate from one another.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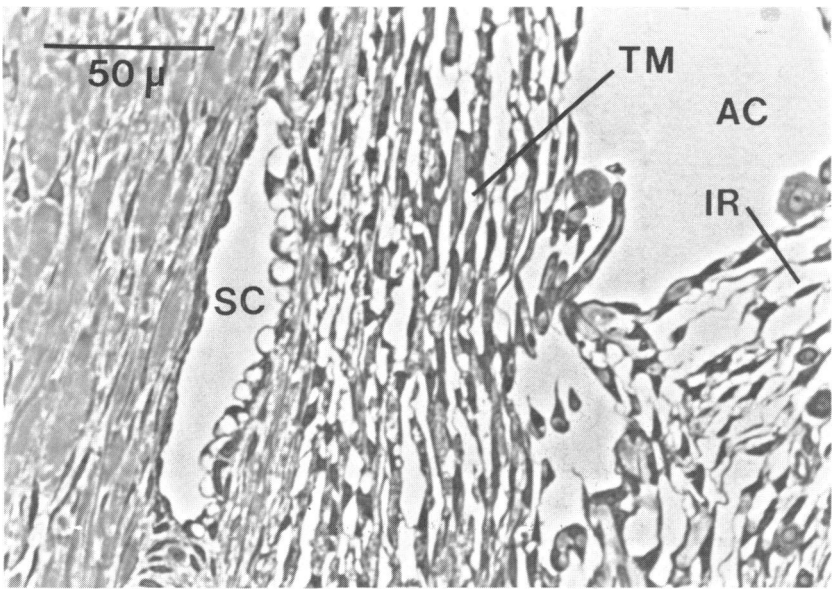

FIGURE 6
Angle structures of a normal eye of a 1400-gram human fetus. The delicate nature of the trabecular beams and the spaces between them is apparent. In this particular specimen there are many vacuoles in the endothelial cells lining the inner wall of Schlemm's canal (paraphenylene diamine, ×460).

INFANTILE GLAUCOMA

In the five surgical specimens from infantile glaucoma, the iris and anterior ciliary body occupy a position in relationship to the trabecular meshwork equivalent to the seventh or eighth month of fetal development (Fig 11). The anterior ends of the ciliary muscle overlap the posterior portion of the trabecular meshwork, as do the ciliary processes.

FIGURE 5
A: Anterior chamber angle of a normal eye of a 32-day-old infant and B: a 10-week-old infant. In A the trabecular meshwork appears as a cellular mass of tissue because the collagenous beams are not seen, and the sheets are packed together so that the spaces between the beams are not apparent. In B the trabecular sheets have splayed apart as an artifact of tissue preparation, making the thickness of the sheets and the existence of spaces between the sheets apparent (hematoxylin and eosin, ×200).

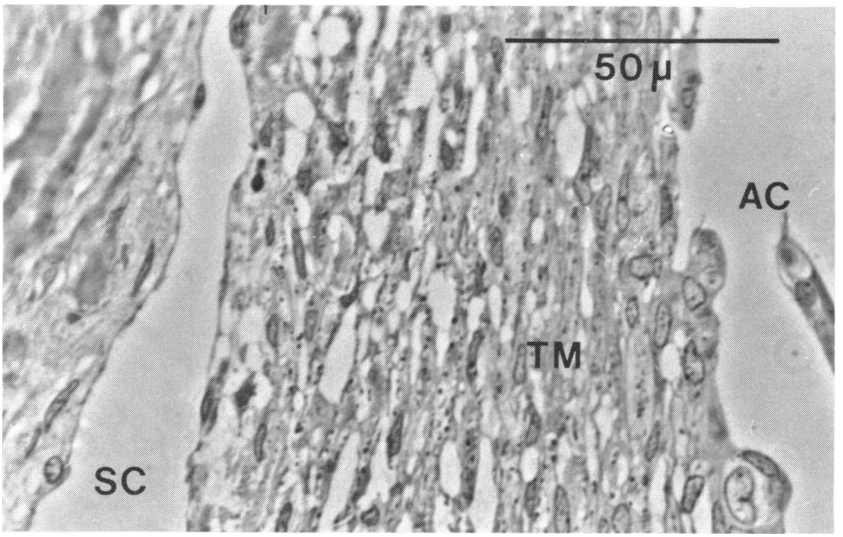

FIGURE 7
Angle structures of the normal eye of an 8-week-old child. A few intertrabecular spaces are seen, and the width of the trabecular beams can be appreciated. In this specimen, vacuoles are not seen in the wall of Schlemm's canal (paraphenylene diamine, ×740).

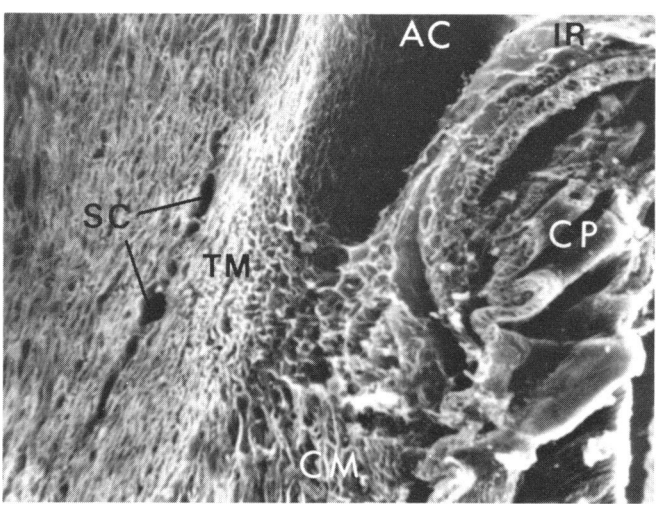

FIGURE 8
Scanning electron micrograph of anterior chamber angle of an infant who died of congenital ichthyosis three days after birth. Gestational age and birth weight are not known, but the angle development is equivalent to the seventh or eighth month. The angle recess has exposed only the anterior third of the trabecular meshwork, and the ciliary processes are still anterior at the level of the trabecular meshwork (×90).

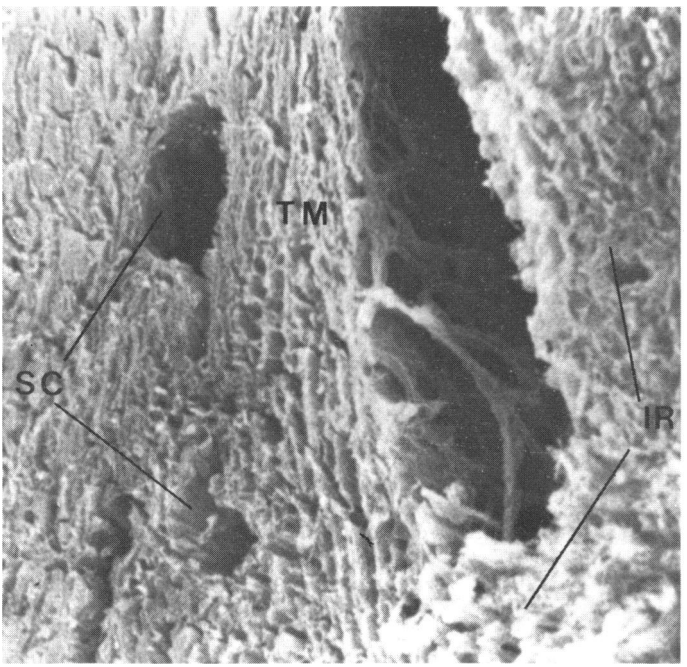

FIGURE 9
Scanning electron micrograph of the anterior chamber angle of a normal eye of a human infant with multiple anomalies who died of aspiration pneumonia seven days after birth. The delicate trabecular beams are well formed, and the angle recess has exposed most of the trabecular meshwork to the anterior chamber (×250).

The trabecular beams are thickened (Fig 12), not only in comparison with those of late fetal development (Fig 6), but also in comparison with the meshwork of a normal meshwork eight months postnatally (Fig 7). Schlemm's canal was present in all specimens and had a normal appearance, except for the absence of vacuoles in the endothelial lining of the inner wall. Adjacent to the inner wall of Schlemm's canal, the juxtacanalicular connective tissue contains an amorphous extracellular substance which had a very pale grey granular appearance by phase-contrast microscopy. By transmission electron microscopy, the material contains collagen and amorphous (presumably proteoglycan) substrate.

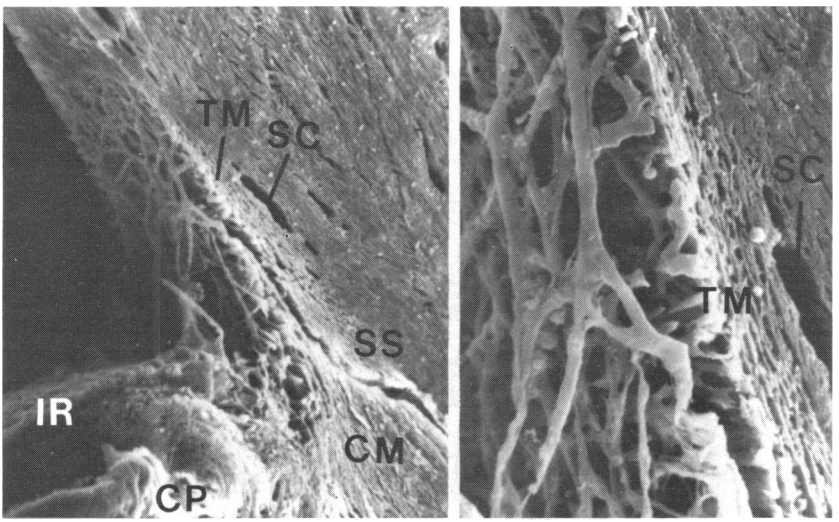

FIGURE 10
Scanning electron micrograph of normal angle of a 4-year-old child. This normal eye was removed at the time of orbital exenteration for rhabdomyosarcoma (×80, ×250).

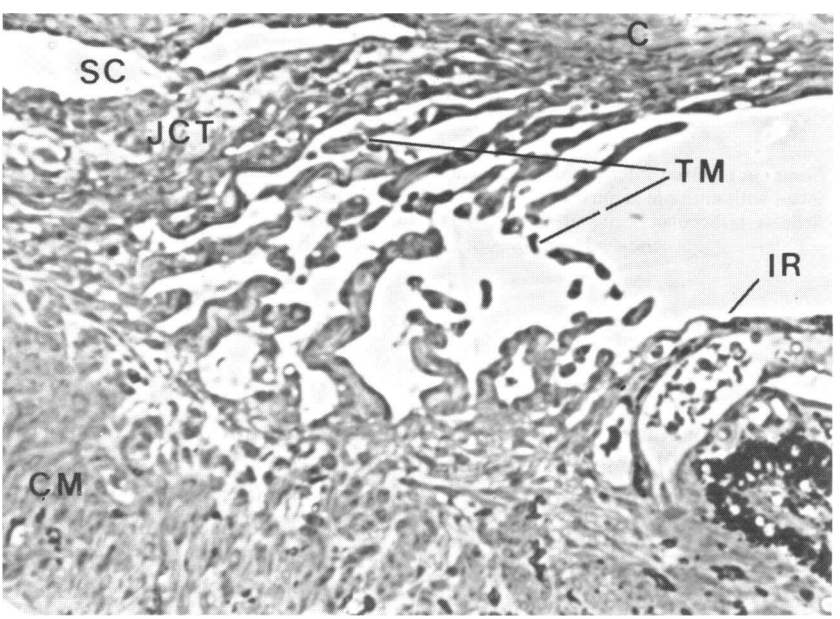

FIGURE 11
Angle structures of infantile glaucoma, Case 1. Because of tissue relaxation after excision of the specimen, the thick trabecular beams are not on the full stretch that they would be in life (paraphenylene diamine, ×200).

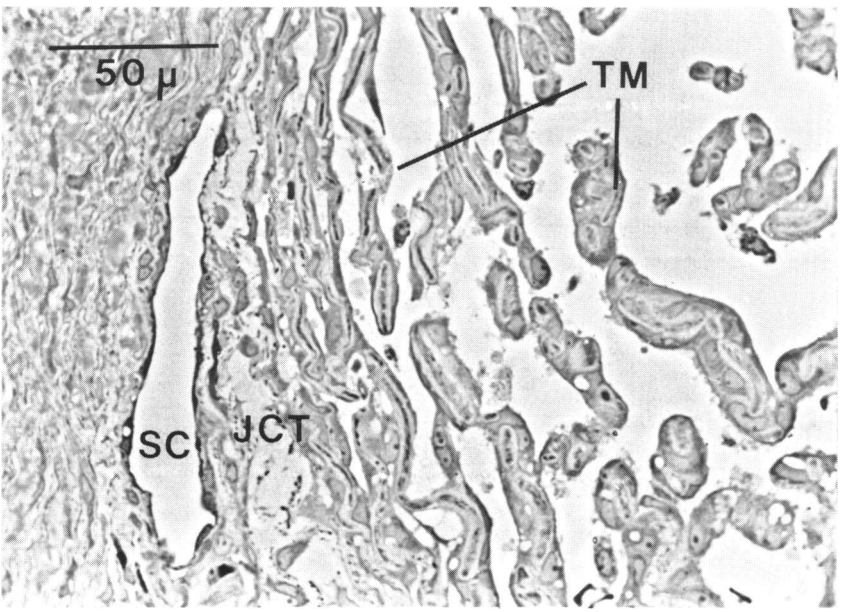

FIGURE 12
Higher powers of the angle structures in the same case (different section) of infantile glaucoma, Case 1. The thickness of the trabecular beams in this section can be compared with Figure 6, which is at the same magnification, and Figure 7, which is at a higher magnification. Amorphous material fills the juxtacanalicular connective tissue [JCT] (paraphenylene diamine, ×460).

By scanning electron microscopy (Figs 13–15), the trabecular meshwork has a reasonably normal appearance, except for the broadened nature of the trabecular beams. A membrane covering the surface was not found in any of the surgical specimens.

The specimen retrieved for scanning electron microscopy from a paraffin block was much different from the others. Schlemm's canal was not identified, and the trabecular meshwork had the appearance of compressed sheets of tissue (Figs 16–18). The surface had a texture like that of compressed trabecular meshwork. One of the tissue blocks was pulled apart following freeze-drying, lifting the most superficial layer of tissue from the trabecular meshwork as a sheet (Fig 19). It could be interpreted that the surface of the trabecular meshwork in this specimen was covered by a membrane, perhaps consisting of the innermost trabecular sheet

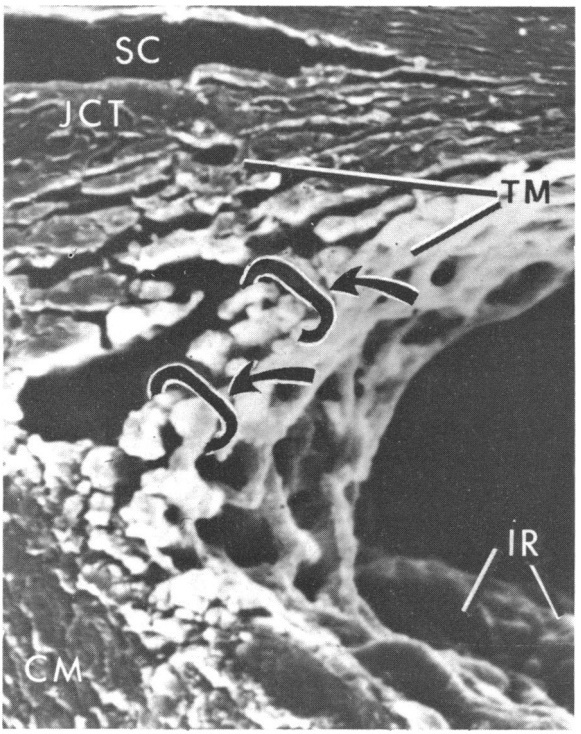

FIGURE 13
Scanning electron micrograph of the angles of infantile glaucoma, Case 1, right eye. The cut surface is equivalent to the view in Figure 11. The innermost sheet of trabecular tissue (encircled, arrows) gave the appearance of a membrane covering the trabecular meshwork when viewed with the dissecting microscope (×300).

which failed to develop the normal openings. However, the same appearance could result if several layers of trabecular tissue were compressed together and became adherent either in life or during fixation and tissue processing. Judging from the compressed appearance of the subjacent meshwork, the latter seems the more likely explanation.

In all five post-mortem specimens examined only in paraffin sections, the iris insertion and anterior ciliary body overlapped the posterior portion of the trabecular meshwork. As in previous reports,[3,7-10,19,20] the degree of failure of the iris and ciliary body to recede posteriorly was

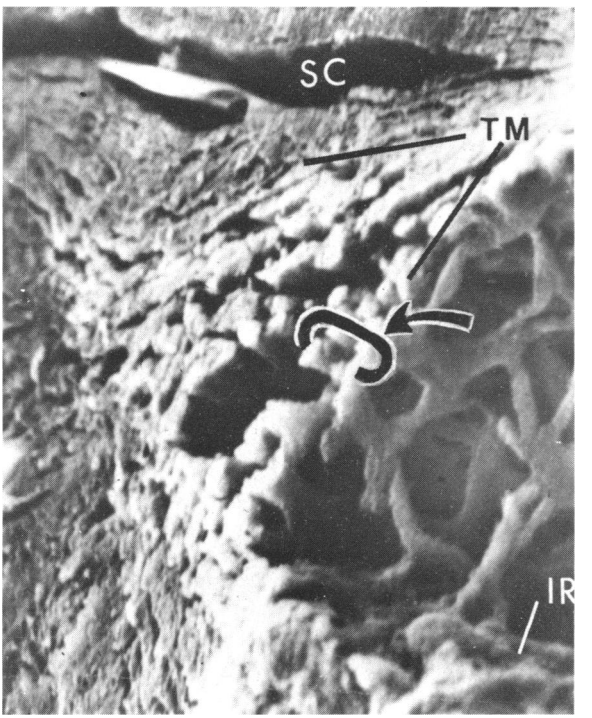

FIGURE 14
Same specimen as Figure 13, viewed from a different angle. The unobstructed openings of the innermost sheet of trabecular tissue can be seen with scanning electron microscopy, but had not been visible in the dissecting microscope (×300).

somewhat variable, so that anywhere from 0.25 to 0.5 of the meshwork was exposed. The trabecular tissue sometimes appeared as a compact mass of trabecular cells (Fig 20A), since the individual trabecular beams could not be visualized with this technique. In other specimens, however, the trabecular sheets were splayed apart, and the spaces between the sheets made the laminated nature of the trabecular meshwork apparent (Fig 20B). In some of these, the innermost sheet, being separated from the underlying trabecular sheets, has the *appearance* of a membrane lining the inner surface of the anterior chamber angle, but in reality would seem to be the innermost trabecular sheet, or perhaps two to three sheets stuck together. The tautness of the trabecular beams and the

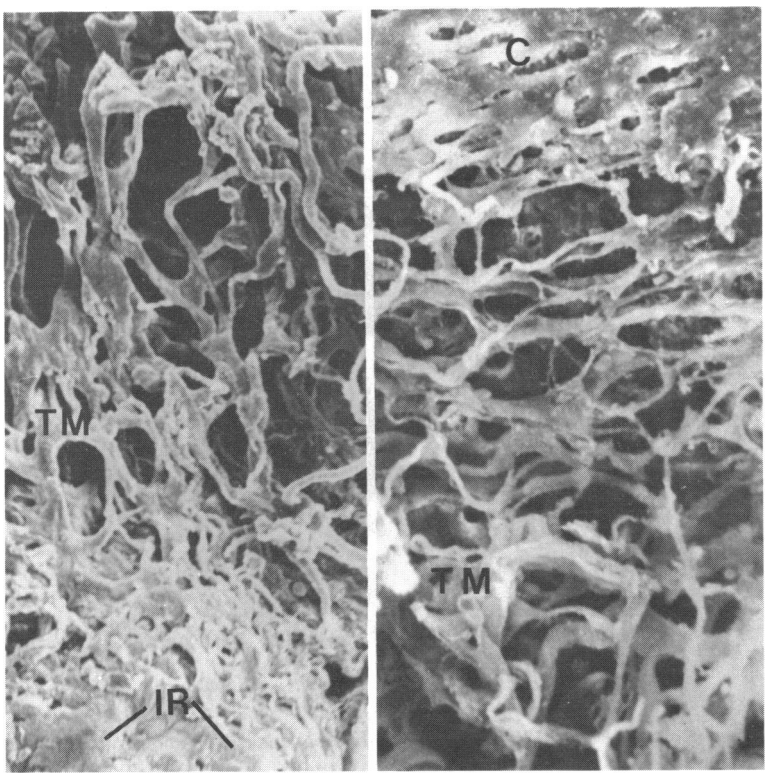

FIGURE 15
Scanning electron micrographs of the surface of the trabecular meshwork of infantile glaucoma, Case 3 (left) and Case 2 (right). There was no membrane covering the surface in either (×400).

traction on the peripheral iris is often evident. The ciliary processes are elongated inward toward the lens. All of these features can be seen in the illustrations of previously published reports.[3,7-10,19,20]

DISCUSSION

In describing the *normal* angle by gonioscopy, Barkan[1] noted, "the angle is clothed by an almost transparent membrane with a shagreened surface." In describing the angle of an infant with glaucoma, he observed that

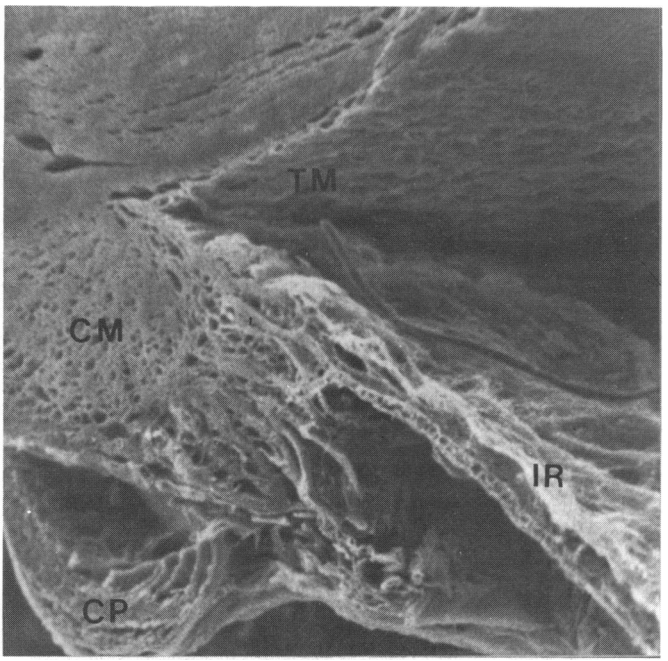

FIGURE 16
Scanning electron micrograph of angle structures of infantile glaucoma, specimen retrieved from paraffin block. The trabecular region is represented by a compacted tissue with a rough surface (×95).

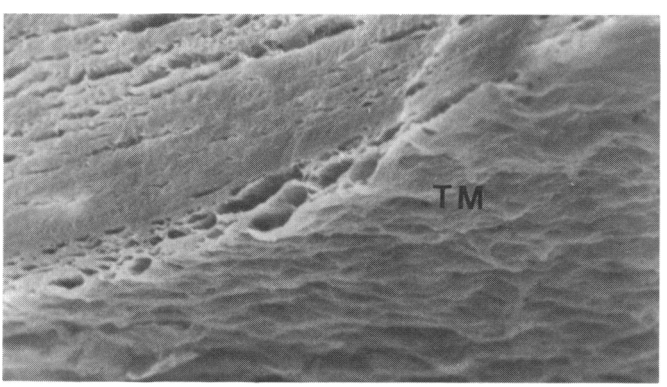

FIGURE 17
Details of the surface in the trabecular region of infantile glaucoma; same specimen as Figure 16 (×240).

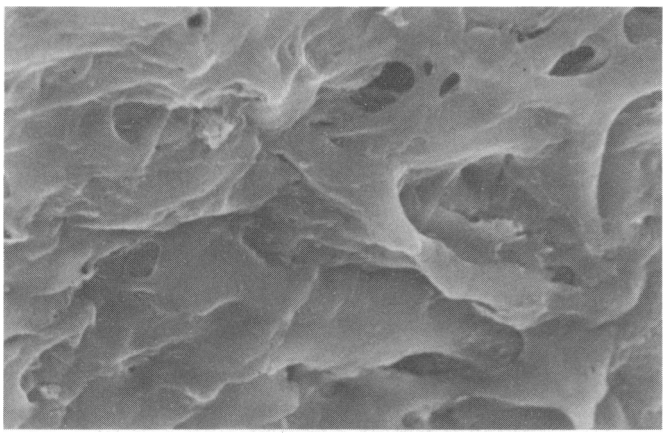

FIGURE 18
Even higher magnification of the surface of the trabecular tissue of infantile glaucoma; same specimen as Figure 17 (×800).

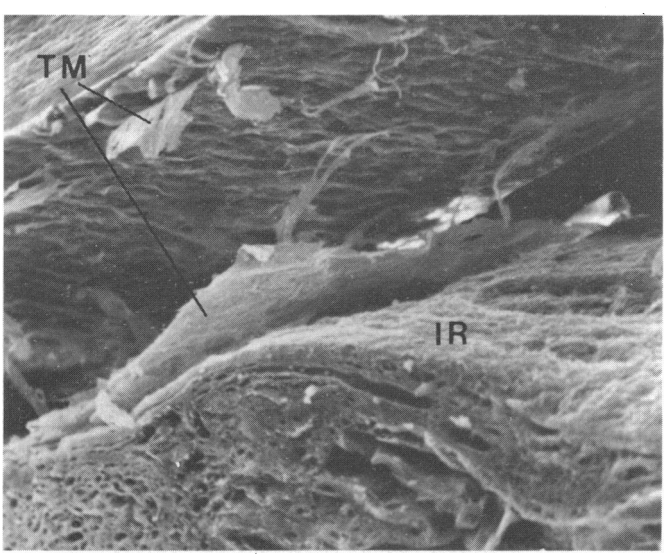

FIGURE 19
Scanning electron micrograph of a block of angle tissue of infantile glaucoma; same cases as Figures 16–18. In this block the surface sheet of tissue is torn away from the underlying tissue, revealing the texture of the trabecular meshwork. The most superficial sheet is imperforate (×80).

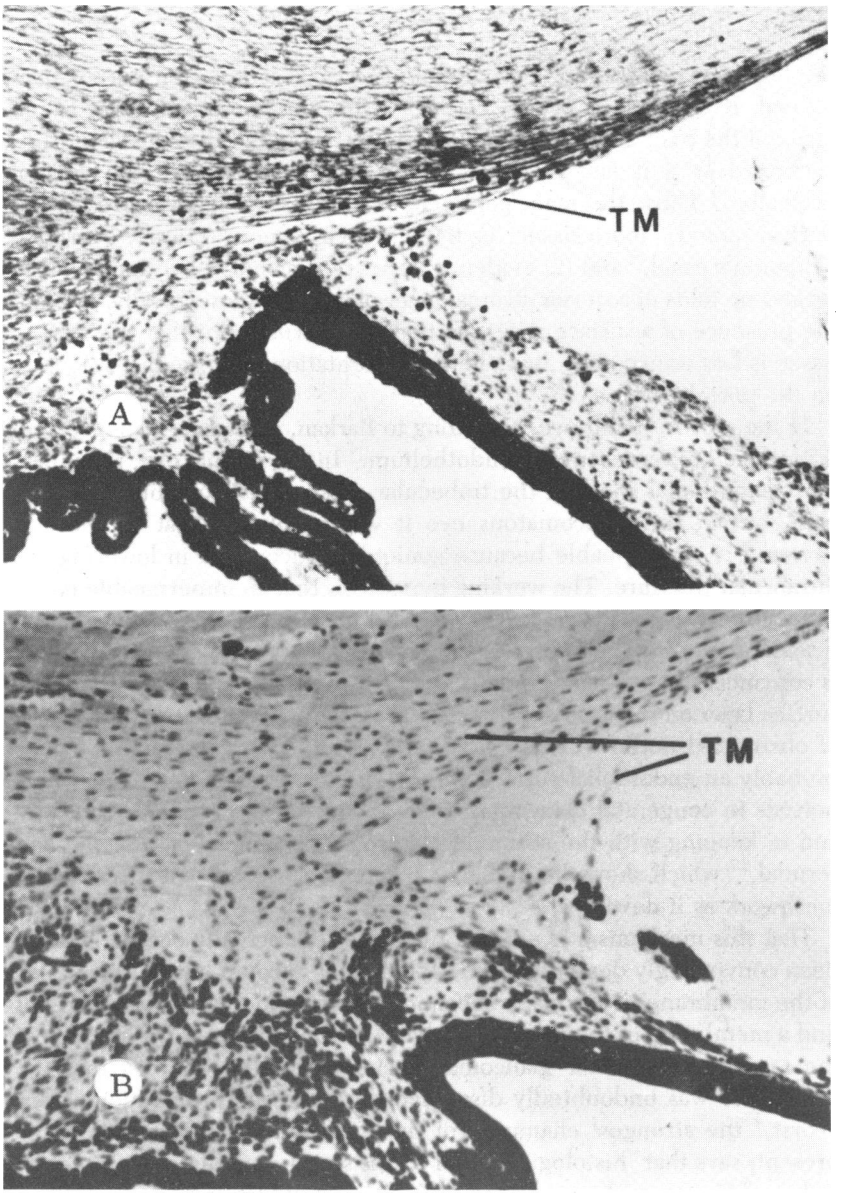

FIGURE 20

Examples of infantile glaucoma. A: The thick trabecular beams can be seen (compared with Figure 5). It would be difficult to say from this type of section whether or not the inner-most sheet of the trabecular meshwork (or, indeed, the deeper sheets) is perforated. B: The innermost sheet of the trabecular tissue is artifactitially separated away, giving the impression of a membrane lining the angle (hematoxylin and eosin, ×125, ×200).

the, "shagreened membrane covering the canal is less transparent than normal. It drops more or less vertically from the line of Schwalbe to the plane of the iris." At the time of goniotomy, he noted that the "iris drops backward as if it had been pulled or pinned up toward the ring of Schwalbe." Thus, the main gonioscopic features, as again expressed by Barkan, are (1) "more tissue, or tissue which is more opaque, overlying Schlemm's canal," and (2) evidence of traction manifest by "anomalously picked-up folds of anterior stromal layer of iris." Gonioscopically, it is not the presence of a surface shagreen that is abnormal, but that the surface tissue is less transparent, has a vertical orientation, and produces traction on the peripheral iris.

In the normal infant eye, according to Barkan,[1] the shagreened surface is "*assumed* to represent the endothelium." In the normal eye, the innermost endothelial sheet of the trabecular meshwork is obviously permeable, but in the glaucomatous eye it was concluded that the surface covering is impermeable because goniotomy succeeded in lowering the intraocular pressure. The working hypothesis that an impermeable membrane produces the glaucoma certainly fits all of the clinical features of the disease, including the improved outflow after goniotomy.[7] Thus, Worst[3,4] is convinced that the glaucoma is due to the "presence of an imperforate surface layer on this persistent mesodermal tissue, which is the only cause of obstructed outflow. This surface membrane, Barkan's membrane, is probably an endothelial surface which normally breaks apart, but which persists in congenital glaucoma." Such a concept has seemed plausible, and in keeping with the scanning electron micrographs of Hansson and Jerndal,[17] which show the breaking apart of a surface layer of trabecular meshwork as it develops.

That this mechanism is not just plausible, but actually occurs, has not been convincingly demonstrated. Maumenee[7-10] could not find evidence of the membrane in the many specimens he examined. I likewise did not find a membrane in the present study. Hansson[21] excused the absence of the membrane in their glaucoma specimens on the grounds that the membrane was undoubtedly disrupted during tissue preparation. Even Worst,[4] the strongest champion of the hypothesis that a membrane is present, says that "histological proof of this structure is almost completely lacking." However, he continues: "the absence of histological proof, however, has little influence on the probability that this concept is valid." The gonioscopic and surgical impression of the presence of a membrane is exceedingly strong!

I believe that the clinical experience of cutting a taut, transparent trabecular tissue has been misinterpreted to be the cutting of a tough

imperforate membrane over the surface of the trabeculum. In reality, the tissue cut is the inner portion of the trabecular meshwork, which is colorless and translucent, with its holes too small to be resolved by gonioscopy. The tension of the iris root produced by this taut tissue is evident by gonioscopy, and becomes more apparent when the tissue is incised (goniotomy), allowing the peripheral iris to fall backward.

By histopathology,[3,19,20] it is a band of tissue under obvious traction stretching from the iris root to Schwalbe's line that is interpreted as a membrane. The illusion of a membrane can be particularly striking in a specimen in which the most superficial sheet or two of trabecular tissue is split from the underlying layers during fixation and tissue preparation. This histopathologic appearance may well be the result when adjacent innermost sheets of the trabecular tissue adhere to each other during fixation and then split off from the layers underneath during tissue processing due to the innate tissue tension in the taut trabecular tissue. However, it is impossible to say from a conventional tissue section that this layer is imperforate. I was first awakened to the illusionary nature of the membrane on one of the first specimens I examined. Under the dissecting microscope there was a definite transparent sheet which could be put on stretch and visualized under the dissecting microscope. The appearance was the same as the "membrane" that we had seen by gonioscopy, and I was quite surprised to find that by scanning electron microscopy the "membrane" was actually a thickened layer of normally-perforated uveal meshwork (Fig 13).

Not only do these thickened trabecular sheets account for the gonioscopic appearance, but premature or excessive formation of the collagenous beams during the last trimester might be the primary developmental defect. We can hypothesize that the thick trabecular sheets prevent the posterior sliding of the uveal tract against the inner surface of the corneoscleral envelope. If the posterior sliding of the ciliary body is prevented, it would result in the anterior position of the ciliary processes, ciliary muscle, and iris in infantile glaucoma, so that the overall configuration in these features resembles that of a seven- or eight-month fetus. Speakman and Leeson[22] mentioned that in infantile glaucoma there seemed to be a "failure of uveal fibers to lengthen," though they attributed this to "incomplete differentiation of the angle" rather than to premature or excessive formation of the meshwork collagen as is being hypothesized now.

We can also speculate that the obstruction to aqueous humor outflow is due to compression of the taut trabecular sheets against one another. It is clear that the trabecular sheets are under tension, both from the histolog-

ic appearance and from the fact that upon incising the meshwork there is a release of traction on the peripheral iris and ciliary body visible gonioscopically at the time of surgery. Maumenee[7-10] attributed the traction on the trabecular sheets to the anteriorly-placed insertion of the ciliary body muscle. However, the traction could simply be the result of the differential growth rate that normally draws the uveal tract backwards while the thickened trabecular bands hold it forward. Naturally, in such an instance, the longitudinal muscle fibers are anteriorly placed; but tone of these muscles may not contribute to the compression of the trabecular sheets. Indeed, it appears that neither pilocarpine nor cycloplegic agents affects the intraocular pressure in infantile glaucoma.[9] Moreover, the anterior position of the longitudinal muscle is not prominent in all cases, the degree of a retained anterior position of the muscle being variable.

It should be noted that the intraocular pressure is often in the mid-range, so that there is an increased resistance to the outflow of aqueous humor, but not a complete obstruction as would occur with a membrane. This adds to the plausibility that compressed trabecular sheets are the explanation for the impaired outflow.

If the resistance is due to compacted trabecular sheets, an incision through the trabecular sheets would relieve the compaction and could account for the rather striking success of goniotomy or trabeculotomy in infantile glaucoma. The only other site of resistance evident in the present studies is the thickened juxtacanalicular connective tissue. While the histologic appearance makes this an attractive site to which to attribute the resistance, it would require very precise placement of the goniotomy incision to cut through this tissue and regularly to enter Schlemm's canal: the typical high success rate for goniotomy would seem impossible to achieve if this were the site of abnormal resistance. Perhaps the formation of thickened juxtacanalicular tissue could simply accompany the excessive premature formation of collagen in the trabecular beams, but not contribute to the abnormal outflow resistance.

The lack of vacuoles in the endothelium of Schlemm's canal is an uncertain finding, since the presence of vacuoles depends upon the conditions of fixation. I presume that the absence is an artifact; but if not, I would interpret the lack of vacuoles not as a cause, but rather as a passive result of reduced aqueous flow through the trabecular meshwork.

The elongation of the ciliary processes is probably the result of inward traction, not because the lens is particularly small, but because the enlarging diameter of the eye in buphthalmous creates traction on the zonules attached to a non-enlarging lens. This traction may also contribute to the rocking forward of the ciliary muscle (and the forward

migration of the ciliary muscle). Maumenee[8] invokes this mechanism to explain the forward position of the ciliary muscle in cases of persistent hyperplastic primary vitreous associated with congenital glaucoma. However, in primary infantile glaucoma, it would seem that the non-yielding trabecular beams are the main cause for the forward traction on the ciliary body.

In conclusion, the findings in this study suggest that the primary defect of congenital glaucoma may be the premature or excessive formation of collagenous beams within the trabecular meshwork. The normal posterior sliding of the ciliary body and peripheral iris may be prevented by the non-yielding collagenous beams. The traction forces generated may cause compaction of the trabecular sheets and obstruction of aqueous humor outflow. Release of the traction by surgery could relieve such an obstruction, although it remains to be explained how an incision in only one quadrant so effectively relieves all the apparent obstruction.

SUMMARY

Tissue from ten eyes with infantile glaucoma and from 40 normal eyes of fetuses and infants without glaucoma were examined by light and electron microscopy. In normal development, the corneoscleral coat grows faster than the uveal tract during the last trimester, leading to a posterior migration of the ciliary body attachment from Schwalbe's line (5th month) to the scleral spur (9th month), and then to a location behind the scleral spur (postnatally).

In infantile glaucoma, the insertion of the anterior ciliary body and iris overlaps the trabecular meshwork, similar to the late fetal position. The trabecular sheets are perforated, and there is no membrane over the surface of the trabecular meshwork. The trabecular beams are thicker than in normal infant eyes. There is both histologic and clinical evidence of traction on the iris root exerted by the thickened trabecular beams. These findings suggest that in congenital glaucoma the thickened beams had prevented the normal posterior migration of the ciliary body and iris root. This traction may compact the thickened trabecular beams, obstructing aqueous humor outflow. Release of the traction by an incision (goniotomy or trabeculotomy) of the thickened meshwork may relieve the obstruction. Of uncertain pathological significance is that there are no vacuoles in the endothelium of Schlemm's canal and there is a broad layer of collagen and amorphous material in the juxtacanalicular connective tissue. The ciliary processes are elongated inward, as if they were pulled by zonular traction (perhaps created by an enlarging diameter of the limbus with a fixed lens diameter).

ADDENDUM

Van Buskirk[23] has published scanning electron microscopic observations of developing monkey trabecular meshwork showing fenestration of the trabecular endothelium as the iris retracts posteriorly from the peripheral cornea. Two studies have been reported showing the thickened trabecular beams in congenital glaucoma.[24,25]

Hoskins *et al*[26] have published scanning electron micrographs from a couple of eyes with infantile glaucoma, noting in one that "thickened and abundant uveal cords appear to hold this iris forward, preventing the normal separation of the corneal scleral trabecular sheath" and in the other that "there are dense cords in the pretrabecular area."

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