THE PATHOGENESIS OF CORNEAL EDEMA INDUCED BY TWEEN 80

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Despite its abundance of collagen, the normal cornea is transparent. This essential visual requirement is, at least in part, related to the degree of corneal hydration.[1-7] Opacification follows the accumulation of fluid within the cornea, and, as the excised cornea is markedly hydrophilic, a characteristic of this tissue in the living subject is its ability to maintain a deturgescent state.[1,2,8-16] Corneal edema complicates numerous ocular disorders that involve the endothelium of the cornea.[1,17-81] This association poses the problem of whether the corneal endothelium normally prevents the contiguous aqueous humor from being imbibed by the cornea, or whether the endothelium contributes to the maintenance of the state of relative dehydration of the normal cornea by an active transport of water and electrolytes from the cornea.

It was observed that the injection of polyoxyethelene sorbitan monooleate (Tween 80, Atlas Chemical Industries, Inc.) in normal saline into the anterior chamber of rabbits' eyes promptly and regularly produced marked corneal edema. As the corneal edema was associated with lesions of the corneal endothelium, this model was utilized to obtain information concerning the mechanism of fluid transportation into the corneal tissues. The resulting morphologic alterations were investigated using light and electron microscopy. Evidence was sought regarding the permeability of the corneal endothelium and of the limbal vasculature by the injection of tracer particles of variable molecular size into the anterior chamber and into the systemic circulation. The findings were viewed in relation to the normal and pathologic states of corneal hydration in human beings.

MATERIALS AND METHODS

Adult albino rabbits, weighing 2–3 kg., were anesthetized by an intraperitoneal injection of urethane dissolved in normal saline (1.5 gm./kg. body weight). Different concentrations of Tween 80 (0.1% to 100%) in normal saline were injected

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into the anterior chambers of rabbits' eyes using sterile 1-ml. tuberculin syringes (needle: 27 gauge, ½A). The anterior chambers were approached by penetrating the cornea or by directing the needle into the aqueous humor from behind the ciliary body and iris without impinging on the cornea. In each instance, 0.1 ml. of aqueous humor was aspirated and replaced by a comparable volume of Tween. In some animals, Tween was injected subconjunctivally at the limbus. To prevent infection, a few drops of 2% boric acid were instilled on the surface of the eye after each instillation of Tween.

A portion of cornea was studied by light and electron microscopy at variable intervals after a unilateral instillation of Tween (5 min. to 14 days). In each instance, the contralateral, unaffected cornea was used for control purposes. Light microscopic observations were performed on stained and unstained preparations of unfixed frozen and formalin-fixed, paraffin-embedded material.

At the time of excision, part of each cornea was sectioned into small cubes measuring less than 2 mm. and fixed in cold sodium-cacodylate-buffered glutaraldehyde (pH 7.4) for 24 hr. It was then postfixed in cold veronal-buffered 2% osmium tetroxide for 1 hr., dehydrated in graded alcohols, infiltrated with styrene, and embedded in Epon. Thin sections were cut with the Servall Porter-Blum microtome, mounted on 300-mesh copper grids, and stained for 10 min. in lead citrate at a high pH at room temperature.[32] Electron-microscopic examinations were made with an RCA EMU-3A electron microscope at 50 kv. Companion sections were stained with thionine azure blue for purposes of orientation.[33]

The effect of intraocular Tween upon the limbal vascular endothelial permeability was studied after the intravenous injection of Evans blue, human gamma globulin, or thorium dioxide. When Tween-induced corneal edema was evident, 10 ml. of 1% Evans blue in normal saline, or 3 ml. of Thorotrast® (Fellows-Testagar) was injected intravenously into some animals. These animals were killed 5–10 min. later. Immediately after the introduction of 0.1 ml. of Tween into the anterior chamber of one eye, human gamma globulin (500 mg. in 5 ml. normal saline) was administered intravenously to rabbits which were killed 1 and 24 hr. later. The anterior segments of the eyes were removed bilaterally and rapidly frozen with Dry Ice in acetone. Frozen sections were cut at 6μ, stained with fluorescein-labeled anti-human gamma globulin, mounted with glycerine-phosphate-buffered saline, and examined under ultraviolet irradiation.

To test the permeability of the corneal endothelium and Descemet's membrane, 0.2 ml. of Thorotrast® or Pelikan black waterproof drawing ink (Günther Wagner) was injected into the anterior chamber of both eyes 24 hr. after the unilateral intraocular instillation of Tween. In each instance an equivalent volume of aqueous humor was withdrawn prior to the injection of the colloidal particles. The corneas were studied by light and electron miscoscopy 30 min. and 24 hr. later.

All experiments on the vascular and corneal endothelial permeability were performed in triplicate.

Results

Within 5 min. of the intraocular instillation of 0.1 ml. of 25–100% Tween, the limbal vessels became congested (Fig. 1). By 2 hr., marked conjunctival and corneal edema consistently appeared on gross examination (Fig. 2). The conjunctival edema was transient and usually disappeared in a few hours. The corneal opacification progressively increased in severity, reaching its greatest intensity within 24 hr. (Fig. 3 and 4). It then remained without change for 1–2 weeks. The lesions were independent of the method by which Tween was introduced into the anterior chamber.

The resulting edema was less pronounced and not as rapidly produced in greater dilutions of Tween. The subconjunctival injection of Tween resulted in severe conjunctival edema and congestion, which were most marked at the site of inoculation. In these experiments the edema spread toward the periphery of the cornea and involved the latter to a minimal degree. In the dosage used, boric acid had no ill effects.

The corneal thickness was increased 3–4 times the normal owing to swelling of the substantia propria (Fig. 5). Less than 2 hr. after the introduction of Tween into the anterior chamber, the blood vessels at the corneal limbus were surrounded by leukocytes. Fibrinoid material was occasionally evident in the lumina and walls of some limbal blood vessels (Fig. 6). The corneal epithelium was not increased in thickness. In view of the limitations in resolution, it was difficult to evaluate the morphology of the cellular constituents of the cornea by light microscopy.

Fine structural alterations were first evident in the cornea after a few minutes exposure to Tween, and in less than 2½ hr. marked corneal endothelial cytolysis was manifest. Many corneal endothelial cells had ill-defined cell membranes and contained intracytoplasmic vacuoles, myelin figures, and osmiophilic debris at this time. Only vestiges of occasional corneal endothelial cells were identifiable 5½ hr. after the intraocular instillation of Tween (Fig. 7 and 8).

Vascular Endothelial Permeability

Less than 5 min. after the intraocular instillation of Tween, a blue discoloration appeared at the periphery of the cornea in animals which had previously received intravenous Evans blue. The dye gradually spread centripetally, and within 30 min. it had stained the entire cornea. The intensity of the hue gradually diminished during the subsequent 24–48 hr. The cornea of the contralateral control eye did not exhibit any color changes. The aqueous humor exhibited a slight blue coloration which was independent of intraocular Tween.

Under the conditions employed, human gamma globulin was demonstrated readily in tissue by the specific fluorescence which was exhibited after staining with fluorescin-labeled anti-human gamma globulin. In the normal eye this protein marker was evident only within blood vessels such as those of the iris (Fig. 9, left). One hour after exposure to intraocular Tween, the human gamma globulin appeared extravascularly in the iridial stoma and at the corneal limbus but did not involve the central cornea. In contrast to the tortuous vascular design of the control eye, the fluorescence of the iris exhibited a diffuse pattern (Fig. 9, center). The entire cornea and iris of Tween-exposed eyes both displayed fluorescence 24 hr. after human gamma globulin (Fig. 9, right).

In the absence of exposure to Tween, particles of thorium were restricted to the lumen of the limbal blood vessels, 5–10 min. after the intravenous administration of this electron-dense marker (Fig. 10). In addition to vascular labeling, the edematous cornea exhibited extravascular spilling of the thorium dioxide after a comparable time interval. Under these circumstances the electron-dense grains were evident in the extravascular interstitial space, within the cytoplasm of pericytes, macrophages, unidentified cells, and, rarely, vascular endothelial cells (Fig. 11 and 12). Figure 13 summarizes the observations on the extravasation of intravascular markers following the introduction of Tween into the anterior chamber.

Corneal Endothelial Permeability

Electron-dense grains of thorium dioxide (7–15 mμ in diameter) regularly appeared in the cytoplasm of the corneal endothelium and within Descemet's membrane following their instillation into the anterior chamber of normal rabbits' eyes (Fig. 14 and 17, top). When the endothelium was not discernible following Tween, the particles tended to aggregate adjacent to Descemet's membrane but were virtually absent within Descemet's membrane (Fig. 15 and 17, bottom). Thorium dioxide accumulated in the filtration angle and in the corneoscleral junction in both Tween-exposed and nonexposed eyes (Fig. 16). When colloidal carbon (42–135 mμ in diameter) was introduced into the anterior chamber, the particles accumulated in sites similar to those of thorium accumulation but did not enter Descemet's membrane in either Tween-exposed or normal eyes.

Discussion

The association of corneal edema with corneal endothelial lesions, as is demonstrated in the present model, has been observed in numerous spontaneous, iatrogenic and experimentally produced ocular conditions in man and other animals.[1,17–31] It is widely believed that an abnormal corneal endothelium is causally related to the accompanying corneal edema. As a consequence, it has been suggested that the edematous fluid may be derived from aqueous humor percolating through Descemet's membrane and a damaged corneal endothelium.[28,34,35] Alternatively, it has been postulated that corneal endothelial lesions interfere with a physiologic dehydrating mechanism of the cornea.[13,36] The latter hypothesis arose largely from the observation that the excised cornea is markedly hydrophilic, particularly after the corneal endothelium has been stripped. Attempts to demonstrate an active transport of water or a solute from the substantia propria and across the corneal endothelium

have so far been unsuccessful, with the result that the entire concept is open to question.[37] Furthermore, the discovery of Dohlman, Brown, and Martola[38] that some types of corneal edema can be treated satisfactorily by suturing a transparent silicone membrane directly to the posterior surface of the cornea, and that the overlying stroma remains clear disputes the concept of a significant corneal endothelial dehydrating pump. If the maintenance of corneal deturgescence is not dependent upon the active transport of water out of the cornea, an explanation is still required for the failure of the normal cornea to imbibe excessive water. Bearing upon this problem, Langham[37] has suggested that the cornea does not normally swell because of a balance between the imbibition pressure of the corneal mucopolysaccharides and the cohesive forces between the structural components of the stroma.

Although it is widely accepted that the limbal vascular plexus contributes significantly to the normal corneal nutritional fluid,[2,39-41] scant attention has been devoted to an increased limbal vascular permeability in the genesis of corneal edema. In this regard Langham[27] demonstrated an increased permeability of the capillary limbal vessels to the water-soluble vital dye Pontamine sky blue in alloxan-induced corneal edema —a type of edema also associated with corneal endothelial lesions.

It is clear that an increased permeability of the limbal vessels contributes significantly to the corneal edema in the present model. The migration of the intravascular markers—Evans blue, human gamma globulin, and thorium dioxide—into the cornea demonstrates the increased permeability of the limbal vessels in the Tween-treated eyes. The diffusion of these substances is evident from the periphery of the cornea and is almost certainly associated with fluid.

The present study is not concerned with the ultrastructural mode of transport of colloidal particles across the corneal endothelium—a problem which has been thoroughly investigated by Kaye and coworkers.[42-44] It is clear that colloidal thorium traverses the corneal endothelium and diffuses through Descemet's membrane in normal rabbits' eyes after being introduced into the anterior chamber. If the percolation of aqueous humor through Descemet's membrane into the corneal stroma is a prime factor in the genesis of the corneal edema, one would expect this to be accompanied by the diffusion of thorium through Descemet's membrane. However, this electron-dense tracer was not visualized in Descemet's membrane in the Tween-induced edematous cornea despite a concentration gradient predisposing to diffusion toward the substantia propria. It is possible that some of the thorium may have been aggregated by and entrapped in necrotic cell debris, but this explanation cannot fully account for the virtual absence of thorium par-

ticles in Descemet's membrane, as many particles were present in the corneoscleral junction and corneal limbus. The passage of thorium dioxide into Descemet's membrane may be dependent upon an active transport mechanism of the corneal endothelium, as has been implicated for other substances.[37] Alternatively, a flow of fluid from the edematous cornea toward the aqueous humor may have impeded the passage of this marker through Descemet's membrane in the Tween-treated rabbits.

The pronounced corneal endothelial cytolysis is presumably due to the combination of the surfactant with cellular membranes.[45–48] The findings in the present study do not in themselves refute the role of corneal endothelial disintegration in the genesis of corneal edema. However, when viewed in relation to the previously mentioned data, they raise the question whether other reported instances of human and experimental corneal edema ascribed to corneal endothelial damage per se are perhaps, at least in part, manifestations of an increased limbal vascular permeability.

Summary

It was observed that the injection of a surfactant, Tween 80, in normal saline into the anterior chambers of rabbits' eyes promptly and regularly produced marked corneal edema which was accompanied by marked corneal endothelial cytolysis and an increased limbal vascular permeability. This model was utilized to obtain information concerning the mechanisms of fluid transportation into the corneal tissues. The findings were viewed in relation to the normal and pathologic states of corneal hydration, and particularly with regard to the possible causal relationship between corneal endothelial lesions and corneal edema.

The migration of intravascular markers—Evans blue, human gamma globulin, and thorium dioxide—into the cornea from the limbal vessels, clearly implies that an increased limbal vascular permeability contributes to corneal edema in the present model. When thorium dioxide was introduced into the anterior chambers of normal rabbits' eyes, the colloidal particles readily traversed the corneal endothelium and Descemet's membrane. In the Tween-exposed eyes, particles of thorium were observed adjacent to Descemet's membrane, but not within the membrane.

The present study does not in itself refute the role of corneal endothelial cytolysis in the genesis of corneal edema. However, when viewed in relation to other studies, it seriously raises the question whether some other reported instances of corneal edema ascribed to endothelial damage are perhaps, at least in part, manifestations of increased limbal vascular permeability, rather than solely expressions of endothelial damage per se.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

Key:

CE	corneal endothelium	Pe	pericyte
DM	Descemet's membrane	R	red blood cell
E	vascular endothelium	SP	corneal substantia propria
EP	corneal epithelium	UC	unidentified cell
I	interstitial space	er	endoplasmic reticulum
IB	iridial blood vessel	m	mitochondria
IS	iridial stroma	n	nucleus
L	lumen		

FIG. 1–4. Within 5 min. of intraocular instillation of 25–100% Tween, conjunctival congestion is evident (Fig. 1). Cornea and conjunctiva regularly manifest marked edema 2 hr. later (Fig. 2). Conjunctival edema usually subsides in a few hours, but cornea remains edematous and markedly opaque for more than 10 days (Fig. 3) in contrast to normal cornea (Fig. 4).

FIG. 5. Cornea manifests marked increase in thickness following intraocular instillation of Tween (bottom). Edematous cornea regularly becomes 3–4 times its normal thickness (top). Hematoxylin and eosin stain. × 110.

FIG. 6. Less than 2 hr. after intraocular instillation of Tween, perivascular infiltrate of leukocytes occurs at corneal limbus, associated with deposition of fibrinoid material in lumina and walls of blood vessels (arrows). Periodic acid-Schiff stain. × 250.

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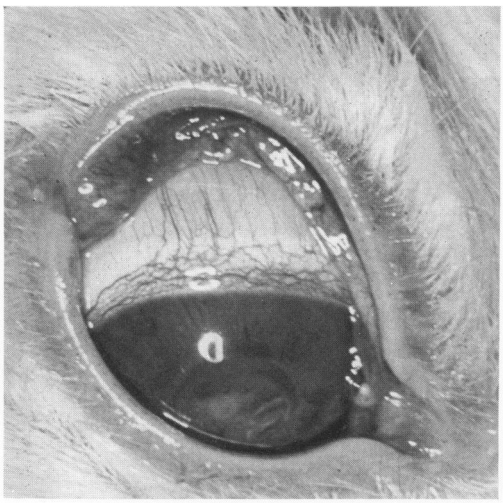

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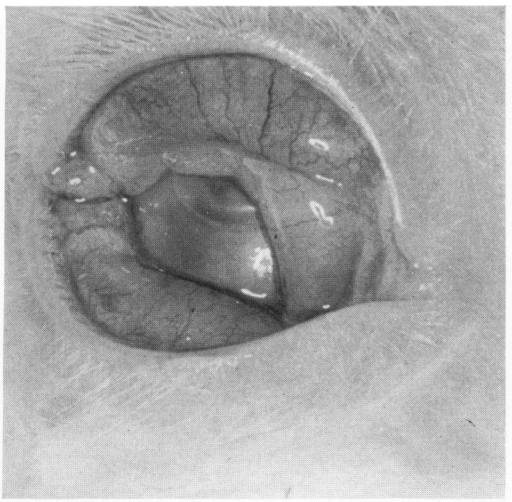

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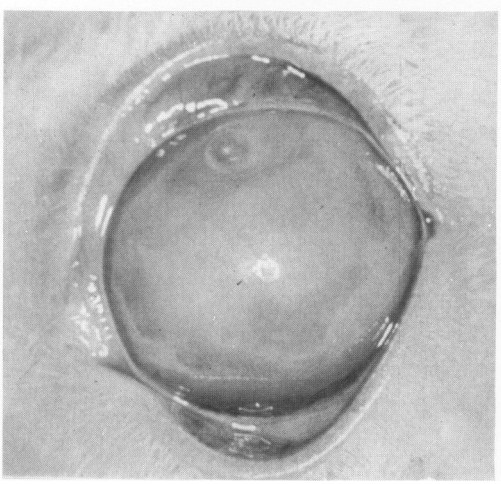

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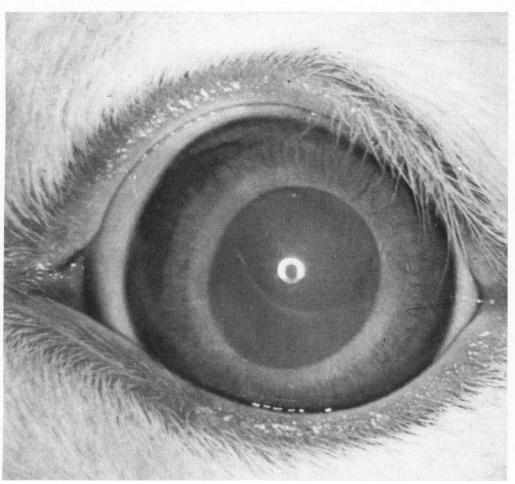

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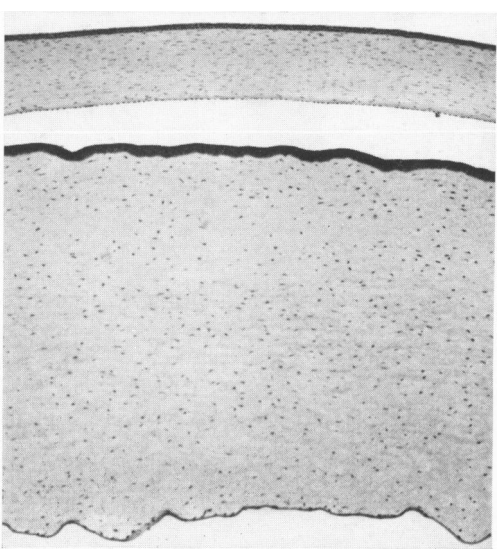

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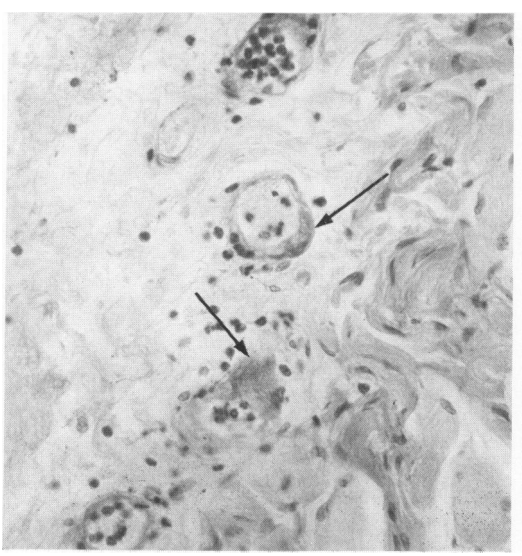
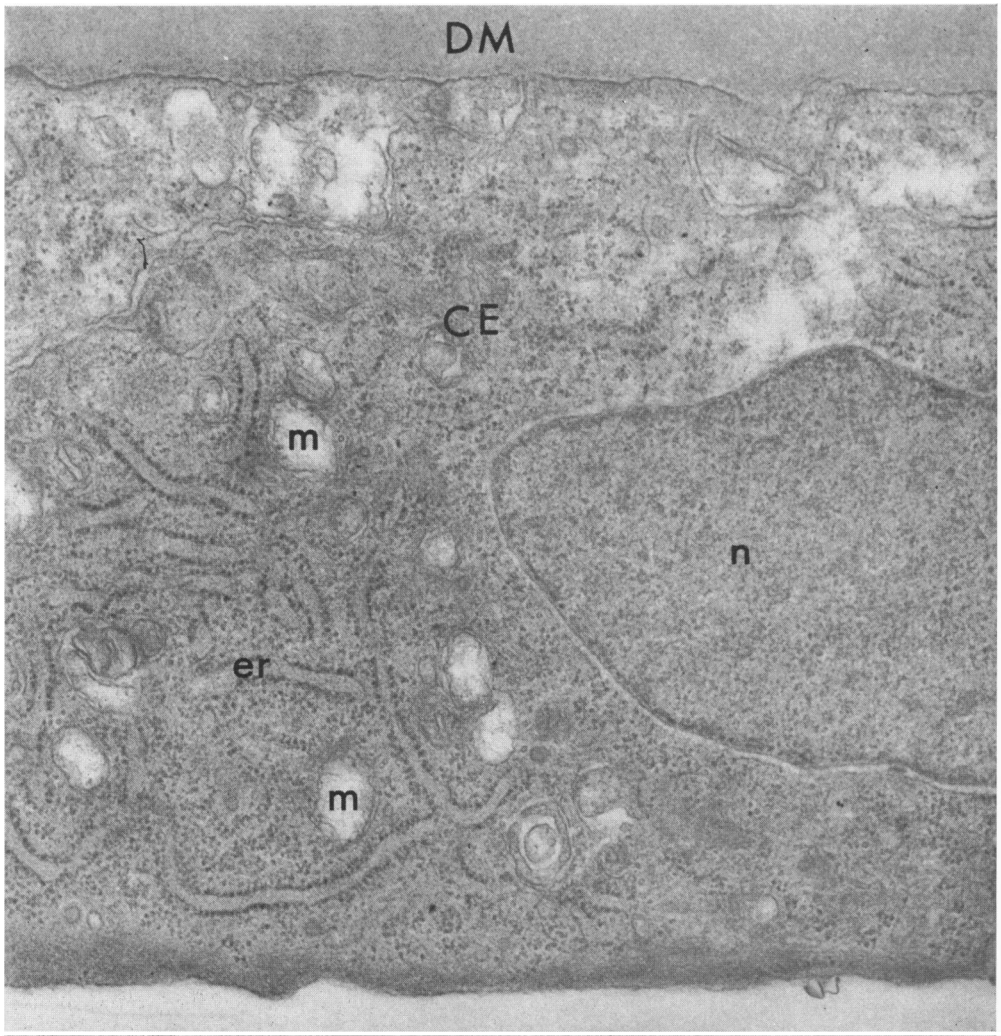


FIG. 7. Normal rabbit corneal endothelium contains prominent rough endoplasmic reticulum and numerous mitochondria. × 39,500.

FIG. 8. Following intraocular instillation of Tween, many corneal endothelial cells disintegrate while only vestiges of others, such as this, remain. × 26,000.



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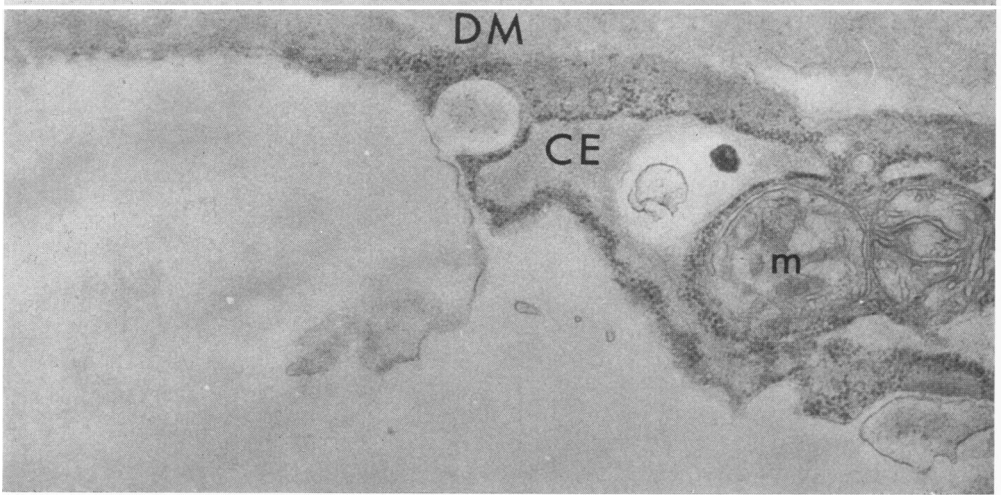

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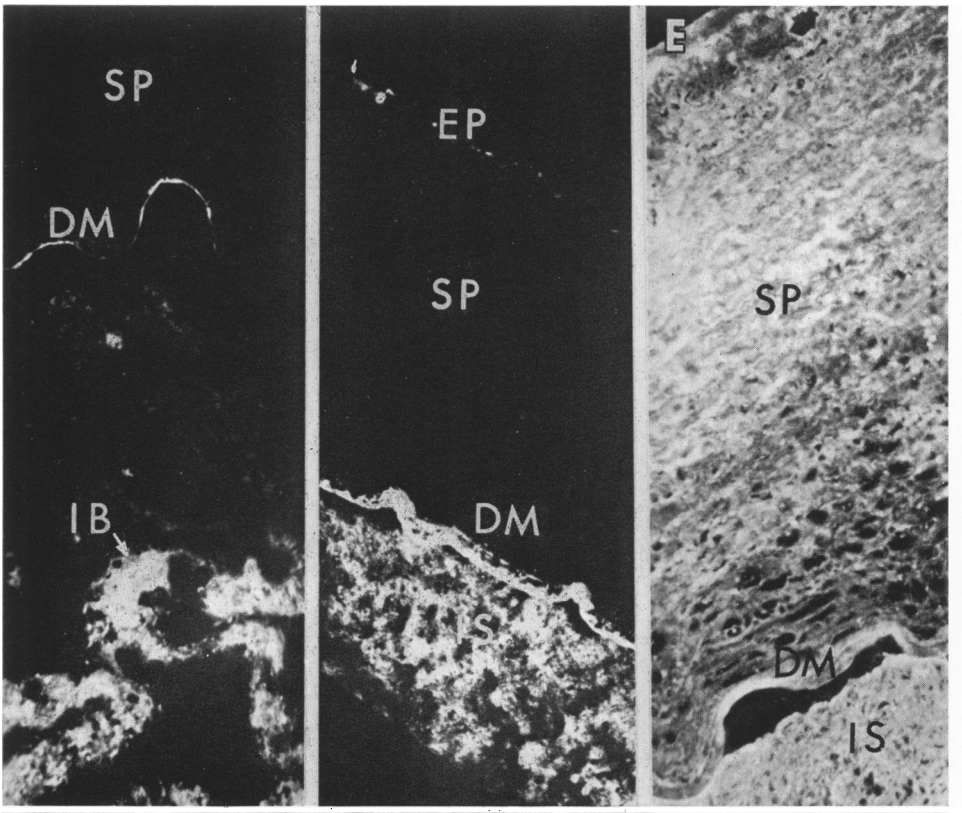
FIG. 9. Following intravenous administration of human gamma globulin, specific fluorescence is exhibited in normal rabbit only in sites of blood vessels, such as those in the iris (left). At 1 hr. after intraocular instillation of Tween and intravenous injection of human gamma globulin, entire iridial stroma manifests diffuse and generalized fluorescence while corneal substantia propria still remains unaffected (center). At this stage diffuse fluorescence is evident also at limbus (not shown in this micrograph). At 24 hr. after introduction of Tween and gamma globulin, the latter is readily detected throughout entire cornea and iridial stroma (right). × 63.

FIG. 10. In the absence of exposure to Tween, particles of thorium are restricted to lumen of limbal blood vessels, 5–10 min. after intravenous administration of this electron-dense marker. × 29,000.



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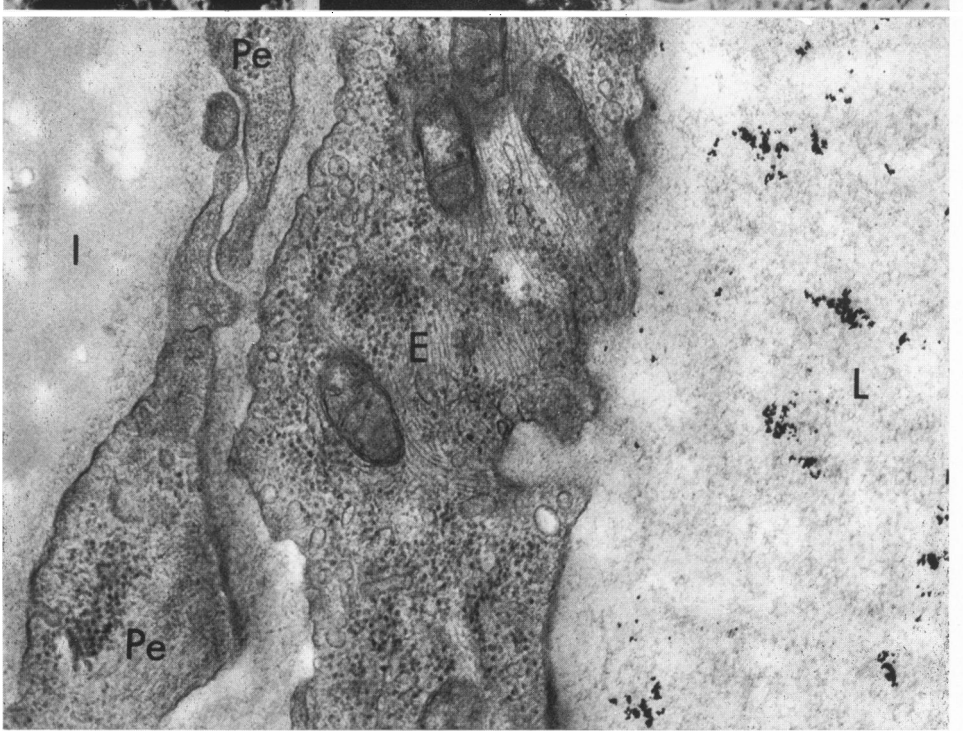

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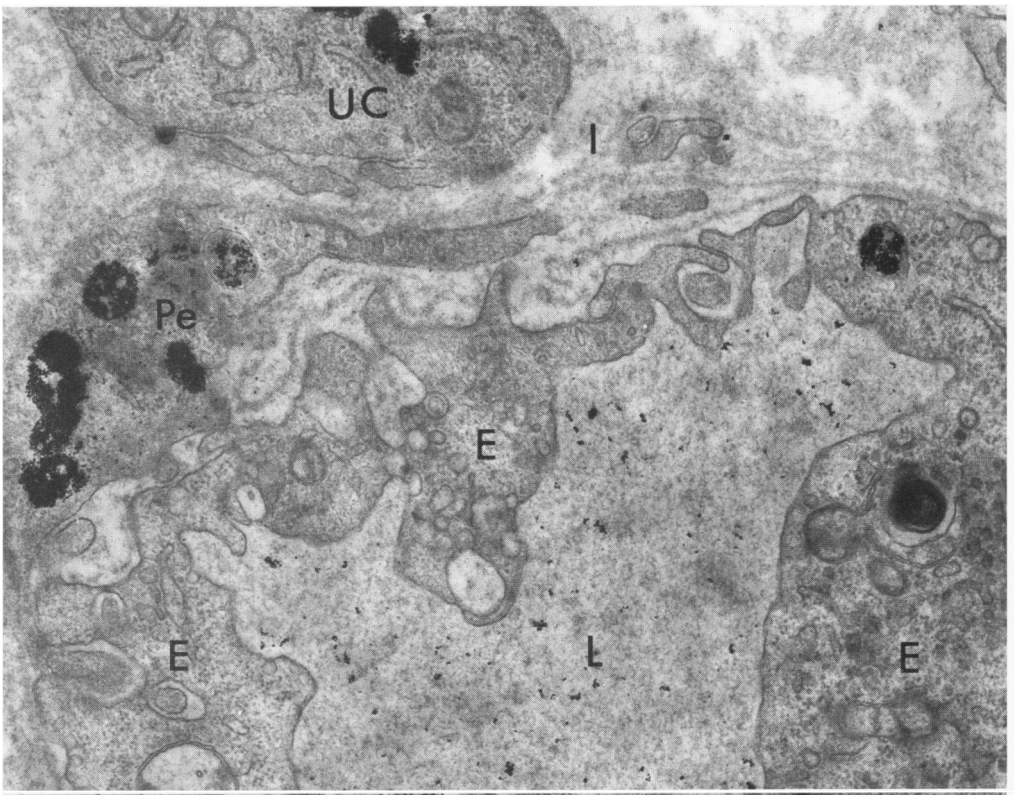
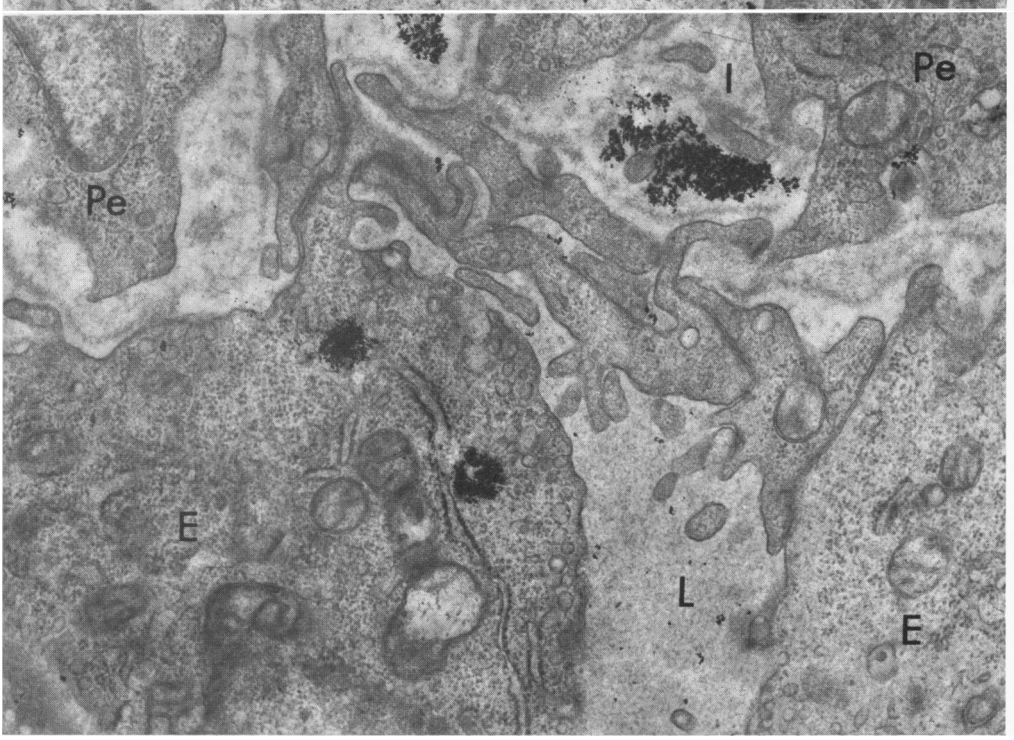
FIG. 11. In less than 30 min. after intraocular instillation of Tween and 5–10 min. after intravenous injection of thorium dioxide, lumina of corneal limbal blood vessels contain numerous grains. In addition to vascular labeling, there is extravascular leakage of electron-dense grains, with the latter appearing in the interstitial space and within cytosomes of vascular endothelium, pericytes, and other contiguous unidentified cells. × 29,000.

FIG. 12. Particles of thorium are shown in lumen of limbal blood vessel, as well as within vascular endothelium, pericytes, and perivascular interstitial tissue under same circumstances as those described for Fig. 11. × 28,500.



11



12

FIG. 13. This diagram summarizes the extravasation of intravascular markers following introduction of Tween into the anterior chamber. In control animals the markers remain within lumina of blood vessels (top). Less than 1 hr. after intraocular instillation of Tween and intravascular administration of thorium dioxide or human gamma globulin, the latter substances appear in perivascular regions of anterior segment of eye (center). By 24 hr. these markers are dispersed throughout entire cornea and iris (bottom).

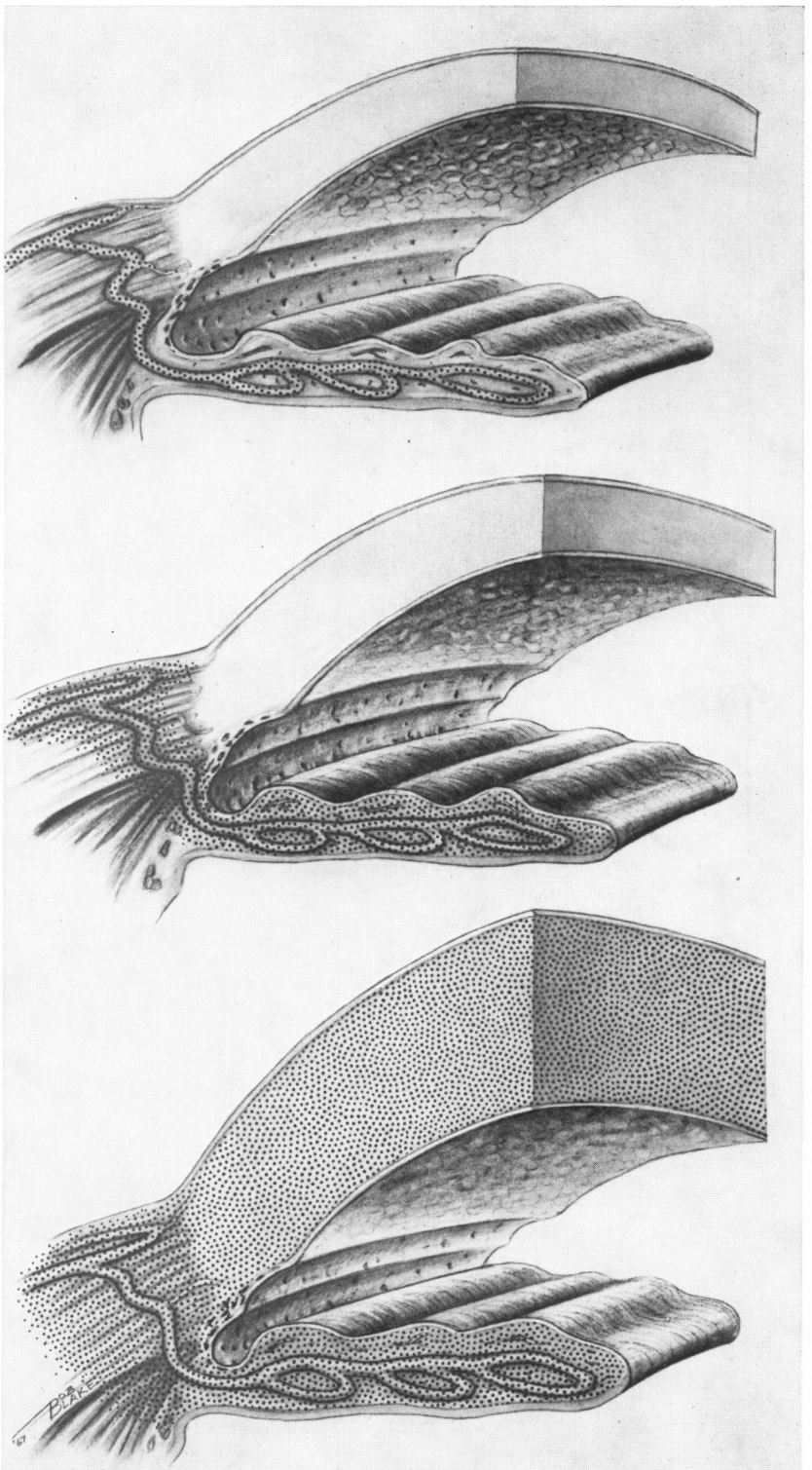


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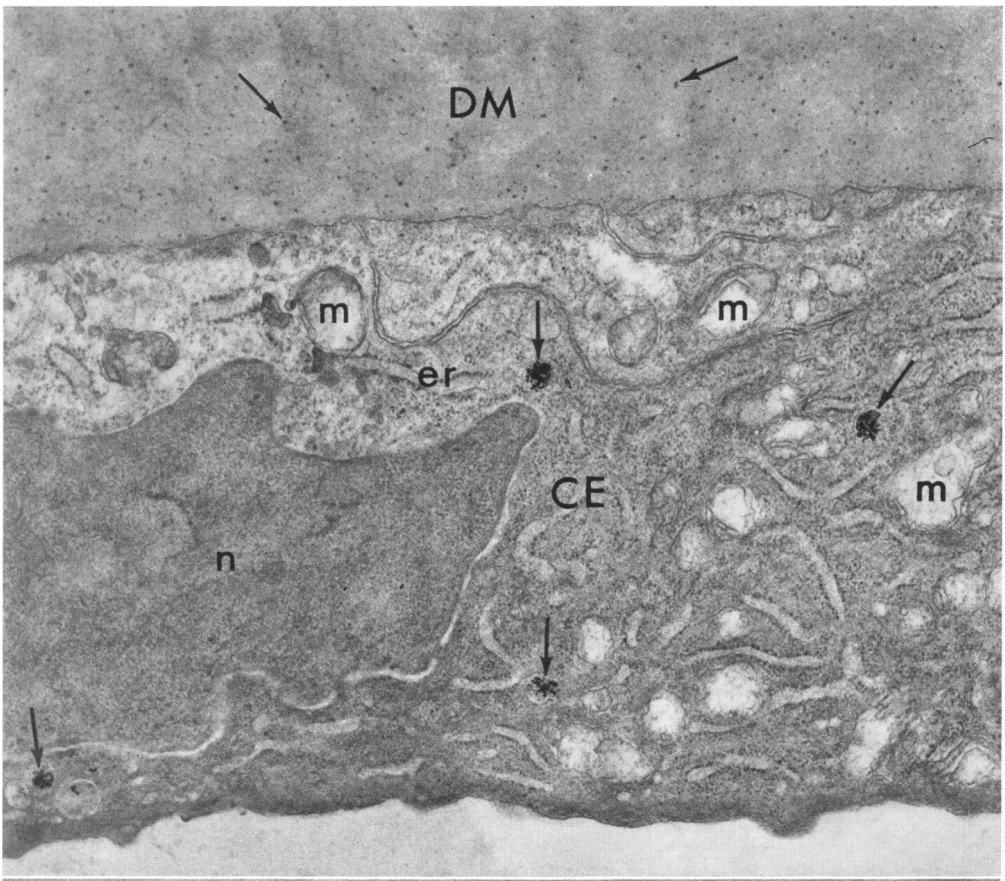
FIG. 14. At 24 hr. after Thorotrast is introduced into anterior chamber of normal rabbit's eye, the particles (arrows) occur within cytoplasm of corneal endothelium, as well as within Descemet's membrane. × 13,500.

FIG. 15. In contrast to the control (Fig. 14), grains of thorium dioxide aggregate adjacent to Descemet's membrane but do not traverse it if Thorotrast is instilled into the aqueous humor 30 min. or 24 hr. after Tween instillation. × 13,500.



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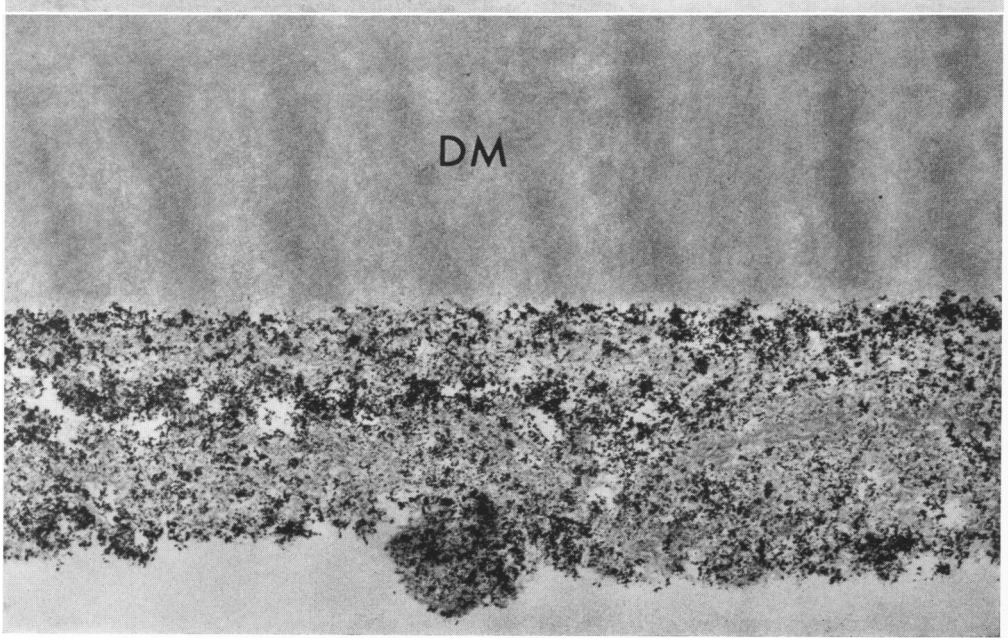
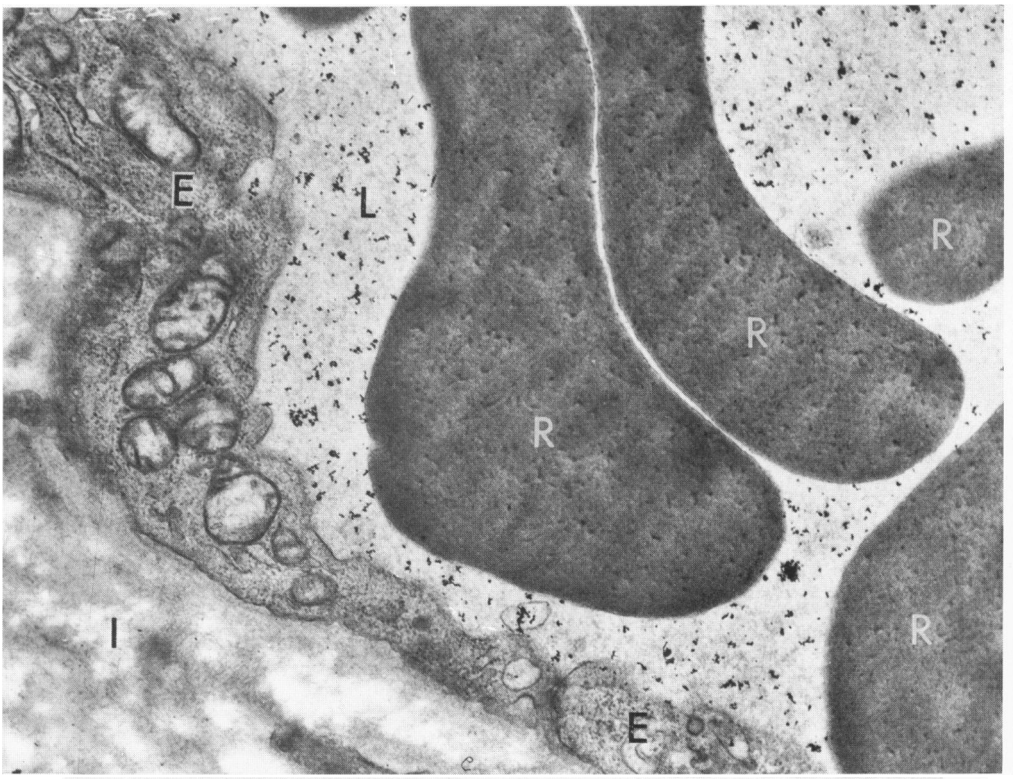


15

FIG. 16. Lumen of limbal blood vessel from same eye as in Fig. 15 contains particles of thorium, demonstrating their migration from anterior chamber into corneal limbus. × 22,000.

FIG. 17. This diagram summarizes findings shown in Fig. 14 and 15 after Thorotrast is introduced into anterior chamber of normal (top) and Tween-exposed (bottom) rabbit's eyes. In both instances, particles appear in the filtration angles.



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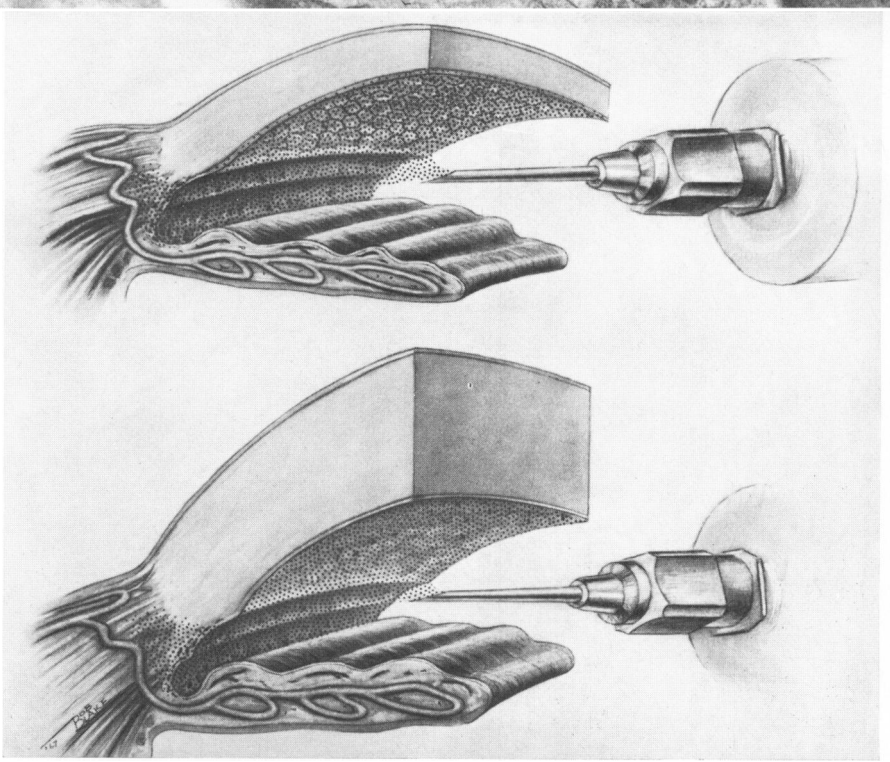

17