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Electron Microscopy of the Wall of Iris Vessels in Eyes with and without Exfoliation Syndrome (Pseudoexfoliation of the Lens Capsule)

AMUND RINGVOLD

University Eye Department, Rikshospitalet, Oslo, Norway
and Anatomical Institute, University of Oslo, Oslo, Norway

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Elektronenmikroskopie der Irisgefäßwände in Augen mit und ohne Exfoliations-Syndrom

Zusammenfassung. Die elektronenmikroskopische Untersuchung der Wände der Irisgefäße bei 4 Augen mit Kataract *mit* und 8 Augen mit Katarakt jedoch *ohne* Exfoliationssyndrom brachte folgende Ergebnisse:

1. Die Irisgefäße der kataraktbefallenen Augen ohne Exfoliationssyndrom sahen aus wie normale Irisgefäße.

2. Die Irisgefäße der kataraktbefallenen Augen mit Exfoliationssyndrom unterscheiden sich von normalen Irisgefäßen: Jeweils auf der Außenseite der Endothelzellen findet sich eine ungewöhnliche extracelluläre Substanz. Diese besteht aus einer fibrillären und einer nichtfibrillären Komponente. In Gefäßen mit beträchtlichen Mengen dieser extracellulären Substanz wurden die Basalmembranen als dünn und als stellenweise unterbrochen erkannt. Der jeweils äußere Teil der Bindegewebshülle der Gefäße zeigte ein normales Aussehen. Hieraus wird abgeleitet, daß das Auftreten der abnormen extracellulären Substanz mit dem Exfoliationssyndrom wesensmäßig verbunden ist und die Morphologie der fibrillären Komponente der extracellulären Substanz mit ähnlichen Befunden im Rahmen früherer Beschreibungen des sogen. Exfoliationssyndromes übereinstimmt.

Summary. An electron microscopic study was performed of the vessel wall of the iris in four eyes with cataracts without the exfoliation syndrome and in eight eyes with cataracts with the exfoliation syndrome. The following results were obtained:

1. Iridial vessels in cataractous eyes without exfoliation syndrome appeared like vessels of the normal iris.

2. Iridial vessels in cataractous eyes with exfoliation syndrome appeared different from normal: An abnormal extracellular material was found outside the endothelial cells. This material consisted of fibrillar and nonfibrillar components. The basement membrane was often thin and sometimes interrupted in the vessels with a great amount of abnormal extracellular material. The outer part of the connective tissue layer was always normal.

It is concluded that the appearance of the abnormal extracellular material is associated with the exfoliation syndrome, and that the morphology of the fibrillar component of this material agrees with previous descriptions of exfoliation material.

Introduction

In eyes with exfoliation syndrome (pseudoexfoliation of the lens capsule) the exfoliation material is found by clinical and light microscopic examinations on the anterior lens capsule, on the zonules, on the anterior hyaloid membrane, on the ciliary body, on the posterior and anterior iris surfaces, on the pupillary margin, and in the trabecular area (Busacca, 1928; Dvorak-Theobald, 1954; Sunde, 1956;

Hörven, 1966). Sometimes exfoliation material is present as small flakes on the corneal endothelium and on pupillary membrane threads (Tarkkanen, 1962; Hörven, 1935).

The ultrastructure of exfoliation material from the posterior iris surface and the zonules was described by Blackstad *et al.* (1960), and later electron microscopic studies showed exfoliation material on and within the lens capsule (Bertelsen *et al.*, 1964; Ashton *et al.*, 1965). Furthermore, granular filaments similar to exfoliation material was demonstrated on the ciliary body, and in the perivascular area of iris vessels in eyes with exfoliation syndrome (Shakib *et al.*, 1965). Such filaments were not found by electron microscopic studies of normal human iris vessels (Purtscher, 1966; Vegge and Ringvold, 1969).

The purpose of the present work is to study the ultrastructure of iris vessels in eyes with exfoliation syndrome, with particular attention on changes associated with this condition. Since the material is from cataractous eyes with exfoliation syndrome, an investigation of iris vessels in cataractous eyes without exfoliation syndrome is included in the study.

Material and Methods

Iris tissue from 12 cataractous human eyes were obtained from the 12 o'clock position by performing a full iridectomy during surgical cataract procedure. Prior to surgery exfoliation material was demonstrated in 8 of these eyes, while the remaining 4 eyes (examined in mydriasis) showed no sign of exfoliation syndrome. No other abnormalities were present in any of these normotensive eyes. The 4 patients without exfoliation syndrome were from 74 to 84 years old, and the 8 patients with exfoliation syndrome were from 54 to 83 years old at the time of operation. All specimens were placed immediately in precooled 1% OsO_4 buffered to pH 7.3 with phosphate buffer, and with few exceptions they were fixed for 1—2 hours. One of the specimens without exfoliation syndrome was fixed for 5 h, and two of the specimens with exfoliation syndrome were fixed for 3 respectively 6 h. Tissue blocks from two of the eyes without exfoliation syndrome were dehydrated in increasing concentrations of alcohol with 1% uranyl acetate added to the first of the two 100% alcohol baths. All other preparations were dehydrated in increasing concentrations of acetone. Araldite was the infiltrating and embedding medium. Sections were made with an LKB Ultrotome, and stained with uranyl acetate followed by lead citrate. Siemens Elmiskop 1 b and 1 A were used.

Results

Cataractous Eyes without Exfoliation Syndrome. In these preparations the vessels showed the same appearance as previously reported from normal human iris vessels (Purtscher, 1966; Vegge and Ringvold, 1969).

The thickness of the *endothelial wall* varied, and the transitions between thicker and thinner parts were usually gradual (Fig. 1). The endothelial cells contained mitochondria, Golgi apparatus, rough and smooth surfaced endoplasmic reticulum in moderate amounts. Zonulae occludentes were present between neighbouring endothelial cells (Fig. 3), and marginal flaps at the intercellular junctions were sometimes seen (Fig. 2).

The *basement membrane* was separated from the endothelial wall by a lamina rara. The thickness of the basement membrane varied up to 2—3 μ, often filling up the space between the endothelium and the cells just outside (Figs. 1, 2). The basement membrane was granular intermingled with irregularly scattered filaments and collagen fibrils (Fig. 4). Granular, moderately dense looking bodies

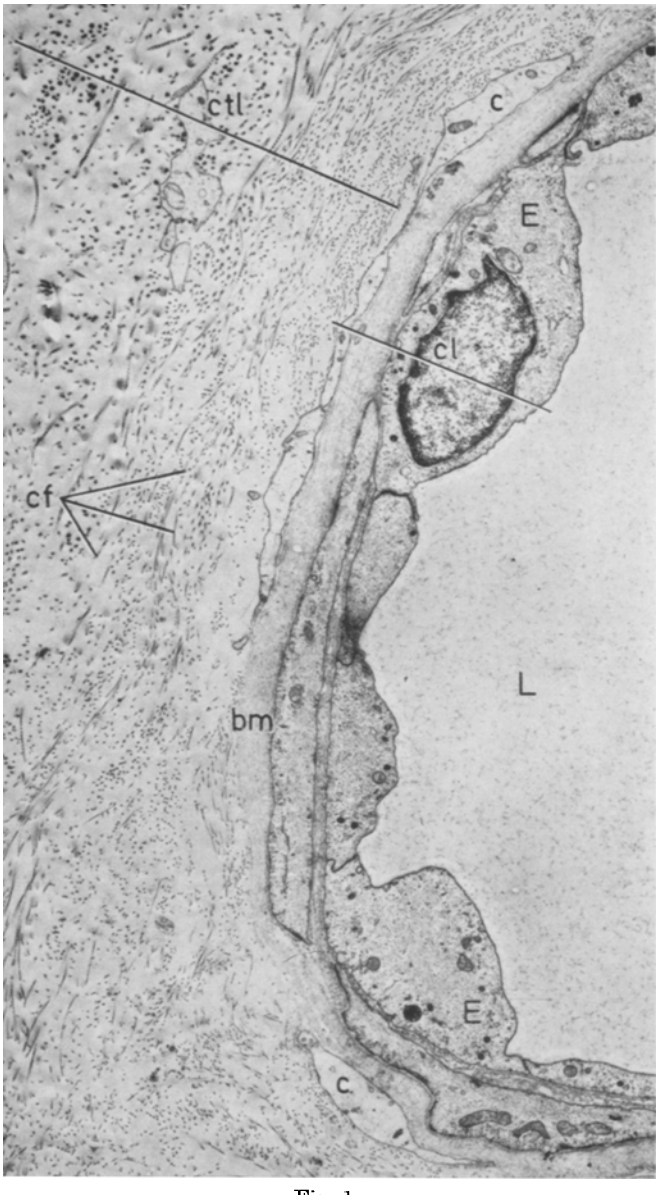

Fig. 1

Key to all figures: Figs. 1—4 are from eyes without exfoliation syndrome. Figs. 5—10 are from eyes with exfoliation syndrome.

abem	abnormal extracellular material	*E*	endothelium
bm	basement membrane	*f*	filaments
C	cells with a pale cytoplasm	*fcb*	fibrils showing cross bands
cf	collagen fibrils	*g*	granules
cl	cellular layer of the vessel wall	*L*	vessel lumen
ctl	connective tissue layer of the vessel wall	*sm*	smooth muscle cell
db	dense body	*zo*	zonula occludens

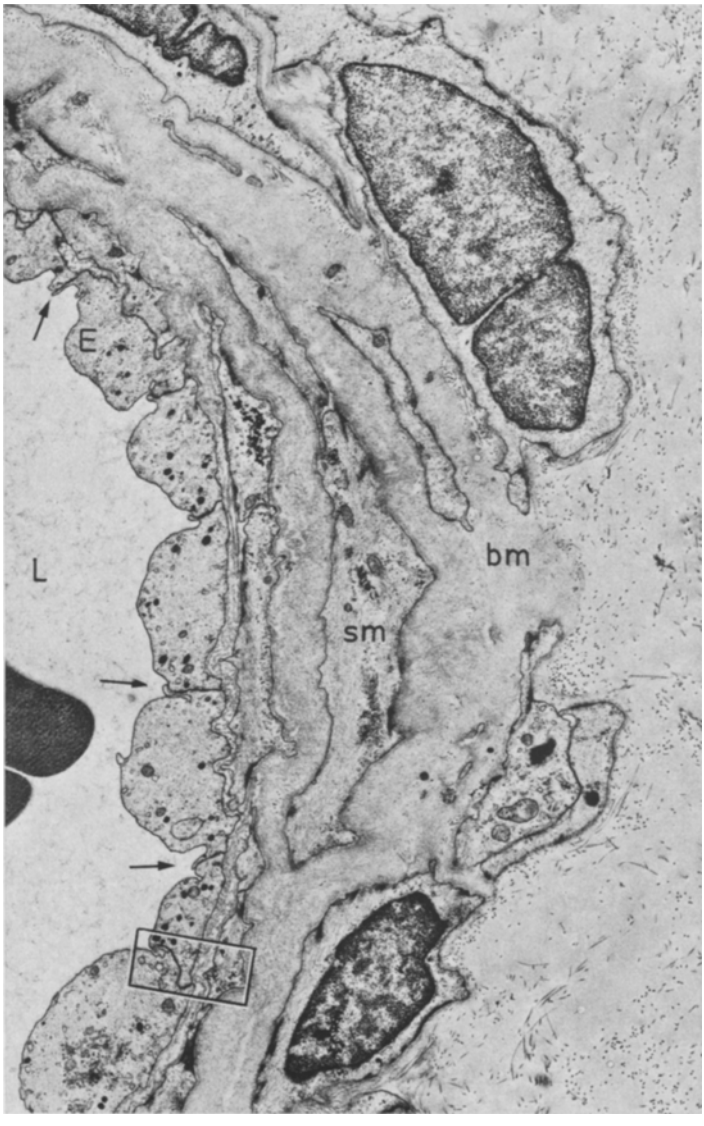

Fig. 2. Marginal flaps at arrows. Note the thick basement membrane. *sm* smooth muscle cell. ×6,900

were found within the basement membrane, and some of them were surrounded by triple-layered membranes (Fig. 4). Only extremely seldom did they appear next to the cellular walls.

Fig. 1. The inner part of the connective tissue layer (*ctl*) is made up of collagen fibrils measuring about 300 Å thickness. Cells (*C*) with a pale cytoplasm and few organelles are sometimes found outside the basement membrane. *L* vessel lumen, *E* endothelium, *bm* basement membrane, *cl* cellular layer of the vessel wall, *cf* collagen fibrils. ×6,900

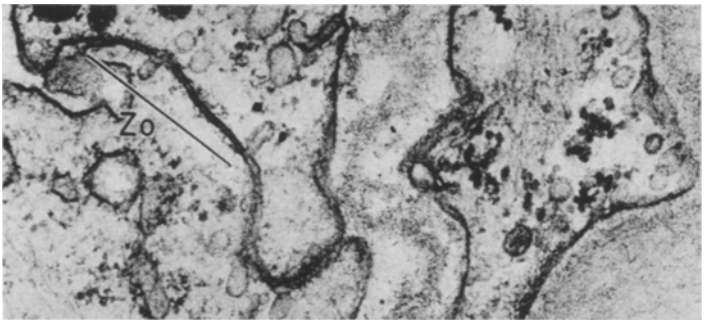

Fig. 3. Detail of a zonula occludens (*zo*) from Fig. 3. ×45,000

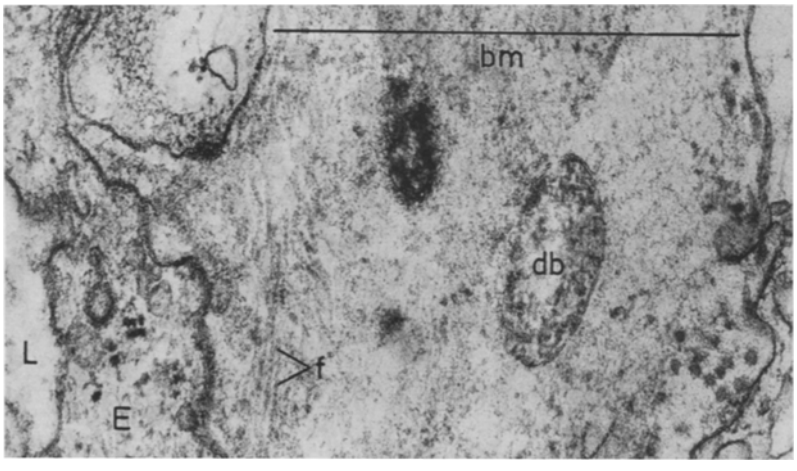

Fig. 4. Dense body (*db*) in the basement membrane surrounded by a triple-layered membrane. ×60,000

Pericytes or muscle cells were mostly present in the vessel wall. The muscle cells formed a discontinuous layer around the larger vessels, and usually not more than two layers of muscle cells were found. Outside the basement membrane cells with a pale cytoplasm and few organelles sometimes appeared (Fig. 1). The inner part of the *connective tissue layer* showed collagen fibrils of about 300 Å thickness (Fig. 1). They ran mainly parallel to the longitudinal axis of the vessel, and among them appeared dense bodies similar to those described above. This thin-fibrillar area was encircled by an outer zone built up of collagen fibrils measuring about 1000 Å thickness. Here too, the fibrillar course mostly followed the longitudinal axis of the vessel, but in both zones several obliquely and circularly running fibrils were observed. The fibrils were more densely packed in this outer zone than in the iris stroma, sometimes appearing in bundles. These bundles seemed to be more frequent in specimens dehydrated in alcohol with uranyl acetate, but general conclusions on this point cannot be drawn on account of this small material. At the outer border of the connective tissue layer some of the collagen fibrils gradually changed their directions radiating into the iris stroma. The connective tissue layer was often indistinct in the smallest vessels.

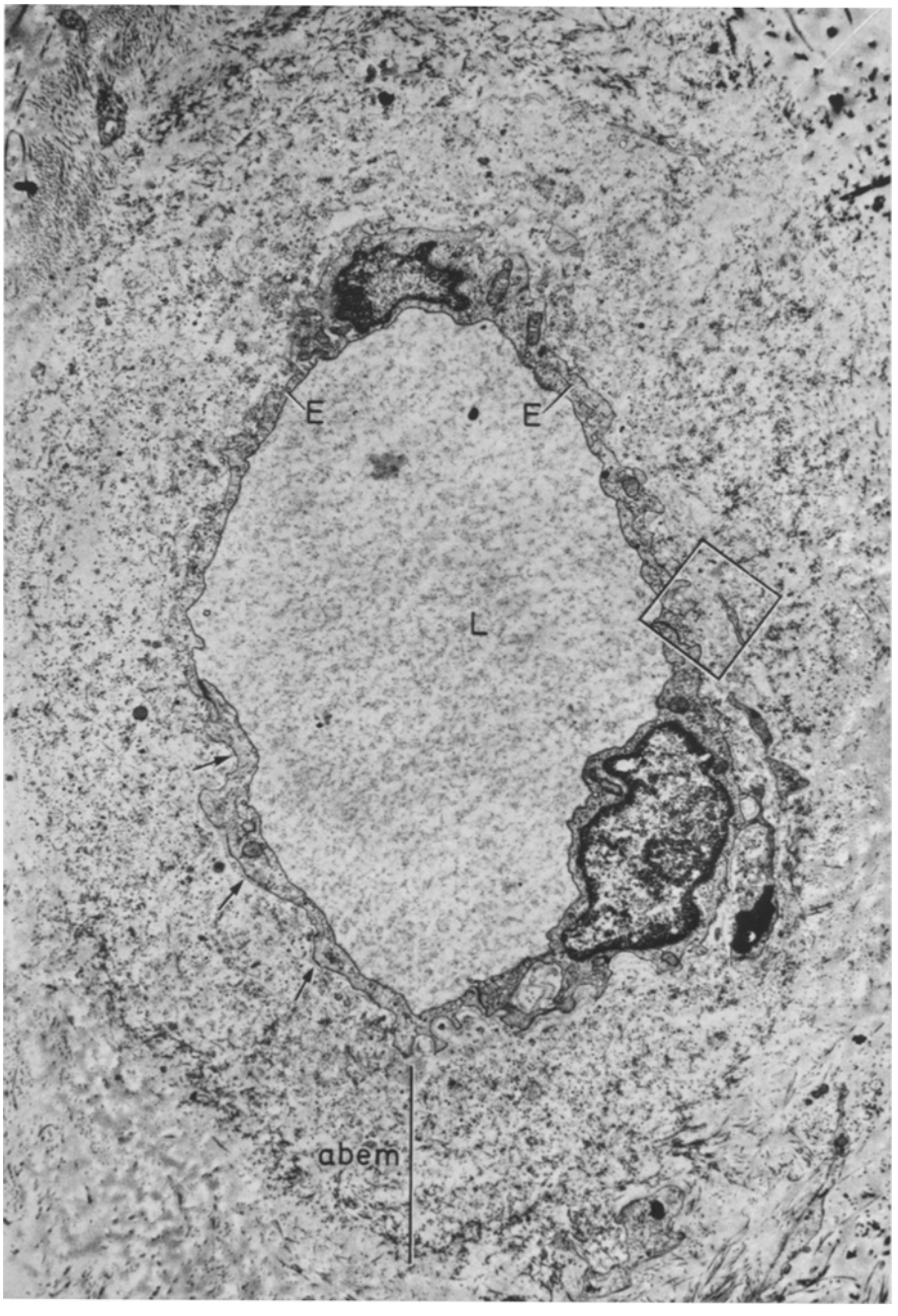

Fig. 5. Endothelial wall surrounded by an abnormal extracellular material (*abem*). Note the thin basement membrane at arrows. ×6,900

Cataractous Eyes with Exfoliation Syndrome. The endothelial cells appeared normal. Outside the endothelial wall an abnormal extracellular material was found in all eyes (Fig. 5). This extracellular material contained fibrillar and nonfibrillar

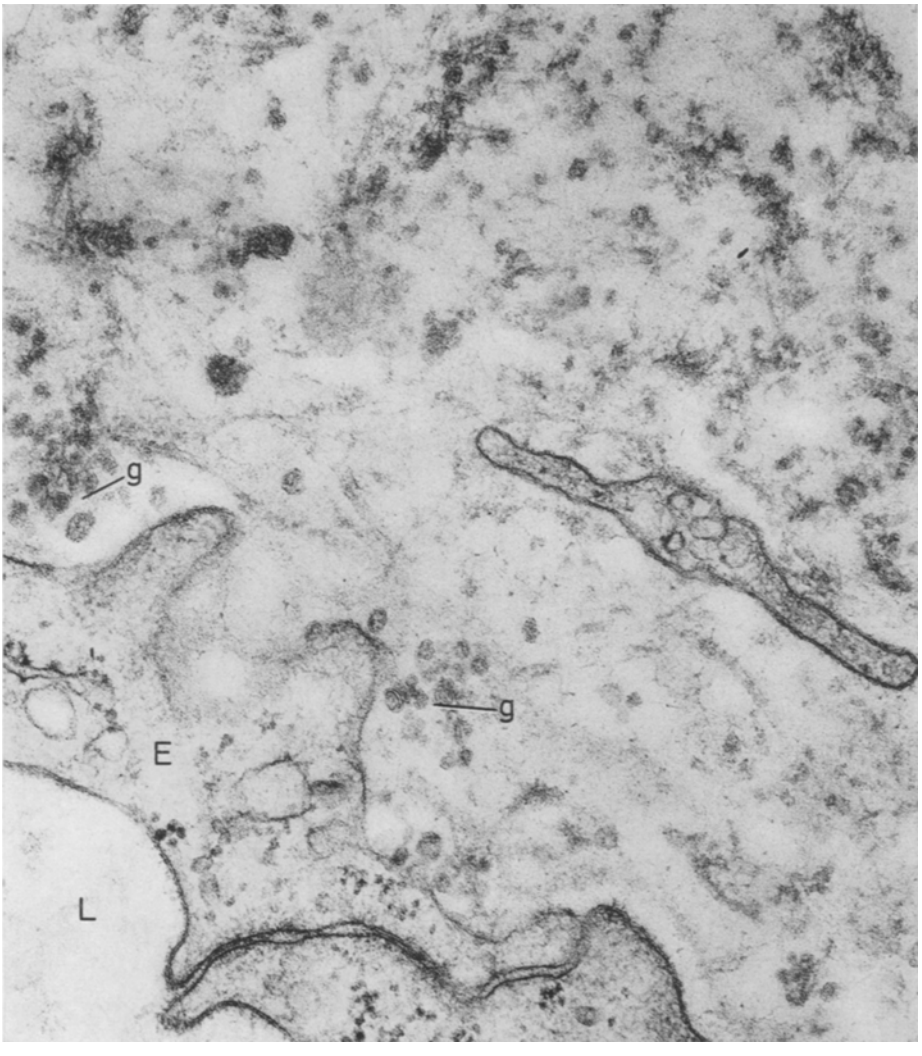

Fig. 6. Detail from Fig. 5. Granules (*g*) limited by triple-layered membranes. ×60,000

components: The fibrillar component showed irregularly distributed fibrils, and their course were usually straight or slightly bent (Fig. 7). The length of the fibrils varied up to 1.2 μ. They were rough surfaced, sometimes ill-defined, sometimes fairly distinctly outlined (Fig. 7). The thickness was subject to great variations along the fibril, and the dimensions were different in fibrils lying next to each other. Estimated at their smallest width the fibrils measured between 70 to 300 Å. Cross bands were found in parts of some fibrils. These were characterized by bands of higher density, crossing the fibrils at various angles, and often protruding beyond the fibril surface on one or both sides (Fig. 7). The distance between cross bands measured 400—560 Å along the middle axis. Fibrils were also found in aggregates with a central structure so dense that details could not be seen.

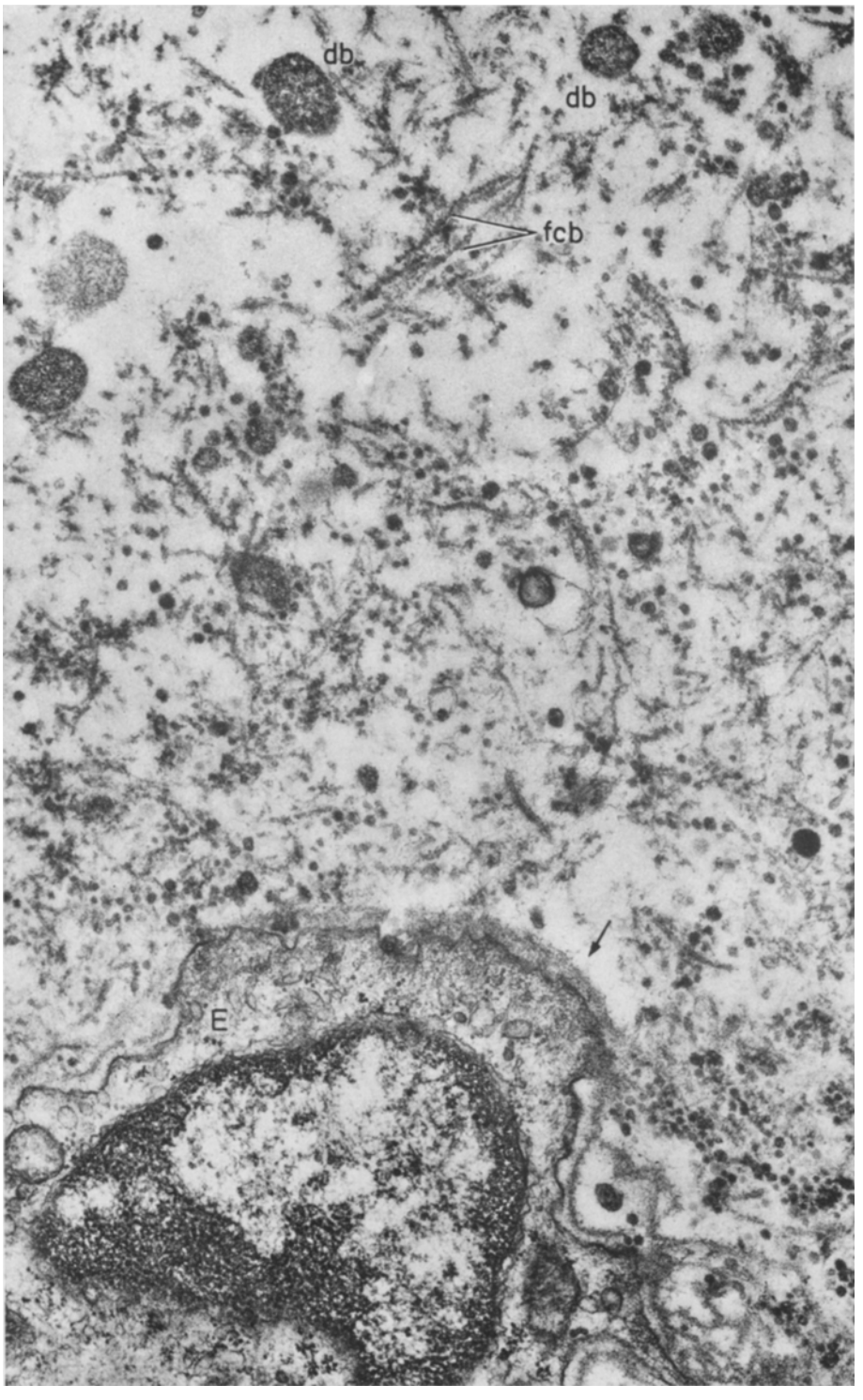

Fig. 7. Straight or slightly bent, rough surfaced fibrils of varying length. Thin basement membrane at arrow. *db* dense body, *fcb* fibrils showing cross bands. ×30,000

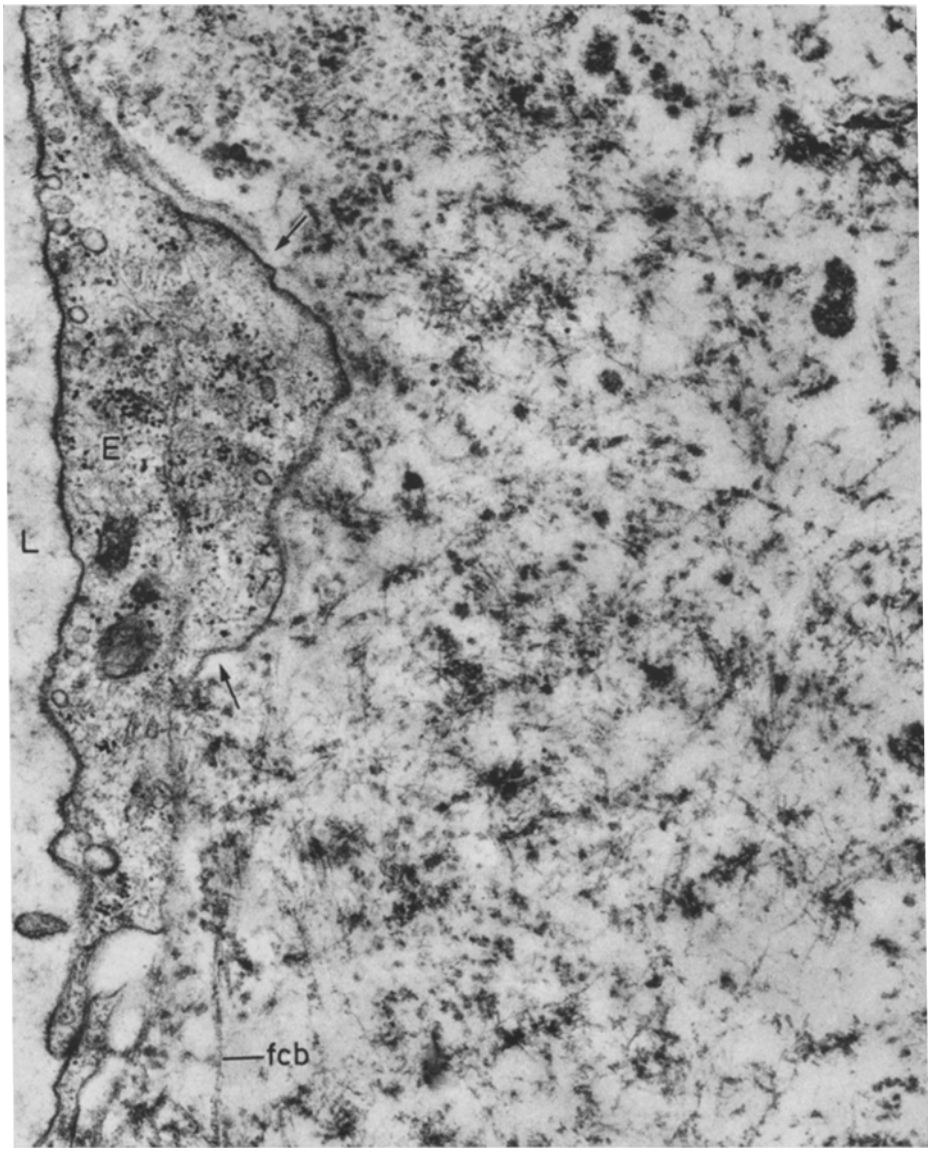

Fig. 8. The basement membrane is missing (arrows). Note the abundance of nonfibrillar elements in the abnormal extracellular material. ×30,000

Among these fibrils, nonfibrillar components as well as collagen fibrils, and dense bodies similar in dimensions and appearance to those described in normal iris vessels (Vegge and Ringvold, 1969) were scattered (Fig. 7). The nonfibrillar elements were mainly round to slightly oval granules sometimes limited by triple-layered membranes (Figs. 6, 7). These granules were usually lying close together, and close to the endothelium, and they measured mostly between 330—500 Å.

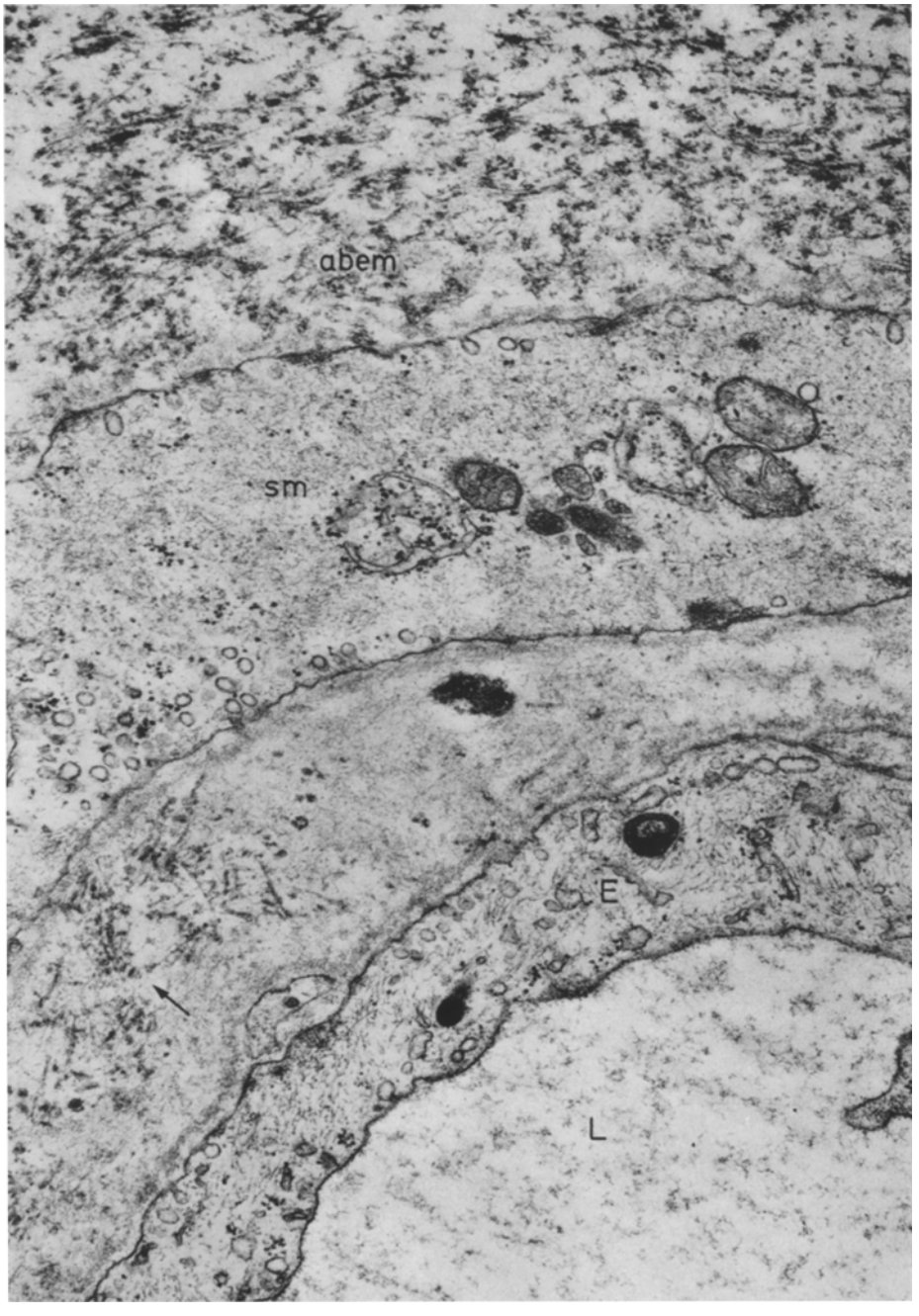

Fig. 9. Abnormal extracellular material lying in close contact to the smooth muscle cell on the abluminal side. The same material is found within the basement membrane at arrow. ×30,000

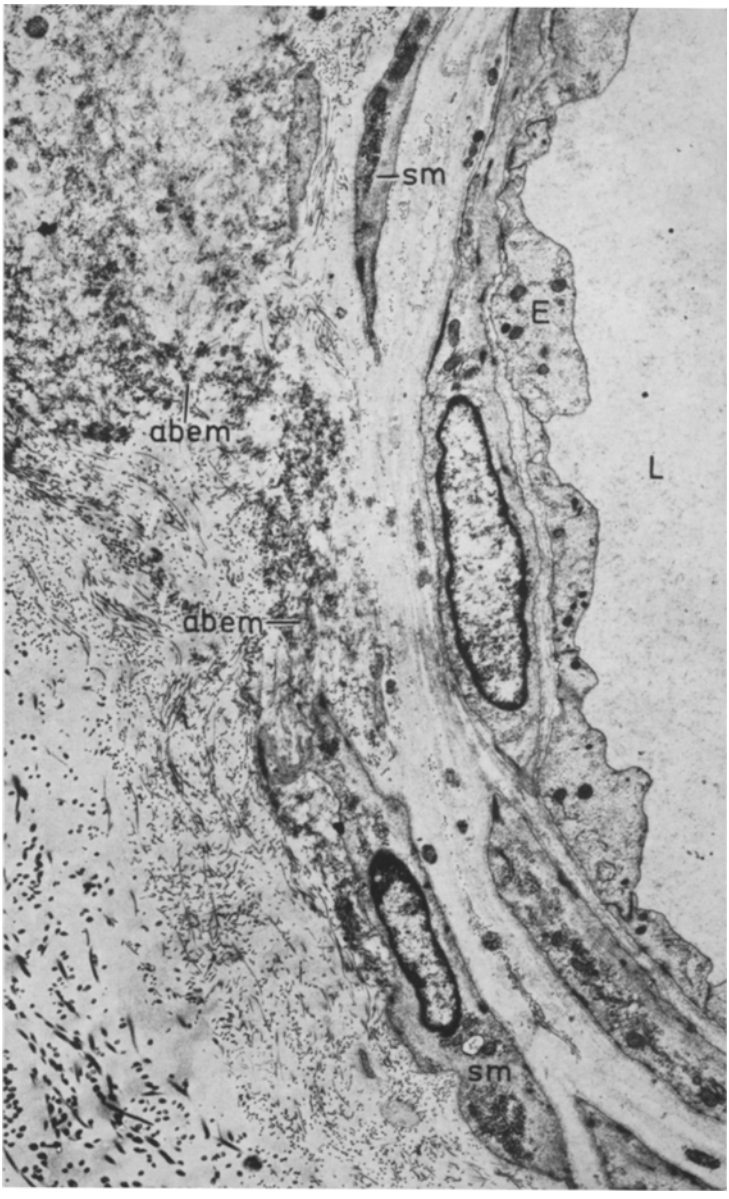

Fig. 10. Abnormal extracellular material present only on the abluminal side of the outer cells No abnormalities are found in the innermost part of the vessel wall. ×6,900

They were far more numerous than the dense bodies. These smaller granules were of varying density, showing an even finely granular appearance with fairly distinct outlines (Figs. 6, 7).

Among the nonfibrillar component were also edged, indistinctly limited elements, and some of the smaller ones were possibly transverse or oblique sections of the

described fibrils. In those vessels that showed the greatest amounts of the abnormal extracellular material, the basement membrane was often thin measuring about 400—500 Å (Figs. 5, 7), and sometimes it was even missing (Fig. 8). Now and then only few of the described fibrils appeared adjoining endothelial cells and pericytes beneath the basement membrane. Usually the basement membrane was separated from the endothelial cells by a lamina rara of normal thickness. Large invaginations of the outer endothelial cell borders, seemingly empty, and with the basement membrane sometimes running straight across them, were also found.

As in normal vessels pericytes and muscle cells were found outside the endothelium, and muscle cells sometimes formed a discontinuous layer around larger vessels. Pericytes and muscle cells were completely surrounded by a basement membrane, which often fused with that of the endothelial cells. The described extracellular material lay in close contact to the pericytes and the muscle cells, and was mostly irregularly scattered in the surrounding basement membrane (Fig. 9). Moderately altered vessels now and then showed such material only on the abluminal side of the cells (Fig. 10).

The abnormal extracellular material was always located in the cellular vessel wall and the innermost part of the connective tissue layer. Only very few collagen fibrils, measuring 300 Å thickness as normal, were found among the extracellular material in the inner part of the connective tissue layer, and round to oval fields of lesser density sometimes appeared in the same region. The thin-fibrillar area of the connective tissue layer was never completely filled up by the described abnormal extracellular material. Outside the abnormal extracellular material the connective tissue layer appeared normal, and bundles of collagen fibrils occured rather frequent in the outer part. The whole connective tissue layer sometimes appeared normal in vessels showing depositions of the abnormal extracellular material in the cellular layer of the vessel wall. The opposite situation was never observed.

In addition to these changed vessels, some normal appearing vessels were also found. They corresponded in all respects to the description of iris vessels in cataractous eyes without exfoliation syndrome. It was a striking feature that normal iris vessels were found close to vessels showing the described changes.

Discussion

To avoid possible influences upon iris vessel morphology caused by raised intraocular pressure all specimens in the present work were taken from eyes with normal intraocular pressure (below 18.9 mm Hg, Schiötz), and with no previous history of ocular hypertension. Such specimens were obtained from cataractous eyes.

The ultrastructure of iris vessels in cataractous eyes without exfoliation syndrome was investigated in order to look for morphologic changes associated with the development of senile cataract. The first part of this study shows no differences between iris vessels in cataractous eyes without exfoliation syndrome and normal human iris vessels (Purtscher, 1966; Vegge and Ringvold, 1969). The described changes in iris vessels in cataractous eyes with exfoliation syndrome are therefore not due to cataract or changes associated with the development of

cataract, but the presence of these changes is a phenomenon associated with exfoliation syndrome.

The morphology of the abnormal appearing extracellular material present around the iris vessels in eyes with exfoliation syndrome as shown in the present study agrees with previous descriptions of exfoliation material on zonular fibres, anterior lens surface, and posterior iris surface (Blackstad *et al.*, 1960; Bertelsen *et al.*, 1964; Ashton *et al.*, 1965) as regards the fibrillar component. The granules found around some of the iris vessels in the present work, have not been described in previous studies of the exfoliation material, and it is not immediately obvious why these granules should be encountered only in conjunction with vessel walls. It is noteworthy that some of the granules were surrounded by triple-layered membranes, which may indicate that these granules are the result of intracellular synthesis.

The presence of exfoliation material in the perivascular area of the iris vessels is a pathologic phenomenon, and this fact makes us ask how this material comes into this location. Gregersen (1958) irrigated the anterior chamber of autopsy eyes and enucleated eyes with a suspension of killed cocci (measuring 0.5—1 μ in diameter) to test the tissue spaces of the human iris and their communication with the anterior chamber. He found the cocci able to find their way into the iris stroma, but only through the crypts. Inside the iris stroma the spread of cocci took place through the cleft of Fuchs, and they were demonstrable only outside the connective tissue layer of the vessel walls. It seems therefore unlikely to assume that the exfoliation material is transported to the perivascular area from other intraocular regions, and this calls attention to the iris vessel wall as a possible source for the production of exfoliation material. On the other hand it is not ruled out that the exfoliation material perhaps enters the eye through the blood stream, although it was never observed within the vessel lumen, and Shakib *et al.* (1965) found no exfoliation material around vessels in the ciliary body.

The presence of great amounts of exfoliation material in the iris vessel wall was associated with basement membrane changes. The simultaneous occurence of these two phenomena may perhaps indicate a biochemical disturbance leading both to changes in the basement membrance synthesis and to the appearance of exfoliation material.

The possibility that the exfoliation material is synthesized in one location, and then carried to an entirely different location where it is subsequently precipitated, must not be ruled out.

Further studies are required to elucidate the chemical nature of the exfoliation material, and to determine its site or sites of synthesis.

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Dr. Amund Ringvold
University Eye Department
Rikshospitalet
Oslo, Norway