Virchows Arch. Abt. A Path. Anat. 350, 95—104 (1970)

Ultrastructure of Exfoliation Material (Busacca deposits)

Amund Ringvold

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

Received February 24, 1970

Summary. The peripheral exfoliation band was scraped from seventeen lenses that were removed because of senile cataract. A suspension of the exfoliation substance, negatively stained with sodium silicotungstate, was studied and compared with the sectioned exfoliation material.

It is concluded that the exfoliation substance probably consists of a collagen material, that perhaps comes from basement membranes.

Zusammenfassung. Bei 17 Augenlinsen, die wegen einer senilen Katarakt extrahiert worden waren, wurde das periphere Exfoliationsband abgeschabt. Suspensionen dieses Materials wurden elektronenmikroskopisch nach einer Negativ-Färbung mit Natrium-Kieselsäure-Wolframsalz untersucht und die Befunde mit geschnittenem Exfoliationsmaterial verglichen. Aus den Untersuchungsergebnissen wird der Schluß gezogen, daß die Exfoliationssubstanz wahrscheinlich aus einem kollagenen Material besteht, welches möglicherweise von der Basalmembran abstammt.

During the last fifty years different techniques have been used to elusidate the origin and the chemical nature of exfoliation material, which is sometimes seen in the anterior segment of the eye. Light microscopic investigations have shown the presence of such material on the anterior lens capsule, on the zonules, on the anterior hyaloid membrane, on the ciliary body, on the posterior and anterior iris surface, on the pupillary margin, and in the trabecular area (Busacca, 1928; Dvorak-Theobald, 1954; Sunde, 1956; Hörven, 1966). Some histochemical studies (Dvorak-Theobald, 1954; Arnesen *et al.*, 1963) conclude that acid mucopolysaccharides are present in exfoliation material, but later works deny this (Bertelsen and Ehlers, 1969). According to Hörven (1966) histochemical methods indicate that the exfoliation material is a glyco- or mucoprotein, perhaps similar in nature to the collagen containing lens capsule.

Electron microscopic investigations of exfoliation material (Blackstad *et al.*, 1960) showed that it consists of two types of fibrils, of which the one appeared identical with fibrils in the vitreous body (Blackstad and Vegge, 1961). The other type was irregularly outlined, sometimes changing into a more or less non-fibrous meshwork. Bertelsen *et al.* (1964) also described two types of fibrils in exfoliation material from the lens. One type, however, was interpreted as deriving from the lens capsule, and had therefore to be distinguished from the exfoliation material proper. Ashton *et al.* (1965) confirmed the description by Blackstad *et al.* (1960) concerning the morphology of exfoliation material as seen in ultrathin sections. Later studies demonstrated small granules among the exfoliation fibrils in iris vessel walls (Ringvold, 1969).

The present work was undertaken in order to study exfoliation material, which had not been exposed to fixatives, with negative staining technique. The results will be correlated to the morphology of exfoliation material as it appears in ultrathin sections.

Material and Methods

Seventeen lenses extracted because of senile cataract were used. They all showed exfoliation material on the anterior lens surface by slit-lamp examination. Chymotrypsin was not utilized at operation, and only lenses with intact capsules were included. After operation the lenses were kept in 0.9% NaCl-solution until they could be taken care of. Within a few hours the saline solution was removed, and the peripheral exfoliation band was carefully rubbed off with a pin under the dissection-microscope before the lens capsule dried. The exfoliation material was then stored at $\div 80°$ C.

Samples of exfoliation material were suspended in 100 microliter 0.9% NaCl-solution or distilled water at 4° C. Carboncoated grids were dipped into the solutions, whereafter negative staining was done with 4% sodium silicotungstate (pH 6.8) in water. The suspensions were never stored for more than 8 days, and unsolved material was present at the bottom of the glas throughout the experiment. Siemens Elmiskop 1 A and 1 b were used.

Results

Both saline and distilled water suspensions gave the same results. The suspensions contained fibrils that differed from each other in appearance.

All the fibrils were straight or slightly bent, usually with blunt ends. Their width varied between 250 and 2,000 Å, and the majority measured in the range of 700—1,500 Å.

Some of the fibrils showed the well-established cross-band pattern of negatively stained collagen fibrils with an axial period of about 625 Å (Fig. 1). Other fibrils also showed cross-banding, but in a pattern and with a period that differed from that of collagen. The period length in these fibrils measured from 330 to 540 Å, but variations within any particular fibril were small. In many of these fibrils the periods consisted of light and dark regions resembling the A-region and the B-region of collagen, and additionally transverse lines were observed within these regions (Figs. 2—5). Longitudinal filaments were seen between cross-bands (Figs. 2 and 4).

Occasionally, fibrils with an indistinct cross-banding were found. In some fibrils segments without cross-banding alternated with cross-banded regions. Such segments were usually wider than the rest of the fibril, and sometimes showed longitudinal filaments particularly clear (Fig. 4). In some instances such filaments could not be observed, and these segments had a granular appearance (Fig. 3). A longitudinal splitting at the fibril ends was often observed, sometimes extending along a greater part of the fibril, thereby changing its appearance into a more or less filamentous mass (Fig. 5).

Most of the exfoliation material, however, did not appear as distinct fibrils. The material was found as aggregates (up to 1.5 μ across) containing irregular filaments (Figs. 6 and 7). These aggregates sometimes showed a structure closely imitating the filamentous regions of cross-banded fibrils (compare Fig. 7 with Fig. 5).

Discussion

According to Sunde (1956) the peripheral exfoliation band is located in front of the zonular attachment on the anterior lens capsule, sometimes with projections towards the insertion of the zonular fibers. Apart from some electron opaque granules in the superficial layer, no abnormalities were observed in the capsule

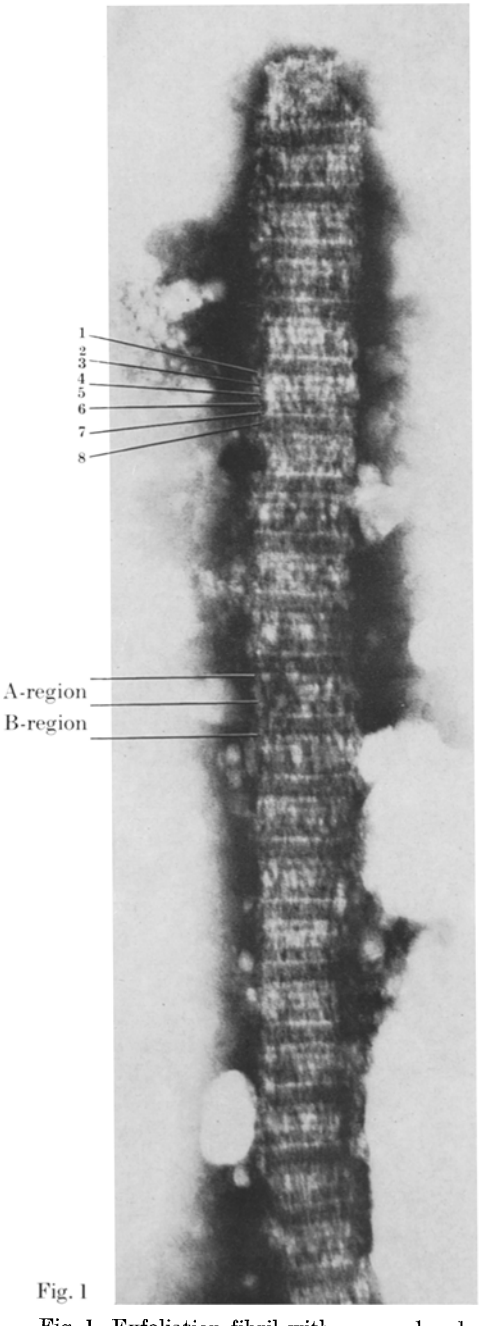

Fig. 1

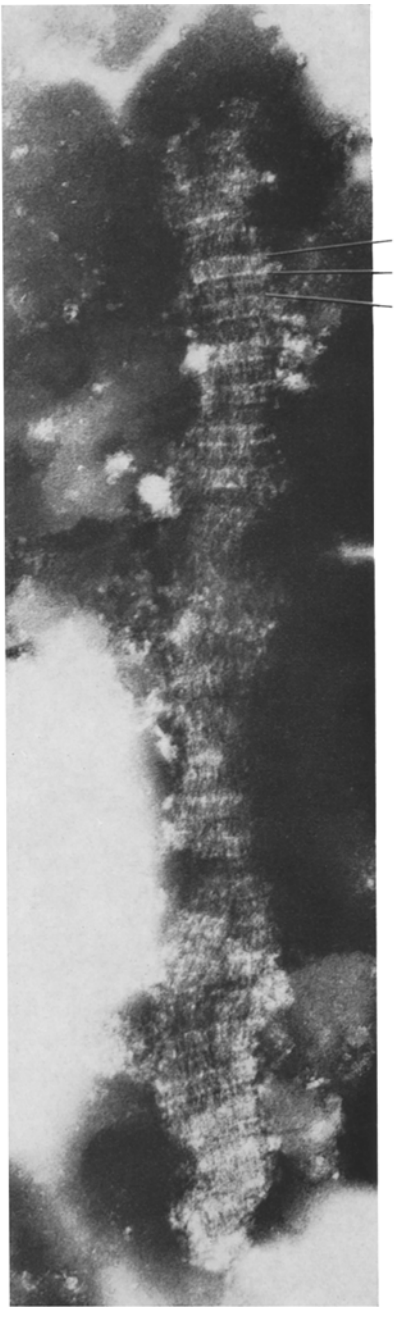

Fig. 2

Fig. 1. Exfoliation fibril with a cross-band pattern corresponding to that of collagen fibrils. The transverse lines are labeled with numbers according to Olsen (1963). The period length is about 625 Å. ×120,000

Fig. 2. Exfoliation fibril with periods clearly subdivided in an A-region and a B-region. Additionally, some transverse lines are seen within these regions. The period length is about 460 Å. ×120,000

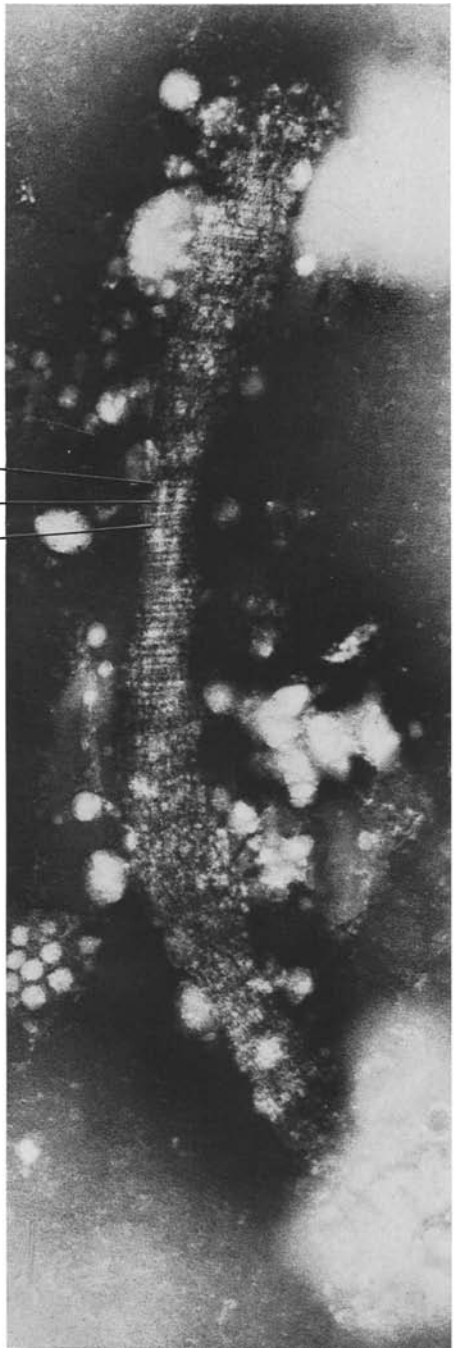

Fig. 3

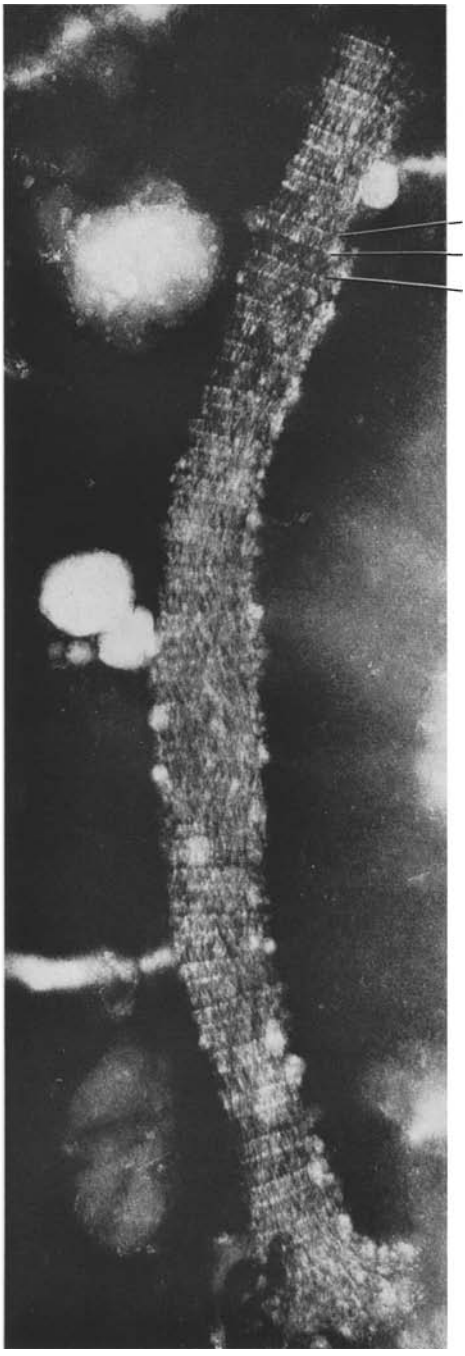

Fig. 4

Fig. 3. Exfoliation fibril with an axial period of about 540 Å length. Note the indistinctly appearing segment within the lower half of the bluntly ending fibril. ×120,000

Fig. 4. Exfoliation fibril with periods consisting of an A-region and a B-region. Transverse lines within these regions are seen. Note the segment without cross-bands showing distinct longitudinal filaments. The axial period measures about 500 Å. ×120,000

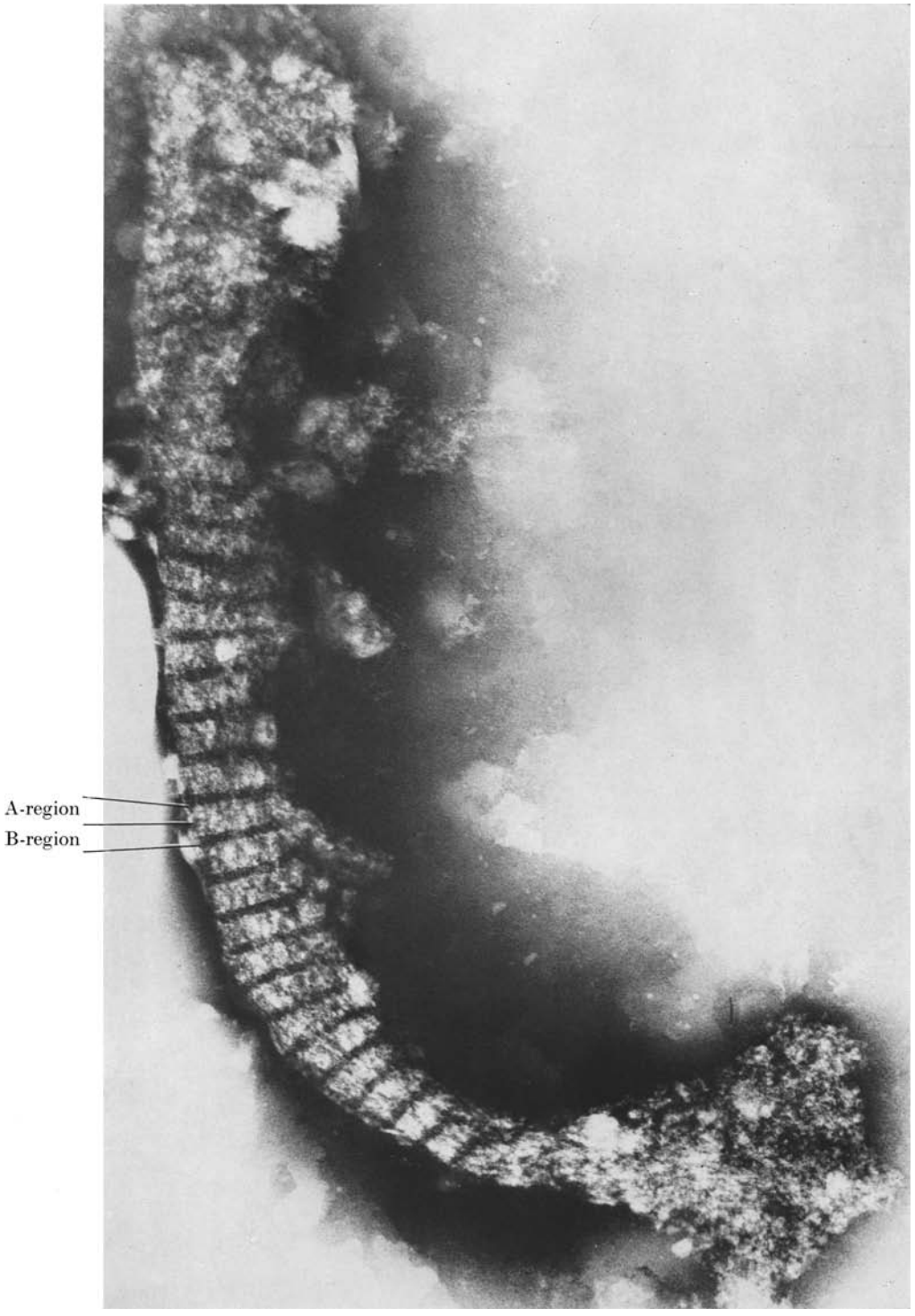

Fig. 5. Exfoliation fibril with an axial period of about 480 Å length. The fibril changes into filamentous masses at both ends. ×120,000

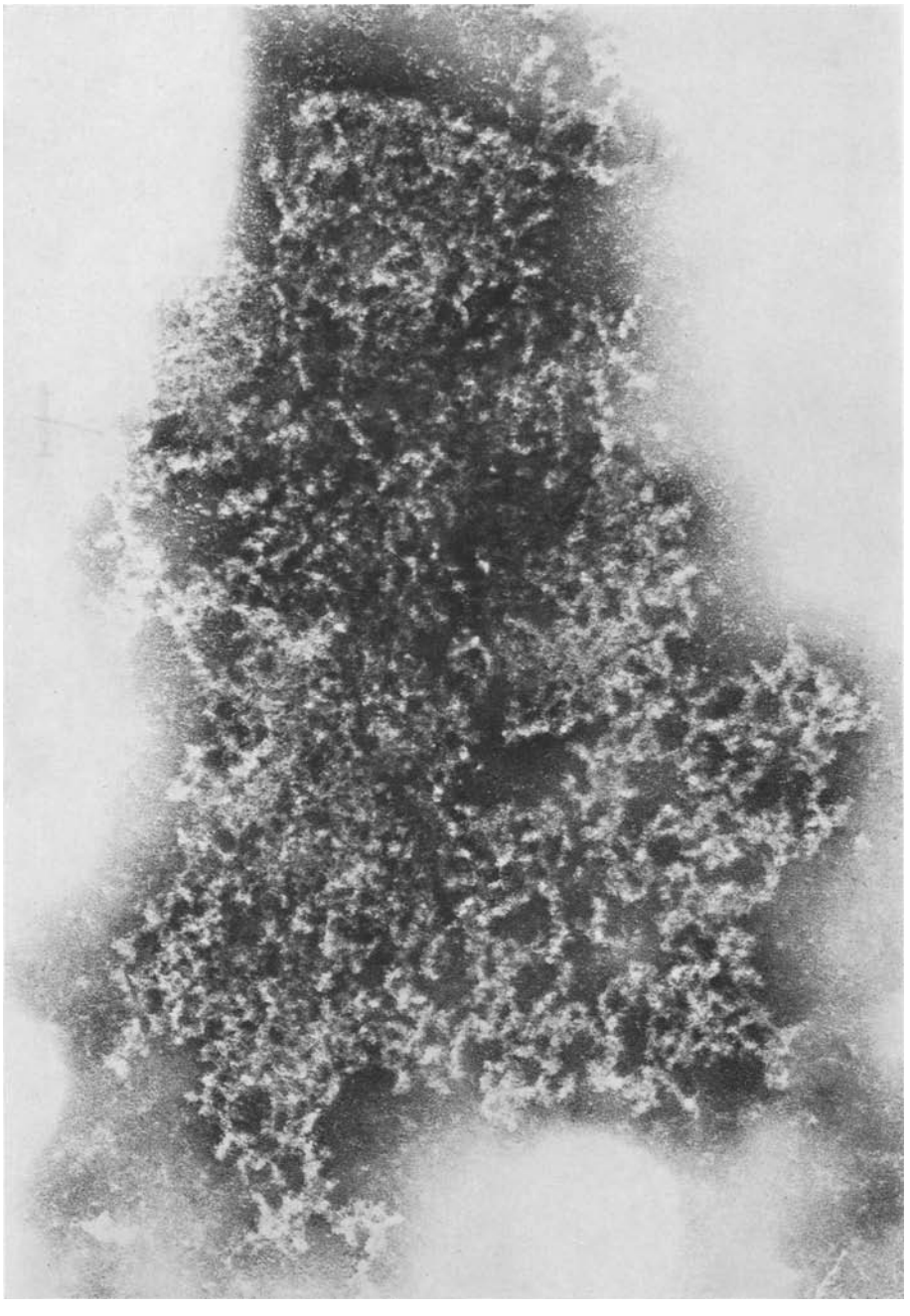

Fig. 6. Filamentous masses consisting of irregularly outlined filaments. ×120,000

beneath this band by Ashton *et al.* (1965). Sunde (1956) demonstrated that the peripheral exfoliation band can be rubbed off without damaging the lens capsule; an observation verified by the present author. In addition, lens capsule material,

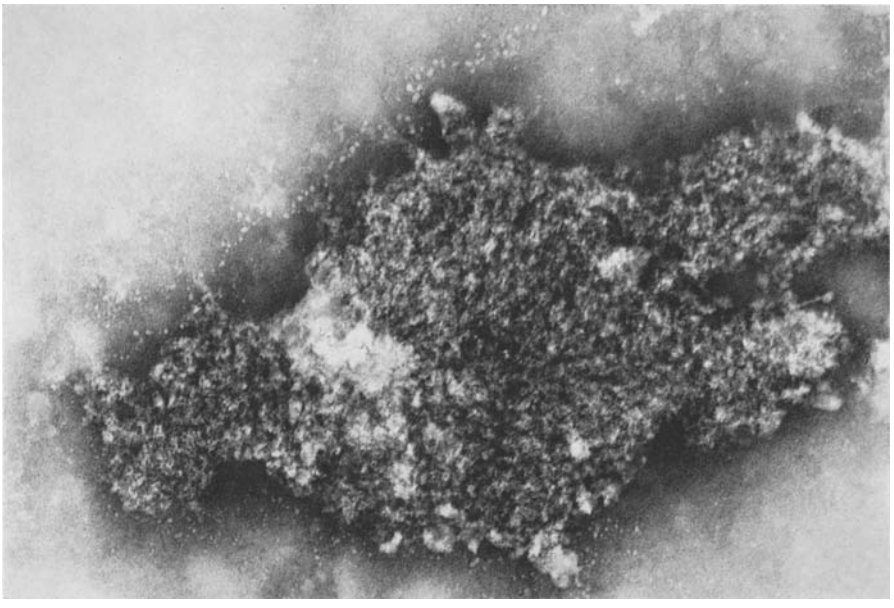

Fig. 7. Filamentous mass imitating filamentous regions in cross-banded fibrils. ×120,000

zonular fibres, and vitreous fibrils were not seen in the present material. The exfoliation material accumulated by rubbing off this band is therefore supposed to be insignificantly contaminated.

The appearance of exfoliation material as seen in ultrathin iris sections is demonstrated for comparison in Fig. 8, and Bertelsen *et al.* (1964) have established that exfoliation material on the posterior iris surface is identical to that on the anterior lens capsule. According to earlier descriptions (Bertelsen *et al.*, 1964; Ashton *et al.*, 1965; Ringvold, 1969) exfoliation fibrils as seen in sections were straight or slightly bent, measuring up to 1.2 μ in length, and up to 375 Å in thickness. Some of them showed cross-bands at intervals of 400—560 Å.

The negatively stained fibrils described in the present paper were found to be straight or slightly bent. They were 0.4—2.2 μ long, mostly 700—1,500 Å thick, and with an axial period between 330—625 Å. This shows that the diameter of the sectioned fibrils is different from that of the negatively stained fibrils. However, the smaller width of the sectioned fibrils may be due to the preparation procedure. Because no other fibrils were found in the present material, it is reasonable to conclude that the fibrils shown in this study are identical to exfoliation fibrils as seen in ultrathin sections.

According to Olsen (1963) each period in negatively stained collagen fibrils consists of two distinct zones called the A-region and the B-region. Usually 7—8 transverse white lines are observed per period, 4—5 in the A-region and 3 in the B-region. Some fibrils in the exfoliation suspension showed cross-bands corresponding to this description, and are therefore considered to be collagen fibrils.

Several of the observed fibrils showed periods similarly subdivided in two distinct zones, and in addition some transverse lines appeared within these regions

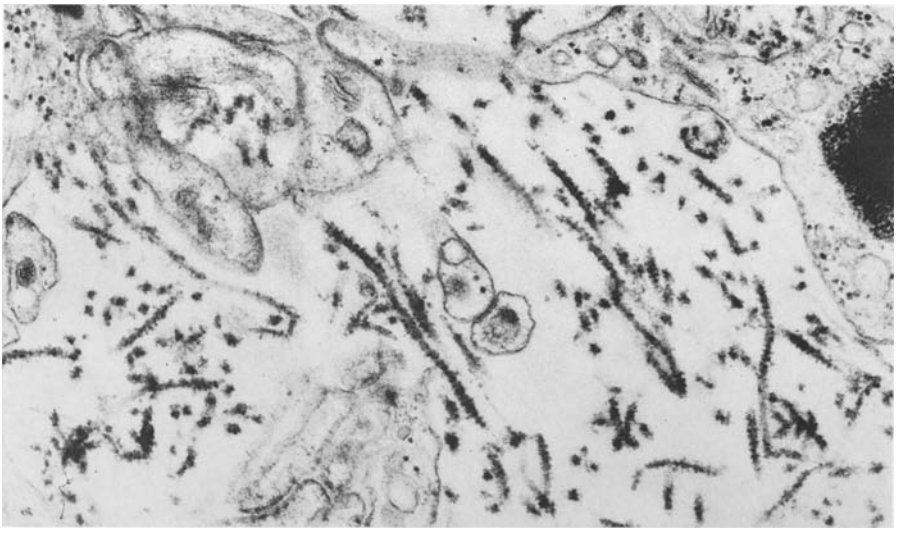

Fig. 8. Sectioned exfoliation material from the posterior iris surface (OsO_4 fixation and araldite embedding). Note straight or slightly bent fibrils with cross-bands. ×30,000

giving the fibrils an appearance closely similar to that of collagen fibrils. It is well-known that the cross-band pattern of aggregated collagen material depends on the precipitation procedure (Kühn and Zimmer, 1961). Because of the resemblance of these fibrils to collagen fibrils it seems therefore probable that they are composed of collagen molecules even though the periods were shorter.

As described some of the fibrils showed indistinct crossbands. No conclusive evidence exists that these fibrils are of the same kind as those discussed above. However, their length and width varied within the same range as the distinctly cross-banded fibrils, and similar to these they showed blunt ends. In addition, it should be kept in mind that more or less indistinct regions appeared within distinctly cross-banded fibrils. It would therefore not be surprising that some fibrils occured lacking a distinct periodic pattern throughout their entire length.

The fact that distinctly cross-banded fibrils sometimes continued into filamentous masses perhaps indicates that the filament aggregates and the fibrils represent different forms of the same material, probably collagen.

Exfoliation material in the wall of iris vessels infiltrated the basement membrane, which additionally appeared abnormally thin or interrupted (Ringvold, 1969). The same material has also been found on and within the internal limiting membrane covering the posterior iris surface and the ciliary body (Bertelsen *et al.*, 1964; Shakib *et al.*, 1965). Furthermore, exfoliation material was observed on the lens surface infiltrating the equatorial part of the capsule (Bertelsen *et al.*, 1964; Ashton *et al.*, 1965). Thus, keeping in mind that the lens capsule is a basement membrane an intimate relationship exists between exfoliation material and at least some of the intraocular basement membranes. The chemical nature of basement membranes may therefore be of considerable interest in the search for the origin of exfoliation material.

Two glycoproteins have been isolated from glomerular basement membrane; one collagen-like rich in hydroxyproline, hydroxylysine, and carbohydrate, the other a glycoprotein which lacked hydroxyproline and hydroxylysine (Kefalides, 1966, 1968). Further, two carbohydrates have been isolated from glomerular basement membrane and lens capsule; one disaccharide linked to the collagen component in the basement membrane (Dische *et al.*, 1965; Kefalides, 1967), the other a heteropolysaccharide linked to another non-collagenous protein (Dische *et al.*, 1965). The mode of interaction between these two glycoproteins is unknown. Kefalides (1967) has made the suggestion that the collagen in basement membranes does not form regular fibrils because of its increased carbohydrate content or its association with another glycoprotein. He was supported in this assumption by the fact that glomerular basement membrane collagen and lens capsule collagen, extracted with pronase, formed fibres of variable length by addition of ATP. Keeping in mind the intimate relationship between exfoliation material and basement membranes, these observations lead to the hypothesis that exfoliation material is derived from basement membrane collagen.

References

Arnesen, K., Sunde, O.A., Schultz-Haudt, S.D.: Histochemical studies of the deposits of Busacca in eyes with glaucoma simplex and so-called senile exfoliation of the anterior lens capsule. Acta ophthal. (Kbh.) **41**, 80—84 (1963).

Ashton, N., Shakib, M., Collyer, R., Blach, R.: Electron microscopic study of pseudo-exfoliation of the lens capsule. I. Lens capsule and zonular fibers. Invest. Ophthal. **4**, 141—153 (1965).

Bertelsen, T.I., Drablös, P.A., Flood, P.R.: The socalled senile exfoliation (pseudoexfoliation) of the anterior lens capsule, a product of the lens epithelium. Fibrillopathia epitheliocapsularis. Acta ophthal. (Kbh.) **42**, 1096—1113 (1964).

— Ehlers, N.: Morphological and histochemical studies on fibrillopathia epitheliocapsularis. Acta ophthal. (Kbh.) **47**, 476—488 (1969).

Blackstad, T.W., Sunde, O.A., Trætteberg, J.: On the ultrastructure of the deposits of Busacca in eyes with glaucoma simplex and so-called senile exfoliation of the anterior lens capsule. Acta ophthal. (Kbh.) **38**, 587—598 (1960).

— Vegge, T.: On the ultrastructure of vitreous body filaments. Acta ophthal. (Kbh.) **39**, 37—53 (1961).

Busacca, A.: Struktur und Bedeutung der Häutchenniederschläge in der vorderen und hinteren Augenkammer. Albrecht v. Graefes Arch. Ophthal. **119**, 135—176 (1928).

Dische, R.M., Pappas, G.D., Grauer, A., Dische, Z.: The carbohydrate of basement membranes of human kidney glomeruli. Biochem. biophys. Res. Commun. **20**, 63—70 (1965).

Dvorak-Theobald, Georgiana: Pseudo-exfoliation of the lens capsule. Amer. J. Ophthal. **37**, 1—12 (1954).

Hörven, I.: Exfoliation syndrome. A histological and histochemical study. Acta ophthal. (Kbh.) **44**, 790—800 (1966).

Kefalides, N.A.: A collagen of unusual composition and a glycoprotein isolated from canine glomerular basement membrane. Biochem. biophys. Res. Commun. **22**, 26—32 (1966).

— Characterization of the collagen from lens capsule and glomerular basement membranes. Proceedings of the VIth Congr. of the Int. Diabetes Federation. Stockholm, July 30 to August 4, 1967.

— Isolation and characterization of the collagen from glomerular basement membrane. Biochemistry **7**, 3103—3112 (1968).

Kühn, K., Zimmer, E.: Über die Anordnung der Tropokollagenmolekeln in den Long-spacing-Kollagenfibrillen. Naturwissenschaften **48**, 219—220 (1961).

— — Über eine neue Form der Long-spacing-Fibrillen des Kollagens. Naturwissenschaften **48**, 220 (1961).

Olsen, B.R.: Electron microscope studies on collagen. I. Native collagen fibrils. Z. Zellforsch. **59**, 184—198 (1963).

Ringvold, A.: Electron microscopy of the wall of iris vessels in eyes with and without exfoliation syndrome (pseudoexfoliation of the lens capsule). Virchows Arch. Abt. A Path. Anat. **348**, 328—341 (1969).

Shakib, M., Ashton, N., Blach, R.: Electron microscopic study of pseudo-exfoliation of the lens capsule. II. Iris and ciliary body. Invest. Ophthal. **4**, 154—161 (1965).

Sunde, O.A.: On the so-called senile exfoliation of the anterior lens capsule. A clinical and anatomical study. Acta ophthal. (Kbh.), Suppl. **45** (1956).

Dr. Amund Ringvold
University Eye Department
Rikshospitalet
Oslo, Norway