MORPHOLOGICAL AND CLINICAL STUDIES ON THE EXFOLIATION SYNDROME AND OPEN ANGLE GLAUCOMA

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This thesis is dedicated to my father,
who suggested this research topic
a long time ago

'It is incident to physicians I am afraid, beyond all other men, to mistake subsequence for consequence'

Samuel Johnson

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PUBLICATIONS & COMMUNICATIONS

The results of the studies described in this thesis have been published, or are being prepared for publication. These publications are listed below.

1. Konstas AGP, Marshall GE, Lee WR. (1989) Investigating the pathogenesis of exfoliation syndrome. **Ophthalmologia,** 1, 326-34.

2. Konstas AG, Marshall GE, Lee WR. (1990) Immunocyto-chemical localisation of collagens (I-V) in the human iris. **Graefe's Archive for Clinical and Experimental Ophthalmology,** 228, 180-86.

3. Konstas AG, Marshall GE, Lee WR. (1990) Immunogold localisation of laminin in normal and exfoliative iris. **British Journal of Ophthalmology,** 74, 450-57.

4. Konstas AGP, Marshall GE, Lee WR. (1991) Iris vasculopathy in exfoliation syndrome: An immunocyto-chemical study. **Acta Ophthalmologica,** 69, 472-483.

5. Konstas AGP, Jay JL. (1992) Modification of trabeculectomy to avoid postoperative hyphaema. The 'anterior guarded fistula' operation. **British Journal of Ophthalmology,** 76, 353-357.

6. Konstas AGP, Dutton GN. (1992) Exfoliation material on IOL's (letter). **Ophthalmology,** 99, 1644.

7. Konstas AGP, Jay JL, Marshall GE, Lee WR. (1993) Prevalence, diagnostic features and response to trabeculectomy in exfoliation glaucoma. **Ophthalmology,** (in press)

8. Konstas AGP, Dimitrakoulias N, Konstas PA. (1993) Exfoliation syndrome and open angle glaucoma. A review. **Klinische Monatsblätter für Augenheilkunde** (accepted for publication)

9. Konstas AGP, Marshall GE, Cameron SA, Lee WR. (1993) Morphology of vasculopathy in exfoliation glaucoma. **Acta Ophthalmologica** (submitted for publication)

10. Konstas AGP, Marshall GE, Koliakos GG, Lee WR. (1993) The nature of exfoliation material. **Ophthalmologia** (in preparation)

11. Konstas AGP, Marshall GE, Lee WR. Exfoliation syndrome. Mini review. **British Journal of Ophthalmology** (in preparation)

PREFACE

Exfoliation syndrome and exfoliation glaucoma are common in patients in Northern Greece with cataract, or open angle glaucoma. The management of these patients is problematic. This has prompted the author's interest in the disorder providing the motivation for this investigation.

This thesis is presented in two parts. Part I deals with morphological studies on the exfoliation syndrome. First, a critical review of the history, pathology, nature, origin, pathogenesis, epidemiology, clinical features and management of the disorder is presented. This is followed by the description and discussion of conventional morphological and immunocytochemical studies.

In Part II two clinical studies have been conducted. First, Scottish surgical patients were investigated prospectively, both clinically and morphologically, in order to ascertain the "true" prevalence of exfoliation glaucoma in this population and highlight differences between this disorder and primary open angle glaucoma. Second, a prospective randomised study has investigated the effect of varying the position of the trabeculectomy fistula on the incidence of postoperative hyphaema. The results are discussed and suggestions for future studies are outlined.

SUMMARY

Exfoliation syndrome may be defined as a condition in which there is a characteristic clinical pattern of deposition of fine white granular material upon and within ocular and orbital tissues. Morphologically, exfoliation material comprises randomly orientated fibrillar aggregates with the staining and ultrastructural characteristics of a protein. The origin and structure of this protein (exfoliation material) are uncertain. Exfoliation syndrome is a common, but poorly understood cause of glaucoma worldwide.

Iris tissue samples from 100 patients operated upon for open angle glaucoma and 31 control subjects with closed angle glaucoma have been subjected to light and electron microscopy. This aimed at establishing a definitive diagnosis for the clinical study described below. Ostensibly normal iris vessels were found close to other vessels with intense perivascular aggregation of exfoliation material. Foci of exfoliation material in the iris stroma exhibited a pattern consistent with 'ghost vessels'. A diffuse distribution would have been anticipated if passive deposition of exfoliation material had taken place.

Collagen types I-V and laminin were sought in 16 normal and 20 exfoliative specimens of iris and/or trabecular meshwork, by means of ultrastructural immunogold localisation. Collagen types I-V were not present in the exfoliation material. In this study the glycoprotein laminin has been identified, for the first time, as an integral component of the exfoliation material. The vascular matrices of exfoliative iris exhibited

increased amounts of collagen types I and IV in less affected vessels. Depletion of collagen types I, IV and laminin was noted in severely affected vessels.

In part II of this thesis the results of two prospective studies are presented. The first investigated the prevalence and clinical characteristics of exfoliation glaucoma. Surgical patients with open angle glaucoma were divided by clinical examination into 3 groups: definite exfoliation (22); possible exfoliation (18) and no exfoliation (60). Subsequent morphological assessment delineated two definitive groups: exfoliation glaucoma (26) and primary open angle glaucoma (74). Exfoliation glaucoma patients more frequently had higher untreated IOP at diagnosis, shorter duration of medical therapy, higher treated IOP before surgery and were operated upon more often for high IOP. They also exhibited significantly lower IOP postoperatively compared with age-matched primary open angle glaucoma patients.

The second study was performed upon 78 patients and studied the effect of varying the position of a surgical fistula upon the incidence of postoperative hyphaema. This study confirmed that by fashioning the fistula entirely in corneal tissue the incidence and severity of postoperative bleeding is reduced resulting in shorter hospital stay. The incidence of postoperative hyphaema was lower in the exfoliation glaucoma group, but not significantly so. Neither the type of dissection, nor the occurence of hyphaema influenced the IOP at 4 months following surgery.

LIST OF ABBREVIATIONS

ECCE	Extracapsular cataract extraction
ECM	Extracellular matrix
EM	Electron microscopy (transimission)
FIG	Figure
ICCE	Intracapsular cataract extraction
IOP	Intraocular pressure
LM	Light microscopy
MINS	Minutes
MM	Millimetre
NM	Nanometre
POAG	Primary open angle glaucoma
SEM	Scanning electron microscopy
S.D.	Standard deviation
TEM	Transmission electron microscopy

PART I MORPHOLOGICAL STUDIES ON THE EXFOLIATION SYNDROME

CHAPTER 1 LITERATURE REVIEW ON THE EXFOLIATION SYNDROME

General introduction

Exfoliation syndrome may be defined as a discrete clinical entity characterised by the production and deposition of fine white granular material, (exfoliation material), upon and within ocular and orbital tissues. The clinical diagnosis of exfoliation syndrome is based on the incidental finding of 'dandruff-like' material upon the pupillary margin, or 'sugar frosting' of the anterior lens capsule. Sight threatening glaucoma and the development of cataract are common (Bertelsen 1966, Jerndal 1986). The socio-economic importance of this age-related disorder is likely to increase due to the increasing life expectancy in Western societies where the disease is most prevalent.

1.1 History and nomenclature

1.1.1 History

The early literature has been reviewed by Sunde (1956) and Tarkkanen (1962). The following description outlines the various stages of development in the understanding of the disorder.

Differences of opinion on the disorder

From the historical point of view, exfoliation syndrome is one of the most controversial subjects in ophthalmic

literature. In their review article in 1973, Layden & Shaffer asserted that 'exfoliation syndrome is a leader amongst the topics in ophthalmology that undergo constant appraisal as to terminology, pathogenesis and clinical implications'. From the clinical standpoint, controversy has arisen concerning both the epidemiological and the clinical features of the condition (Jerndal 1986, Forsius 1988). Even the nomenclature of exfoliation syndrome remains debatable. Another problem has been that anecdotal findings reported by eminent ophthalmologists have been accorded undue weight.

The first accounts

The Finnish ophthalmologist Lindberg is credited as having been the first author to describe the clinical presence of exfoliation material. In 1917 he found 'small greyish-white, or bluish-grey flakes which originate from the edge of the pupillary margin' in the iris of 30 out of 60 patients with glaucoma, examined as part of his doctoral thesis work in Petersburg. Lindberg thought these flakes were 'old inflammatory exudates'. In 1923, the Norwegian, Malling observed 33 cases with 'greyish-white formations on the anterior surface of the lens' and assumed that these arose as a 'membrane-like exudate on the surface of the lens'.

In 1925, Vogt provided the first detailed clinical description of the disorder and accorded the name 'superficial exfoliation of the anterior capsule of the lens'. He was the first to implicate exfoliation material as the underlying cause for a secondary glaucoma and to suggest that exfoliation glaucoma had a worse prognosis than primary

open angle glaucoma (POAG), (Vogt 1930a).

Busacca (1927) was the first author to provide a morphological account and exfoliation material was subsequently referred to by some authors as 'Busacca's deposits' (Sobhy Bey 1932, Sunde 1956). Trantas (1929) was the first to suggest that the condition was a more generalised disorder affecting all the 'hyaline membranes' of the eye. Malling (1938) proposed a vascular aetiology for the 'atrophic exfoliative-depigmentation phenomenon'.

The first British cases of exfoliation syndrome and glaucoma were reported by Goulden (1925) and Foster (1933). Goulden (1925) presented a 63 year old female with 'rupture of the zonular lamella complicating ordinary senile cataract'.

Further developments

The publication of an important paper by Dvorak-Theobald (1954) revised the views of many ophthalmologists that exfoliation syndrome is a disorder of the lens capsule. Dvorak-Theobald provided histological evidence that contradicted Vogt's theory and came to the conclusion that exfoliation material was a deposit on the lens. She introduced the term 'pseudoexfoliation of the lens capsule' to stress the unknown origin of the exfoliative aggregates and to differentiate the condition from 'true exfoliation of the lens capsule'. The latter may rarely be found in patients exposed to prolonged and excessive heat (i.e. glass-blowers). Blackstad and coworkers (1960) conducted the first ultrastructural study of the exfoliation material.

Detailed accounts of the clinical picture of the condition have been provided, among others, by Hörven (1937), Örgen (1949), Hörven (1966) and Sugar (1984). Barkan (1936) first identified flakes of exfoliation material by gonioscopy. Gradle & Sugar (1940) were the first to draw attention to the pigmentary changes in the disorder. Irvine (1940) was the first to study two different ethnic populations and to recognise varied ethnic prevalences. Tarkkanen (1962) carried out the first clinical study on 418 patients with exfoliation syndrome/glaucoma. He established that dilatation of the pupil, using mydriatics, is essential for the diagnosis of the condition. A characteristic gonioscopic feature of the exfoliation syndrome, termed Sampaolesi's line (defined in section 1.9), has been documented by Sampaolesi (1959). Vannas (1969) first demonstrated striking vascular abnormalities in affected eyes by employing fluorescein angiography of the iris. Bartholomew (1971) demonstrated an early 'pre-granular' stage in the evolution of the disorder.

1.1.2 Nomenclature

The condition was first referred to both as senile exfoliation of the lens capsule and as 'exfoliatio superficialis capsulae anterioris' by Vogt (1925). The term pseudoexfoliation syndrome has somehow evolved from this term to accommodate the full clinical picture of the condition. Bertelsen and coworkers (1964) have proposed the term fibrillopathia epitheliocapsularis. The term exfoliation syndrome was originally put forward by Sunde (1956). Pseudoexfoliative fibrillopathy (Streeten et al 1990) is yet

another term which has recently been coined. Nowadays, as there is evidence of multifocal aggregation and possibly origin, a retreat to the simple designation exfoliation syndrome is gaining popularity (Garner 1990).

None of the terms used in the literature is satisfactory because they each simply describe a specific pathological feature of the condition and, on the basis of current information, are obsolete. The terms exfoliation syndrome/glaucoma are used throughout this thesis in preference to the terms pseudoexfoliation syndrome/glaucoma because there is no current need to distinguish the condition from 'true exfoliation of the lens', a condition which is rare and does not cause glaucoma. It has also been shown that true exfoliation of the lens capsule does occur in a number of patients with the condition (Jerndal 1986). Clearly there is a need for a uniform terminology for this condition.

1.2 Pathology of exfoliation syndrome

The motivation for the morphological studies was to identify the nature and source of exfoliation substance. The appropriate literature is discussed in section 1.4.

1.2.1 Macroscopic appearance

Exfoliation syndrome may be difficult to recognize macroscopically. It is seen on the anterior lens capsule in two areas, a central disc (Figs 1.1 & 1.2) in the pupillary

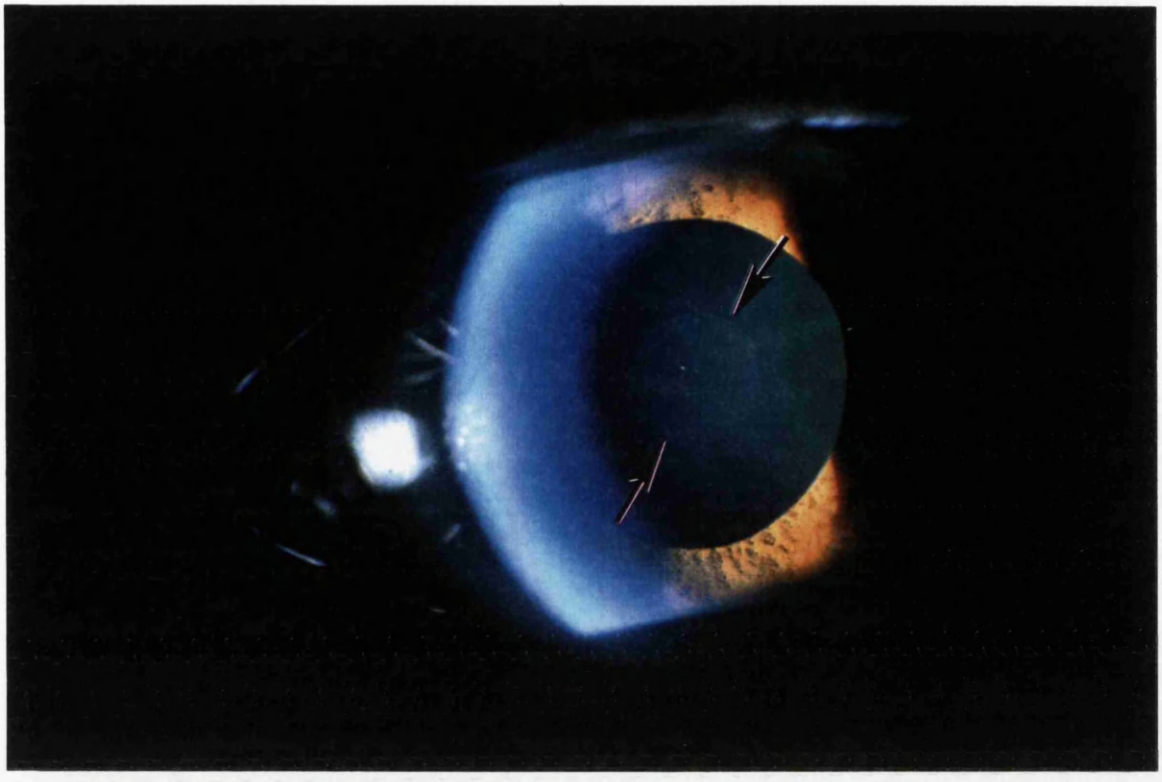


Figure 1.1: Slit-lamp photograph of a patient with exfoliation glaucoma to illustrate the central disc (arrows). discrete flecks of exfoliation material can be discerned in the centre of the disc.

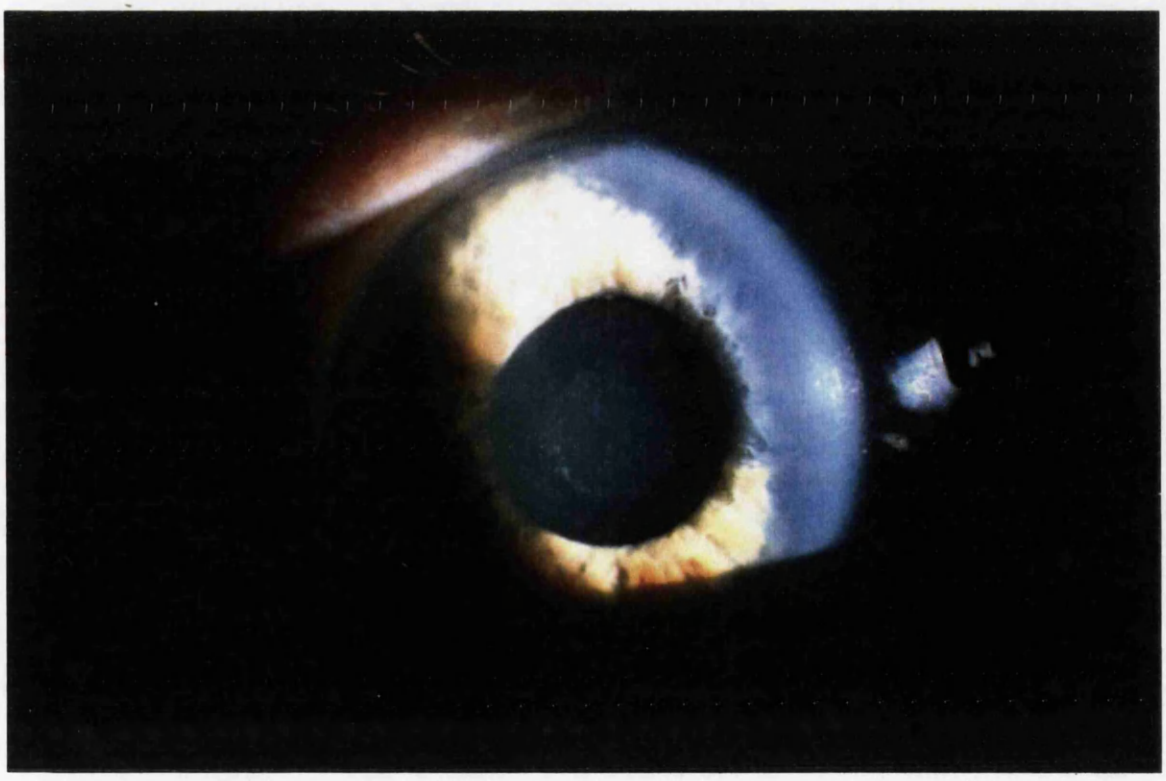


Figure 1.2: Slit-lamp photograph of a patient with exfoliation glaucoma. The edge of central disc appears more prominent.

region and a band around the periphery, referred to as the granular zone, (see section 1.7.2). The central disc possesses a flat grey-whitish uniform appearance, which sometimes is difficult to distinguish from the lens capsule. In some cases the central disc may be conspicuous with a clearly defined edge which is sometimes curled inwards (Fig 1.2). A far more reliable indicator for the presence of exfoliation is the granular zone (Figs 1.3 & 1.4).

Feathery excrescences of exfoliation material are commonly found along the posterior and less frequently along the anterior surface of the iris (Vogt 1938a, Hörven 1966), (Fig 1.5). The ciliary processes (Fig 1.6) and the zonular fibres are similarly coated (Trantas 1929, Hörven 1936). The trabecular meshwork in affected eyes exhibits dense pigmentation (Fig 1.7). The 'frosting' of the ciliary body and the zonules in the exfoliation syndrome is helpful in establishing the diagnosis (Figs 1.6 & 1.8).

1.2.2 Light microscopic appearance

Exfoliation material is described as fluffy, fibrillar, or fibrillogranular, eosinophilic, extracellular material, forming tree-like structures (Sunde 1956, Tarkkanen 1962, Larsen 1969, Duke-Elder 1969, Morrison & Green 1988), (Figs 1.9 & 1.10). The best descriptions offered in the literature for the light microscopic (LM) appearance of exfoliation aggregates are 'iron filings lining up on a magnet' (Yanoff & Fine 1989) and 'bush shaped' accretions (Ringvold 1970a, Morrison & Green 1988). Exfoliation aggregates on the

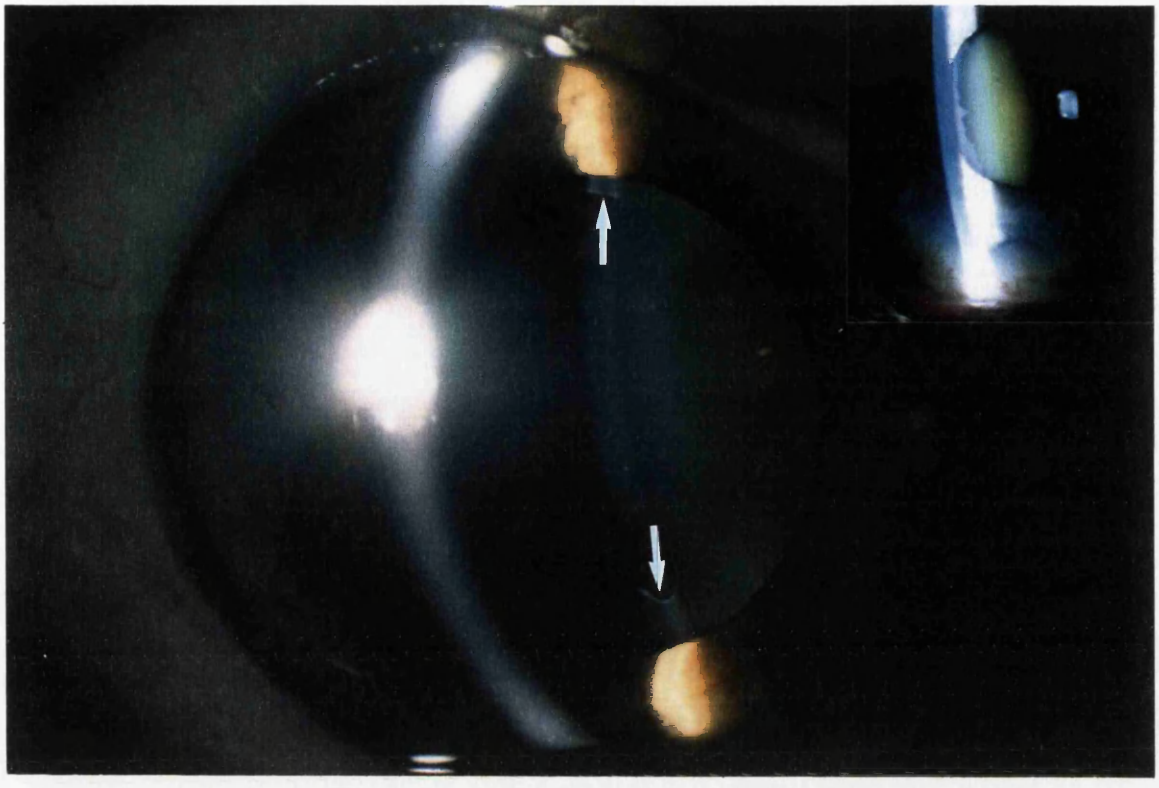


Figure 1.3: Slit-lamp photograph of an eye with exfoliation syndrome. Note the granular zone extending from the pupil margin to the arrows. The inset shows this zone with a frosted appearance.

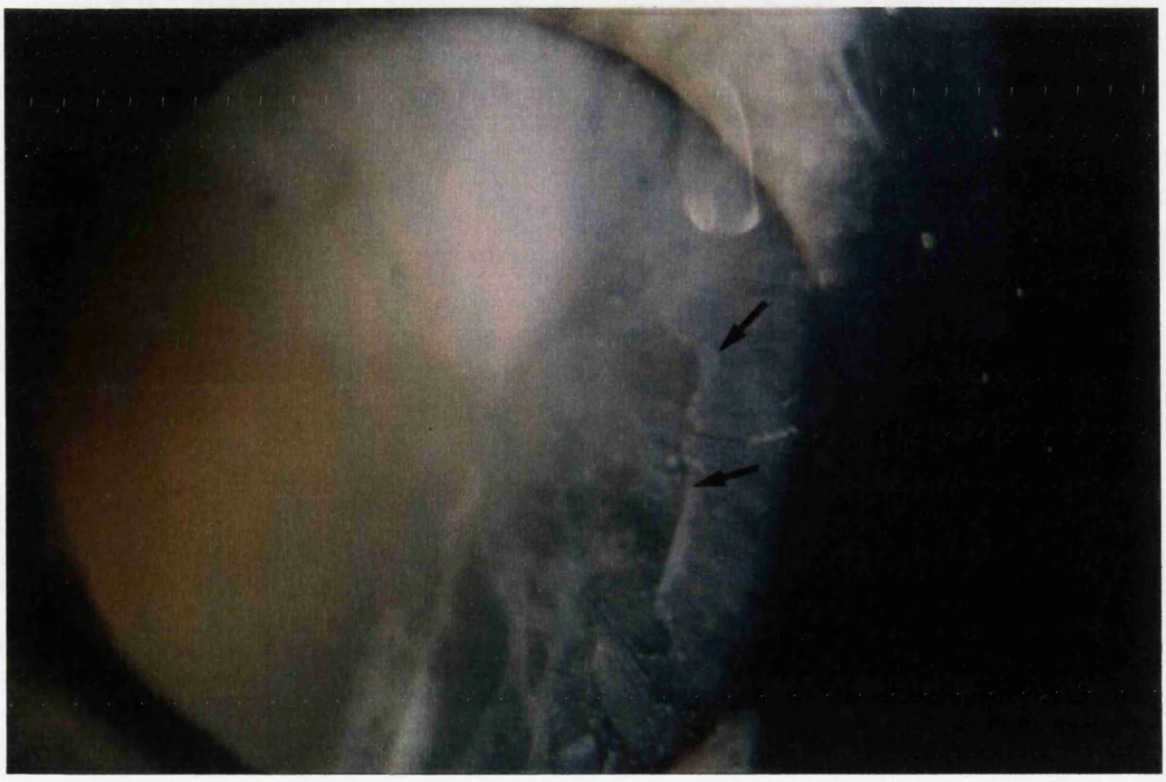


Figure 1.4: Slit-lamp photograph of an eye with exfoliation glaucoma and cataract. The granular zone extends from the pupil margin to the arrows and its inner edge is curled.

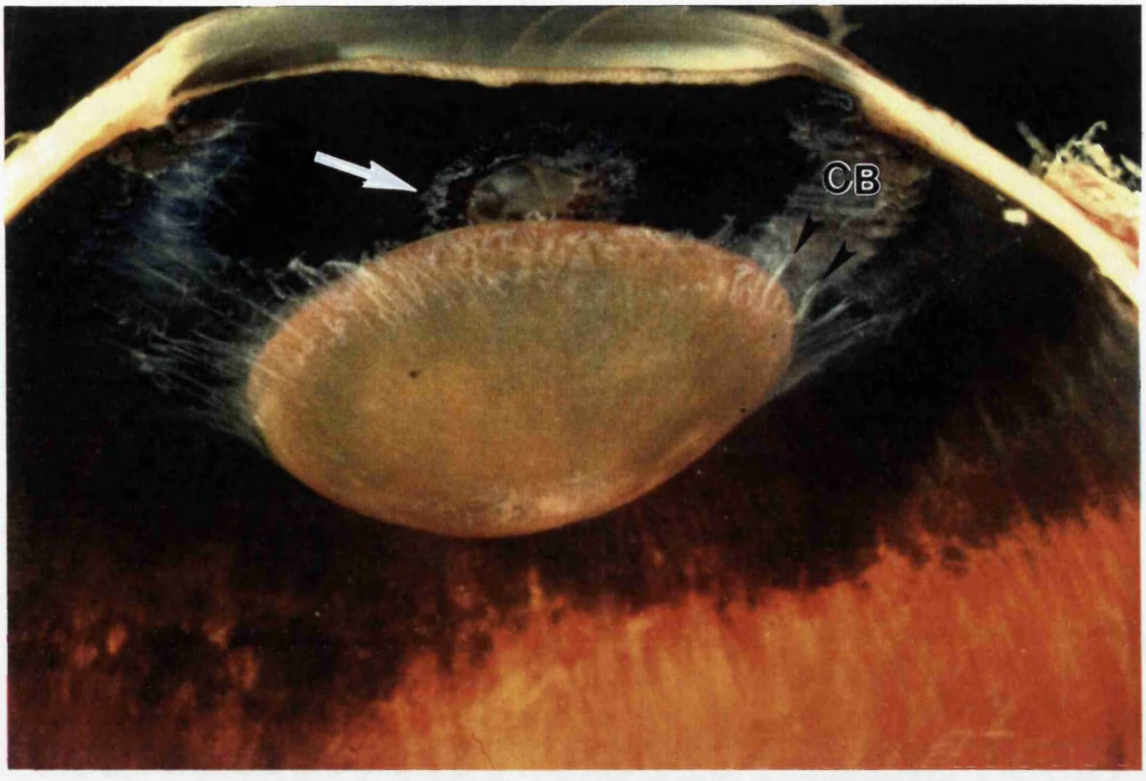


Figure 1.5: Macroscopic appearance of a globe with exfoliation glaucoma. Exfoliation material is deposited on the posterior surface of the pupillary region (arrow), ciliary body (CB) and zonules (arrowheads). Subluxation of the lens is a sectioning artefact.

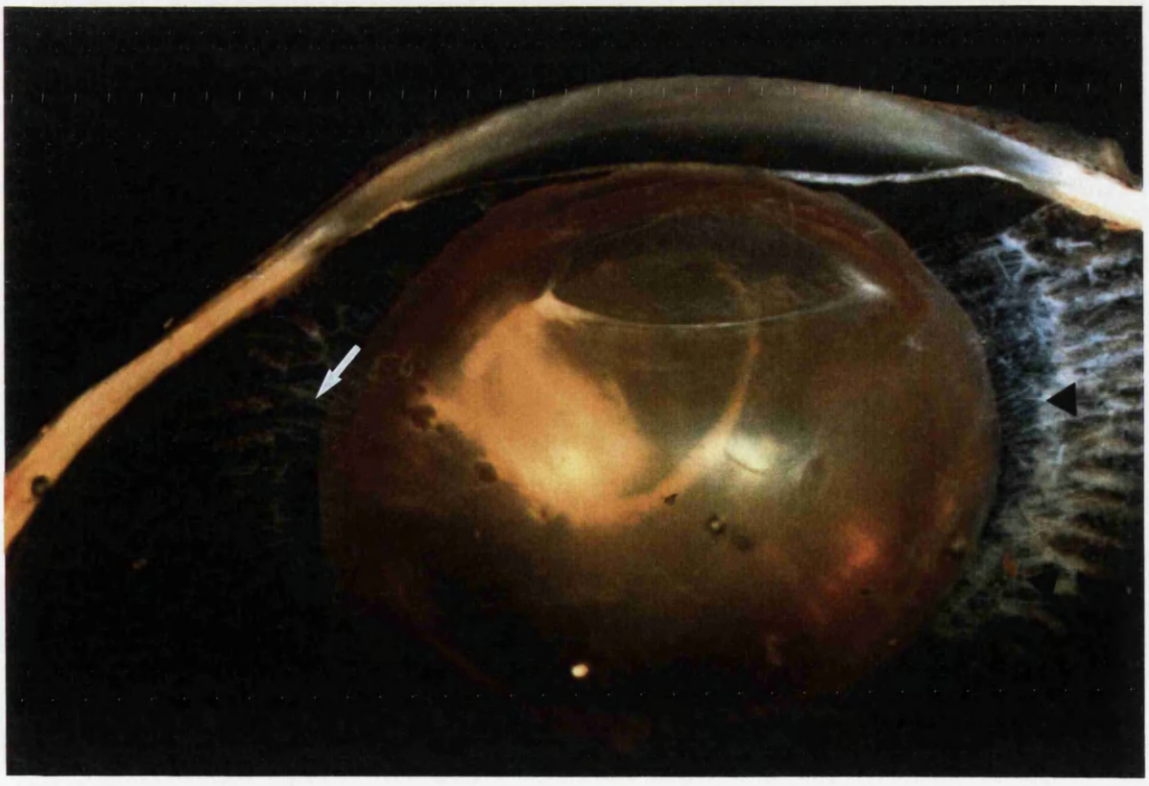


Figure 1.6 Colour photograph of anterior segment of an eye with exfoliation glaucoma. Note uneven deposition of white granular material (exfoliation material) upon the zonules (arrow) and ciliary body (arrowheads).

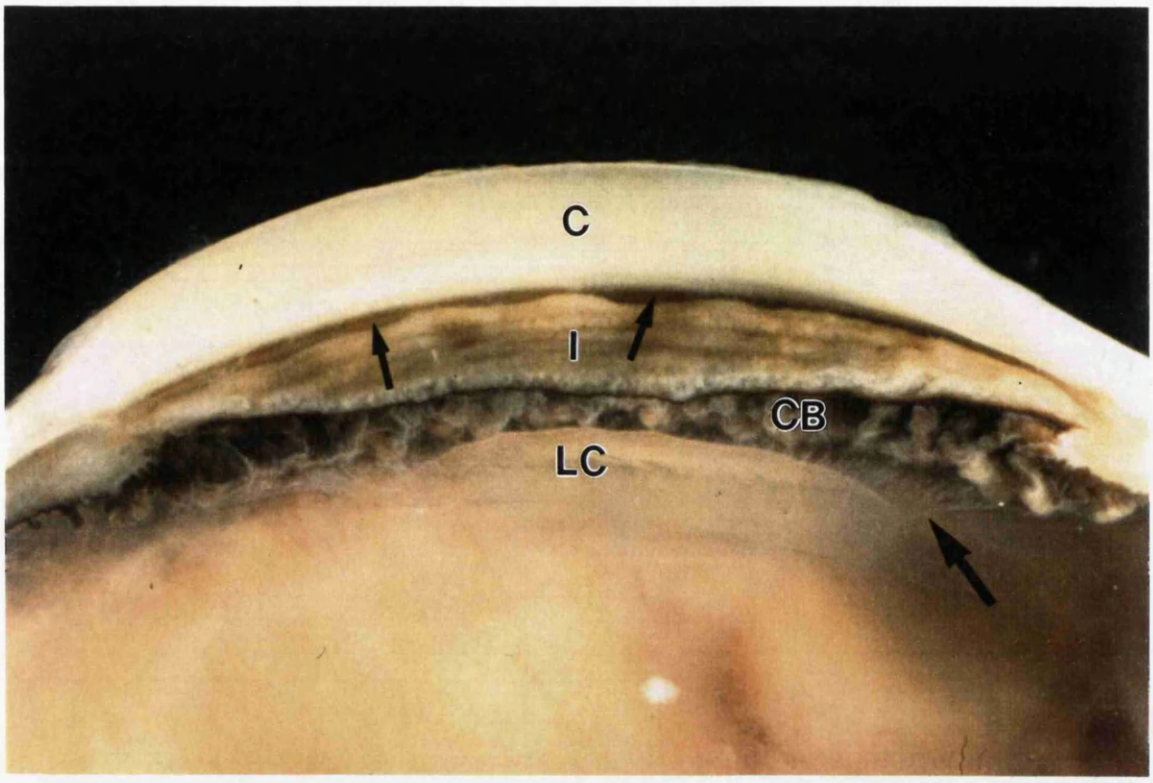


Figure 1.7 Macroscopic appearance of the anterior chamber angle in a globe with exfoliation glaucoma. Note dense pigmentation of the trabecular meshwork (arrows). C:cornea; I:iris; CB: ciliary body; LC: lens capsule; zonules are arrowed.

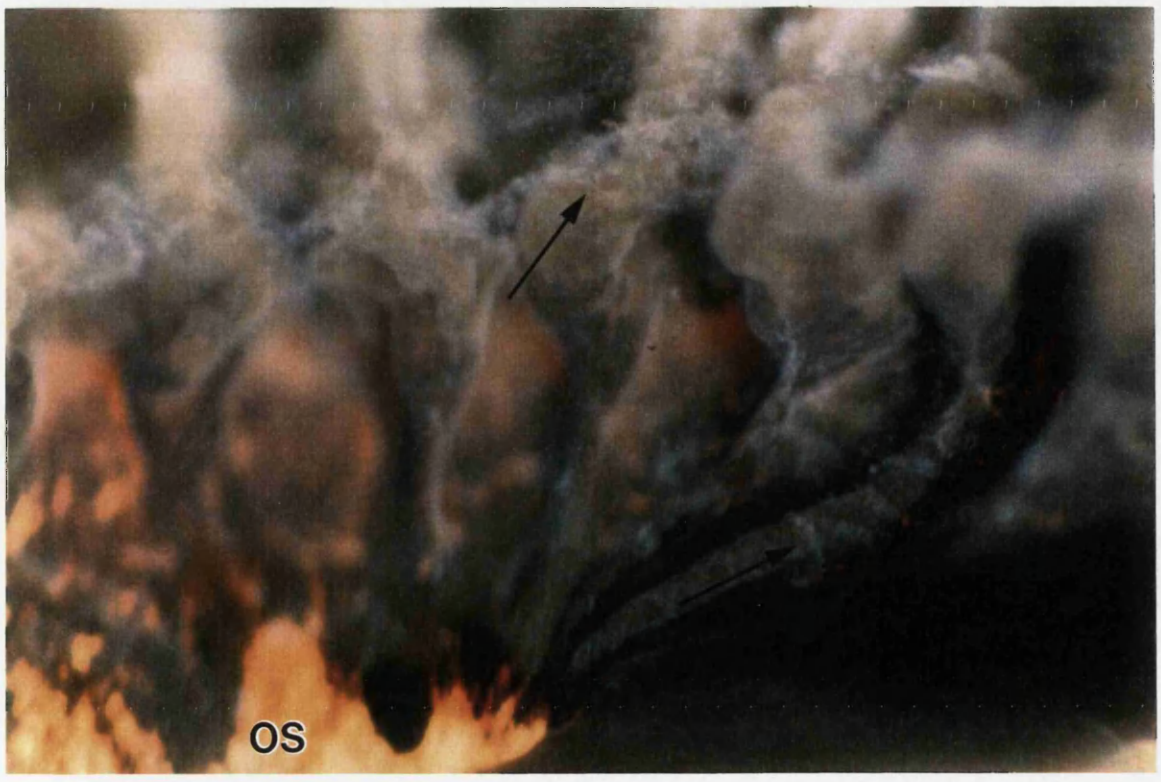


Figure 1.8: Macroscopic photograph of exfoliation material deposition upon the ciliary processes (arrows), which occurs in bands. OS: ora serrata.

central disc of the anterior lens capsule appear as sparse, short 'vegetations' whereas, on the granular zone they have a tall broad bush-shaped appearance (Fig 1.10). Exfoliation material has been shown to be loosely attached to the anterior lens capsule, zonules and anterior vitreous face and firmly adherent to the equatorial lens capsule, pigment epithelium of the iris and the non-pigmented ciliary epithelium (reviewed by Morrison & Green 1988).

Conjunctiva and cornea

LM has not revealed exfoliation material in the conjunctiva (Layden & Shaffer 1974). Exfoliation material has been reported to adhere to and even to be endocytosed by the corneal endothelium (Dvorak-Theobald 1954). However, Sunde (1956) did not detect exfoliation material on the cornea.

Trabecular meshwork

Dvorak-Theobald (1954) studied 3 enucleated eyes and found exfoliation material both on the inner surface of the trabecular meshwork and amongst the intertrabecular spaces. Another study demonstrated exfoliation material around Schlemm's canal in all 4 cases of advanced exfoliation glaucoma (Kurosawa et al 1983).

The iris

Sunde (1956) found exfoliation deposits in all 23 cases he studied. The LM appearance of exfoliation deposits in the iris (Fig 1.9) is similar to that in other affected tissues and comprises aggregation of exfoliation material principally in association with blood vessels (Ringvold 1969; 1970a;

1970c) and upon the pigment epithelium (Hörven 1937, Gifford 1957) and sporadically, within iris crypts in the anterior border layer (Vogt 1925, Sunde 1956). Ringvold (1970c) demonstrated exfoliation material in 113 out of 499 iris vessels in 8 iridectomy specimens. The affected vessels showed, in toluidine blue stained sections, exfoliation material as a light blue material, unevenly distributed in the normally unstained vascular matrix outside the vascular cells).

Ciliary processes

Exfoliation material deposition on the ciliary processes has been described in a number of studies (Bussaca 1928, Dvorak-Theobald 1954, Larsen 1969, Sugar et al 1976, Morrison & Green 1988). The ciliary processes are frequently coated with feathery eosinophilic excrescences, which adhere firmly to the underlying non-pigmented epithelium and its limiting membrane (Fig 1.8).

Zonules

Zonular involvement in the exfoliation syndrome was established in 1937 by Hörven, who felt that the 'zonular changes are of constant nature and form an integral part of the clinical picture of exfoliation'. Gradle & Sugar (1940) and Dvorak-Theobald (1954) confirmed Hörven's observations. Gifford (1957) found an intimate association between exfoliation material and the zonules whereas, the lens capsule was ostensibly normal.

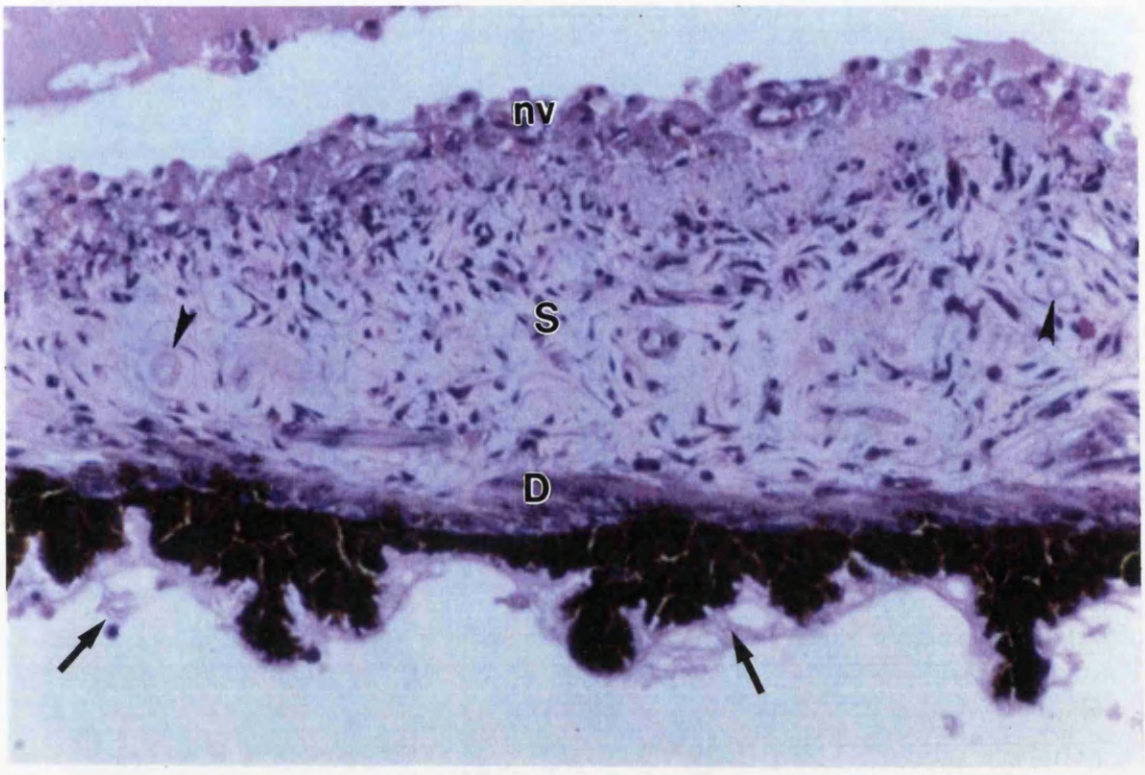


Figure 1.9: Histological section of exfoliative iris. Exfoliation material (arrows) covers the posterior surface of the pigmented epithelium, which is serrated. S:iris stroma; D:dilator muscle; vessels are arrowheaded. The anterior border layer is bounded by a neovascular membrane(nv).

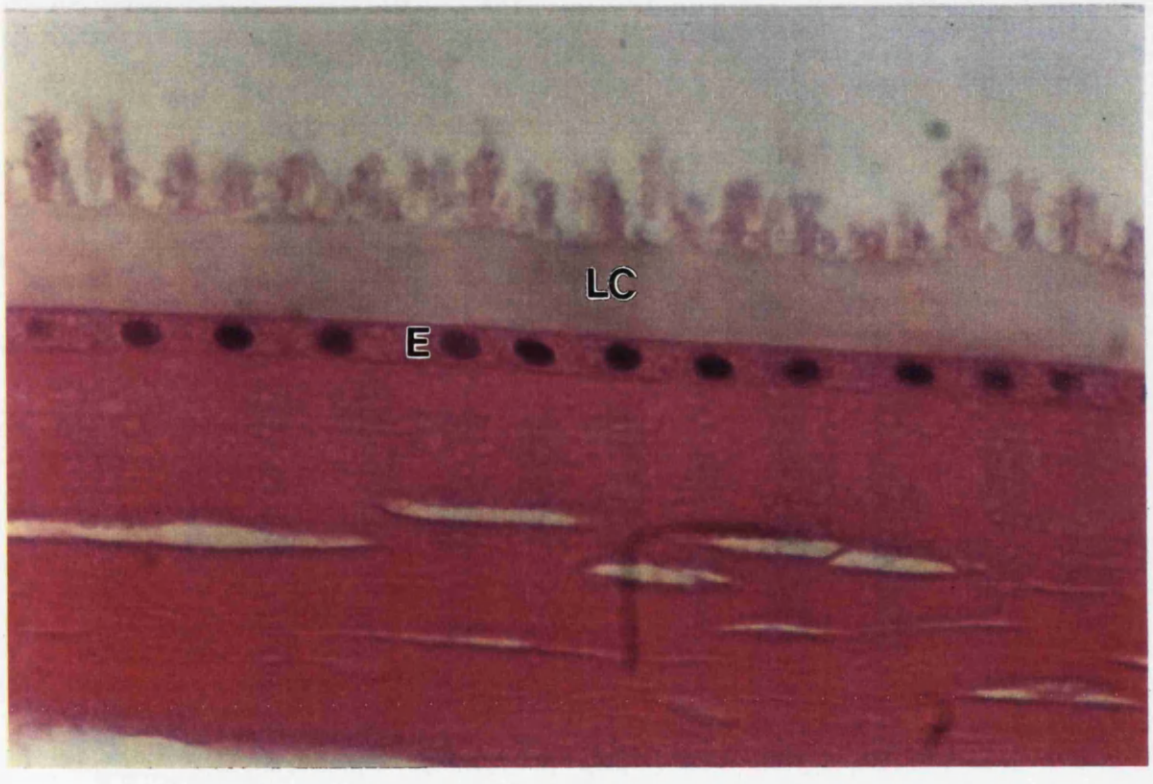


Figure 1.10: Histological section of anterior exfoliative lens capsule (LC). Exfoliation aggregates present on its surface are 'bushy' in appearance. E:lens epithelium.

The lens

Bussaca (1928) described vacuoles in the superficial and deep layers of the lens capsule. Dvorak-Theobald (1954) and others (Sunde 1956, Gifford 1957, Hörven 1966) have noted that in enucleated eyes exfoliation deposits can easily be removed mechanically leaving behind a 'normal' lens capsule. However, other investigators have provided evidence of underlying alterations in the lens capsule in association with the exfoliation syndrome namely an amorphous layer close to the lens epithelium, which was not present in normal controls (Bertelsen et al 1964, Dark et al 1969). Larsen (1969) studied 100 autopsy eyes and found this amorphous layer in all 12 eyes with the exfoliation syndrome and in none amongst the controls. Gifford (1957) demonstrated thickening of the anterior lens capsule in 2 out of 6 lenses. 'True exfoliation' (peeling off) of the anterior layer of the lens has also been observed (Gifford 1957, Dark et al 1977, Morrison & Green 1988).

Other tissues

The anterior vitreous face may contain exfoliation deposits, which appear as radial stripes (Sunde 1956, Larsen 1969). Exfoliation material has not been detected by LM beyond the boundaries of structures bathed by the aqueous.

1.2.3 Ultrastructural appearance

Electron microscopy (EM) has made it feasible to examine exfoliation material in greater detail (Figs 1.11 & 1.12). In the first transmission electron microscopic (TEM) study

Blackstad and coworkers (1960) described exfoliation material as 'heterogeneous deposits consisting of numerous fibres of varying form and size that most often lie in accumulations with ill-defined outlines'.

Several investigations have described exfoliation material since Blackstad's early study (including Ringvold 1969; 1970b, Rohen & Witmer 1972, Witmer & Rohen 1976, Dark et al 1977, Harnisch 1977, Davanger 1978; 1980, and Dark & Streeten 1990). The dominant ultrastructural feature of exfoliation material is the presence of straight, or slightly bent exfoliation fibres with blunt (Ringvold 1970b, Dark & Streeten 1982), or split (Harnisch 1977) ends. The fibres are randomly orientated (Ringvold 1969; 1970a, Seland 1988) and electron-dense in nature (Dark et al 1977, Harnisch 1977). Their width has been reported to vary between 20-80 nm (Nishi et al 1976, Harnisch 1977, Takei & Mizuno 1978, Seland 1979; 1988, Davanger 1980). Their length ranges between 100 and 700 nm (Harnisch 1977, Shimizu 1985), but has been reported to vary up to 5 microns (Davanger 1975). Exfoliation fibres are most often identified by a distinctive aggregation pattern resembling 'meshwork' of randomly distributed, incompletely separated fibres. Davanger (1980) has suggested that the fibres may enlarge by the accumulation of new material on their surface. To date, there is no information in the literature concerning the effects of conventional processing on the morphology of exfoliation fibres. It is conceivable that fixation and osmication of exfoliative tissues may influence considerably the morphology and dimensions of exfoliation material.

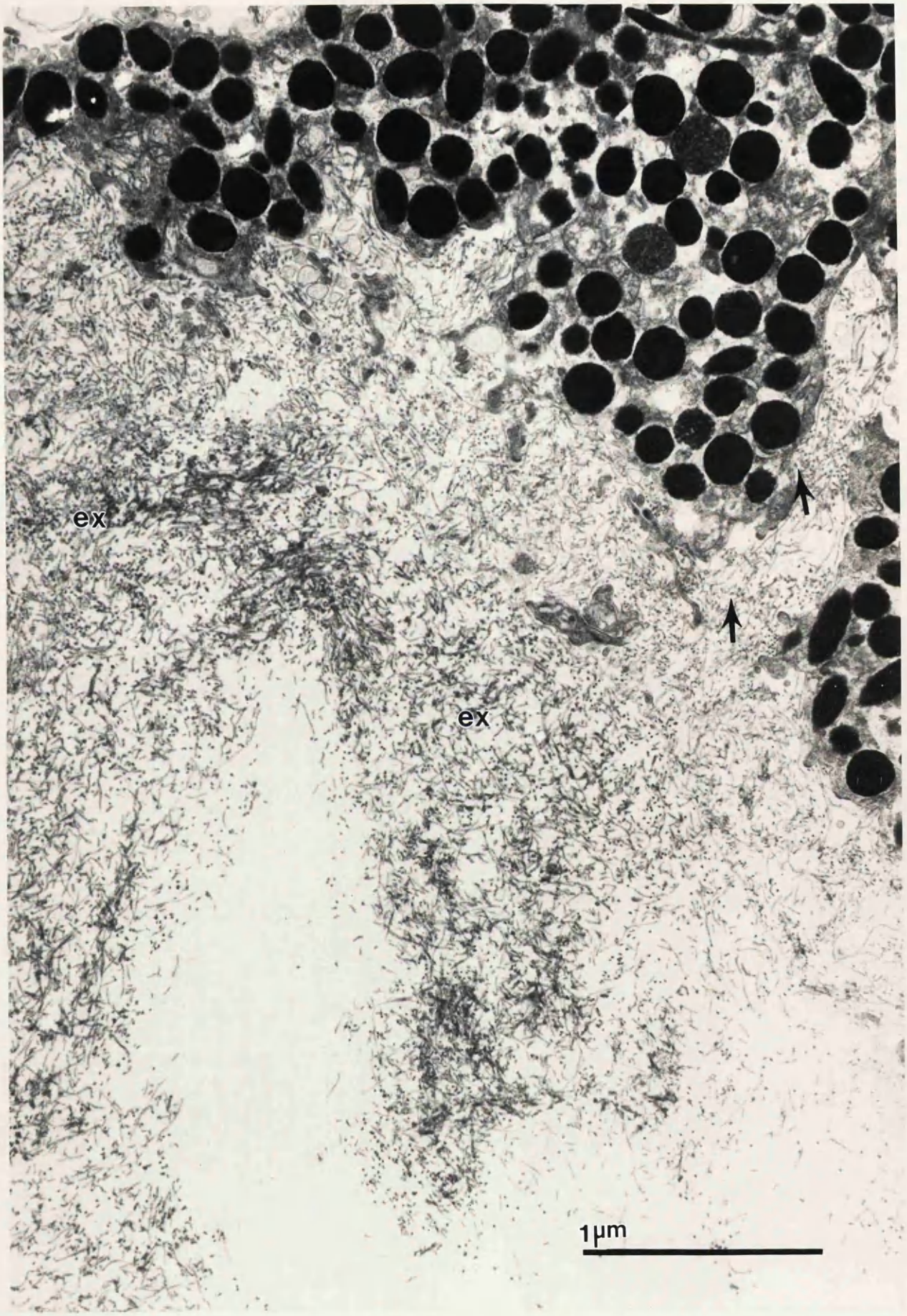


Figure 1.11: Transmission electron micrograph of exfoliation material aggregates(ex) on the basal surface of the iris posterior pigmented epithelium (PPE). The basement membrane is absent and exfoliation material is present between cells (arrows).

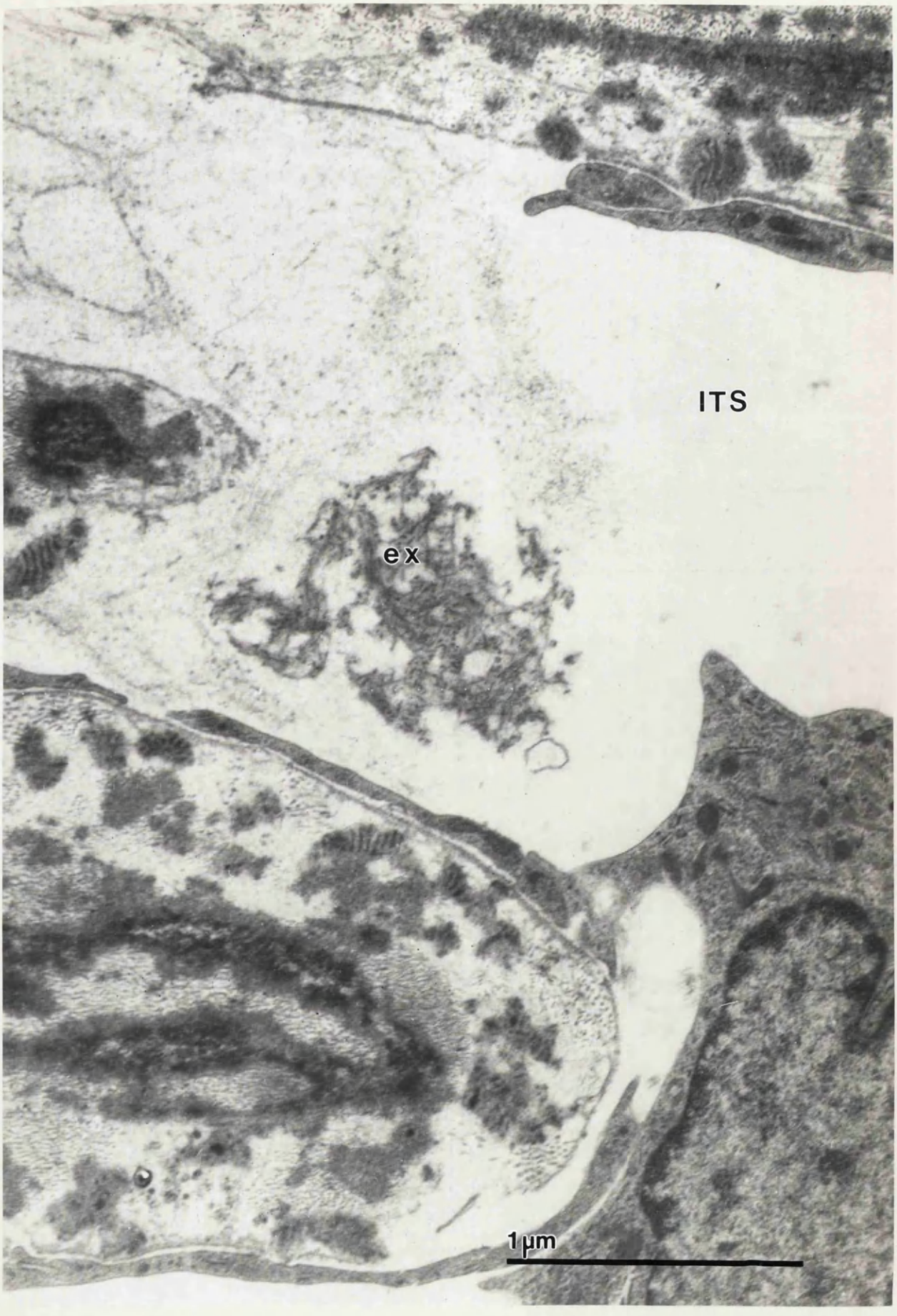


Figure 1.12: Transmission electron micrograph of exfoliation material in the trabecular meshwork of a patient with exfoliation glaucoma. An aggregate of exfoliation material (ex) lies in the intertrabecular space (ITS).

Periodicity

There is a lack of agreement as to the precise periodicity of exfoliation material. Overall, exfoliation fibres have been shown to display a major banding between 17-56 nm (Blackstad et al 1960, Bertelsen et al 1964, Ringvold 1969, Davanger 1980, Garner & Alexander 1984, Dark & Streeten 1982, Roh et al 1987) and a more inconsistent micro-periodicity of approximately 4-10 nm (Takei & Mizuno 1978, Dark & Streeten 1982). Streeten and coworkers (1990) found considerable variation in the structure of exfoliation fibres in different ocular and extraocular sites. Exfoliation fibres were divided into type A fibres (long, thin fibres up to 0.6 microns long) and type B fibres (short, thicker with unspecified length). A periodicity of 20-25 nm was more prominent on type A fibres, whereas type B fibres appeared to contain an increased amount of densely osmiophilic coating. Streeten and coworkers (1990) also observed that in the iris stroma exfoliation fibres tended to be shorter and less clearly banded, in comparison with the lenticular aggregates.

Other structural components

Two other structural components of exfoliation material have been described (Davanger & Pedersen 1975, Davanger 1978; 1980a; 1980b). A filament with a uniform width of 10 nm and a length up to 1 micron and an amorphous interfibrillar matrix in which exfoliation fibres and filaments are embedded. Davanger (1980a) employed lanthanum to enhance the structural features. He hypothesised that the basic unit of exfoliation material, the exfoliation fibril (fibre), may be formed by lateral aggregation of these filaments.

Ultrastructural distribution of exfoliation material

The distribution of exfoliation material and the associated matrix changes are described in relation to the tissue involved:

a) Conjunctiva

Exfoliation material was first localised in the conjunctival stroma by Ringvold (1972). Subsequent reports have shown that the involvement of the conjunctiva is a distinct feature (Ringvold 1973c, Speakman & Ghosh 1976, Roh et al 1987, Prince et al 1987, Streeten et al 1987; 1990). Ringvold (1972; 1973c) observed exfoliation material as densely packed aggregates which measured up to 22 microns across, consisted of irregularly arranged exfoliative fibres and contained elastic elements, collagen and some granular material. Abundant exfoliation material deposits adjacent to multilayered vascular basement membranes were also observed (Ringvold 1972).

Speakman & Ghosh (1976) found exfoliation material in the loose connective tissue of the conjunctiva, adjacent to the endothelium of thin-walled lymphatics and occasionally in close apposition to the walls of blood vessels. Roh and coworkers (1987) found no evidence of exfoliation material deposition near blood vessels but found 'poorly orientated microfibrils in close proximity to fibroblasts and elastic/collagen fibres'. Streeten and coworkers (1987) established a close relationship between exfoliation aggregates and stromal fibroblasts and elastosis. In contrast with previous studies they stated that 'exfoliative

fibres did not appear to be specifically related to collagen and exfoliative clumps did not contain visible collagen'.

The conflicting evidence on the exfoliative conjunctiva may be attributable to a difference in the material employed; Ringvold (1972) studied enucleated eyes, whereas, Roh et al (1987) and Streeten et al (1987) studied small tissue samples (i.e. conjunctival biopsies). Alternatively, the appearance of exfoliation material within and adjacent to conjunctival vascular basement membranes established by Ringvold (1972; 1973c) and Speakman & Ghosh (1976) may be a late phenomenon in the evolution of the disease.

b) Cornea

In a study on the cornea from eyes with exfoliation syndrome Sampaolesi and coworkers (1988) studied the accumulation of pigment upon the corneal endothelium. They suggested that the pigment seen biomicroscopically consisted of melanin granules located close to the cell nucleus within the endothelial cells.

c) Trabecular meshwork

In the first investigation on the exfoliation outflow system Ringvold & Vegge (1971) noted exfoliation material in the outflow system of 3 enucleated eyes. In the first eye with normal intraocular pressure (IOP), exfoliation material was present only in the inner intertrabecular spaces as clumps of varying size and density, often touching the trabecular endothelial cells. In the second eye, (with ocular hypertension), exfoliation material was also found in

asymmetric vacuoles inside the endothelial lining of Schlemm's canal; within the cribriform layer and in the outer walls of Schlemm's canal. Most giant vacuoles appeared empty, but significantly, some were filled with exfoliation material. In the third eye, (which had exfoliation glaucoma with bullous keratopathy), the amount of exfoliation material was greater than in the other two eyes. The trabecular beams were disorganised often lacking endothelial cells. In that eye the endothelial lining of Schlemm's canal showed areas of marked thinning and the cribriform layer was packed with exfoliation material. No giant vacuoles were seen.

Subsequent reports have demonstrated exfoliation material in the uveal meshwork (Sampaolesi & Argento 1977, Kurosawa 1981, Richardson & Epstein 1981), within the cribriform layer (Benedikt & Roll 1979, Shimizu et al 1980, Kurosawa et al 1983, Ito & Inomata 1985, Lutjen-Drecoll et al 1986, Rohen & Jikihara 1988) and in the outer wall of Schlemm's canal (Benedikt & Roll 1979, Kurosawa 1981, Kurosawa et al 1983). Some, but not all, published studies have found that some of the giant vacuoles in the lining endothelium of Schlemm's canal contain exfoliation material (Ringvold & Vegge 1971, Benedikt & Roll 1979, Kurosawa 1981, Richardson & Epstein 1981, Dark & Streeten 1982). To date, only one study has localised exfoliation material in the external collector channels (Kurosawa et al 1983). Figure 1.12 illustrates an aggregate of exfoliation material in the trabecular meshwork.

d) Iris

There are 14 TEM studies which report the ultrastructural features of the exfoliative iris (Shakib et al 1965, Ringvold 1969; 1970a; 1970c; 1973a, Anastasi et al 1974, Ghosh & Speakman 1974, Shimizu et al 1980, Ringvold & Davanger 1981, Shimizu & Futa 1985, Shimizu 1985, Ito & Inomata 1985, Spinelli et al 1985, Sugino 1990); (Fig 1.11). The ultrastructural features of the exfoliative iris are the subject of the first part of this thesis and the relevant literature is discussed in sections 5.2.4 and 6.4.

e) Ciliary body

Shakib and coworkers (1965) were the first to describe the ultrastructural changes of the exfoliative ciliary body based on their observations in one enucleated eye. They found exfoliation material in the surface convolutions and within the basement membrane (internal limiting membrane) of the non-pigmented ciliary epithelium. Exfoliation material was not observed in the cytoplasm, or nuclei of intact cells. It is interesting to note that in that study the pigmented and the non-pigmented epithelial cells and the blood vessels (no location given) were normal; no evidence of exfoliation material was found in the perivascular tissue or stroma.

In a subsequent investigation Ghosh & Speakman (1973) studied an eye enucleated for painful absolute glaucoma. They found similar features, but also noted marked localised thickening of the basement membrane of the non-pigmented ciliary epithelium in places where exfoliation material had accumulated. There were numerous uninvolved portions of the

basement membrane of the non-pigmented ciliary epithelium, with abrupt transitions between diseased and exfoliation free basement membrane. In their study the thickest most heavily stained exfoliation fibres were located in the middle third of the basement membrane. The inner third (i.e. the one in direct communication with the posterior chamber) contained only a few exfoliative fibres. This finding implies that exfoliation material may be actively synthesised by the non-pigmented epithelium of the ciliary body.

Davanger (1975) has provided the only SEM study of the exfoliative ciliary body to date. He examined 3 enucleated eyes to visualise 'plaques' of exfoliation material attached to parts of the ciliary body, particularly to the surface of the ridges of the ciliary processes.

f) Zonules

There are four studies of the zonular fibres at the site of their attachment to the lens (Ashton et al 1965, Takei & Mizuno 1978, Futa & Furuyoshi 1989, Chijiiwa et al 1989). These studies have shown the zonules to be encrusted with accumulations of exfoliation material (Ashton et al 1965, Takei & Mizuno 1978) and degeneration of the zonular fibres in association with heavy deposition of exfoliation material (Futa & Furuyoshi 1989, Chijiiwa et al 1989). The latter feature was observed in zonules of lenses obtained from exfoliation glaucoma patients with clinical evidence of phakodonesis.

g) Lens

The literature on the fine structure of the exfoliative lens has recently been reviewed by Seland (1988). In the surface layer of the anterior lens capsule exfoliation material is often mixed with degraded cell products, of which iris pigment granules are the most frequent (Bertelsen et al 1964, Dark et al 1977, Takei & Mizuno 1978). In a recent study, Dark & Streeten (1990) have suggested that the presence of a 'precapsular film' on the ageing lens capsule is a conceivable precursor of the exfoliation syndrome development on the lens. By SEM, this film appeared as a friable incomplete fibrillar layer, with rolling of the edges suggesting loose attachment. However, the importance of this finding was limited by the observation of some degree of precapsular film in control tissue.

The description of the lenticular features of the disease are conflicting. For example, the 'amorphous layer' observed by Bertelsen and coworkers (1964) could not be confirmed by Benedikt (1973). Seland (1979; 1988) documented an increased thickness of the exfoliative capsule. However, another study on 105 cataractous lenses showed no statistical difference in the capsular thickness between exfoliation-positive and exfoliation-negative lenses (Ruotsalainen & Tarkkanen 1987). Previous published work has stressed the importance of numerous fibrillogranular lenticular inclusions (Dark et al 1969, Bertelsen & Seland 1971) and lens epithelial pits (Bertelsen et al 1964, Dark et al 1969, Ueno et al 1987) in the evolution of exfoliation syndrome. However, all these features have been documented in clinically normal elderly

patients (Ghosh & Speakman 1972, Dickson & Ramsey 1979, Seland 1988). From the information available, only long term follow up of the development and progression of the lenticular changes in the exfoliation syndrome could delineate the complete spectrum and specificity of the pathological features that relate to the disease alone, as contrasted to those attributable to ageing, or cataract.

h) Vitreous body

Only one SEM study has documented the involvement of the vitreous cavity in the exfoliation syndrome (Davanger 1975b). Exfoliation material accumulated in an unspecified number of cases upon the anterior vitreous face of aphakic eyes in the form of radial stripes, presumably as a consequence of deposition from the aqueous humour.

i) Orbital sites

Formerly, exfoliation syndrome was considered principally a disease of the anterior segment of the eye. In the last two years however, two studies have identified exfoliation material, (or material morphologically similar to exfoliation material), in a number of previously unrecognised extraocular sites. First, Küchle and coworkers (1991) reported involvement of the recti muscles, vortex veins, and optic nerve sheath in a single case. Schlötzer-Schrehardt and coworkers (1991a) reported the presence of exfoliation material in the orbital connective tissue septae and central retinal vessels. These authors found unevenly distributed deposits of exfoliation material among normal connective-tissue components such as elastic fibres, collagen fibres and

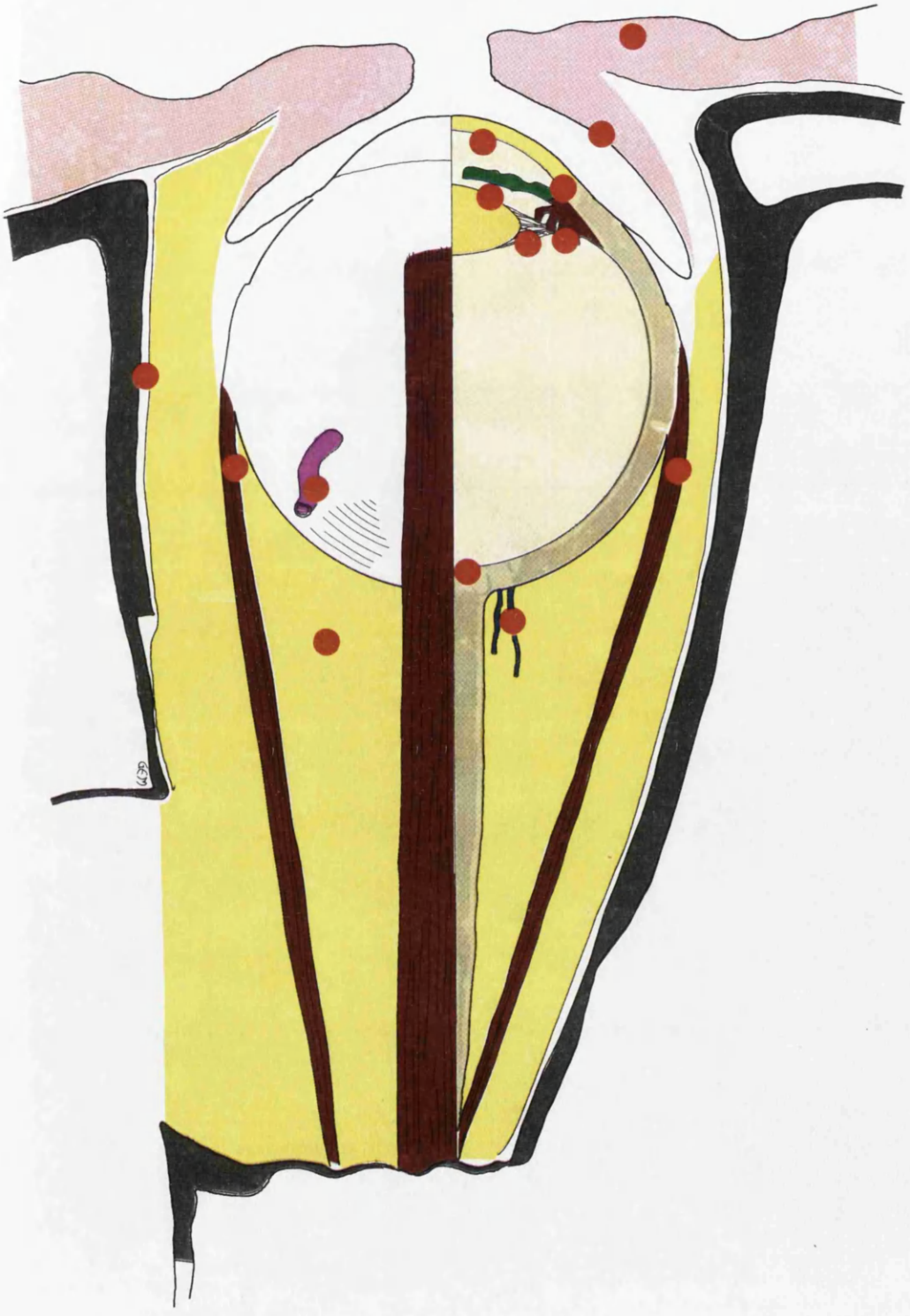


Figure 1.13: Summary diagram indicating the various sites (red circles) in which exfoliation material has been documented by transmission electron microscopy.

fibroblasts. In all 5 eyes exfoliation material aggregates were not evident in extrabulbar locations by LM, but could only be discerned by TEM examination of serial sections. Schlötzer-Schrehardt and coworkers (1991a; 1991b) have stated that in 2 cases exfoliation material localised in extrabulbar locations by TEM, could not be identified in intraocular locations (ciliary body and iris). The authors have speculated that the development of extraocular exfoliation syndrome may precede its intraocular appearance.

1.2.4 Is exfoliation syndrome a systemic disease?

In a recent study Sugino (1990) has reported typical exfoliation material in the dermis of the lateral canthus in 3 out of 9 cases of exfoliation positive patients. He confirmed the diagnosis in all 9 patients by examination of the iris. He also noted 'immature' forms of exfoliation material in the other 6 cases and suggested that exfoliation syndrome may be a systemic degenerative disease of the body. In an attempt to resolve the question of whether exfoliation syndrome is a local, or systemic disorder Streeten and coworkers (1990) carried out an ultrastructural examination of skin biopsy specimens from one to three areas in 13 patients with ocular signs of exfoliation syndrome. A 'fibrillopathy' closely resembling that seen in the affected eye was found in 11 out of 13 patients. Exfoliation material occurred primarily along elastic fibres. The authors concluded that exfoliation syndrome is a systemic disorder.

Figure 1.13 summarises the ocular and extraocular locations in which exfoliation material has been documented by TEM. Although recent evidence suggests that exfoliation material, or material which morphologically resembles exfoliation material, can be found in the skin of patients with exfoliation syndrome uncertainties remain about the significance of these findings. As conceded by Streeten and coworkers (1990) the diagnostic criteria employed in their study for the detection of exfoliation material must be supported by immunocytochemical, or biochemical data.

1.3 Nature of exfoliation material

The nature of exfoliation material has been extensively investigated, but remains obscure (reviewed by Dickson & Ramsey 1979, Jerndal 1986, Morrison & Green 1988, Ringvold 1988). Published work has indicated that exfoliation material is a complex compound (Davanger & Pedersen 1975, Dark et al 1977, Baba 1983, Streeten et al 1984, Ringvold 1988), which may even vary in its morphology in different regions of the eye (Streeten et al 1987; 1990).

1.3.1 Histochemical studies

The first histochemical description of exfoliation material was included in Dvorak-Theobald's paper (1954). The author states that 'since exfoliation material is moderately positive with PAS and Millon tests it may be assumed that mucopolysaccharides (glycosaminoglycans) and tyrosine are present'. In a subsequent study, Arnesen and coworkers

(1963) digested paraffin sections of enucleated eyes with exfoliation glaucoma with collagenase. It was found that although the lens capsule disappeared, exfoliation deposits remained unaltered. They suggested that the exfoliation deposits are chemically different from the lens capsule and probably do not consist of collagen. They also found faint staining with Alcian Blue and therefore lent their support to Dvorak-Theobald's hypothesis that glycosaminoglycans are present in the exfoliation material.

Bertelsen and coworkers (1964) found these reports unconvincing because 'glycosaminoglycans are PAS negative and if they were present in large quantity then the Alcian Blue reaction should have been strongly blue'. On the other hand, they asserted 'mucoproteins and glycoproteins stain weakly blue with Alcian Blue'. Bertelsen & Ehlers (1969) investigated the histochemical properties of exfoliation material aggregates on the lens surface of 7 lenses from patients with exfoliation syndrome, who had undergone intracapsular cataract extraction. In their study the abnormal material stained lightly with toluidine blue and was completely negative with Sudan black B (stain for lipids), Congo-red (for amyloid), Alcian blue (for glycosaminoglycans), and elastin/Weigert reticulin stain (for elastic fibres). The excrescences were positive with Millon stain (for tyrosine), Acid fuchscin -van Gieson- stain (for collagen) and reticulin stain (for reticulin fibres). The same authors carried out a number of enzyme digestion experiments in 5 exfoliative lenses fixed in a mixture of acetic acid and ethanol. Exfoliation aggregates and

fibrillogranular inclusions within the lens were resistant to proteolytic enzymes: pepsin, trypsin, chymotrypsin, collagenase and papain (Bertelsen & Ehlers 1969).

Periodic acid-Schiff stain

Periodic acid-Schiff stain (PAS), which demonstrates the presence of mucoproteins, glycoproteins and lipoproteins has reacted positively with exfoliation material in some studies, but the PAS reaction has not been uniformly reported. Although initial studies reported it to be positive (Dvorak-Theobald 1954, Hörven 1966), Bertelsen and coworkers (1964) found it to be 'mostly negative'. In an ensuing study Bertelsen & Ehlers (1969) reported that the excrescences on the surface of the lens capsule were weakly positive (red) with PAS staining. Dark and coworkers (1969) reported exfoliation material to be PAS positive, Alcian blue negative and strongly positive with Chrome haematoxylin. They noted variable colouration with their tests which they attributed to the compound nature of the abnormal substance.

Exfoliation material and amyloid

Ringvold & Husby (1973) reported positive staining of exfoliation aggregates on the ciliary body and on the posterior iris surface after staining with Sirius red and Congo red. Ringvold & Husby (1973) have argued that exfoliation material should be classified amongst the amyloid-like substances. Indeed, in an TEM investigation Meretoja & Tarkkanen (1977) demonstrated amyloid-like deposits in 19 selected specimens (17 enucleated eyes, one autopsy, one iridectomy) from eyes with exfoliation syndrome.

Amyloid material is difficult to distinguish histologically, but can be histochemically differentiated from exfoliation material (Schwartz et al 1982). Subsequent reports have been unable to confirm the previous positive histochemical results for amyloid in the exfoliation material. Dark and coworkers (1977) studied sections from 3 cataractous lenses with exfoliation syndrome and reported that Congo red dichroism, the most critical histochemical test for amyloid, was not present. Several studies since have confirmed that exfoliation aggregates do not stain with Congo red (Garner & Alexander 1984, Streeten et al 1984; Li et al 1989). Thus today, there is little support for the view that exfoliation syndrome is a type of amyloidosis. Nevertheless, it is conceivable that exfoliation material is an amyloid-like substance which does not stain with Congo red.

1.3.2 Biochemical and tissue culture studies

Amino acid analysis

A preliminary amino acid analysis of exfoliation material was performed by Ringvold in 1973. The author collected exfoliation material from the anterior surface of 18 lenses with demonstrable deposits. The amino acid analysis according to this study showed that exfoliation material was rich in glutamic acid and aspartic acid. It contained glycine, proline, serine and alanine; and was deficient in 4-hydroxyproline. Ringvold (1973) suggested that this profile was consistent with the presence of proteins and made it unlikely that collagen was a significant component of exfoliation material (as collagen is rich in

4-hydroxyproline). He was of the opinion that exfoliation material had a similar amino acid profile to amyloid, the microfibrillar component of elastic fibres and the non-collagenous proteins of the basement membranes.

Aqueous humour studies

Baba (1983) provided evidence of an abnormal electrophoretic pattern (peculiar tailing and a biphasic pattern in albumin) in 3 aqueous samples from eyes with exfoliation syndrome. The author speculated that his observations indicated an abnormal overproduction of glycosaminoglycans in the exfoliation syndrome. Hannappel and coworkers (1985) studied the amino acid pattern in the aqueous humour of 5 patients with exfoliation syndrome, in comparison with 27 POAG patients and 30 controls (cataract patients). All 21 amino acids studied showed higher levels in POAG than in exfoliation, or control patients. Ringvold and coworkers (1989) have studied 4 exfoliative lens capsules with SDS-PAGE electrophoresis and established the presence of two polypeptides (molecular weight estimated between 14,400 and 20,000), not present in their controls. The significance of these findings was difficult to assess, but the authors have speculated that either exfoliation syndrome generated unknown products, or these polypeptides represented fragments from established proteins. In the same study, 15 pooled aqueous samples collected during cataract surgery from patients with exfoliation syndrome were analysed. No definitive differences with the controls could be obtained.

Tissue culture studies

Only one tissue culture study has been performed to date on the exfoliation syndrome. Ringvold & Nicolaissen (1990) studied by TEM samples of iris tissue from 8 exfoliation positive patients and 6 normal controls. The iridectomy specimens were cultured on tissue culture plastic and on biological basement membranes. Extracellular fibrillar material closely resembling exfoliation material appeared to originate from the newly divided cells in the exfoliation-positive iris tissue after four weeks.

1.3.3 Immunohistochemical studies

Immunoperoxidase studies

Harnisch and coworkers (1981), employed the indirect immunoperoxidase technique to study 3 lenses from patients with the exfoliation syndrome. The authors treated semi-thin frozen sections with antibodies against basement membrane proteoglycans (BM_1PG isolated from EHS-sarcoma) and found positive staining for basement membrane proteoglycans. The authors considered that their findings supported the hypothesis that the disorder is caused by a disturbance in the biosynthesis of basement membranes.

Streeten and coworkers (1986), employed indirect LM and EM peroxidase techniques with polyclonal and monoclonal antibodies to study 10 lenses with the clinical diagnosis of exfoliation syndrome. Exfoliation aggregates stained with zonular antisera (most intense staining, mainly in the outer portions of large exfoliation fibres), bovine elastic

microfibrillar protein (less diffuse staining, less penetration) and monoclonal fibrillin antibody (diffuse stain). Streeten and coworkers (1986) offer evidence which indicates that the ocular zonules share similar histochemical properties and some component, or components with the exfoliation material. The significance of these results however, remains uncertain. Firstly, the peroxidase method results in a diffuse aggregate which makes the precise localisation of the antibody difficult. Secondly, as conceded in the paper, the antibodies employed may have labelled more than one antigen. Finally, in support of their hypothesis, the authors claim that 'we have found no cross-reactivity (unpublished work) between exfoliation material and three basement membrane components: type IV collagen, laminin and basement membrane proteoglycan'. Since at that time, the distribution of these three important ECM components in the normal ocular tissues was unknown, their comment is surprising. It is reasonable to assume that the specific immunohistochemical localisation of collagen type IV, laminin and basement membrane proteoglycan would have been of considerable interest to the scientific community. On the other hand, failure to establish the distribution of these three basement membrane components in the normal ocular tissues invalidates the negative results obtained for exfoliation material.

1.3.4 Immunocytochemical studies

Elastin and exfoliation material

In the first immunogold study, to investigate the nature of exfoliation material, Li and coworkers (1988) have shown the presence of elastin and tropoelastin in exfoliation material. Specimens were examined from 12 patients with exfoliation syndrome/glaucoma diagnosed clinically and histologically. Evidence for elastin and tropoelastin-associated epitopes in exfoliation material was seen in both the intraocular and conjunctival sites. Some variability in binding affinity was seen in the different ocular sites and immunogold labelling was seen more often on the periphery than in the centre of the exfoliation fibres.

Neither elastin, nor the elastic microfibrillar protein are known to be circulating proteins. Indeed, the secretion of these molecules in the extracellular space is rapidly followed by cross-linking and insolubility (Ruggeri & Motta 1984, Ringvold 1992 personal communication). Therefore, this observation implies secretion of these molecules by local ocular cells, especially basement membrane-producing cells. As the authors concede, 'all of the cells in juxtaposition to exfoliation material in the posterior chamber are epithelial cells, not known to engage in elastogenesis normally' (Li et al 1988). To substantiate this hypothesis Li and coworkers (1988) quote three lines of evidence. First, unpublished observations of elastin epitopes in cultured ciliary non-pigmented epithelial cells. Second, the likelihood that zonular fibres are synthesised by the non-pigmented

epithelium of the ciliary body. Thirdly, the appearance of microfibrils resembling elastic microfibrils, or oxytalan in proximity to basement membranes (e.g. Descemet's membrane) in some unspecified disorders. These authors have concluded that exfoliation syndrome arises from abnormal regulation of extracellular matrix (ECM) synthesis (Li et al 1988).

Amyloid P and exfoliation material

In a recent review article Ringvold (1988) has suggested that in retrospect, the observed cross-reaction between anti-amyloid serum and exfoliation material could have been attributable to the presence of amyloid-P, a less prominent component of all types of amyloid which constitutes 5-10% of the dry weight of amyloid substance. In addition, Ringvold states that 'the same substance is part of the normal basement membrane and it may be part of the exfoliation material if this is an abnormal basement membrane product'.

Indeed, Li and coworkers (1989) have recently demonstrated by immunoelectron microscopy the presence of amyloid P protein in exfoliation aggregates from 14 ocular and conjunctival specimens from patients with exfoliation syndrome. In their study amyloid was not found by Congo red stain, or ultrastructural examination. Moreover, immunostaining of exfoliation material for other common amyloid proteins, including amyloid A, prealbumin and immunoglobulin light chains was negative in most cases. Immunoelectron microscopy on Lowicryl sections revealed weak labelling for amyloid P mostly on the edges of exfoliation fibres. No labelling for amyloid P was seen on oxytalan fibres, in the zonules and in

the lens capsule. The authors were of the opinion that the consistent presence of amyloid P, a minor serum component, within exfoliation aggregates indicated a specific association. They proposed that there may be similar binding sites on exfoliation fibres with those on elastic fibres. However, no direct data was obtained in any of their specimens to support this hypothesis.

There are two limitations to the study by Li and coworkers (1989). First, as the authors themselves point out, it is well documented that a varied degree of iris vasculopathy is a common feature of the exfoliation syndrome. This increased permeability of the walls of iris vessels and the consequent leakage of protein could account for the presence and binding of amyloid P. Second, amyloid P is present in basement membranes. The authors refute this possible source of amyloid P since 'they did not find it in the lens capsule'. However, lens capsule is a highly specialised basement membrane with a unique ECM composition (Marshall et al 1991d). It is the vascular basement membranes that are the likely sites of amyloid P, but there is no mention of these in the paper.

1.4 Origin of exfoliation material

Several anatomical sites have been proposed for the origin of exfoliation material, but the published reports to date have not established the principal source(s) with any certainty.

The lens

Bertelsen and coworkers (1964) and Dark and Streeten (1969) supported the exclusive lenticular origin of exfoliation material with the fine structural observation of fibrillogranular inclusions apparently emanating from the diseased epithelium. The morphological similarity between these inclusions and exfoliation fibrils prompted these investigators to suggest a lenticular origin for exfoliation material. The supporters of the lens epithelial origin believed that exfoliation material originates from a metabolically abnormal lens epithelium and subsequently migrates through the lens capsule.

The uvea

In 1951, Weekers and coworkers proposed that the primary change in exfoliation syndrome was an atrophy of the the pigmented epithelium of the iris and thus exfoliation material was depigmented iris tissue. Sunde (1956) highlighted the importance of the close proximity of the iris in the formation of the peripheral band of exfoliation material on the lens. He showed that patients with an iris coloboma and exfoliation syndrome exhibited a discontinuous band of 'lenticular' exfoliation material at the site of the iris coloboma.

Sugar in a series of publications (Sugar et al 1976, Sugar 1976; 1980; 1984) provided evidence suggesting that the iris may be the source of exfoliation material. He pointed out that the pattern of exfoliation deposition upon the lens was dependent upon the configuration of the iris. In a case report he demonstrated changes in the typical circular pattern of exfoliation material deposition on the lens capsule of a patient with post-traumatic alteration of the iris shape (Sugar 1976). He considered that exfoliation material could not originate from the lens as it could not pass through the thick lens capsule. Most investigators in the subsequent literature appear to have tacitly accepted that the iris may be an additional source of exfoliation material (reviewed by Morrison & Green 1988). The ciliary epithelium has also been considered a source of exfoliation material, although the evidence for this is more tentative (section 1.2.3).

The morphological evidence put forward by the supporters of the uveal origin of the exfoliation material had one inherent weakness. It does not exclude the possibility of exfoliation material originating from the lens epithelium subsequently being transferred to uveal locations via the aqueous circulation. The exclusive lenticular origin of the exfoliation material started to lose favour only when clinical and ultrastructural studies supplied comprehensive evidence of exfoliation material synthesis in the absence of the lens. Exfoliation material synthesis was shown to commence (Fellner & Benedikt 1971) and continue (Radian & Radian 1975, Sugar 1980, Caccamise 1981) long after removal

of the lens by intracapsular cataract extraction. In a case report, Ghosh & Speakman (1983) confirmed, by TEM examination the absence of exfoliation material from the lens removed from the uninvolved eye of a patient with unilateral exfoliation syndrome. Five years later, the patient developed exfoliation syndrome in the originally uninvolved eye. Nine years later, when the eye was removed at autopsy, typical exfoliation fibres were found in the iris, the ciliary body and the anterior vitreous. Furthermore, in a recent iris tissue culture study, Ringvold & Nicolaissen (1990) provided evidence to suggest in vitro synthesis of exfoliation material in the iris.

A comparable pattern of exfoliation material distribution to that found on the human lens has been shown to arise on the surface of plastic intraocular lenses following extracapsular cataract surgery (Ringvold & Bore 1990). Published evidence thus favours the synthesis of exfoliation material in the iris, but the precise pathway remains uncertain. The plausible role of the ciliary epithelium in the synthesis of exfoliation material requires further elucidation.

Other sites of synthesis

Ringvold & Vegge (1971) proposed that the trabecular meshwork may be an independent source of exfoliation material. The case for local synthesis of exfoliation material in the trabecular meshwork has not been substantiated since that study. Ringvold (1972; 1973) provided TEM evidence for the presence of exfoliation material in the limbal (1972) and palpebral (1973) conjunctiva. He considered it unlikely that

exfoliation material was transported to the palpebral conjunctiva, as this tissue is neither exposed to the aqueous humour, nor to the blood supply of the anterior segment. He proposed that the conjunctiva is an additional source of exfoliation material. Indeed, Prince and coworkers (1987) have proposed that the synthesis of exfoliation material in the conjunctiva may precede the clinically obvious disease.

A recent report by Schlötzer-Schrehardt and coworkers (1991a) has provided evidence of exfoliation material in a number of extrabulbar locations including the orbit (section 1.2.3). They have proposed that synthesis of exfoliation material outside the globe may precede its intraocular appearance. On reviewing the literature on the origin of exfoliation material, Dark & Streeten (1982) highlighted a potential complicating factor: exfoliation material may aggregate in locations remote from the original site of synthesis. This question can only be resolved when accurate biochemical data are obtained on the composition of exfoliation material in a variety of anatomical locations.

1.5 Pathogenesis

1.5.1 Pathogenesis of exfoliation syndrome

The theories concerning the pathogenesis of exfoliation syndrome have been extensively reviewed (Hörven 1937, Dickson & Ramsey 1979, Dark & Streeten 1982, Jerndal 1986, Ringvold 1988, Morrison & Green 1988). Although there are no known published studies on animals, exfoliation syndrome is

considered to be a disease peculiar to man. On the basis of studies published on the subject, the risk to individuals is related to ageing (section 1.6.8).

The basement membrane disorder theory

Anomalous basement membrane synthesis may well underly the pathogenesis of exfoliation syndrome (Bertelsen et al 1964, Anastasi et al 1974, Sugar et al 1976, Seland 1988, Morrison & Green 1988). Vascular basement membrane abnormalities accompany the perivascular accumulation of exfoliation material (Ringvold 1969; 1970a; 1988, Ringvold & Vegge 1971, Harnisch 1977, Eagle et al 1979, Garner 1990) and basement membrane proteoglycan components have been identified in such material (Harnisch et al 1981). However, morphologically only specific basement membranes are involved (e.g. vascular basement membranes) but the basement membrane of the cornea and retina have never been implicated (Seland 1985).

To summarise, most workers in the field still favour the hypothesis that exfoliation syndrome is a form of basement membrane disorder (Ringvold 1969; 1988, Layden & Shaffer 1973, Sugar et al 1976, Dickson & Ramsey 1979, Harnisch et al 1981, Layden 1982, Seland 1985, Yanoff & Fine 1989, Garner 1990), but the evidence for this is still circumstantial.

The elastosis theory

Streeten and coworkers (Streeten et al 1984; 1986a; 1987 & Li et al 1988) have postulated that the exfoliation process is a type of elastosis with elastic microfibrils, or an abnormal related fibril, forming the basis for the exfoliative fibres.

These authors have proposed that exfoliation material derives from abnormal polymerisation of glycoproteins associated with the zonular-elastic microfibrillar system. This theory relied upon histochemical and immunohistochemical similarities between exfoliation material, the ocular zonules (Streeten et al 1986a; 1986b) and elastic components (Li et al 1988). With regard to the theory that exfoliation syndrome is a basement membrane disorder, Streeten and coworkers (1986) have stated that 'the proximity between unhealthy basement membranes and exfoliation material appears to implicate the basement membrane-producing cells in the pathogenesis of exfoliation syndrome, but does not necessarily mean that exfoliation material is composed of basement membrane in any usual sense'. Thus, although there is no unified concept concerning the pathogenesis of exfoliation syndrome, there is at least general agreement on the involvement of local basement membrane cells in the pathogenesis of the disorder.

Other theories on the pathogenesis of the disease

Schlötzer-Schrehardt and coworkers (1991a) have suggested that exfoliation syndrome may result from a disordered synthesis and\or assembly of connective-tissue microfibrils. Their theory was based on the observed association between connective tissue components and exfoliation material. There is some evidence that exfoliation syndrome may be an environmental disease (Taylor 1979), or that at least environmental factors may influence its pathogenesis (Taylor 1979; 1980, Khanzada 1985, Johnson et al 1989). Taylor (1979) found a strong occupational association, with

Australian Aborigine stockmen exhibiting a significantly higher prevalence of the disorder when compared with an age matched control Aboriginal population. He also established that exfoliation syndrome increased at lower latitudes and higher levels of solar radiation. He suggested that solar ultraviolet irradiation may induce protein denaturation or synthesis of abnormal basement membrane precursors (Taylor 1979; 1980).

Genetic factors (Teikari 1987), a traumatic cause (Sugar 1976), a vascular aetiology (Anastasi et al 1974, Spinelli et al 1985) and an unspecified immunological background (Krishnan et al 1986) have also been implicated in the pathogenesis of the disease. The evidence offered for these theories remains unconvincing. To date, it appears that the triggering mechanism for the development of exfoliation syndrome and the pathway by which exfoliation material is synthesised remains as obscure as when the condition was discovered seventy-four years ago by Lindberg (1917).

1.5.2 Pathogenesis of exfoliation glaucoma

It has been established that aqueous outflow may be obstructed by cells and particulate matter carried into the trabecular meshwork along with the aqueous (Spencer 1985). What remains uncertain, however, is the precise role of exfoliation material in exfoliation glaucoma and why a significant number of patients with exfoliation syndrome do not ultimately develop exfoliation glaucoma (Tarkkanen 1986).

Some authors (Trantas 1929, Cebon & Smith 1976, Tarkkanen 1984, Wollensak et al 1992), are convinced that exfoliation syndrome does not cause glaucoma; that either it is a coincidental feature of ageing, or its presence merely aggravates pre-existing POAG. However, the majority of workers in the field support the view that exfoliation glaucoma is a true secondary open-angle glaucoma (Vogt 1930, Dvorak-Theobald 1954, Ringvold & Vegge 1971, Rohen & Witmer 1972, Linner & Rohen 1974, Harnisch 1977, Rohen 1983, Lutjen-Drecoll et al 1986).

Lütjen-Drecoll and coworkers (1986) have shown a significant difference between the ultrastructural appearance of the outflow system in POAG and exfoliation glaucoma. By quantitative analysis of the 'plaque material' in the cribriform layer and outer wall of Schlemm's canal (38 and 26 specimens respectively) they established disease-related differences. In exfoliation glaucoma, the total amount of 'plaque material' was significantly less than that detected in POAG and comparable to that found in normal autopsy control eyes.

An important question in the pathogenesis of exfoliation glaucoma is whether the increased resistance is due to trabecular endothelial phagocytosis. As stated previously, Dvorak-Theobald (1954) considered that the endothelium of the trabecular meshwork phagocytosed exfoliation material. With one exception (Ringvold & Vegge 1971), her observation, was not confirmed by subsequent studies, which indicated that unlike pigment, exfoliation material is not phagocytosed by

the trabecular endothelial cells (reviewed by Spencer 1985). Richardson & Epstein (1981) employed TEM and SEM to study two autopsy eyes from a patient with exfoliation glaucoma. In both eyes the intertrabecular spaces were open and relatively free from exfoliation material, but massive deposits of exfoliation material accumulated in the cribriform layer and obstructed Schlemm's canal.

Ringvold & Vegge (1971) found a close relationship between the degree of exfoliation material deposition within the outflow system and the disease stage. Extrapolating from their findings the authors have suggested that the accumulation of exfoliation material within the outflow system is the principal mechanism underlying the development of exfoliation glaucoma. Kurosawa (1981) was in broad agreement with Ringvold & Vegge. He considered that exfoliation material may occlude the outflow system at various anatomical levels.

On the other hand, Benedikt & Roll (1979) in a TEM study of the outflow system of a non-glaucomatous eye with exfoliation syndrome, found that extensive infiltration with exfoliation material had not caused glaucoma. They endorsed the view that exfoliation material does not play any significant role in the pathogenesis of exfoliation glaucoma. Their observations on this single case however, stand in isolation from those of other investigators who consider that exfoliation material deposition within the cribriform layer obstructs the outflow system (Ringvold & Vegge 1971, Kurosawa 1981, Richardson & Epstein 1981, Lutjen-Drecoll et al 1986).

1.6 Epidemiology

In the early literature, the reported prevalence of exfoliation syndrome varied greatly in different ethnic groups. Early authors, such as Busacca (1927), Trantas (1929), Rehsteiner (1929), Baumgart (1933), Hörven (1937), Irvine (1940), Lemoine (1950), Leydhecker (1960), Forsius & Eriksson (1961), Joannides and coworkers (1961) and Clements (1968) considered the condition to be common in some countries (Finland, Norway, Äland Islands, Isle of Man, Russia, Greece, Italy and India) and rare in other countries (Sweden, Denmark, Britain, France, Germany and the United States).

In a retrospective study, Lemoine (1950) found 29 patients with exfoliation glaucoma in Boston, USA, out of a total of 816 glaucomatous patients (4%). Leydhecker (1960) has claimed that he could not find one single case of exfoliation glaucoma amongst 500 German glaucoma patients in Bonn. In contrast, the reported proportions of exfoliation glaucoma in Scandinavian, Greek and Indian studies ranged between 24% and 79% (Lindberg 1917, Malling 1923, Trantas 1929, Irvine 1940, Thomassen 1949, Joannides et al 1961, Klouman 1967, Krause 1973). Thomassen (1949) studied two cohorts of glaucoma patients (including operated cases) after dilatation of the pupil and gonioscopy. In the University Department of Ophthalmology in Oslo, Norway, 45 out of 57 cases (79%) had exfoliation glaucoma. In Moorfields Hospital, London, England, only one out of 50 cases (2%) was reported as showing evidence of exfoliation (Thomassen 1949).

Differences of opinion

In the literature of the late sixties and early seventies, the notion that exfoliation syndrome varied greatly between different countries came under close scrutiny, especially by the Norwegian ophthalmologist Aasved. Bertelsen (1966) first succinctly pointed out that 'the opinion regarding the frequency of the disease in Sweden depends largely on the interest which the particular ophthalmologist takes in the entity'. To further demonstrate this point, Bertelsen gave as an example the first report on the prevalence of the disorder in Sweden by Strömberg (1962). He found 22 patients with the condition among 213 individuals with ocular hypertension detected in mass screening of the population over the age of 40 in a Swedish town. Another ophthalmic surgeon working in the same area was of the opinion that exfoliation syndrome did not exist in that district, until he became acquainted with Strömberg's report (Bertelsen 1966). Aasved (1969, 1971) challenged the prevailing concept that exfoliation syndrome showed considerable geographic variation. First, he refuted the evidence presented to that date on the prevalence of exfoliation syndrome as unrepresentative, since it was not based on random population samples. Writing in 1971, Aasved suggested that he could find only 7 population studies in the literature; of these only 2 (Bertelsen et al 1965, Hollows & Graham 1966) had screened more than 600 individuals.

Motivated by disbelief in the reported differences in the prevalence of exfoliation syndrome in neighbouring European countries, Aasved carried out a prospective personal survey

of the prevalence of exfoliation syndrome in three cohorts over the age of 60 in Bergen, Norway; Bonn, Germany and Birmingham, England. He established that the prevalence of exfoliation syndrome was similar in the three ethnic groups- Bergen 6.3%, Bonn 4.7%, Birmingham 4.0%- (Aasved et al 1969, Aasved 1971d; 1979b).

Thus, Aasved provided convincing epidemiological evidence to suggest that the prevalence of the disorder in population groups is first, much higher than previously thought and second, similar in all geographic areas. Subsequent studies, mostly in ophthalmic cohorts, in various ethnic groups have either supported (Sood 1968, Sveinsson 1974, Kinoshita 1979, Hiller et al 1982, Krause et al 1988), or contradicted (Forsius 1979; 1988, Ringvold et al 1988, Cashwell & Shields 1988, Aine 1988, Stefaniotou 1990) Aasved's view. The most recent literature does suggest that the prevalence of the condition may vary significantly even within the same country and remarkable differences exist within and between various ethnic groups. However, none of these studies is strictly comparable, or of similar scale to those of Aasved. Thus, Tarkkanen (1986) has asserted that 'the prevalence of exfoliation syndrome is similar in those countries that population-based surveys have been conducted'.

Geographic distribution

Worldwide, the reported prevalence of exfoliation syndrome varies from 16% in a Greek population cohort (700) over the age of 50 years (Stefaniotou 1989), to 0% amongst 96 Eskimos of similar age (Forsius 1979). Forsius (1988) has reviewed

the epidemiological data available and has highlighted some inconsistencies and factors that may give rise to regional and geographic variations. Significantly, little is known about the prevalence of the disorder in many countries worldwide. Moreover, many published reports are unsatisfactory. Extrapolating the true prevalence of exfoliation syndrome from retrospective studies on selected ophthalmic patients is a tenuous exercise. The following review focuses on the most relevant studies and emphasises up-to-date published reports on the subject.

In a population based study in Middle-Norway, Ringvold and coworkers (1987; 1988) found the prevalence of exfoliation syndrome in individuals over the age of 64 to be different in three separate municipalities. They established a marked variation in the geographic distribution of exfoliation syndrome between coastal (10.2%) and inland areas (21% and 19.6%). Hollows & Graham (1966) investigated a defined population of 4,231 individuals in Wales, 40-75 years old. The overall prevalence rate for exfoliation syndrome in the total population was 0.22% (10 out of 4,231). Bartholomew (1979) investigated specifically the prevalence of exfoliation syndrome/glaucoma in three Scottish cohorts in Edinburgh. The prevalence of exfoliation syndrome in a consecutive in-patient population with cataract was 2.5% (6 out of 247 patients); the prevalence of exfoliation syndrome in a consecutive out-patient cohort was 1.5% (18 out of 1,200 patients) and the prevalence of exfoliation glaucoma in a newly diagnosed glaucoma cohort was 5% (number unspecified).

Forsius (1979) has documented a high prevalence of exfoliation syndrome in four different populations above 60 years of age in Oulu, Finland, Reykjavik, Iceland, Åland Islands and Novosibirsk, Russia. The prevalence of exfoliation syndrome in this study varied between 29.8% in Finland and 12.4% in Russia. In a subsequent study in a Russian glaucoma group it was found that 37.7% of patients below the age of 70 years and 70% above that age suffered from exfoliation glaucoma (Forsius 1979). Frolova & Khamitova (1984) screened 3,400 Russians over the age of 40 years, engaged in sedentary work, during the course of a regular medical check-up. They detected exfoliation syndrome in 200 individuals (5.9%) and recommended obligatory screening for exfoliation syndrome in regular medical check-ups of subjects over the age of 40 years.

In a prospective study conducted by 30 ophthalmologists in private practice in France the total prevalence among 4,042 ophthalmic patients older than 50 years was 5.5% (Colin et al 1988). Significant regional differences were observed within two regions (Brittany and South-East France showing higher prevalences of 16.3% and 8% respectively). Montanes and coworkers (1990) documented in a prospective study a high prevalence for exfoliation glaucoma in the North-West of Spain (44.5% of the open angle glaucoma cohort).

In a population study in South Africa, Bartholomew (1971) examined 2,584 Pondos, a Bantu tribe, over the age of 30. He found typical exfoliation syndrome in 132 individuals (5.1%) and early changes (termed pregranular exfoliation) in 76,

predominantly younger individuals (2.9%). In another study the same author (1973) studied 9 rural black tribes (Bantus and Swahilis) on field tours and found exfoliation syndrome to be present to a varied extent in 8 of these tribes (total prevalence in 4,156 individuals of all ages was 0.55%).

More recently, Shiose and coworkers (1991) carried out a prospective population survey to establish the prevalence of the glaucomas in seven districts throughout Japan (8,126 persons over the age of 40). Exfoliation glaucoma was found in 13 individuals (0.16%) whereas, POAG was detected in 47 individuals (0.58%).

In the only American population study to date, conducted in Framingham, United States, 1,906 patients age between 52 and 85 were screened for the presence of exfoliation syndrome. The overall prevalence of the disorder was 1.8% (36 out of 1,906 individuals screened). In the age group 75-85 the prevalence of the condition was 5.0%. As the ophthalmic examination in this study was conducted by residents in ophthalmology, presumably with little expertise, or special interest in the condition, it is conceivable that the true figure in that population may have been higher.

Controversy concerning geographic variation

Exfoliation syndrome is a common condition, but its true prevalence is particularly difficult to assess because of the insidious onset and subtle signs (Gifford 1958, Tarkkanen 1962). The diagnostic sensitivity increases when the condition is actively sought by an experienced observer

(Aasved 1969, Jerndal 1986). Although the clinical description of advanced exfoliation syndrome is well established, little clinical data exist as to the early changes of the disorder (Jerndal 1985; 1986, Dark & Streeten 1990). These have not been adequately documented, but may be particularly subtle and confined to the mid-periphery of the lens (Bartholomew 1971 Jerndal 1985, Dark & Streeten 1990). Exfoliation syndrome and the ensuing exfoliation glaucoma have a worldwide prevalence and an age related incidence. The prevalence of the disorder is generally underestimated. A clear geographic variation within and between various ethnic groups is possible, but further large prospective population studies employing similar methodology are required to prove this hypothesis. Several reports have indicated that, as yet, unidentified genetic, ethnic, or environmental factors may contribute to the varied prevalence of the condition.

Other epidemiological features

Age

It is well established that exfoliation syndrome is age-dependent (Aasved 1969; 1971, Tarkkanen 1986, Forsius 1988). The youngest patients recorded in the world literature have been 22 (Sugar 1976); 27 (Aminisarduei & Ferkrat 1986); 31 (Hörven & Huchinson 1967, Küchle & Naumann 1992); 32 (Khanzada 1985); 35 (Hörven & Huchinson 1967, Taylor et al 1977) and 39 (Joannides et al 1961).

In 25 published reports in the Western and Japanese literature the youngest patient was over the age of 50 years.

On the other hand, a distinctly younger pattern exists in some racial groups. In the Pondo tribe of South African Bantu, Bartholomew (1973) documented an overall prevalence of 6.4% in the 30 to 39 age group (10 out of 157 persons), principally due to the inclusion of a pre-granular stage in the evolution of the disorder (8 out of 157 cases; 5%). Along with the Australian Aboriginal cohort reported by Taylor and coworkers (1977) and an Icelandic cohort reported by Als (1980) an 'earlier involvement pattern' possibly influenced by genetic, or environmental factors has been documented.

Sex

The reported series on the relationship between exfoliation syndrome and sex are often irreconcilable. Some authors have shown a higher prevalence for females (for example, Aasved 1979, Ekstrom 1987, Esmail 1991). Two studies where the data were population corrected and subsequently compared with age matched controls, show females being affected by the disorder more frequently than males (Aasved 1969, Hiller et al 1982). However, this preponderance of females has not been confirmed in other populations (Bartholomew 1973, Taylor 1979, Forsius 1979, Heriot et al 1983, Summanen & Asbjörn 1988) and large ophthalmic cohorts (Colin et al 1988, Montanes et al 1989). In some clinical reports a marked preponderance of males with the condition has been reported (Örgen 1949, Zlatar 1965, Sood 1968, Bartholomew 1979, Khanzada 1985, Mohammad & Kazmi 1986). More specifically, Sood (1968) reported a prevalence of 1.2% in female patients and 2.1% in males in his ophthalmic clinic in India. In Pakistan, males were affected

TABLE 1.1 YOUNGEST PATIENTS WITH EXFOLIATION REPORTED IN THE LITERATURE

AGE AT DIAGNOSIS (years)	AUTHOR	COUNTRY	RELEVANT OCULAR HISTORY
22	Sugar 1976	U.S.A.	11 months old corneal injury; iris prolapse excised
27	Aminisarduei 1986	Iran	No data provided
31	Hörven et al 1967	U.S.A.	Juvenile glaucoma; iridencleisis
32	Khanzada 1985	Pakistan	No data provided
35	Taylor et al 1977	Australia	No data provided
35	Hörven et al 1967	U.S.A.	Keratoconus; corneal graft at the age of 24
37	Küchle et al 1992	Germany	Keratoconus; corneal graft
38	Summanen et al 1988	Saudi Arabia	No data provided
39	Ioannides et al 1961	Greece	No data provided
30-39 10 cases)	Bartholomew 1973	South Africa	No data provided
<40 2 cases)	Åls 1980	Iceland	No data provided
40	Forsius et al 1974	Iceland	No data provided
41	Sugar et al 1976	U.S.A	No data provided
42	Küchle et al 1992	Germany	Keratoconus; corneal graft
43	Konstas 1992	Scotland	Keratoconus; corneal graft; heterochromia

three times more frequently than females (Mohammad & Kazmi 1986). Cultural and social factors influencing the proportions of males and females presenting to ophthalmologists were not discussed by these authors.

1.7 Diagnosis

1.7.1 Symptoms

Exfoliation syndrome is usually characterised by the lack of symptoms, for throughout its long and insidious course the condition is clinically silent (Duke-Elder 1969). Symptoms may only arise with the development of cataract, or when exfoliation glaucoma ensues. Cataract will give rise to blurring of vision. Exfoliation glaucoma, in a similar fashion to the other glaucomas, may remain asymptomatic and undetected until serious visual disability occurs. However, in a number of cases it may give rise to subjective symptoms: dull eye ache, or 'tension' (due to high IOP); difficulty in reading, diminished peripheral or night vision and ultimately failure of vision due to advanced glaucomatous atrophy of the optic nerve head. In a small proportion of cases exfoliation glaucoma may present in a more dramatic fashion with headache or pain owing to the development of 'acute open angle glaucoma' with an IOP in excess of 50 mm Hg (Jerndal 1986, Brooks & Gillies 1988).

1.7.2 The classic diagnostic signs

Pupil

The objective diagnostic signs of exfoliation syndrome are diagramatically shown in Figures 1.14 & 1.15. The pupillary margin is encrusted with 'dandruff-like' discrete particles of exfoliation material (Figs 1.14 & 1.16). In the advanced stage, exfoliation aggregates may be seen upon the anterior iris surface and within the iris crypts. Exfoliation material on the lens surface is the most commonly recognised feature of the disorder. Three distinct zones have been documented (reviewed by Sugar et al 1976). In the centre of the anterior lens surface a white, ring-shaped, subtle opacification resembling a cellophane-like membrane termed the central disc is seen (cf Figs 1.1 & 1.2).

Biomicroscopically, the central disc ranges in size between 1-2.5 mm (Prince & Ritch 1986) and is best visualised with bright illumination after dilatation of the pupil since its border may be hidden under the iris. In many cases the border of the central disc appears well-demarcated and in some cases it may be curled inwards (Jerndal & Svedbergh 1978); (Fig 1.15). On the edge, or the surface of the central disc minute exfoliation material particles may be seen (cf Fig 1.1). It has been suggested that the size and shape of the central disc depend upon the size of the pupil i.e. a miosed pupil would have a smaller central disc whereas distortion in the shape of the pupillary rim would change the shape of the central disc (Sunde 1956, Sugar 1984, Prince & Ritch 1986). The central disc may be absent in some 10 to 18

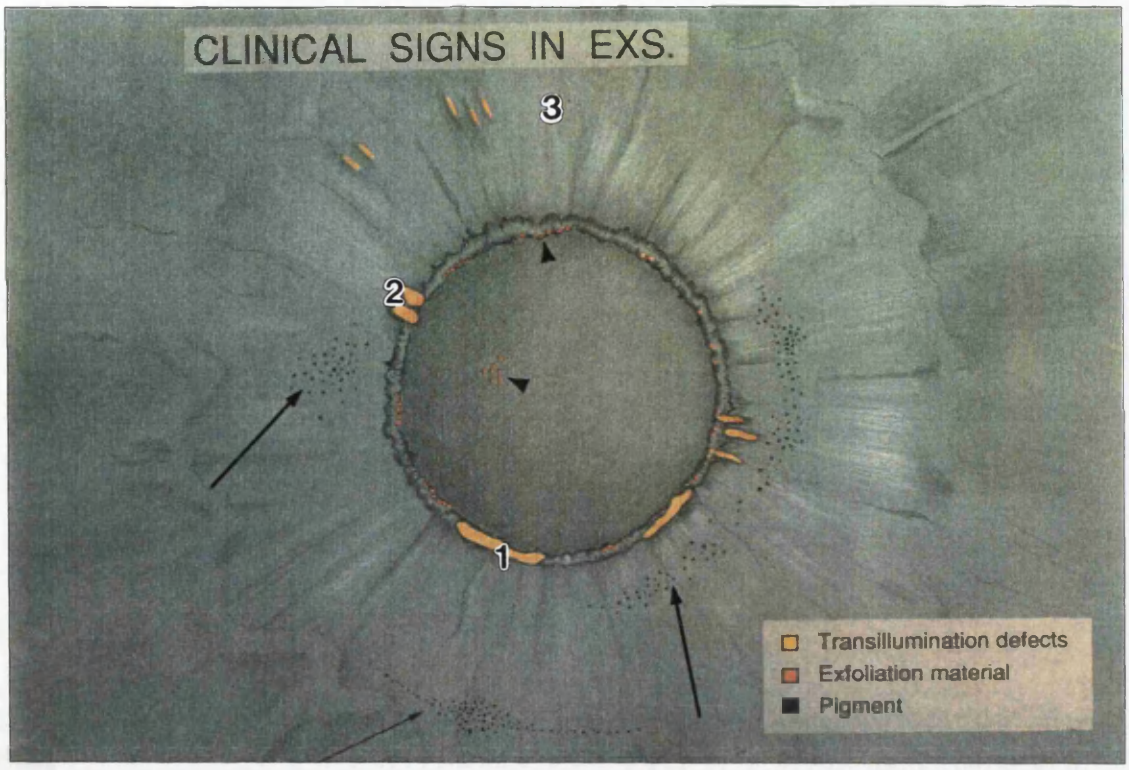


Figure 1.14: Diagramatical representation of clinical signs in exfoliation syndrome prior to dilation of the pupil. Transillumination defects (orange) are observed in pupillary ruff(1), peripupillary(2) and mid-peripheral iris(3). Pigment deposit (arrows) and exfoliation material(arrowheads) are also shown.

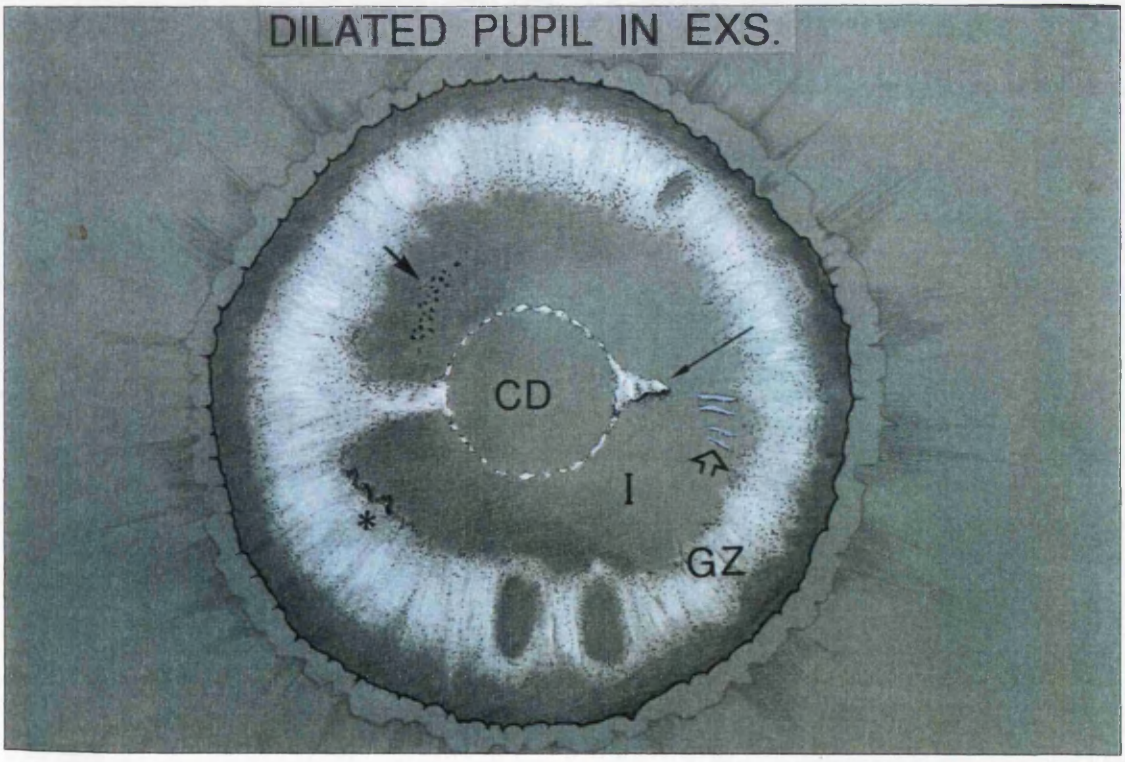


Figure 1.15: Diagramatical representation of clinical signs on the exfoliative lens (dilated pupil). CD:central disc; I:intermediate zone; GZ:granular zone; ⁎:rolled edge of GZ; long arrow:peeling of lens capsule; thick arrow:radial striae; short arrow:pigment.

per cent of cases (Joannides et al 1961, Tarkkanen 1962, Sood 1968, Aasved 1971b; 1971d, Kinoshita 1979, Sugar 1984).

The periphery of the lens capsule exhibits a granulated circular band of variable width resembling 'sugar frosting', called the granular zone (Figs 1.3, 1.4 & 1.15). This zone may exhibit holes and occasionally communicates with the central disc via bridges of exfoliation material (Fig 1.15). Several suggestions have been made to explain the development of the granular zone (for example Sunde 1956, Gifford 1957, Bertelsen 1966, Sugar et al 1976, Dark et al 1977, Ringvold & Bore 1990). None of these, however, is entirely convincing.

A clear intermediate zone of normal lens capsule separates the central disc from the peripheral granular zone (Fig 1.15). It is thought that the clear zone is formed by the 'cleaning' action of the iris which constantly moves from the dilated to the constricted position (Sugar et al 1976).

1.7.3 Pigmentary signs

A close relationship exists between the development of exfoliation material deposition and progressive atrophy of the posterior pigmented layer of the affected iris. This process is more pronounced in the pigment epithelium of the pupillary and peripupillary iris (Aasved 1973), (Fig 1.17) but recently it has been shown that the peripheral iris may also be involved (Repo et al 1990).

The precise mechanism of the pigment release remains obscure.

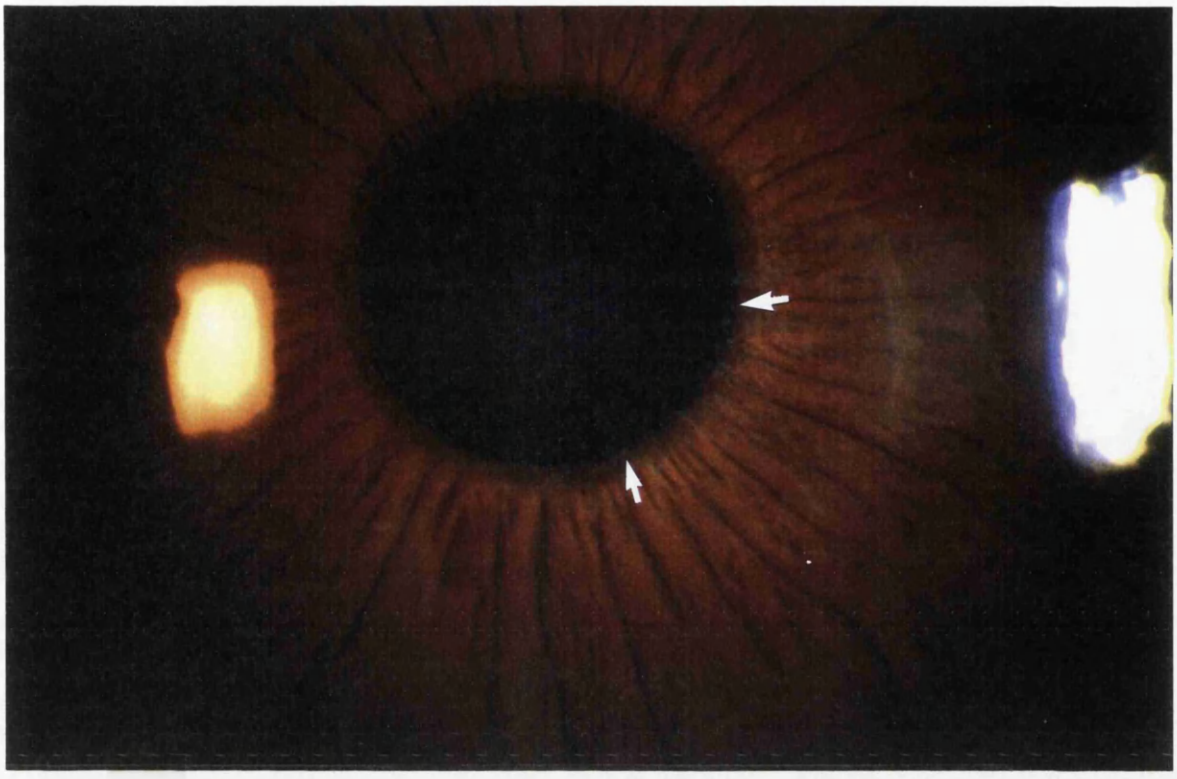


Figure1.16: Biomicroscopical appearance of an eye with exfoliation syndrome. Note deposition of exfoliation material upon the pupillary margin (arrows).

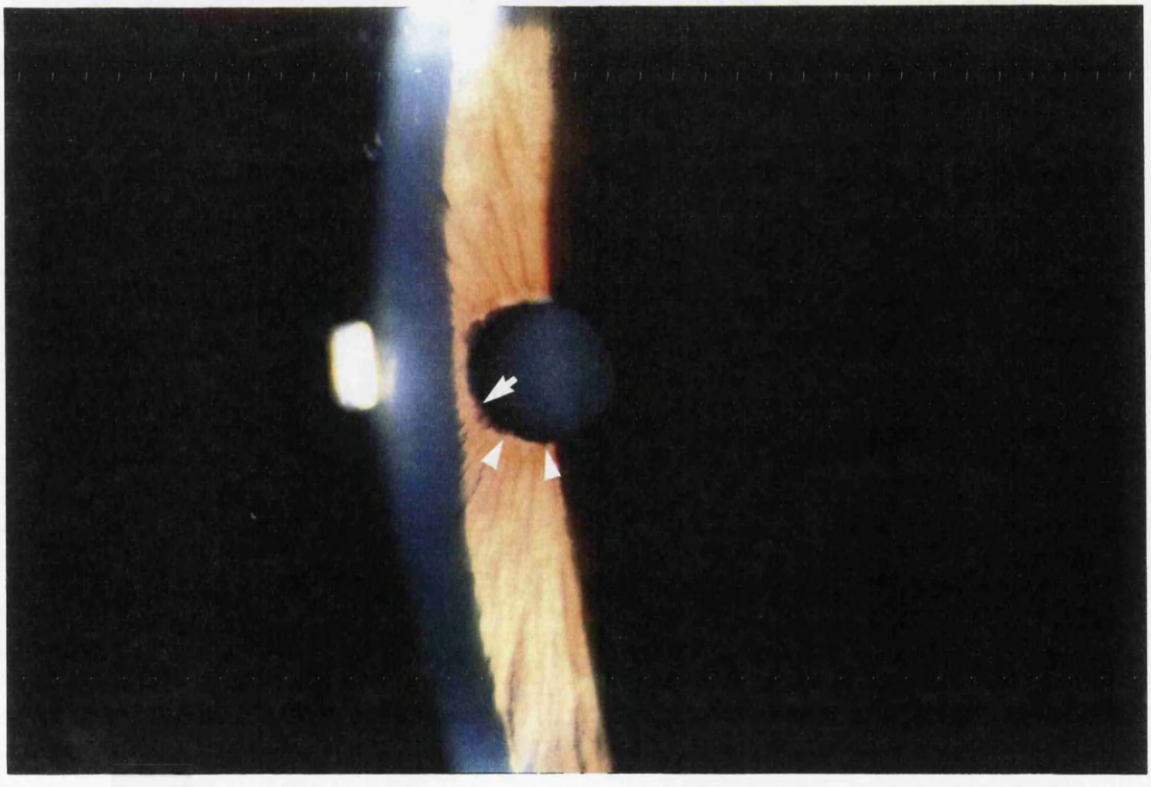


Figure 1.17: A slit-lamp photograph of an eye with exfoliation syndrome to illustrate subtle deposition of exfoliation material (arrow). A section of loss of the pupillary ruff is shown (arrowheads).

It has been suggested that pigmentary liberation is caused by a primary vascular disturbance in the iris (Vannas 1969), or increased mechanical friction between the 'defective', uneven anterior lens surface and the affected pigment epithelium (Aasved 1973). In any case, the pigment granules released, move along with the aqueous humour and subsequently some of them adhere to and within various ocular structures, e.g. anterior iris surface, corneal endothelium, filtration angle (Wishart et al 1985, Prince & Ritch 1986, Jerndal 1986, Prince et al 1987). Krause and coworkers (1973) were the first to provide controlled data on the subject of pigment liberation from the iris. It has now become well recognised that this process gives rise to a number of characteristic diagnostic features (Madden & Crowley 1982, Prince & Ritch 1986, Prince et al 1987, Montanes et al 1990b, Repo et al 1990, Konstas & Dutton 1991).

Pigmentary signs in the iris

The pigment deposition upon the surface of the iris consists either of distinctly particulate large granules, localised to the iris sphincter (Figs 1.14 & 1.15), or diffuse small pigment particles scattered all over the peripheral iris. Occasionally, a discrete cluster of pigment may be noted at the 6 o' clock position at the inferior border of the iris (Jerndal & Svedbergh 1978); (Fig 1.14).

A variable degree of iris atrophy is a consistent feature in the exfoliation syndrome. This atrophy involves initially the pupillary ruff (Fig 1.17). Aasved (1973) demonstrated a higher prevalence of pupillary ruff defects in individuals

with exfoliation syndrome (74%) when compared with normal controls (6.1%). However, the significance of these findings was diminished by the fact that in his study the results for the normal population were not age-corrected; the exfoliation group was older (Aasved 1973).

In a more advanced stage of the disorder, the iris sphincter becomes visible and the entire circumference of the pupillary margin transilluminates in a characteristic fashion (Jerndal 1986). In most cases, the 'moth eaten' pattern is confined to the iris sphicter area. However, in a recent prospective study, Repo and coworkers (1990) found that 45% of cases with the condition demonstrated generalised peripheral iris transilluminance; this compared with 18% in an age matched control population (Repo et al 1990).

Pigment in the anterior chamber

After dilatation of the pupil a shower of loose pigment may be seen in the anterior chamber of affected eyes. Writing in 1965, Kristensen described a mydriasis-induced excessive pigment liberation with a concurrent rise in IOP in two patients with exfoliation glaucoma. This phenomenon may also occur in normal aged individuals, but it has been shown that in the exfoliation syndrome the incidence and volume of pigment released following pupillary dilatation, is much greater (Kristensen 1965, Aasved 1973, Valle 1976, Roth & Epstein 1980, Mapstone 1981).

Pigmentary signs in the cornea

A characteristic deposition of fine pigment granules may

occur upon the central region of the corneal endothelium (Sugar et al 1976, Brooks et al 1988, Montanes et al 1990b). Although, the pattern seen is usually diffuse, a Krukenberg spindle pattern may also develop in a few cases (Tarkkanen 1962, Jerndal & Svedbergh 1978, Prince & Ritch 1986). Tarkkanen (1962) studied 418 patients with the condition and found a Krukenberg spindle pattern in between 4 and 16% of the various subgroups with exfoliation.

Importance of pigmentary signs

Historically, the diagnosis of the condition has always been made on the basis of the typical appearance of exfoliation material upon the pupillary margin and/or the lens surface. Krause and coworkers (1973) have shown that patients with the condition exhibit significantly more dense pigmentation gonioscopically, compared with normals. On the other hand, no such difference was noted by these authors between the two eyes of patients with unilateral exfoliation syndrome. They interpreted the latter finding to be consistent with increased pigmentation being the first stage of the disorder (Krause et al 1973). Recently, these more subtle signs have been stressed as important early cinical features which should alert the ophthalmologist to search for the condition (Jerndal 1986; 1989, Prince & Ritch 1986, Rouhiainen & Teräsvirta 1990). It has now been suggested that the pigmentary signs not only accompany, but precede the fully established condition (Wishart et al 1985, Jerndal 1986; Prince et al 1987, Konstas & Dutton 1991).

Importance of early diagnosis

There are four reasons why early diagnosis is optimal for clinical practice. First, the early recognition of exfoliation syndrome is important because these individuals may develop exfoliation glaucoma (Hansen & Sellevold 1969, Odland & Aasved 1971, Jerndal 1986, Slagsvold 1986, Henry et al 1987). Documenting the presence of exfoliation syndrome early in these patients will determine their subsequent management. Second, the distinction between exfoliation glaucoma and POAG will influence treatment since exfoliation glaucoma is different from POAG with regard to treatment, prognosis and follow-up (see sections 1.12.1, 10.1 & 10.3). Third, the distinction between exfoliation glaucoma and POAG is essential prior to research into the pathogenesis, or the clinical features of glaucoma. Many published studies fail to make this important distinction. Finally, a strong association exists between the development of lens opacities and the exfoliation syndrome. It has been shown that the rate of peroperative and postoperative complications in cataract surgery is significantly higher in the presence of exfoliation syndrome (section 1.11).

1.8 Development of the disorder

The evolution of the exfoliation syndrome is still poorly understood. Evidently, it evolves slowly, over many years (Gifford 1957; 1958, Duke-Elder 1969, Jerndal 1986). Gifford (1957) has postulated that the entire development of exfoliation syndrome was 'a matter of 10 to 20 years rather than 3 to 5 years'. Individuals with bilateral involvement

are on average 2 to 7 years older than those with unilateral involvement (Aasved 1971b, Madden & Crowley 1982, Slagsvold 1986). Similarly, it has been shown that a significant proportion of individuals with long-standing exfoliation syndrome develop exfoliation glaucoma. Whether exfoliation glaucoma can occur without an interval of 'normality', i.e. without the initial development of the exfoliation syndrome, is not known.

Theories on early development

The biomicroscopic changes, which describe the early stages of the disorder, remain ill defined and only few studies have dealt with this subject (Bartholomew 1971, Jerndal 1985, Dark & Streeten 1990). In 1971, Bartholomew first provided clues of an early stage in the development of the condition. Whilst studying the condition among the Bantu in South Africa, he noticed grey, radial striations on the peripheral, anterior lens capsule of some young Bantu patients. Subsequently, in a few of these cases he noted that these lines became more confluent and eventually merged into the classic granular zone of exfoliation material. Bartholomew considered these lines to comprise a prodromal stage, so called 'pregranular stage', in the evolution towards the fully established condition (i.e. presence of exfoliation material, 'granular stage'). In his view, these early changes were 'imprints' created by the corresponding iris folds and were pathognomonic, superseded later in life by the 'granular' stage. Bartholomew (1973) documented 8 patients with 'pregranular exfoliation', in the 30-39 age group among the Pondo tribe in South Africa. Later, in support of this

theory, he observed identical striations in a small number of Scottish patients (Bartholomew 1979). Significantly, similar striations were recently noted in Caucasians at an early stage of the condition by Dark & Streeten (1990).

An alternative hypothesis for the early development of the condition has been suggested by Jerndal (1985; 1986; 1989). According to his theory, in stage 1, the posterior layer of the iris epithelium begins to lose pigment and tiny dots of pigment appear in radial clusters upon the lens capsule. Stage 2 is characterised by progressive loss of the pupillary ruff, transillumination, and an incomplete granular zone with the first aggregates of exfoliation material often intermingled with pigment. Stage 3 shows the completed granular zone and advanced transillumination of the iris.

Preclinical stage

A number of workers have recently shown that further insight into the development of the disorder might be gained by ultrastructural studies on tissues from exfoliation patients who do not demonstrate clinical evidence of exfoliation material on biomicroscopy. First, Speakman & Ghosh (1976) found exfoliation material in conjunctival biopsy specimens from both eyes in 7 patients with clinical evidence of unilateral exfoliation syndrome. Similar evidence was obtained from ultrastructural studies in conjunctival specimens from patients who only exhibited pigmentary signs (these are described in section 1.7.3). Thus, the concept that there is a preclinical (histological) stage in some patients is now firmly established. Employing specular

microscopy, Miyake and coworkers (1989) demonstrated comparable morphological alterations (polymegethism and pleomorphism) in the corneal endothelium of both eyes in patients who clinically demonstrated only unilateral exfoliation. This study concluded that corneal endothelial changes are consistent with early manifestations of the condition and that the fellow 'normal' eye is in fact at a preclinical stage of the disorder.

1.9 Gonioscopic features

The gonioscopic features in exfoliation syndrome/glaucoma (Figs 1.18 & 1.19) are important in the early diagnosis of the disorder (Gradle & Sugar 1940) and gonioscopy is considered of value in assessing the risk of IOP elevation (Sampaolesi 1959, Jerndal 1986, Montanes et al 1990b).

Sampaolesi's line

Sampaolesi's line is defined as a single wavy pigmented line, superior to Schwalbe's line and situated between 4 and 8 o' clock position in the angle (Sampaolesi 1959); (Fig 1.19). This line may extend onto the posterior face of the inferior corneal endothelium. Occasionally, more than one line of pigment, each parallel to the other, are observed (Sampaolesi et al 1988). Sampaolesi's line is found in between 63 and 98% of patients with the condition (Mizuno & Muroi 1979, Konstas & Allan 1989, Montanes et al 1990b, Konstas & Dutton 1991). A significant correlation between increased IOP and Sampaolesi's line has recently been confirmed in a study of 263 cases (Montanes et al 1990b).

Pigment in the angle

It has been suggested that increased pigmentation may be the earliest detectable sign of the exfoliation syndrome (Wishart et al 1985). Although few published studies actually record their assessment of pigmentation, a densely pigmented trabecular band has been consistently reported (Gradle & Sugar 1940, Rouhiainen & Teräsvirta 1990). In appearance the angle in exfoliation syndrome is characterised by diffuse, brown/black pigmentation of heterogeneous distribution (Jerndal & Svedbergh 1978, Montanes et al 1990b). Considerably more pigmentation is seen upon the inferior angle (Prince & Ritch 1986, Sampaolesi et al 1988), with maximum pigmentation localised at the 6 o' clock position (Wishart et al 1985); (Figs 1.18 & 1.20). The intensity of angle pigmentation correlates well with the severity of exfoliation glaucoma (Montanes et al 1990b, Rouhiainen & Teräsvirta 1990). Whether increased angle pigmentation is attributable to enhanced shedding of pigment cells, or reflects ineffective pigment removal is not known.

Exfoliation material in the angle

Exfoliation material may be seen gonioscopically in the angle (Barkan 1936, Gradle & Sugar 1940) in between 5.6 and 50% of cases (Thomassen 1949, Joannides et al 1961, Tarkkanen 1962, Konstas & Dutton 1991). Exfoliation material in the angle is not pathognomonic for raised IOP, since it is as commonly found in normotensive patients with exfoliation syndrome. When present, it has no typical pattern of appearance, but is usually observed in the inferior angle sometimes mingled with pigment (Sampaolesi et al 1988).

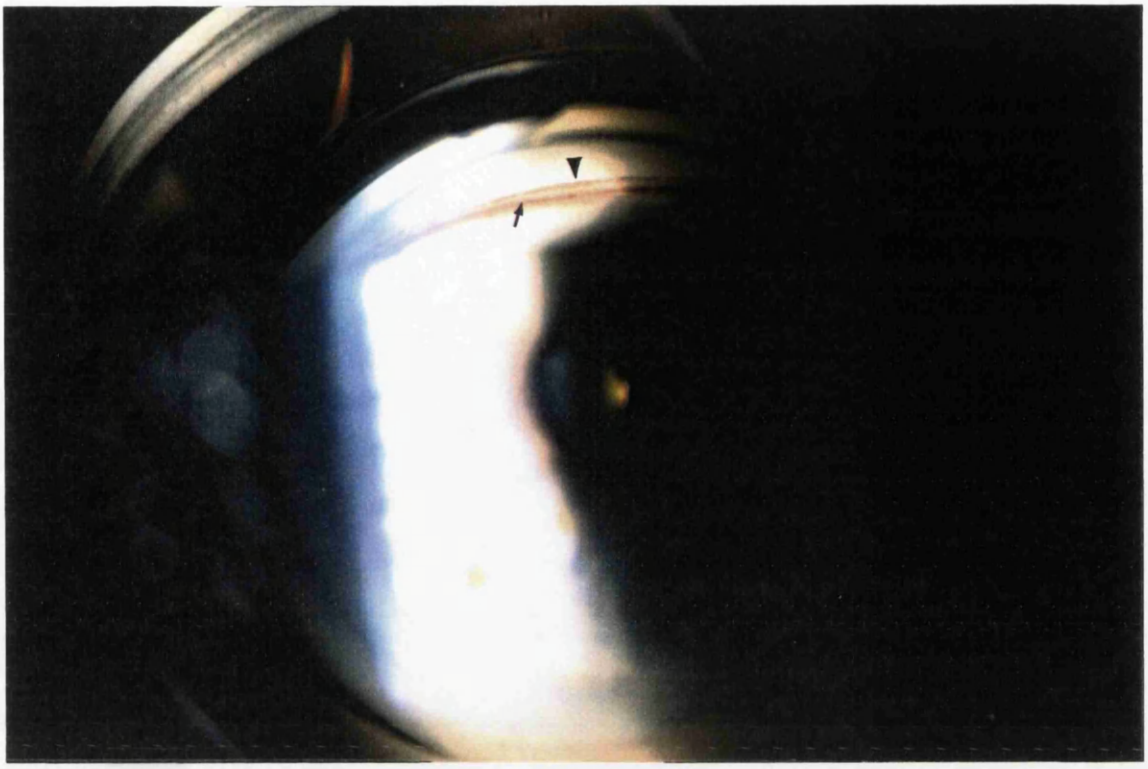


Figure 1.18: Typical gonioscopic appearance of the inferior angle in a patient with exfoliation glaucoma. The angle exhibits moderate pigmentation(arrow). Sampaolesi's line is present(arrowhead).

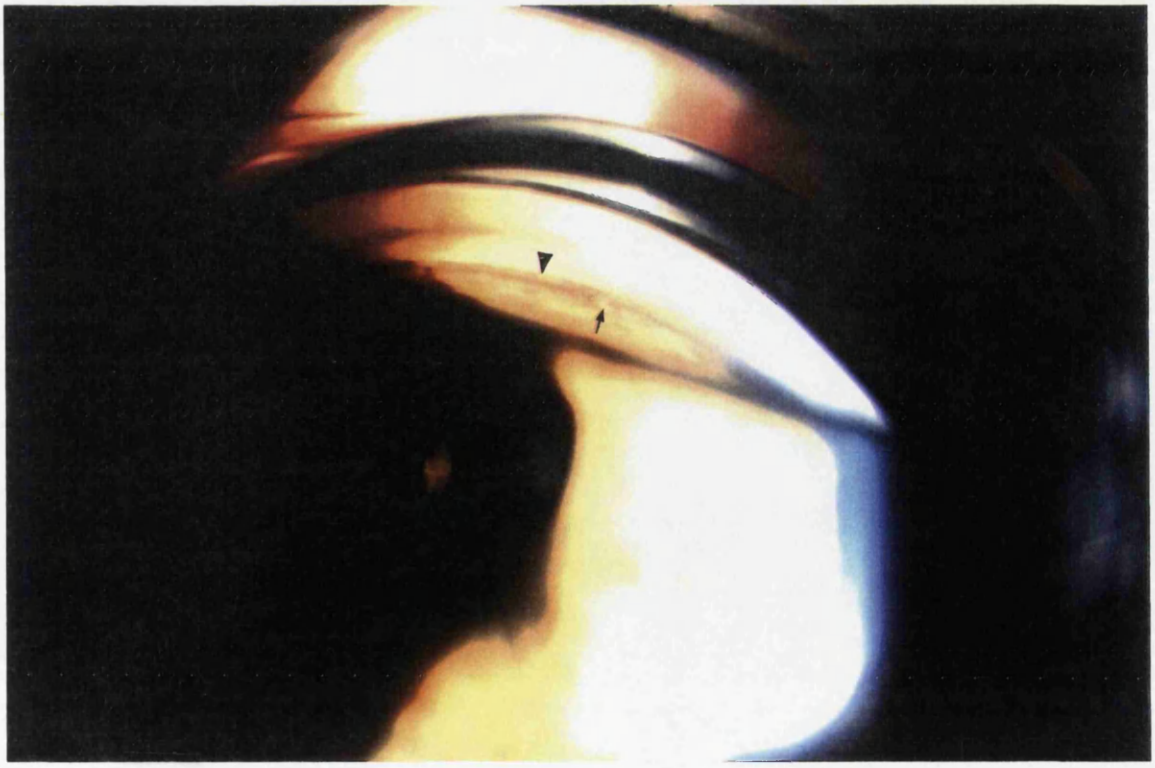


Figure 1.19: Gonioscopic photograph of the inferior angle in an eye with exfoliation glaucoma. Note exfoliation material in the angle (arrow) and Sampaolesi's line (arrowhead).

1.10 Other clinical features

Fluorescein angiographic studies

Vannas (1969) was the first to use fluorescein angiography to describe the anterior segment changes of exfoliation syndrome/glaucoma. He documented extensive vascular changes in the iris of 15 out of 20 cases with exfoliation syndrome and 40 out of 41 cases with exfoliation glaucoma. In the 15 cases with exfoliation syndrome, the findings comprised 1) early and pronounced fluorescence at the pupillary border, 2) the presence of numerous, thin, tortuous new vessels in the pupillary and ciliary portion of the iris and 3) profuse leakage of fluorescein from these newly formed neovascular 'whorls' (Vannas 1969). In cases with exfoliation glaucoma the angiographic findings were even more pronounced with vascular changes being more widespread, often involving both the pupillary and ciliary portion of the iris. In 33 exfoliation glaucoma cases, Vannas (1969) was impressed with the profuse leakage of fluorescein from the iris vasculature, the presence of a rich neovascular network, the occurence of microaneurysm-like buds, the phenomenon of venous stasis and the absence of radial veins. Although fluorescein extravasation was also noted at the pupillary margin of 33% of the normal control eyes, none of the controls showed evidence of neovascularisation, or leakage of stain at the ciliary portion of the iris (Vannas 1969).

In a more recent study, Brooks & Gillies (1987) confirmed Vannas' findings. They performed iris angiography in 25 patients with exfoliation syndrome and found a varied degree

of microneovascularisation (defined as a more subtle degree of neovascularisation than that seen in neovascular glaucoma) in 87% of their patients. This was accompanied by diminished iris perfusion. The authors concluded that these features are specifically related to the condition (Brooks & Gillies 1987). Furthermore, they described the development of hypoperfusion and microneovascularisation in two previously unaffected eyes after the clinical appearance of exfoliation syndrome (Brooks & Gillies 1987).

1.11 Exfoliation syndrome and cataract

Presence of cataract

Most published reports have suggested a consistent yet unexplained relationship between exfoliation syndrome and cataract (Gradle & Sugar 1940, Hörven 1966, Taylor et al 1977, Roth & Epstein 1980, Madden & Crowley 1982, Forsius 1988, Konstas 1989, Ayed et al 1990). The reported clinical figures for the prevalence of the condition in cataract cohorts vary between 2.5 and 44% (Lindberg 1917, Hörven 1937, Irvine 1940, Bartholomew 1979, Guzek et al 1987, Forsius 1988, Olivius et al 1989, Wålinder et al 1989). Similarly, the prevalence of lens opacities in selected exfoliation cohorts differs according to the study between 20 and 93% (Trantas 1929, Tarkkanen 1962, Hörven 1966, Layden & Shaffer 1974, Taylor 1980, Madden & Crowley 1982, Khanzada 1985, Konstas & Allan 1989, Stefaniotou 1989, Montanes et al 1989).

Taylor (1980) believed that exfoliation syndrome was positively associated with the development of cataract and

suggested a radiation effect linkage. Seland & Chylack (1982) have offered some evidence suggesting a link between exfoliation syndrome and the pattern of cataract formation. They analysed the type of cataract seen in 431 lenses with the disorder and 1,776 senile cataract controls. In patients with exfoliation, cortical cataracts were significantly less common, and nuclear cataracts more common.

1.11.1 Cataract surgery and the exfoliation syndrome

Surgical removal of a cataract may be indicated in patients with exfoliation syndrome for improved visual acuity.

Lens subluxation

Published work has indicated that patients with exfoliation syndrome may have spontaneous, or intraoperative lens dislocation (Bartholomew 1970, Sood et al 1973). This complication may occur as a consequence of increased friability of the zonules, or may be due to their weakened attachment to the ciliary body. Hörven & Hutchinson (1967) documented 9 cases with lens displacement out of 635 cases (1.4%) in the United States.

The first to draw attention to the common occurence of phakodonesis in exfoliation patients was Bartholomew (1970), who pointed out that phakodonesis was an important sign of incipient lens displacement. He reported zonular fragility, phakodonesis and spontaneous rupture leading to subluxation of the lens in 6 Bantu patients with exfoliation. In India Sood and coworkers (1973) found 11 cases with phakodonesis

out of 17 cases with exfoliation detected in the course of 6 months. In two of these cases the lens had dislocated into the vitreous cavity (Sood et al 1973). More recently, Jofe (1988) has suggested that in many exfoliation patients with cataract only a slight degree of phakodonesis may be seen. Detection in these cases is facilitated with biomicroscopic examination for lens movements prior to pupillary dilatation.

ICCE and exfoliation syndrome

Gillies (1973) reviewed 30 cases with exfoliation and stated that 'cataract extraction was difficult in these patients; the lens capsule seemed very liable to break and large hyphaemas frequently occured'. Krause & Tarkkanen (1978) observed 'remnants of posterior synechiae' in 5 out of 100 extracted cataractous lenses with the condition. Dark (1979) described clinically and histologically, adhesions involving the pigment epithelium of the iris and the peripheral anterior lens capsule in 6 cases with exfoliation glaucoma. In that study, extended bonding of the iris pigment epithelium to the pre-equatorial lens capsule by exfoliation fibres was confirmed ultrastructurally (Dark 1979).

ECCE and exfoliation syndrome

Currently, ECCE is the most popular technique of cataract extraction. There are several reports that indicate that exfoliation syndrome may adversely influence ECCE (Raita & Setala 1986, Hovding 1988, Carpel 1988, Pedersen et al 1990, Ruusuvaara et al 1990, Assia et al 1991). However, in the current literature, there are few controlled observations on the surgical complications in elderly patients with

exfoliation syndrome. In a prospective consecutive study of 1,000 ECCE cases in Saudi Arabia, Guzek and coworkers (1987) found a four-fold increase in the risk of zonular breaks when exfoliation syndrome was present (25% of their patients). Naumann and coworkers (1988) reported a five-fold increase in the incidence of vitreous loss in ECCE performed in patients with exfoliation syndrome (4 out of 46 cases in the exfoliation group, (9%), versus 23 out of of 1,205 in senile cataract controls, (1.8%). Similarly, Küchle and coworkers (1989) found exfoliation syndrome to be a significant risk factor for vitreous loss, but not for capsular breaks. In their study, vitreous loss was observed in 8 out of 72 cases with exfoliation (11.1%), and in 32 out of 1,970 controls (1.6%). Capsular breaks, without vitreous loss, occured in 4.2% of the exfoliation cases and 2.8% of the control cataract group (Küchle et al 1989).

Skuta and coworkers (1987) reported 5 patients with exfoliation glaucoma who developed extensive zonular dialyses during ECCE. They speculated that weakness of the lens zonules, or their attachments to the ciliary processes accounted for this complication. In a prospective study in a German cataract population, Konstas and coworkers (1993) have studied the prevalence of exfoliation syndrome and its influence in the surgical outcome. Out of 1,023 consecutive German patients 51 (5.2%) were found to have exfoliation syndrome. Patients with exfoliation syndrome were more likely to be operated upon under general anesthesia, by the most senior surgeon, have iris surgery and expression of the nucleus with a loop instead of phacoemulsification. In spite

of these measures, they had a significantly higher chance for vitreous loss (5.9% versus 0.9% for the controls, $p<0.01$).

The increased incidence of zonular rupture and vitreous loss during cataract surgery in eyes with exfoliation may also be attributable to insufficient pupillary dilatation. Delivering a large nucleus through a poorly dilated pupil, requires a significant increase in pressure applied, which in turn would increase the risk of zonular rupture and vitreous loss (Carpel 1988, Konstas et al 1992). This hypothesis is consistent with the information provided by Guzek and coworkers (1987) who found that decreasing pupil size was the only significant risk for vitreous loss in ECCE.

1.12 Exfoliation glaucoma

Relationship with exfoliation syndrome

The percentage of patients with exfoliation syndrome who have glaucoma (exfoliation glaucoma), or ocular hypertension on initial examination ranges from 22 to 94% (Kozart & Yanoff 1982, Ruprecht et al 1985, Jerndal 1986, Slagsvold 1986, Forsius 1988, Konstas 1989). Exfoliation glaucoma may ensue at any time after the development of exfoliation syndrome. A retrospective study has shown that in patients with exfoliation syndrome and normal IOP, 5% developed raised IOP over 5 years while 15% did so over 10 years (Henry et al 1987).

Exfoliation glaucoma has uniformly been considered as a severe form of chronic open angle glaucoma (Smith 1979, Sugar

1984, Tarkkanen 1984, Jerndal 1986, Thorburn 1988, Naumann et al 1988). Peräsalo & Raitta (1987) found exfoliation glaucoma to be a major cause of visual impairment in an institutionalised geriatric Finnish cohort 66 to 100 years old. Raivio (1987) has estimated that the number of patients with glaucoma in Finland would increase by 40% by the year 2010 owing to the ageing population. He also calculated that a 40% increase of resources and glaucoma care facilities would be required for glaucoma patients by the year 2010.

1.12.1 Course and clinical attributes

The natural history of exfoliation glaucoma without treatment has not been described. This is because most patients are treated, which presumably influences the disease process. Prospective data concerning the long term outcome of treated exfoliation glaucoma is presently not available. Specific clinical attributes distinguish exfoliation glaucoma from POAG (Kristensen 1965, Hörven 1966, Olivius & Polland 1980, Tarkkanen 1984, Blika et al 1984, Sugar 1984, Bertelsen 1985, Lutjen-Drecoll et al 1986, Repo et al 1990). Asymmetry in the clinical manifestations of the disorder is the rule rather than the exception (Johnson & Brubaker 1982).

<u>The fellow eye in unilateral exfoliation glaucoma</u>
Several studies have confirmed a significant risk of the unaffected eye developing exfoliation glaucoma, related to the duration of the condition (Pohjanpelto 1973; 1986, Ruprecht et al 1985, Prince et al 1987, Konstas & Allan 1989, Streeten et al 1990, Montanes et al 1990b). A retrospective

study has indicated that 21-26% of patients with bilateral exfoliation syndrome and unilateral exfoliation glaucoma may develop exfoliation glaucoma in the fellow eye within 5 years (Hansen & Sellevold 1969). In another retrospective series of 519 patients with exfoliation, Brooks & Gillies (1988) established that in unilateral exfoliation glaucoma the presence of exfoliation syndrome in the fellow eye was a serious risk factor. Raised IOP developed in approximately three quarters of these eyes. In addition, exfoliation glaucoma developed in approximately 25% of eyes with ocular hypertension at presentation (Brooks & Gillies 1988).

IOP and exfoliation

A consistent, yet unexplained, feature has been observed in patients with exfoliation syndrome. They exhibit a higher IOP compared with age matched control subjects (Aasved 1971c; 1979a, Krause et al 1973, Hiller et al 1982). Whether this clinical feature is explained by the incomplete handling of the influx of pigment and/or exfoliation material by the self-cleaning filter mechanism of the angle, is speculative. It is conceivable that exfoliation syndrome increases outflow resistance even in 'normal' eyes.

Patients with exfoliation glaucoma typically present with elevated IOP, often considerably so (Tarkkanen 1965, Gillies & West 1977, Bertelsen 1985, Jerndal 1986; 1990). Tarkkanen (1965) reported that over 60% of the affected eyes in patients with unilateral exfoliation glaucoma exhibited an IOP higher than 35 mm Hg, at diagnosis. Lindblom & Thorburn (1984b) surveyed the hospital records of a well defined

glaucoma population in Hälsingland, Sweden. Their cohort consisted of 245 cases with exfoliation glaucoma and 75 cases with POAG (3:1 ratio). Exfoliation glaucoma patients had unilateral disease more often at diagnosis (31% versus 54% for POAG). Both glaucomas showed the same degree of visual field loss at diagnosis, despite the fact that the mean IOP at diagnosis was considerably higher in the exfoliation glaucoma group (42.9 mm Hg for exfoliation glaucoma compared to 34.8 mm for POAG). In exfoliation glaucoma the authors noted a significant increase in the mean IOP with every stage of progression in their classification for glaucomatous damage (Lindblom & Thorburn 1984b). This was not observed in the POAG cases. It is remarkable that, according to their findings, the mean IOP at diagnosis for patients with legal blindness due to advanced exfoliation glaucoma (11 patients) was almost 60 mm Hg.

Few cases of low tension exfoliation glaucoma have been reported to date (Bertelsen 1985). In one study, only 2 out of 245 patients with exfoliation glaucoma and visual field loss had an IOP below 20 mm Hg at diagnosis (Lindblom & Thorburn 1984a). There is nearly universal agreement in the literature that exfoliation glaucoma is a hypertensive glaucoma (reviewed by Jerndal 1986). Indeed, a number of studies have described an acute form of exfoliation glaucoma. Up to 25% of patients with exfoliation glaucoma may present with an acute rise in IOP, frequently in excess of 50 mm Hg, and a varied degree of corneal oedema (Gillies & West 1977, Brooks & Gillies 1988, Gillies & Brooks 1988). The majority of these cases have open angles, although some cases of acute

angle closure glaucoma with exfoliation have also been described (Gillies 1978, Bartholomew 1981, Gillies & Brooks 1988).

Extreme cases of so called 'absolute exfoliation glaucoma' can occasionally present with high IOP and no perception of light (Gillies & West 1977, Gillies 1978). In an Australian series, 5 cases of absolute exfoliation glaucoma were identified in a cohort of 72 cases with acute open angle exfoliation glaucoma (Gillies & Brooks 1988). In the same series, a marked preponderance of males with acute exfoliation glaucoma was noted. Bartholomew (1981) described 8 cases with definite acute angle closure glaucoma in association with exfoliation. He suggested that the underlying mechanism was that of secondary angle closure due to the formation of iridocapsular synechiae.

Steroid response

Among the several differences between exfoliation glaucoma and POAG one of the most interesting is the lack of a change in IOP following topical steroids. Steroid-induced ocular hypertension occurs in approximately a third of the normal population, but occurs in the majority of patients with POAG (reviewed by Armaly 1986). It is reversible, reproducable and genetically determined. The trabecular meshwork is the site of the pathology responsible for the IOP elevation and the response is abolished following filtering surgery (Armaly 1986). Exfoliation glaucoma differs markedly from POAG by exhibiting the same frequency of steroid response as that of the normal population (Armaly 1986).

Prognosis

In a retrospective study, Olivius & Thorburn (1978) reported that after 5 years more glaucomatous damage had occurred in the exfoliation glaucoma patients (102 eyes) than in the POAG group (58 eyes) in spite of recourse to surgery more often and earlier. After 5 years the exfoliation glaucoma group exhibited severe visual field loss in 48% of cases compared with only 19% in the POAG group. Pohjanpelto (1985) studied retrospectively the fate of visual fields in 42 eyes with exfoliation glaucoma and 46 eyes with POAG. At the end of the follow up period (mean 10 years), 71% of the eyes in the exfoliation glaucoma group and 82% of the eyes in the POAG group had deteriorated. Approximately 40% of the eyes with exfoliation glaucoma and 26% of the eyes with POAG had become legally blind.

No comment however, can be made on the prognosis of exfoliation glaucoma until controlled data are obtained. To date, no prospective comparative studies have been conducted comparing an age matched POAG sample with an exfoliation glaucoma sample of comparable severity to determine whether exfoliation glaucoma has a better, or worse prognosis than POAG.

1.12.2 Treatment of exfoliation glaucoma

Medical therapy

Eye drops have conventionally been considered the first line of treatment in the glaucomas (Jay 1991). Several authors have made the anecdotal observation that, in comparison with

POAG, the response to medical therapy is poorer in patients with exfoliation glaucoma (Vogt 1930, Gradle & Sugar 1947, Joannides et al 1961, Hörven 1966, Aasved 1971, Tarkkanen 1965; 1984, Haydon 1986). Another feature stressed by some writers is that an initial good response to medical therapy is often followed by failure of IOP control (Gillies 1978, Tarkkanen 1986, Jerndal 1986; 1990, Brooks & Gillies 1988). However, insufficient control of the IOP with medical therapy and failure after initial success in controlling the IOP have also been documented in a significant proportion of POAG patients (Jay & Murray 1988, Stewart 1990, Jay 1991).

Airaksinen (1979) compared the hypotensive effect of timolol to that of pilocarpine in 10 patients with POAG and 5 patients with exfoliation glaucoma. He concluded that in exfoliation glaucoma the hypotensive effect of timolol was initially 'good', but that later 'IOP increased gradually so miotics had to be added'. Aasved and coworkers (1979) found that the percentage of initial successful control (defined as IOP<22 mm Hg) with timolol in patients with exfoliation glaucoma was only 11%. Blika & Saunte (1982) reported that after 3 years on timolol drops alone successful control was obtained in 33% of the POAG cases (130 eyes) compared with 8% of the exfoliation glaucoma cases (50 eyes). Their material was age matched, but collected retrospectively, selective and the analysis of the results flawed by the analysis of eyes instead of patients.

Granström (1985) documented retrospectively a greater risk of visual field loss in patients with exfoliation glaucoma, compared with POAG patients, treated with pilocarpine 4% three times a day. Öhrström & Kättström (1981) studied the interaction of timolol and adrenaline in a prospective double-blind randomised trial on 10 patients with POAG and 10 patients with exfoliation glaucoma. They found a significantly increased hypotensive effect with adrenaline; in patients with exfoliation glaucoma.

Drawbacks of medical therapy

It is well recognised that between 28 and 58% of patients with open angle glaucoma treated with drops fail to administer their drops (Granstrom 1985, Kass 1985, Kass et al 1986; 1987, Stewart 1990, Winfield et al 1990). Winfield and coworkers (1990) found that in elderly patients a high prevalence of non-compliance was compounded by physical inability to administer eye drops. It has also been shown that ophthalmologists consistently fail to predict the patients who will not comply with their medications (Kass et al 1987). Indeed the problem may be underestimated since data on compliance may be biased. It has been suggested that the inclusion of a patient in a compliance trial improves their compliance by 30% (Novack et al 1988).

There is no specific data on the compliance of patients with exfoliation glaucoma. It is reasonable to assume that the advanced age compounded with the presence of chronic physical and mental ailments would further adversely influence the rate of non-compliance. Drug side effects are common, often

limit therapeutic choices and may even be life threatening, especially with beta blockers, or carbonic anhydrase inhibitors (Kass 1985, Konstas 1989, Jay 1991). It has been shown that troublesome side effects, especially when they appear suddenly, may result in poor compliance (Kass 1985, Kass et al 1987). Less obvious disadvantages of long term medical therapy have recently been identified. Sherwood and coworkers (1989) found increased numbers of inflammatory cells in conjunctival biopsies from eyes on long term medical therapy when compared with biopsies from eyes treated by early trabeculectomy. However, the greatest risk associated with long term medical therapy is the progressive loss of visual field during unsuccessful attempts to establish medical control (Jay & Murray 1988, Jay & Allan 1989). Although, the reported disadvantages of long term medical therapy have been identified in studies of predominantly POAG patients these disadvantages may be even more pertinent in the older patients with exfoliation glaucoma.

Laser therapy

Argon Laser Trabeculoplasty (ALT) is used in the hope of avoiding surgical intervention (Wise & Witter 1979, Tuulonen 1983, Tuulonen et al 1985, Pohjanpelto 1985, Grinich et al 1987, Hetherington 1988, Svedbergh 1988, Jerndal 1990, Reiss et al 1991). This technique involves placing multiple thermal burns, evenly spaced over part or the whole circumference of the trabecular meshwork (Reiss et al 1991). ALT is thought to lower the IOP by increasing the facility of outflow (Stewart 1990). Although its exact therapeutic mechanism is not fully understood, its success in lowering

IOP has been confirmed by many authors (Wise & Witter 1979, Logan et al 1983, Svedbergh & Sherwood 1985, Lund & Zink 1988, Ticho & Nesher 1989, Psilas et al 1989). The success rate has been variably defined and comparisons between published reports are difficult (Reiss et al 1991). In general the response to ALT may be described in two time intervals: initial (up to one year) and long-term (more than one year). Initial results have shown that 71 to 97% of phakic patients obtain an IOP of 21 mm Hg or less after ALT (Wise & Witter 1979, Grinich et al 1985, Ticho & Nesher 1989, Psilas et al 1989, Stewart 1990, Reiss et al 1991). The average drop in IOP is between 5 and 14 mm Hg with a greater reduction of IOP in patients with a higher baseline IOP.

ALT in exfoliation glaucoma

ALT in exfoliation glaucoma has certain characteristic attributes (Svedbergh 1988). Whilst treating the inferior angle, excess pigmentation often obscures the location of the trabecular meshwork. The most efficient treatment parameters for ALT in exfoliation glaucoma remain uncertain. One study showed a 20% decrease in the IOP using either 500, 700 or 900 mW (Rouhiainen et al 1987). Future modifications of ALT in exfoliation glaucoma may reduce the complication rate while still providing an adequate IOP reduction.

Several studies have suggested that ALT is more successful in exfoliation glaucoma than POAG (Thomas et al 1984, Ticho & Nesher 1989). Most authors have ascribed this to the increased pigmentation of the trabecular meshwork (Thomas et al 1984, Sherwood & Svedbergh 1985, Stangos et al 1985, Ticho

& Nesher 1989, Psilas et al 1989). For example, Ticho & Nescher (1989) reported four factors which favour the use of ALT; older age, lower pre-treatment IOP level, exfoliation glaucoma and pigmented meshwork. Indeed, advancing age and exfoliation glaucoma are factors consistently reported to influence positively the outcome of ALT (Tuulonen et al 1985; 1989, Ticho & Nesher 1989). Sherwood & Svedbergh (1985) reported a 70% success rate in 55 eyes with exfoliation glaucoma and late failure only in 2 cases.

This view however, is not universally shared (Logan et al 1983, Higginbotham & Richardson 1986, Lund & Zink 1988). Lund & Zink (1988) did not observe a significant difference in the response to ALT between exfoliation glaucoma and POAG. Higginbotham & Richardson (1986) reported that despite having a large immediate IOP response to ALT the treatment subsequently failed at a faster rate. Another series reported a similar response for exfoliation glaucoma to that for POAG, with a one year success rate of 73%, decreasing to 54% after 3 years (Grinich et al 1985). Furthermore, a high rate of failure in exfoliation glaucoma, compared to POAG, has been reported with longer follow up periods (Higginbotham & Richardson 1986, Raitta & Setälä 1986, Svedbergh 1988). In 1988 Svedbergh conducted a 5 year retrospective analysis of 74 patients treated by ALT and found that late failures were significantly more common in the exfoliation glaucoma group (69% compared to 45% in POAG after 5 years) although the failure rate was similar at the end of the first year (19%).

Primary ALT

Current interest in ALT has moved from its use as a means of avoiding surgery in cases escaping from medical control to direct comparison with medical therapy as a first line of treatment in newly diagnosed glaucoma cases (primary ALT). Thomas and coworkers (1984) reported a 97% success rate with primary ALT in a small number of individuals with exfoliation glaucoma in Egypt. They were able to taper medication in 41% of the exfoliation glaucoma patients and considered primary ALT of great benefit in certain socioeconomic circumstances and in cases of suspected poor compliance (Thomas et al 1984). In a randomised prospective trial Tuulonen and coworkers (1989) showed, after one year of follow-up, better preservation of the neuroretinal rim area, but no significant difference in visual field loss between primary laser therapy and primary medical therapy.

Surgical treatment

For the purposes of this investigation surgical treatment refers exclusively to trabeculectomy which is currently the most popular surgical procedure. Drainage surgery is the most effective means of reducing the IOP from the initial level to the physiological range (Jay 1991). Controversy still exists over the indications for performing glaucoma filtration surgery. Numerous authors including Migdal & Hitchings (1986), Jay and Murray (1988) and Watson and coworkers (1990) have established the greater effectiveness of surgical treatment in lowering IOP. Even when medical therapy is judged to control the glaucomatous progression the mean IOP obtained is significantly higher than that after

surgical treatment (Wilson 1977, Jay & Murray 1988, Watson & Barnett 1975, Watson et al 1990). Moreover, the diurnal variation in IOP is almost eliminated by trabeculectomy (Jay 1991). In contrast, medical therapy has no effect on the diurnal variation whereas ALT has only a minimal effect (Migdal & Hitchings 1986, Reiss et al 1991). The indications, results and specific attributes of trabeculectomy in exfoliation glaucoma are discussed in chapter 10.

1.13 Associated conditions

Several conditions have been reported to be associated with exfoliation syndrome. These include Fuchs' heterochromic cyclitis (Jain et al 1983), a rare corneal dystrophy with oculocutaneous pigment disturbance (Meretoja 1985) and nanophthalmos (Diehl et al 1989, Jin & Anderson 1990). It is thought by some that exfoliation syndrome is associated with droplet keratopathy (Taylor 1979, Johnson et al 1989), but this view is not universally shared (Forsius 1988). In a study of 33 Indian patients with Fuchs' heterochromic cyclitis exfoliation syndrome was seen in 2 patients (Jain et al 1983). The authors speculated that there could be a link between the two conditions namely iris pigmentary changes.

Pigmentary glaucoma

Differences and similarities exist between exfoliation glaucoma and pigmentary glaucoma (Sugar 1984). Similarities include liberation/dispersion of pigment and the trabecular pigment ring. Differences include the younger age of affliction in pigmentary glaucoma, the refractive status and

the possibility of self-limitation in pigmentary glaucoma. Layden and coworkers (1990) recently described 5 patients who had pigment dispersion syndrome and who subsequently developed exfoliation. The concurrent occurence of these two conditions increased the difficulty of medical management of their IOP.

Closed angle glaucoma

The anterior chamber depth is usually found to be normal in exfoliation syndrome\glaucoma (Hörven 1966, Forsius et al 1974, Bartholomew 1980, Montanes et al 1990b, Stefaniotou et al 1990, Konstas & Dutton 1991) and concurrent angle closure glaucoma is probably coincidental (Gradle & Sugar 1940, Tarkkanen 1962, Lowe 1964, Hörven 1966, Forsius et al 1974). However, a high prevalence of angle narrowness in association with the condition has been reported in two studies (Layden and Shaffer 1973, Wishart et al 1985).

1.14 Plan of proposed morphological investigation

From the foregoing review of the literature, the following topics were selected for research and this choice was influenced by 1) the availability of material and immunocytochemical technology and 2) the surgical policy for the treatment of exfoliation glaucoma.

Part I of this investigation deals with the morphology of the iris in exfoliation glaucoma and the nature of exfoliation material. Part II describes two prospective clinical investigations in Scottish patients with exfoliation glaucoma and POAG. It was not considered appropriate to deal with conventional morphology of the normal ocular tissues. However, in section 4.1 a brief description of the morphology of the normal iris has been given. The specific aims of part I of this investigation are:

(1) To establish a definitive diagnosis for the presence of exfoliation material in the open angle glaucoma population studied in part II.
(2) To provide more information on the conventional morphological changes seen in the iris of patients with exfoliation glaucoma.
(3) To document the fine structural distribution of collagens I-V in the normal and exfoliative iris.
(4) To study the distribution of the glycoprotein laminin in the normal and exfoliative iris.
(5) To evaluate changes in the vascular matrix of exfoliative iris vessels in the various stages of the disease.

CHAPTER 2 THE NORMAL EXTRACELLULAR MATRIX

2.1 Introduction

A brief description of the current state of knowledge in the structure and functional role of the extracellular matrix (ECM) is provided in the following chapter. Due to the immense breadth of research on this topic a comprehensive review is impractical. The following description focuses on the ECM components investigated in this thesis (collagen types I-V and laminin) and highlights only functional features of relevance to the present study.

2.2 Definition, structure and role of the ECM

Definition

The extracellular matrix (ECM) is an intricate meshwork of interacting extracellular molecules that acts as a scaffold to which cells attach (Mecham 1986, Adair & Mecham 1990, Alberts et al 1991). The ECM serves as a universal 'glue', and is the major constituent of structures such as cartilage, tendons and bone. The ECM macromolecules are thought to be secreted by local cells, particularly fibroblasts, and assemble into an organised meshwork in the extracellular space of most tissues. In more specialized structures, ECM macromolecules are secreted by specific cells, such as the chondroblasts forming cartilage and the osteoblasts forming bone. Normal development and tissue repair in the higher animals rely on the elaboration of appropriate matrices by specific cell types (Majack & Bornstein 1985).

Structure of ECM

Two of the main classes of extracellular macromolecules are the collagens, which strengthen and facilitate the organisation of the ECM and those of glycosaminoglycans, (forms of polysaccharide), which are frequently linked to protein to form proteoglycans. Collagens are distinctive fibrous proteins which impart stiffness and a high degree of tensile strength to the ECM. The glycosaminoglycan molecules (formerly known as mucopolysaccharides) form a highly hydrated gel-like 'ground substance' in which collagen fibres are embedded. Their role is to allow diffusion of nutrients, metabolites and hormones between the blood and the tissue cells and block diffusion of large molecules, due to their mesh-like structure. Proteoglycans coexist with collagens with whom they interact (Tawara et al 1989). Indeed, proteoglycans have been shown to influence the assembly of the collagen fibrils, their growth and their three-dimensional arrangement (Ruggeri & Motta 1984, Adair & Mecham 1990). Moreover, cell culture studies have shown that regulation of the production of specific collagen molecules can be influenced by glycosaminoglycans (Pratt et al 1985, Majack & Bornstein 1985, Streuli & Bissell 1990).

Functional role of the ECM

Until recently, ECM was considered a rather inert structural network that stabilised the physical structure of tissues. It is now known that the ECM is directly involved in a number of critically important biological roles, influencing the biochemical function of cells (for reviews see Abrahamson 1986, Mecham 1986, Adair & Mecham 1990). Though many studies

have been designed to elucidate the complex role that ECM plays in regulating the behaviour of the cells it supports, our understanding is still incomplete. It is clear however, that a variety of ECM components characterise different tissues. Their presence and their elaborate interactions determine the physico-chemical characteristics of each tissue (Abbot 1988, Engel 1989). Determining the ECM distribution in different tissues is relevant to understanding the function of these tissues and pathological processes that may occur in these tissues. For example, an immunofluorescent study of the human optic nerve head has demonstrated that the lamina cribrosa contained a specialised ECM that did not resemble that of the sclera (Hernandez et al 1986). The authors suggested that this was related to the specialised function of the lamina cribrosa.

Similarly, in the last decade, the disciplines of cell and molecular biology have made great progress in the <u>in vitro</u> documentation of the influence of the ECM on cell behaviour. The mechanisms by which the synthetic capacity of cells can be modified by the subtle ECM changes and by its interaction with modulating and growth factors have also aroused great interest (Pratt et al 1985, Krieg et al 1985, Glaser 1988). Cell culture studies suggest that ECM components provide signals that the overlying cells are capable of detecting and responding to (Pratt et al 1985). Furthermore, it seems that the cells, in turn, can modify the surrounding matrix, thus creating dynamic interplay between cells and their enclosing ECM (Mecham 1986). However, it is important to realise that none of the <u>in vitro</u> assays so far described can reproduce

the entire set of events occuring in vivo.

2.3 Glycoproteins

Glycoproteins comprise significant yet little known constituents of the ECM. Some are unique to basement membranes. Two of the best studied glycoproteins are laminin (dealt in detail later) and fibronectin. These high molecular weight glycoproteins are widely distributed and possess a variety of biological functions. Fibronectin is an ECM glycoprotein that promotes cell adhesion and exists in large aggregates in the extracellular space. Fibronectin, as well as two other lesser known macromolecules thrombospondin and amyloid P, have been detected in some basement membranes by immunofluorescence and immunoelectron microscopy and may be important constituents during embryonic development or tissue repair (Charonis & Tsilibary 1990). However, because of their presence in the plasma they are not considered to be intrinsic components of differentiated basement membranes.

2.4 Collagens

Definition

Collagen derives from the Greek words meaning 'to produce glue'. It comprises a family of highly characteristic fibrous proteins which are rich in the amino acids glycine and proline (Alberts et al 1991). A collagen can also be defined as an extracellular structural protein whose functional properties depend significantly upon a triple helical domain (Burgeson 1988). Collagens are found in all

multicellular animals and constitute approximately 25% of the total protein in mammals.

Structure

Collagens are major constituents of the extracellular space with common properties that allow them to be regarded as a single class of structural proteins. The regular form of fibrous collagens indicates that they derive from a single molecular subunit, despite the fact that the banded fibres vary in diameter from tissue to tissue (Mayne & Burgeson 1987, Burgeson 1988). The basic structural unit of collagen consists of three polypeptide chains whose precise composition depends on the type of collagen. Collagen molecules have an unusual amino acid composition in comparison with other proteins. Glycine and proline are present in high proportions and collagens contain two amino acids that are found in few other proteins, namely 4-hydroxyproline and 5-hydroxylysine. Moreover, the amino acid sequence of collagens is remarkably regular: nearly every third residue is glycine (Stryer 1988). Glycine residues are of critical importance as they are small enough to fit into the crowded interior of the triple helix. Consequently, glycine allows different polypeptide strands to come together. Individual collagen polypeptide chains, synthesized by ribosomes, are injected into the lumen of the rough endoplasmic reticulum as pro-a-chains. Three pro-a-chains then wrap around each other to form a triple stranded helical molecule. When this tropocollagen molecule is secreted by the cell to the exterior, it combines with others to form collagen fibrils.

Classification of collagens

Collagens can be classified by several criteria: length of molecule, molecular weight, flexibility of the molecule and ultimately by supramolecular structure. Collagens can be classified into three groups according to supramolecular structure: the fibrous collagens, the non-fibrous collagens and the filamentous collagens (Bailey 1987).

Fibrous Collagens

Fibrous collagens are seen with the TEM as thick fibrils with a characteristic 67nm periodicity (Fleischmajer et al 1990a). There is considerable variation in fibril diameter, being 30-40 nm in the cornea and up to 200nm in the sclera (Bailey 1987). Size and distribution may be uniform within a tissue (e.g. cornea) or highly variable (e.g. sclera).

Collagen types I, II and III show considerable homology and consist of a single uninterrupted helical domain, almost 300 nm in length (Miller 1985). Data from several sources indicate that collagen types I, II and III are the principal constituents of collagen fibres (Montes et al 1984, Miller 1985, Ben-Zvi et al 1986, Mayne & Burgeson 1987, Linsenmayer et al 1990a). Ultrastructurally, type I collagen fibrils reach full growth at about 100-500 nm in diameter, whereas type III collagen fibrils do not grow beyond about 60 nm (Keene et al 1987, Fleischmajer et al 1990a). Recently, Keene and coworkers (1987) showed by immunoelectron microscopy that antibodies against type III collagen label all fibrils with 67 nm periodicity regardless of their diameter. This study and that of Fleischmajer and coworkers

(1990a), suggest that some collagen fibrils contain both type I and type III collagens. The relationship between these two collagens was further studied by Fleischmajer and coworkers (1990b) who employed single and double immunoelectron microscopy to show that type III collagen coats the periphery of intact human dermal collagen fibres. In the same study, collagen type I was shown throughout the interior of the fibril; however, its demonstration required disruption of the collagen fibrils. The authors considered this as evidence that the epitopes for collagen type I in the adult human skin are normally masked from type I collagen antibodies (Fleischmajer et al 1990b).

Collagen type V occurs both in association with basement membranes and, quite separately, in fibrous stroma such as cornea (Mayne & Burgeson 1987, Marshall et al 1991b). The overall impression is that collagen type V has one, or more functions as a connector between basement membrane and stroma, but may also be found in collagen fibres in intimate molecular association with collagen type I (Birk et al 1986, Birk et al 1988). It is suggested, on the basis of studies on the chick cornea, that type V collagen co-exists with type I collagen and may control fibril diameter (Linsenmayer et al 1990). In the same study, it was hypothesised that a high content of type V collagen within a heterotypic fibril would result in a fibril of small diameter.

Increased synthesis and accumulation of collagen type I has been demonstrated in diet-induced rabbit atherosclerosis (DeLuca et al 1990). The atheroma formation and luminal

narrowing were associated with enhanced levels of type I collagen mRNA and increased rates of collagen type I synthesis. Degradation of collagen types I, II and III in the ECM by collagenases may be a feature of chronic inflammatory disorders, such as rheumatoid arthritis. In the inflammatory synovium production of collagenase, (synthesised by fibroblasts and chondrocytes), has been shown to be responsible for the degradation of these collagens and distortion of the architecture and function of the joints (Krane et al 1990).

Non-Fibrous Collagens

Non-fibrous collagens comprise the principle collagens of the basement membranes which separate fibrous stromal tissue from cells. Basement membranes vary in thickness from 25nm in capillaries to 200 microns in the lens capsule. Only type IV collagen has been classified as a non-fibrous collagen and has been localised in all basement membranes examined so far. This nonfibrillar collagen has been purified from authentic basement membranes as well as from ECM synthesised by tumours and cell cultures (Abrahamson 1986, Charonis & Tsilibary 1990). Collagen type IV molecules self-assemble to form extended complexes that comprise the structural backbone of basement membranes (Timpl et al 1981).

Most immunoelectron microscopic studies localise type IV collagen to the lamina densa and lamina lucida, although some restrict it to the lamina densa (Kennedy et al 1986, Abrahamson 1986, Grant & Leblond 1988, Essner & Lin 1988). Recently, it has been reported (Timpl et al 1990) that type

IV collagen and laminin form polymers and interact with each other through an entactin/nidogen bridge. The supramolecular architecture of type IV collagen in basement membranes is probably that of a fine mesh (Yurchenco 1990). Other components bind to type IV and this in turn places them closer together and narrows considerably the 'pore size' of the mesh.

2.5 Laminin

The glycoprotein laminin was first purified from the basement membrane-like matrix of the EHS sarcoma (Timpl et al 1979). It is a major intrinsic structural basement membrane component, the only one, together with collagen type IV, which forms polymers. Structurally, laminin is composed of three chains designated A (molecular weight 400,000), B1 (210,000) and B2 (200,000). These chains are held together by disulfide bonds to form a cruciform-like structure (Kleinman et al 1990). Current concepts suggest that there may be various molecular forms of laminin. In the developing embryo, the B chains are first synthesised at the two-cell stage, while the A chain does not appear until the 16-cell stage. In adult tissues, the A chain appears to be reduced in amount or lacking, and the ratio of the B1 to B2 chains varies depending on the organ studied (Kleinman et al 1990). As recently reviewed, there is support for the view that multiple forms of laminin may serve to regulate cell behaviour at different times during development and repair (Kleinman et al 1990, Charonis & Tsilibary 1990).

Laminin has so far been found only in basement membranes (Yurchenko 1990). Several studies have shown that numerous biological functions of basement membranes depend on laminin (Sakashita et al 1980, Laurie et al 1982, Martin & Timpl 1987, Schittny et al 1988, Grant & Leblond 1988, Herbst et al 1988, Panayotou et al 1989, Coopman et al 1991). More specifically, laminin contributes to the support, binding and adhesion of cells, to the selective molecular sieving and to the regulation of cell migration, growth and differentiation. In addition, laminin promotes phagocytosis, the production of collagenase IV and enhances the metastatic phenotype of malignant cells (Kleinman et al 1990).

Laminin interacts with many basement membrane components and recent progress has been made in identifying the portions of laminin with which they interact (Yurchenco 1990, Kleinman et al 1990). Collagen type IV has been shown to bind to native laminin. As removal of laminin's amino-terminal (short arm) globules reduces collagen type IV binding, this implicates these regions as major sites of the interaction. Laminin exists as a 1:1 complex with nidogen (entactin), a sulphated glycoprotein which comprises an intrinsic basement membrane component (Martin & Timpl 1987). The laminin-nidogen interaction is probably the strongest interaction between basement membrane macromolecules (Charonis & Tsilibary 1990).

Laminin has been found to bind heparin and heparan sulfate proteoglycan (Sakashita et al 1980, Martin & Timpl 1987, Charonis & Tsilibary 1990, Kleinman et al 1990). Electron

microscopic observations have suggested that the main domain of laminin involved in this binding is the globule of the long arm (Charonis & Tsilibary 1990). Heparin was shown to induce a specific alteration of laminin polymerisation in which self-assemply was accelerated (Yurchenco 1990). Various other molecules, including plasminogen, sulphatides and integrin receptors have been found to bind laminin; the binding sites and the functional significance remain obscure (reviewed by Kleinman et al 1990).

Laminin exhibits many biological activities. Published work has stressed the importance of laminin in the adhesion and migration of normal epithelial and malignant cells (Timpl et al 1979, Abrahamson 1986, Herbst et al 1988, Zabrenetzky et al 1990, Timpl et al 1990). Tumour cells attach and grow better when cultured on laminin substrates and demonstrate a more malignant behaviour. They also display increased synthesis of collagenase IV, an enzyme responsible for the invasive behaviour of tumour cells (Kleinman et al 1990). In a recent in vitro study, Coopman and coworkers (1991) have furnished data suggesting that amongst several purified basement membrane components only laminin can function as a stop signal for cell migration. Within laminin, the activity was associated with the P1 fragment. The authors concluded that laminin is the major determinant of the barrier function of the basement membrane to which some cell types (i.e. malignant tumour cells) may have become insensitive.

The presence of laminin ensures the formation of functional and specialised ECM (Hernandez et al 1986). The past few

years have seen the publication of numerous reports on the presence and function of laminin and a complex picture is beginning to emerge concerning the role of this macromolecule. However, the majority of these studies have been conducted on animal tissue. It may not be appropriate to extrapolate from one species to another as diverse biological matrices may be employed in different species. Moreover, it is now becoming apparent that specialised organs such as the eye are characterised by specialised ECM.

CHAPTER 3 MATERIALS AND METHODS FOR PART I

3.1 Conventional morphological studies

3.1.1 Clinical source of material

Tissue from the iris and trabecular tissue were obtained from surgical patients.

Open angle glaucoma group

In a prospective clinico-morphological study 100 consecutive patients with open angle glaucoma admitted for filtration surgery were examined by the author by comprehensive clinical examination for the presence of exfoliation material (section 9.1). Pathological examination of iris tissue obtained from all 100 patients provided a definitive diagnosis (exfoliation glaucoma or primary open angle glaucoma (POAG) in every case. Subsequently, all the exfoliation glaucoma specimens (26) and an equal number of age-matched POAG specimens were used for the detailed description of the range and characteristics of pathological changes in exfoliation glaucoma.

Closed angle glaucoma group

Iris tissue from 31 consecutive patients who were deemed to warrant trabeculectomy for closed angle glaucoma were also collected. These patients were clinically examined by the author in the same fashion as the open angle glaucoma cases for the presence of exfoliation material (section 9.1.1). Iris tissue from these patients was examined morphologically for the presence of exfoliation material. These 31 cases

were included in this study as controls to determine whether the prevalence of exfoliation material varies in the two glaucoma groups.

3.1.2 Methods for conventional studies

Clinicopathological examination

The open angle glaucoma group was investigated by comprehensive clinical examination (section 9.1.2) and was classified into 3 clinical groups: exfoliation glaucoma, possible exfoliation glaucoma and POAG. LM and TEM examination was performed on iris tissue from all patients with exfoliation glaucoma and possible exfoliation. All of the 60 iris specimens from those with POAG were examined by LM. Sixteen specimens were subsequently examined by TEM either because LM indicated the possibility of exfoliation (4 cases), or gonioscopy had not been performed (12 cases). In addition, TEM was also employed upon a random sample of group III iris specimens (18 cases).

All 31 specimens from the closed angle glaucoma group had LM assessment and 15 specimens, chosen at random, also had TEM examination for the presence of exfoliation material.

Conventional processing

Following surgery iridectomy specimens were fixed in buffered glutaraldehyde 2.5% (pH 7.6) for several days at 4°C. They were then washed in three changes of cacodylate buffer for 20 minutes; post-fixed in equal parts of 2% osmium tetroxide and cacodylate buffer for one hour at room temperature; washed

several times in cacodylate buffer and dehydrated through the following graded series of ethanol:

25% ethanol	10 mins
50% ethanol	10 mins
75% ethanol	10 mins
95% ethanol	10 mins
Absolute alcohol (4 times)	20-30 mins

The tissue was then cleared with two 5 min changes of propylene oxide and infiltrated overnight with a 50:50 mixture of araldite and propylene oxide. Infiltration was continued with a 75:25 mixture of fresh araldite and propylene oxide for 6hrs on a rotivator at room temperature before blocking out into labelled rubber moulds; polymerization was carried out for 24-48hrs at 60°C.

Cutting and staining

Iris tissue was cut in a tangential plane so as to section the majority of iris vessels in transverse section. Semithin sections 1-3 micron thick were cut with a glass knife mounted on an LKB Ultrotome nova III stained with 1% toluidine blue and examined by LM. Ultrathin sections were cut from the selected iris blocks and mounted on 150 mesh, or 200 mesh copper grids. Post-staining with freshly made saturated aqueous uranyl acetate (15 mins) and Reynolds lead citrate (15 mins) was performed at room temperature. To facilitate recognition of exfoliation material at deeper tissue planes serial ultrathin sections were cut approximately every 20-30 microns. An attempt was made to cut at least 50% of the

block to identify and delineate exfoliation material deposits. At least five levels from every block were submitted for ultrastructural examination in a Philips 301 or a Jeol 1200 EXII transmission electron microscope.

Coding for masked morphological assessment

In order to allow unbiased morphological assessment all specimens were coded. The specimens were examined with a dissecting microscope at a x50 magnification and allocated a file number from the Ophthalmic Pathology Laboratory. LM evaluation of coded iris sections stained with toluidine blue was conducted on all specimens. The histological identification of exfoliation material in the iris was conducted according to the criteria described by Ringvold (1970c). Ultrastructural assessment was conducted on serial sections from coded iris specimens from 22 exfoliation glaucoma cases, 18 possible exfoliation glaucoma cases and 34 POAG cases (see section 10.2.1).

3.2 Immunocytochemical studies

3.2.1 Introduction

Definition

Immunocytochemistry provides a means of identification of a tissue component in situ by means of a specific antigen-antibody reaction in which the antibody is bound to a visible label (reviewed by Polak & Varndell 1984, Beesley 1985; 1987) For the purposes of this study, the terms immunocytochemistry and immunogold localisation refer exclusively to the

identification of tissue components at the EM level.

Gold conjungates

The interest in the use of gold-tagged antibodies as an immunocytochemical marker has greatly increased for a number of reasons. First, the visibility and discrete nature of gold particles is much higher than that of other labels such as ferritin. As gold atoms in the gold particles occur in their elemental form, the electron density due to their high atomic weight is augmented by the density of atoms within the particles. This permits the localization of antigenic sites occuring in regions with non-homogenous electron density. Gold probes, unlike other antibody markers, can therefore be used in conjunction with optimally contrasted thin sections as they do not obscure the ultrastructural details of the labelled structures. This has permitted very fine localisation of antigens. Secondly, the particulate nature of the gold probe allows the indirect estimation of the amount of antigenic sites. Individual gold particles are assumed to be in the numbers proportional to the number of antigenic sites.

Antigenicity vs ultrastructure

The quality of ultrastructure and the preservation of antigenicity is greatly influenced by the type of fixative, its concentration, the duration and temperature of fixation (Beesley 1987). Unfortunately, ultrastructural preservation and the preservation of antigenicity are often in diametric opposition. Fixation protocols that preserve fine structure may destroy antigenicity and vice versa (Beesley 1987). Only

the most robust antigens survive the standard fixation regime of 2% glutaraldehyde followed by 1% osmium tetroxide, which normally ensures satisfactory ultrastructural preservation. More sensitive antigens are preserved with low concentrations of paraformaldehyde, but the concomitant loss of ultrastructure is a problem. It is thus important to experiment with numerous fixation regimes for a particular antibody and tissue in order to establish the optimum compromise between antigenic and ultrastructural preservation. The basic investigations for the development of the immunogold technique in this laboratory were conducted by Dr G.E. Marshall.

Labelling of resin embedded sections

All the preparatory steps in embedding biological material adversely affect antigenicity. Such embedding procedures elicit conformational changes of proteins by exposing them to denaturing fixatives, organic solvents and high temperatures (during resin polymerization). The influence of the embedding medium on the protein conformation is not well understood, but the environment provided should be as polar as possible to minimize disruption of protein conformation. The use of London Resin white (LR white) resin for embedding partially dehydrated tissue (up to 70% alcohol) is beneficial in that a hydration shell is maintained around antigenic epitopes. This results in increased antigenic conservation.

3.2.2 Advantages of immunocytochemistry in the study of the ECM components in the eye

Introduction

The precise localisation and role of individual ECM components in the eye in health and disease were not explored in the past due to limitations of the experimental research tools available (reviewed by Marshall 1991). The immunocytochemical (immunoelectron) localisation of ECM components is a sensitive technique which allows fine structural localisation of antigens.

Advantages of immunocytochemistry

Immunohistochemistry at the LM level has insufficient resolution to unravel the structural components of normal, or diseased ophthalmic tissues and to resolve pathological substances (e.g. fibrillar and extrafibrillar exfoliation material), into its constituent parts. In addition, immunohistochemistry may be affected by relocation of the antigen (frozen sections) and diffusion of the label into surrounding tissues (immunoperoxidase technique). In the immunoperoxidase method at the EM level problems encountered at the LM are compounded by the difficulty in distinguishing between peroxidase labelling and background staining. Thus, interpreting the results is difficult.

In contrast to immunohistochemistry, immunogold particles employed in immunocytochemistry are localised in very close proximity to the antigen (i.e. attach themselves to the primary antibody, which in turn interacts with antigenic

epitopes), allowing precise localisation of individual constituents. Other advantages include the potential for semi-quantitation (calculation of relative value of concentration of antigen), multiple labelling (two or three different sizes of particles can be attached to the antibodies in the same tissue) and the permanence of the results, unlike immunofluorescence which quenches or fades.

Disadvantages of immunocytochemistry

Disadvantages of immunocytochemistry when compared to immunohistochemistry include 1) the lack of macrodistribution of a given antigen, since only a very small tissue area is studied, 2) the demand for significantly more technical skill and 3) the significant increase in time and work in evaluating the immunocytochemical results.

Immunogold studies in the eye

The immunogold ultrastructural technique has been employed over the last decade for the identification of specific ECM components (collagens, laminin, fibronectin) mainly in animal tissues (Burgeson 1987;1988, Charonis & Chilibary 1990, Yurchenco 1990). It is only recently that some investigators have utilised the immunogold technique at the EM level in the study of the ECM in the human eye (Morrison et al 1989, Das et al 1990a; 1990b, Marshall et al 1990a; 1991a; 1991b; 1992a; 1992b). Most immunogold studies have investigated normal tissues; the potential for addressing biological problems is easily apparent.

Antibodies

The immunocytochemical studies conducted in this work have been based on commercial antibodies. This is because it proved difficult to secure consistently reliable private sources. Attempts in this laboratory to employ antibodies from private sources (for example collagen type VII) proved disappointing. It was thought that the use of commercial antibodies would aid future comparisons and independent confirmation of results obtained herein by other studies. However, constant use of control material was essential.

3.2.3 Pathological material

Normal tissue

For the purposes of the immunocytochemical studies 16 surgically enucleated eyes with ostensibly normal anterior segments of patients aged 52-81 years were obtained, principally from the eye theatre of the Tennent Institute of Ophthalmology, Western Infirmary, Glasgow. Normal iris tissue and control tissue (cornea and trabecular meshwork) was taken from these 16 eyes at the time of the first gross examination. The details for the eyes selected for this study are indicated in Appendix I. The anterior segment was ostensibly normal as determined by routine paraffin histology and LM examination of London Resin (LR) white semithin sections (courtesy of Professor W.R. Lee).

Exfoliative cases

Exfoliative tissue (iridectomies and trabeculectomies) were obtained from 20 patients aged 65-85 years. The exfoliative iris tissue for the immunocytochemical studies comprised peripheral iridectomies from 12 patients who underwent trabeculectomy for exfoliation glaucoma and 3 patients with exfoliation syndrome who underwent intracapsular cataract extraction. In addition, trabecular meshwork tissue from 6 patients with exfoliation glaucoma was employed in the study of laminin and collagen type I (chapters 5 & 6). The details of the 20 exfoliative cases used in this study are provided in Appendix II. All but 3 patients were examined preoperatively by the author (slit-lamp microscopy and gonioscopy) and the diagnosis was made on the basis of exfoliation material deposits on the lens capsule and pupil. The diagnosis for the other 3 patients was made by the referring clinician.

3.2.4 Methods for immunocytochemical studies

Fixation

The concentration of fixative and type of buffer used for each individual eye is listed in Appendices I and II. One of two buffers was employed in the fixation regime: 0.15M sodium cacodylate pH 7.2, or 0.1M sodium phosphate pH 7.2. The fixative used for each eye was freshly prepared by adding 4g of paraformaldehyde (PFA) to 100ml of preheated buffer. A few drops of 4M NaOH was added to the phosphate/cacodylate buffer solution to complete dissolution of the PFA. The solution was cooled with cold running tap water after

complete dissolution and the pH readjusted with 0.1M HCl. The relevant volume of 25% glutaraldehyde was then added to the solution.

Dissection of specimens

Normal specimens

Enucleated eyes were immersed in fixative within a matter of minutes after enucleation and left for 10-15 mins for the sclera to harden: this facilitates the orientation of the calottes. On removal of the portion relevant for pathological investigation the remainder of the globe was dissected and the normal tissue fixed for a total of 2hrs before washing three times in buffer. The anterior segment was then divided in a radial manner with a razor blade. Corneal blocks were dissected out approximately 3mm anterior to the chamber angle and iris tissue blocks at about 1.5mm adjacent to the angle thus leaving blocks containing trabecular meshwork and part of the ciliary body. Thus anterior segment control blocks routinely contained trabecular meshwork, peripheral iris and anterior pars plicata (ciliary body).

Exfoliative specimens

The iris specimens were embedded without dissection as described in the next paragraph. The trabeculectomy specimens generally measured 2 by 2 mm and were cut in a meridian plane. The trabecular meshwork was easy to recognise due to the heavy pigmentation.

LR White plastic embedding

Blocks allocated for LR white embedding were dehydrated in two 1 hour changes of 70% alcohol and infiltrated with LR white hard grade resin (two 1 hour changes). In a number of cases, for convenience, tissue blocks were left overnight in the second LR white change. Dehydration and infiltration were conducted on a modified TAAB type N rotator at 4°C in a domestic fridge. The blocks were then deposited in OO size gelatin capsules filled with LR white resin and the capsules sealed (gelatin capsules are mandatory because plastic capsules are permeable to air and LR white resin will not polymerize in the presence of oxygen). Attempts made to maintain the orientation of the blocks in the capsules by inserting the capsules into a home made capsule holder either in the vertical or horizontal position were unsuccessful. Polymerization was conducted at 48°to 49°C for 48 hours in a Mark II TAAB embedding oven.

LR white sectioning

Semithin sections (1 and 2 microns) were cut on a LKB Ultratome nova (Cambridge Instruments), mounted on glass slides and stained with toluidine blue. A trapezoid was then cut round the desired area of tissue. Ultrathin sectioning was more satisfactory at higher cutting speeds than the normal (5mm/sec as compared to 2mm/sec). It was essential that the block face should be kept dry as wetting greatly impaired the sectioning process. Acetone was never added to the distilled water in the trough as it had a deleterious effect on antigenicity. The refractive index of LR white ultrathin sections appeared to be different from that of

Araldite or Epon: interference patterns were higher up the scale in sections of comparable thickness. Ultrathin sections were mounted on the matt side of 200 mesh nickel grids. In order to ensure adhesion of the sections to the grids, the grids were left to dry for 15 mins in the grid box before rehydration on distilled water. Rehydration was conducted on microtitre plates with one grid per well in the pattern in which they came out of the grid box.

Immunocytochemistry

Antibodies

The anti-collagen antibodies (types I-V) were raised in goats against human and bovine collagens and were supplied by Southern Biotechnologies (UK distributors, Bionuclear Services Ltd, 24 Westleigh Drive, Sonning Common, Reading RG4 9BL). All were affinity-purified and cross-absorbed against the remaining four purified human collagens. The specificity of these antibodies to human antigens was confirmed by the supplier using indirect enzyme-linked immunosorbent assay. Polyvalent rabbit antibodies against laminin were supplied by Heyl (Berlin, Germany) and had been raised in rabbits by multiple injections of human laminin. The serum was tested by the supplier with the following immunological assays: ELISA, immunoblot and immunohistology (fluorescence, APAAP).

Antibody dilutions

The antibody dilutions in TRIS buffer plus 1% BSA, was determined by a number of preliminary experiments and were between 1:30 and 1:60 for collagen types I,II,III and V and between 1:30 and 1:120 for collagen type IV. Laminin

antibodies were used at dilutions between 1:20 and 1:60. It was found that staining intensity increased with antibody concentrations below 1:20, but the non-specific labelling was unacceptably high. Types I-V collagen goat antibodies were visualized with 10nm rabbit anti-goat immunogold conjugate. Laminin rabbit antibodies were visualized with 10nm goat anti-rabbit immunogold (Biocell Laboratories, Cardiff Business Technology Centre, Senghenydd Rd, Cardiff CF2 4AY).

With the delivery of a new batch of primary antibody a range of dilutions was tested in the immunocytochemical procedure against tissue known to contain the antigen under investigation to determine the dilution of the primary antibody which produced optimal labelling. This dilution was, as a rule, the minimum dilution of the normal serum control at which there an acceptably low level of nonspecific labelling. The dilution range was effectively covered by having a factor of two to three between subsequent dilutions with the minimum dilution at 1:20. Dilutions were adjusted for the season as it was found that, particularly in the summer, the room temperature could exceed 25°C. High temperatures appeared to increase specific and non-specific labelling. The possibility of standardization by conducting incubation of the primary antibody at 37°C was considered but was not pursued for practical reasons. A typical dilution range experiment would be 1:20, 1:60, 1:90, 1:120, 1:160. In exceptional circumstances, lower and higher dilutions would be used. Having determined the preferred dilution it was found that use of that dilution level generally produced satisfactory labelling in all subsequent immunocytochemical

runs within the lifetime of an antibody batch. A dilution test was also performed with the immunogold label without the primary antibody. Very little nonspecific labelling was found within the range 1:20 to 1:100. The dilution range was also confirmed by inserting the normal serum before immunogold labelling.

Normal serum in primary antibody

Inclusion in the primary antibody solution of 1% bovine serum albumin (BSA), which was essentially immunoglobulin free, helped to prevent non-specific staining by competing with the primary antiserum for non-specific binding sites in the tissue.

Immunocytochemical buffer

The buffer used throughout the immunocytochemical procedure was 50mM TRIS pH 7.2 with the addition of 0.5M NaCl and 0.05% Tween 20 to reduce nonspecific antibody labelling (Marshall 1991). The addition of 0.5M NaCl and Tween 20 to the immunocytochemical buffer produced a harsh environment for antibody binding. Carbon Formvar coated grids were used to promote section retention by increasing the area for adhesion. However, with these grids, section retention was promoted at the expense of specific immunogold labelling and with an increase in the amount of unwanted deposit.

Transfer of grids

Transfer of grids from one solution to another was done using gold loops, whose diameter was slightly smaller than the outside diameter of the grid. Designated loops were used for

each particular antibody. Transfer of fluid via the loop was largely eliminated by making a small break in the loop. The use of forceps to transfer grids was avoided: not only this proved technically difficult, but also there was greatly increased contamination from one puddle to another due to capillarity between the tips of the forceps. Buffer and distilled water washes were performed by transferring the grids on loops through a series of 1.5 ml puddles on parafilm, depositing the grid onto each puddle for the time indicated. Each antibody had its own separate series of puddle washes.

Immunogold labelling

On completion of ultrathin sectioning, free aldehyde groups were quenched by incubation on 0.5M NH_4 in 50mM TRIS buffer pH 7.4 for 20-30 mins, again using a microtitre plate. The grids were then washed four times in distilled water (2 mins each) and pre-incubated overnight on 1% bovine serum albumin (essentially immunoglobulin free) in the buffer at 4°C. Primary antibody labelling was conducted at room temperature for 2 hours on 20 l drops of antibody diluted in the immuno-buffer with the addition of 1% BSA. Antibody labelling was performed on 20µl drops on parafilm. Evaporation of the drops was prevented by covering the group of drops with a glass trough which also enclosed a wad of cotton wool saturated with distilled water. The grids were then passed through a series of six buffer puddles (approximately 4 mins each) to remove unbound antibody and deposited on 1% BSA dissolved in the buffer for 5 mins. Immunogold labelling was conducted for 1 hour at room temperature on 20µl drops of the

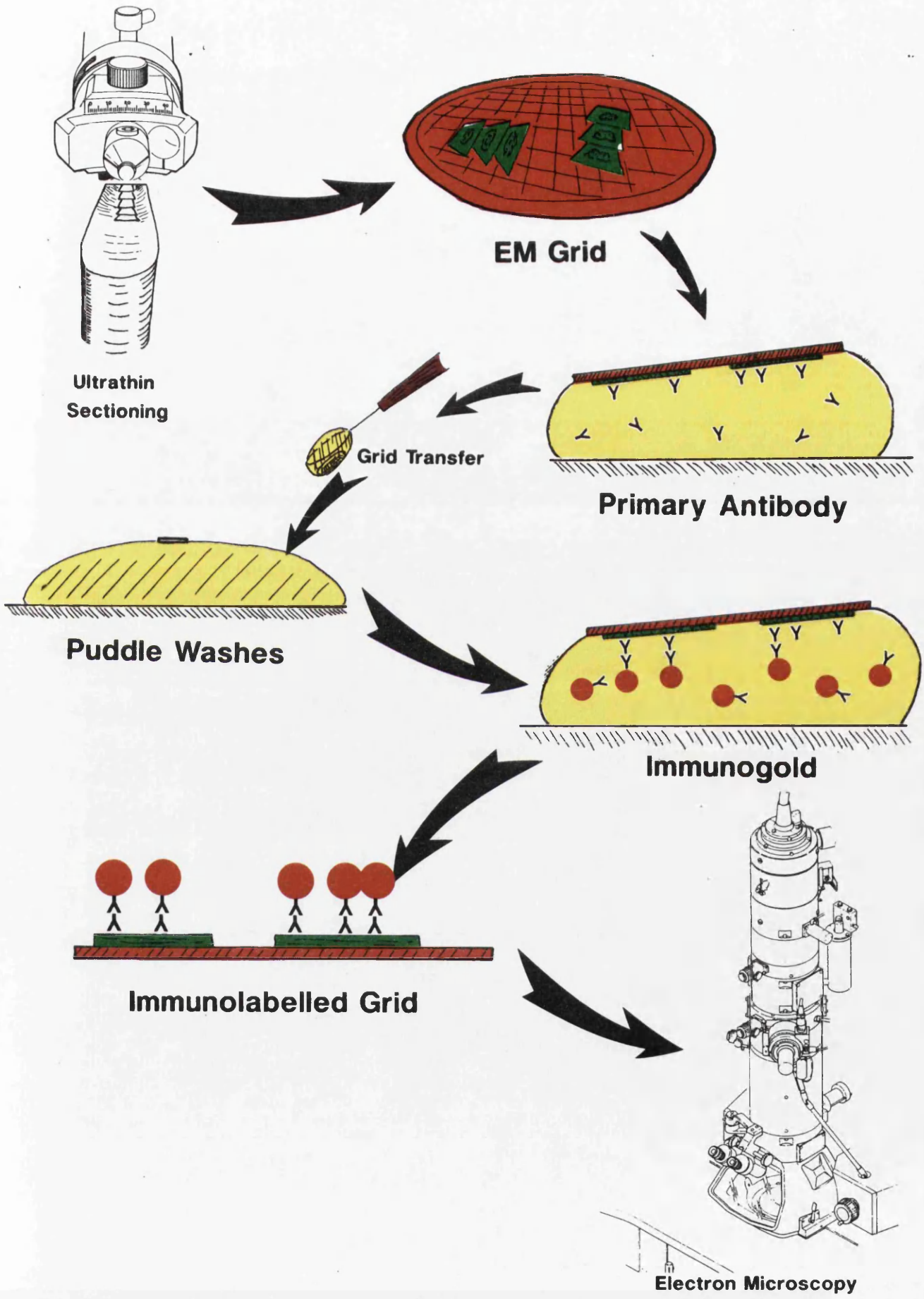


Figure 3.1: Summary of immunogold labelling post-embedding procedure for transmission electron microscopy.

gold conjugate diluted in the immuno-buffer with the addition of 1% BSA. Unbound gold conjugate was removed with three washes (4 mins each) of immuno-buffer plus 0.2% BSA followed by three washes (4 mins each) with immuno-buffer. The sections were then fixed in 2.5% glutaraldehyde in 0.15M sodium cacodylate buffer pH 7.2 for 5 mins in a modified grid box used for staining and washed three times (1 min each) in distilled water.

Staining was performed with freshly made saturated uranyl acetate in distilled water for 15 mins using the grid box stainer in a light tight container. This was followed by three quick distilled water washes. Figure 3.1 summarises the immunogold labelling procedure and Figure 3.2 illustrates the criteria for positive labelling.

3.2.5 Immunocytochemical controls

It is well recognised that with immunocytochemistry it is technically difficult to obtain reproducible and reliable results. Hence, it is essential to have control systems to consolidate information. With post-embedding immunolabelling controls may be employed on sections from the same tissue block and on different tissue blocks.

Negative controls

Two types of negative controls were incorporated into the immunocytochemical procedure, (1) omission of the primary antibody, (2) substitution of the primary antibody with non-immune serum from the same species in which the primary was

raised. The first negative control was found of little significance in that non-specific labelling was always found to be minimal. The normal goat (Sigma-S 2007) and rabbit sera (Sigma-S 2632) controls were conducted at dilutions identical to those used for the corresponding antibodies. Figure 3.3. shows the criteria for negative serum controls in the iris. In this laboratory, the reliability of the immunocytochemical results was repeatedly tested by comparing labelling obtained with ultrathin frozen sections and LR white embedded tissue sections from adjacent tissue blocks.

Positive controls

The use of positive controls strengthens the validity of the results. Therefore, for each ECM component studied, (collagen types I-V and laminin), an appropriate control age-matched tissue was selected as a positive control (Fig 3.4). A number of control tissues were identified as potentially suitable positive controls. These included aged cornea, trabecular meshwork and lens capsule. The selected tissues have been previously studied and the antigen in question had a well documented distribution (Marshall et al 1990a; 1990b; 1991a; 1991c). One or more positive controls were used in parallel with the study of the normal and exfoliative iris.

Evaluation of immunogold labelling

Positive immunogold labelling

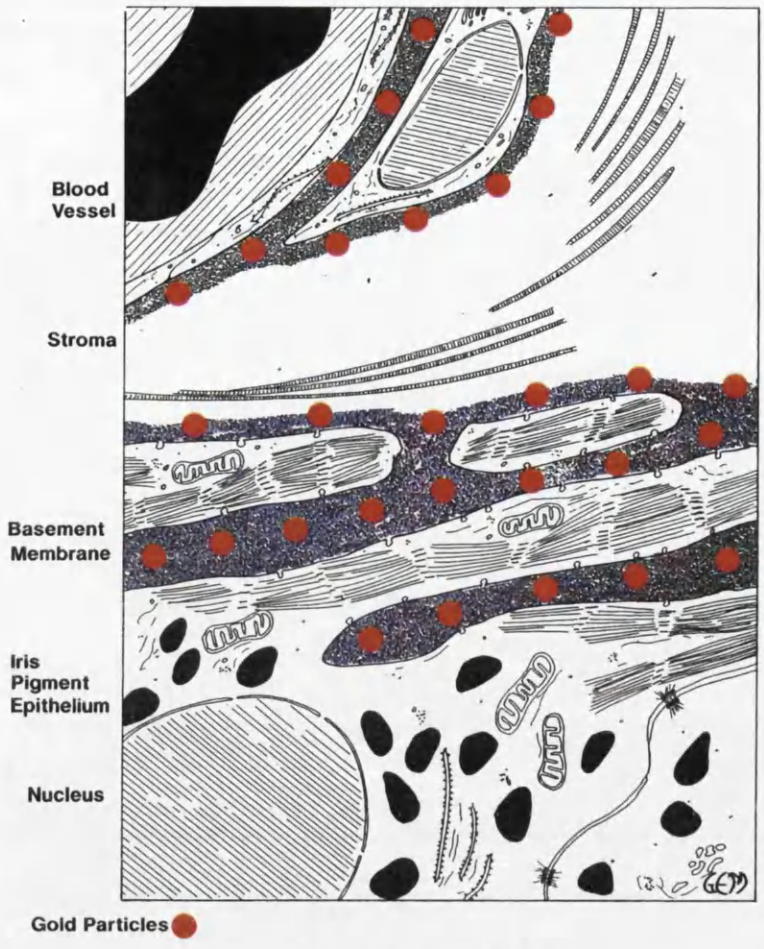
Electron microscopy was performed on a Philips 301 transmission electron microscope at 80kV. An antigen was considered to be positively localized if the immunogold particles were restricted to discrete structures. Such

labelling, particularly if it was weak, was not considered a positive result if a significant number of immunogold particles were present on what was termed internal negative controls which were the first structures to exhibit nonspecific labelling. These internal negative controls were cell nuclei, mitochondria, pigment granules and red blood cells. Little attention was paid to accumulations of immunogold particles associated either with folds and holes in the sections or with contaminating deposits that were usually homogenous and of low electron density. Such labelling was regarded as spurious.

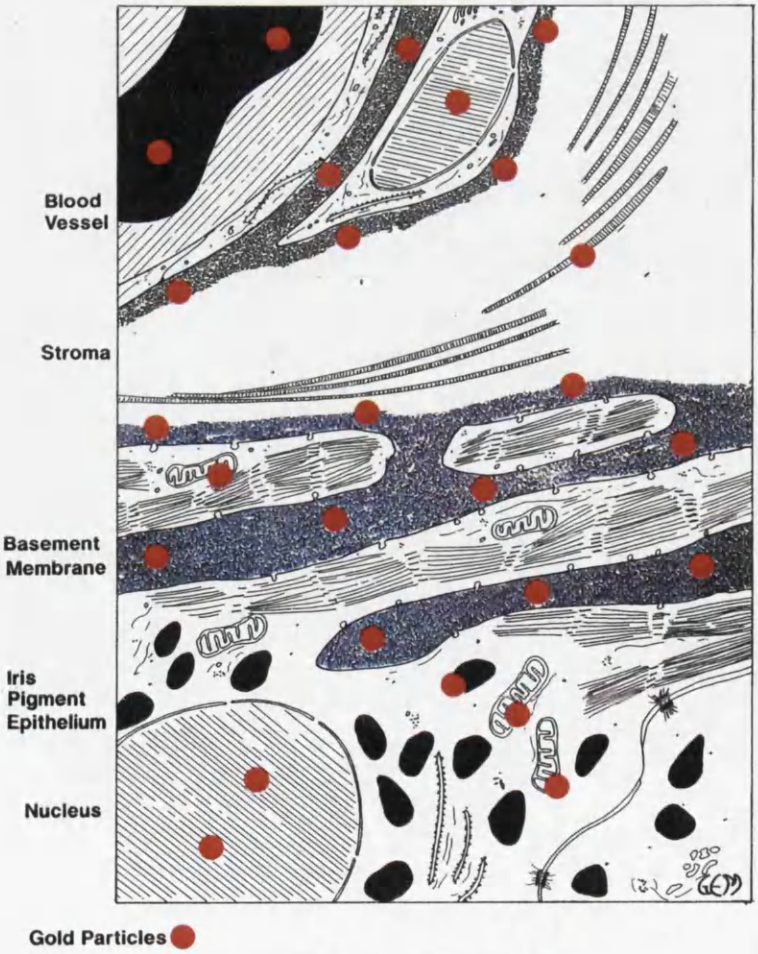
With regard to the interpretation of the results, it must be emphasised that with indirect immunolabelling the gold particle may be up to 20 nm away from the labelled antigenic epitope. Postembedding immunolabelling only labels antigenic epitopes on the surface of a thin tissue section. Therefore, the density of immunolabelling depends upon the number of epitopes exposed on the surface of the section.

Negative immunogold labelling

In order to ensure that the absence of labelling was due to the absence of the antigen and not to failure of the immunocytochemical technique, a tissue in which the antigen was known to be present was included in the immunorun which is refered to as the positive control (Fig 3.4). With most of the antigens another positive control was normally present within the same tissue section (internal positive control).



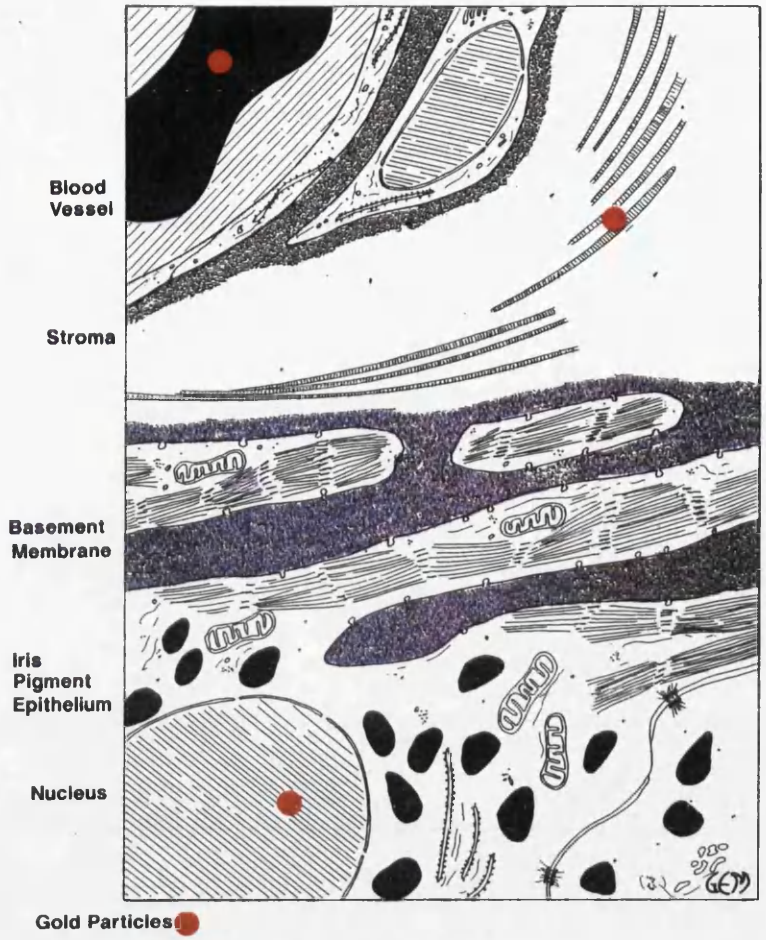
Ideal Positive Labelling



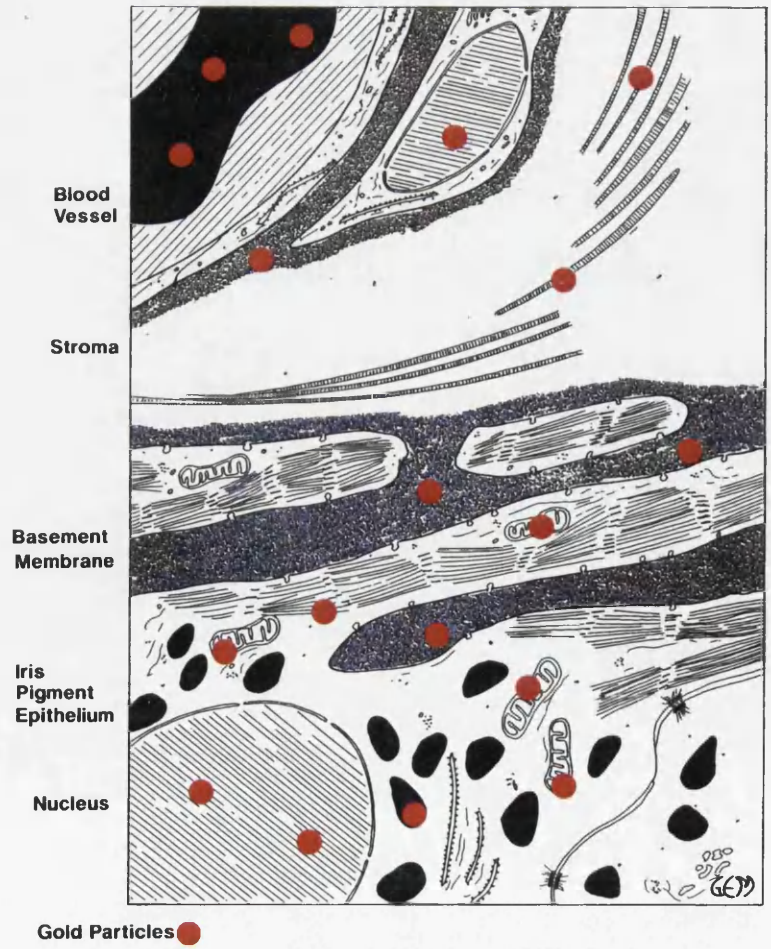
Nonideal Positive Labelling

(unacceptable)

Figure 3.2: Criteria for positive labelling of collagen type IV in iris.



Ideal Serum Control



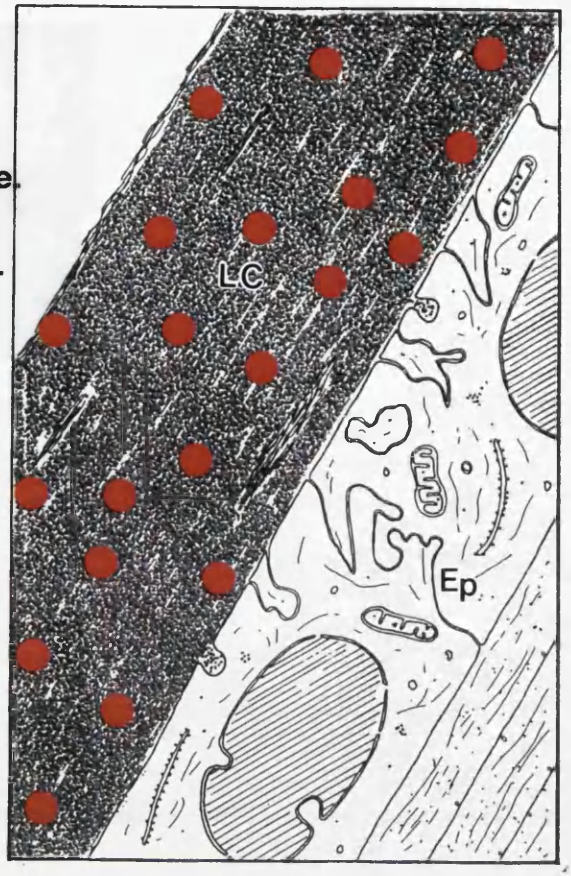
Nonideal Serum Control

(unacceptable)

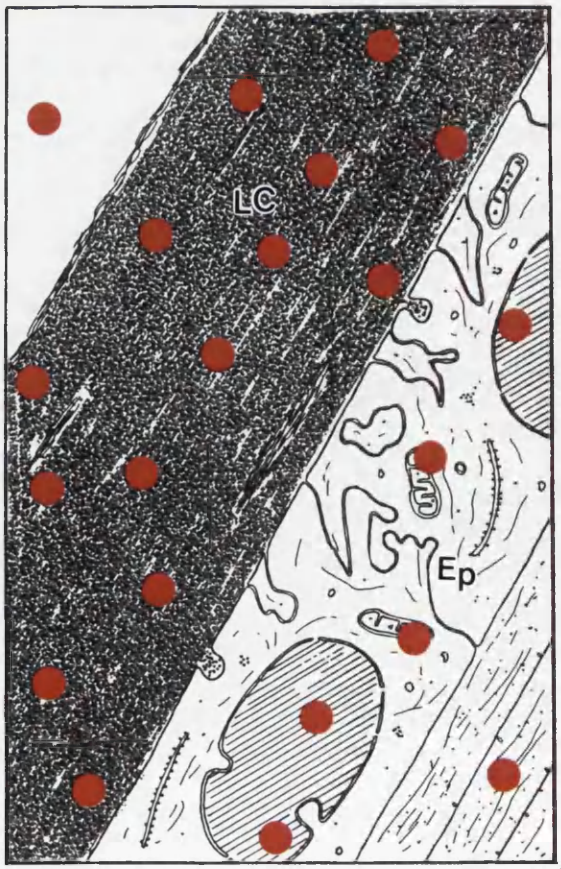
Figure 3.3: Criteria for negative serum controls in iris.

Figure 3.4: Criteria for positive tissue controls. Collagen type IV in the human lens capsule.

LC:lens capsule; Ep:lens epithelium.



Ideal Positive Control



Nonideal Positive Control

(unacceptable)

CHAPTER 4 CONVENTIONAL MORPHOLOGICAL STUDIES ON THE EXFOLIATIVE IRIS

4.1 The choice of iris for this investigation

This section provides background information for chapters 4, 5, 6 and 7. Iris tissue was used to provide a) an accurate description of conventional morphology in exfoliation glaucoma (chapter 4) and b) information on the precise distribution of collagens I-V and laminin in the normal and exfoliative iris (chapters 5, 6, 7).

The choice of iris for the present investigation was the result of preliminary studies and practical limitations in the study of other tissues. Extensive review of the pertinent literature (see sections 1.2.3, 1.4 & 6.2.3) has highlighted the superiority of iris to other affected tissues.

Preliminary studies

To evaluate the suitability of exfoliative iris as a tissue for immunocytochemical studies and to accustom the author to the morphological appearance of exfoliation aggregates in the iris, preliminary conventional LM and TEM studies were conducted upon a number of peripheral iridectomy specimens from Greece. Fifteen iris specimens were randomly selected from a group of Greek surgical iridectomies with exfoliation syndrome and exfoliation glaucoma. All the 15 specimens contained exfoliation material, principally in association with iris vessels. On the basis of these preliminary

observations, it was considered that a fruitful line of research could be undertaken by studying exfoliative iris. In both conventional morphological and immunocytochemical studies it was considered necessary to include an appropriate number of aged-matched control irides. An important distinction however, should be drawn on the control tissue employed. In the conventional morphological studies the control tissue comprised surgical iridectomies from patients with POAG, whereas, in the immunogold studies control tissue from enucleated eyes was used (see Appendix I).

4.2 The normal iris

Structure

The iris forms the most anterior portion of the uveal tract. Anatomically, it forms a delicate and mobile diaphragm between the posterior and anterior chamber. Functionally, it corresponds to the aperture in optical systems (Hogan et al 1971). Amongst the ocular structures, the iris is unique in possessing atypical blood vessels, an ability for contractile movement and a stroma which is freely permeable to aqueous humour via openings (Fuch's crypts) along the anterior surface of the iris. The iris can be divided into 3 regions: (1) anterior border layer, (2) stroma and sphincter muscle and (3) iris pigment epithelium. The gross anatomy of the iris is illustrated in Fig 4.1.

The anterior border layer consists of a single layer of fibroblasts whose long branching processes interconnect with each other and beneath which there are several layers of

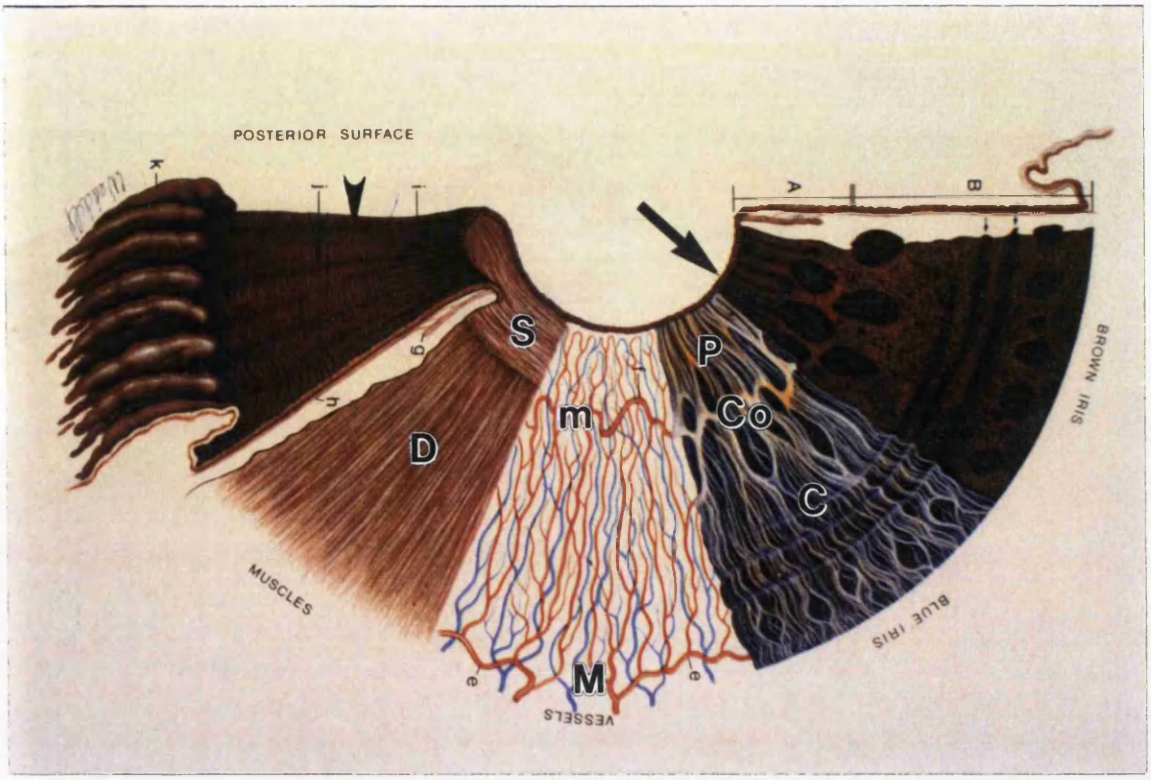


Fig 4.1: Diagram of iris gross anatomy. The collarette(Co) divides the iris into pupillary(P) and ciliary zone(C). The pigmentary ruff is arrowed. Radial branches of the major arterial circle(M) form minor arterial circle(m) from which capillaries radiate to the pupillary margin.

D:dilator muscle; S:sphincter muscle. Radial contraction furrows on posterior surface of the iris are arrowheaded.

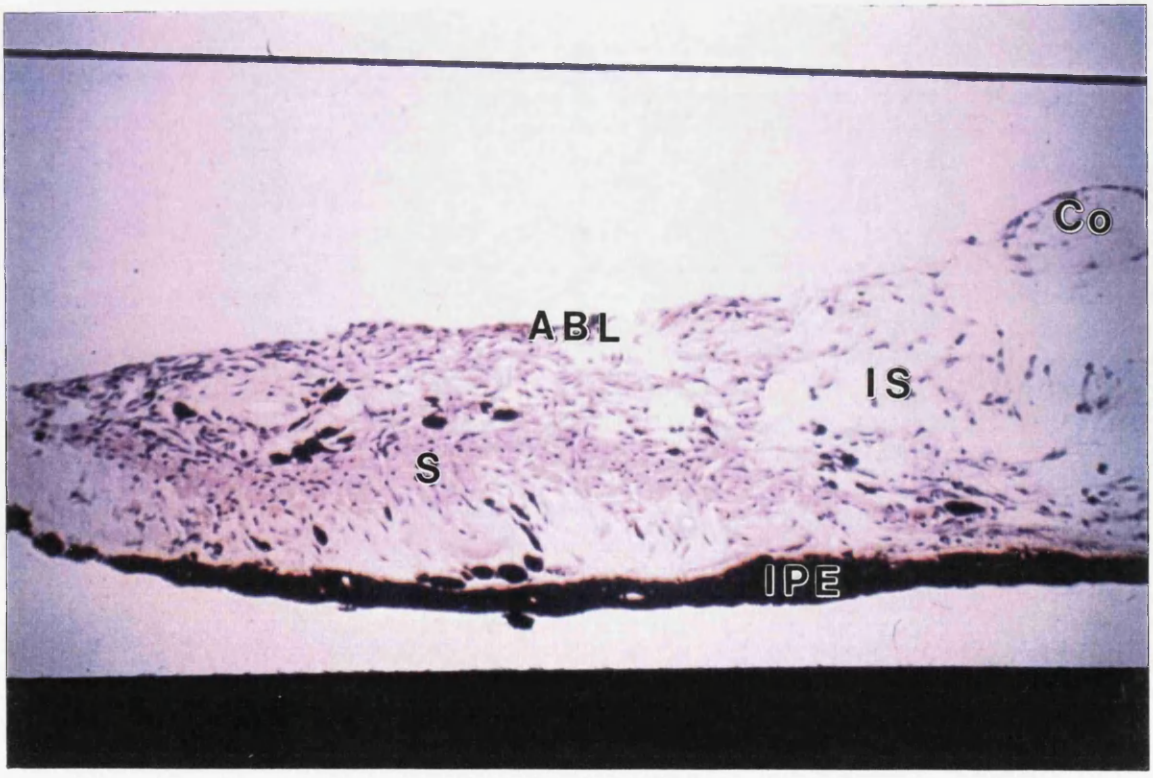


Fig 4.2: Histological section of the pupillary zone in normal human iris. ABL:anterior border layer; Co:collarette; IS:iris stroma; S:sphincter muscle; IPE:iris pigment epithelium. LM.

melanocytes. This layer forms the anterior surface of the iris and is frequently interrupted with gaps (Fuch's crypts). The thickest part of the iris is in the region of the collarette which is located about 1.5 mm from the pupillary margin (Fig 4.1). The collarette divides the iris into two zones: a narrow pupillary zone and a wider ciliary zone, the latter is marked by circular contraction furrows. The rounded irregular openings (Fuch's crypts) which are located on the anterior surface of the iris are continuous with spaces in the iris stroma. A pigmentary ruff is present at the pupillary edge and is a continuation of the posterior iris pigment epithelium (Figs 4.1 & 4.2).

The iris stroma is derived from mesenchyme and consists of highly vascularised connective tissue (Fig 4.3). Striated collagen fibrils are the major component of the stroma and provide mechanical support for this tissue which undergoes constant movement. The collagen fibrils have a diameter of about 60 nm, a periodicity of 65 nm and are aggregated into small and large bundles, which cross at wide angles to form spaces of various sizes. The collagen fibrils are more abundant in heavier sheaths around blood vessels and nerves. The cells most commonly found in the stroma are fibroblasts and melanocytes, but clump cells, mast cells, macrophages and lymphocytes are also present.

The iris pigment epithelium, is derived from the neural ectoderm and is composed of two layers of pigmented cells, the anterior and the posterior pigmented epithelia. These layers meet at their apices. Cells of the anterior pigmented

epithelium possess an apical epithelial portion and a basal muscular portion (Fig 4.3). They are therefore classified as myoepithelial cells. The muscular extensions of the basal portion are embedded in a basement membrane and are orientated in a radial direction from the iris root towards the pupil and collectively form the dilator muscle (Hogan et al 1971). The cells of the posterior pigmented epithelium are rectangular, varying in shape only in the folds and contraction furrows of the posterior surface (Fig 4.1). Their cytoplasm contains a large number of melanin granules. A thin basement membrane is found adjacent to the basal cell membrane (Fig 4.3).

Blood supply

The iris receives its arterial blood supply from the major arterial circle of the iris, which lies in the stroma of the ciliary body near the iris root (Fig 4.1). Branches from the major circle (arterioles) enter the periphery of the iris through its root. These branches occupy the anterior iris stroma as they extend towards the collarette. Arterioles rapidly diminish in size as they enter the stroma and travel in a radial manner toward the pupil eventually forming an incomplete minor arterial circle in the region of the iris collarette (Fig 4.1). Iris blood vessels are believed to have a slight 'corkscrew' shape: this allows them to accommodate rapid changes in the radius of the iris during dilation and constriction. Iris vessels possess a thick collagenous adventitia with the striated collagen fibrils arranged in a helical manner around the vessel. Blood vessels are surrounded invariably by a basement membrane,

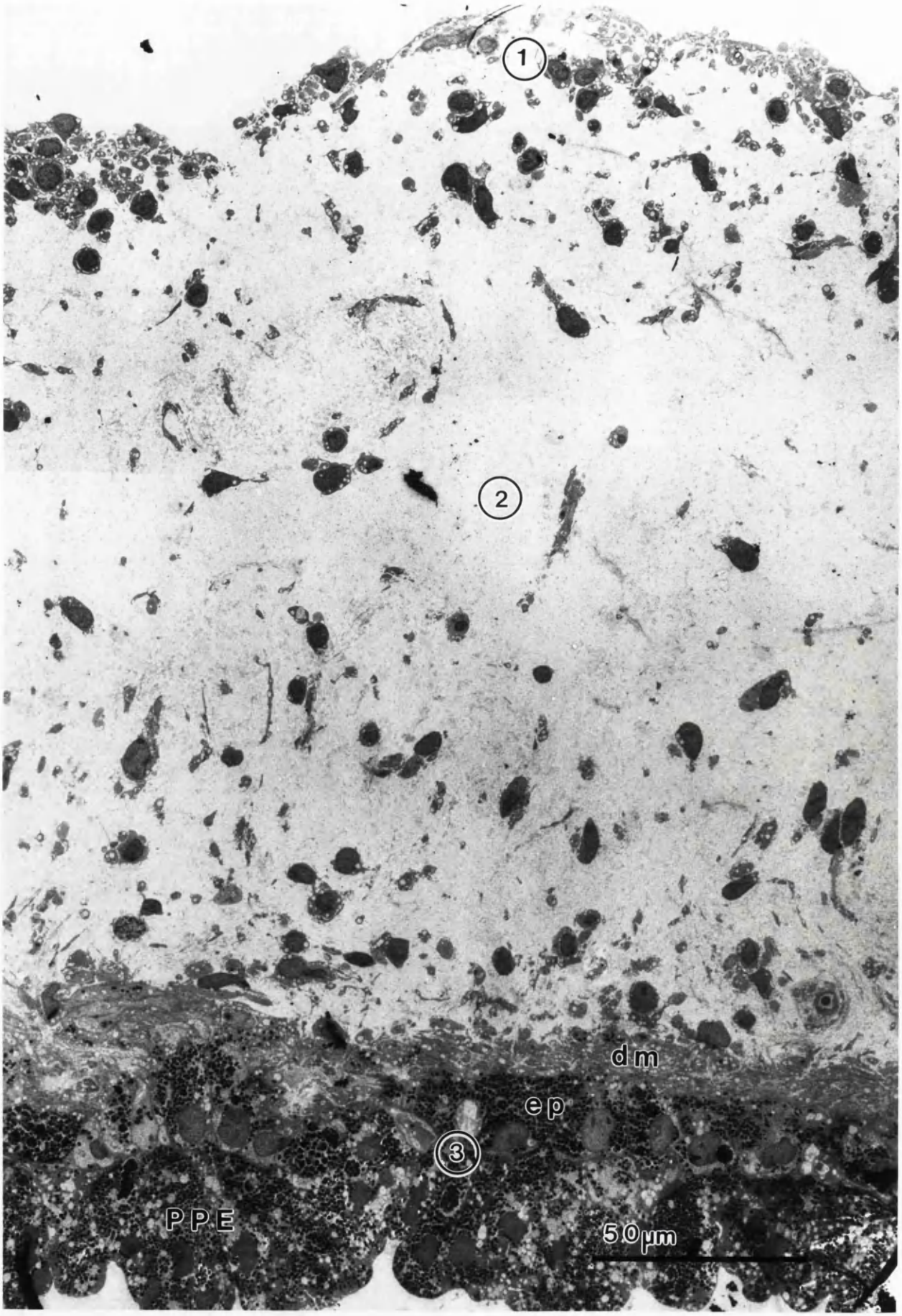


Fig 4.3: Transmission electron micrograph illustrating the 3 regions of normal human iris (ciliary portion). (1) anterior border layer (2) iris stroma (3) iris pigment epithelium. The basal muscular portion, (dilator muscle:dm) and the apical epithelial portion (ep) of the anterior pigmented epithelium are clearly discernable. PPE:posterior pigmented epithelium.

which is up to 3 microns in thickness; muscular tissue is sparse and elastic fibres are absent (Ringvold 1970a). The iris venous drainage follows the route of the arterial supply, the larger venules lie in the anterior stroma and the smaller ones near the dilator muscle. The collector trunks from these venules enter the ciliary body, where the blood then drains into the vortex system and the ciliary plexus (Hogan et al 1971). There is a close anatomic relationship between the iris and the lens which has important functional repercussions. The central third of the iris is in loose contact with the anterior lens capsule. Only the flow of aqueous prevents constant contact and this area of iris-lens contact may be more extensive in an eye with a shallow chamber and in partial mydriasis. In the exfoliation syndrome, adhesions may form between the pigmented epithelium and the lens capsule hindering pupillary dilatation.

Terminology

Iris vessels possess a unique architecture which differs from systemic vessels. For example differentiation between arterioles and venules and pericytes and myocytes is difficult due to the prominent vascular coat. In the present investigation, as in chapters 5, 6 & 7, no distinction was drawn between pericytes and myocytes: they were collectively termed supporting, or cohort cells. In the study of the exfoliation iris vasculopathy the prevailing term 'vascular basement membrane' employed by some authors (Ringvold 1969; 1970a; 1970b, Ghosh & Speakman 1974) to describe the matrix surrounding the endothelial and contractile cells has been avoided. The term 'vascular matrix' has been chosen because

of the difficulty encountered in applying the conventional nomenclature to diseased vessels.

4.3 Introduction: exfoliative iris

Numerous morphological studies have highlighted the extensive involvement of iris in the exfoliation syndrome (sections 1.2.2, 1.4 & 4.1). Most investigators tacitly accept that the iris may be one of the sources of exfoliation material synthesis (section 1.4). However it remains uncertain which precise subcompartment(s) of the iris is responsible for the synthesis of exfoliation material. Furthermore, little attention has been paid to the morphological sequence by which a healthy iris vessel is converted into a non-functioning 'ghost vessel'. This chapter provides information on these topics.

4.4 Results

The clinical significance and the diagnostic value of iris biopsy are discussed in chapter 10.

Light microscopic features

The typical LM appearance of a surgical iris specimen is shown in Figure 4.4. Artefactual problems encountered in many iris specimens included fragmentation of the posterior pigmented epithelium (Figs 4.4 to 4.6). This was more common in the exfoliation glaucoma specimens. In some cases a portion of well preserved pigmented epithelium was enclosed in the centre of the specimen. In contrast, the anterior

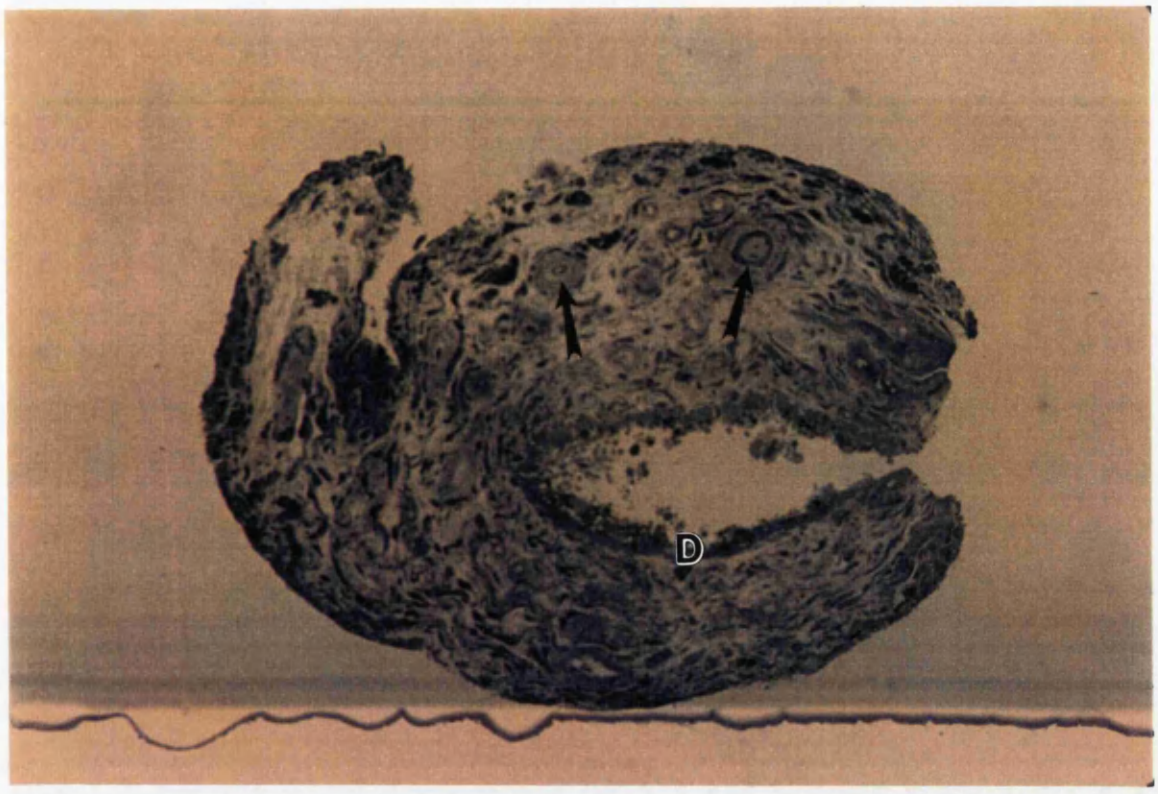


Fig 4.4: A typical iridectomy specimen from a patient with exfoliation glaucoma. Although dilator muscle is present (D), the bulk of the associated posterior pigmented epithelium is absent. Exfoliative vessels are distinguished by increased density of their perivascular matrix (arrows). Araldite, LM.

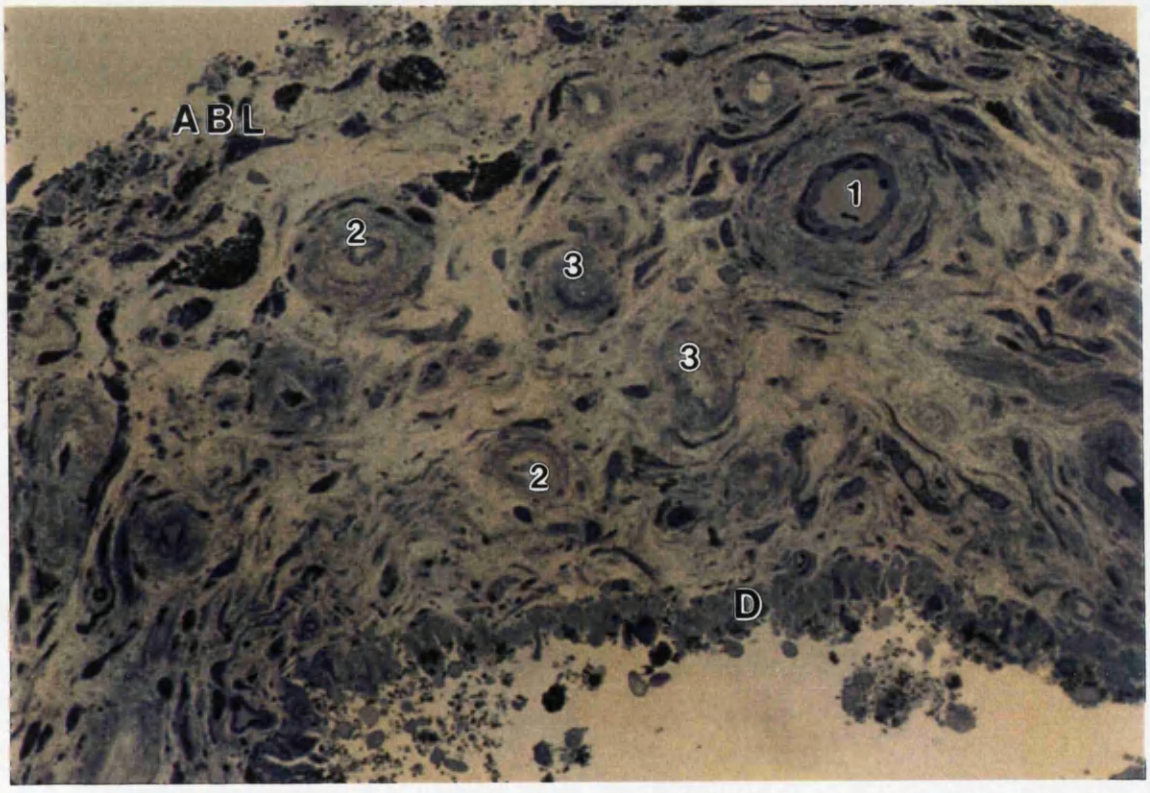


Fig 4.5: Higher magnification of Fig 4.4 illustrating three stages of exfoliation vasculopathy; 1:arteriole with dense perivascular matrix but healthy wall, 2:dense perivascular matrix associated with degenerate vascular elements, 3:ghost vessel where vascular elements are absent. ABL:anterior border layer; D:dilator muscle. Araldite, LM.

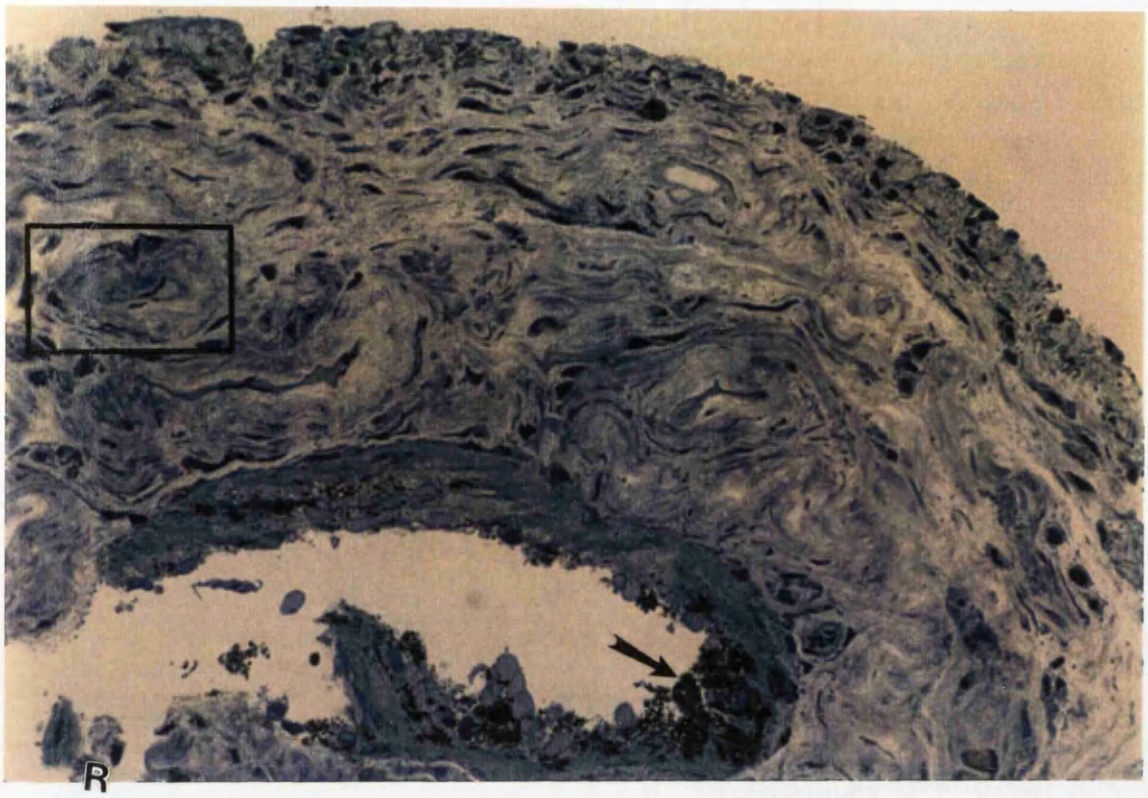


Fig 4.6: Exfoliative iris. The increased density of the iris stroma and collapse of vessels may be due to preparation. A small portion of the posterior pigmented epithelium is preserved (arrow). Araldite, LM.

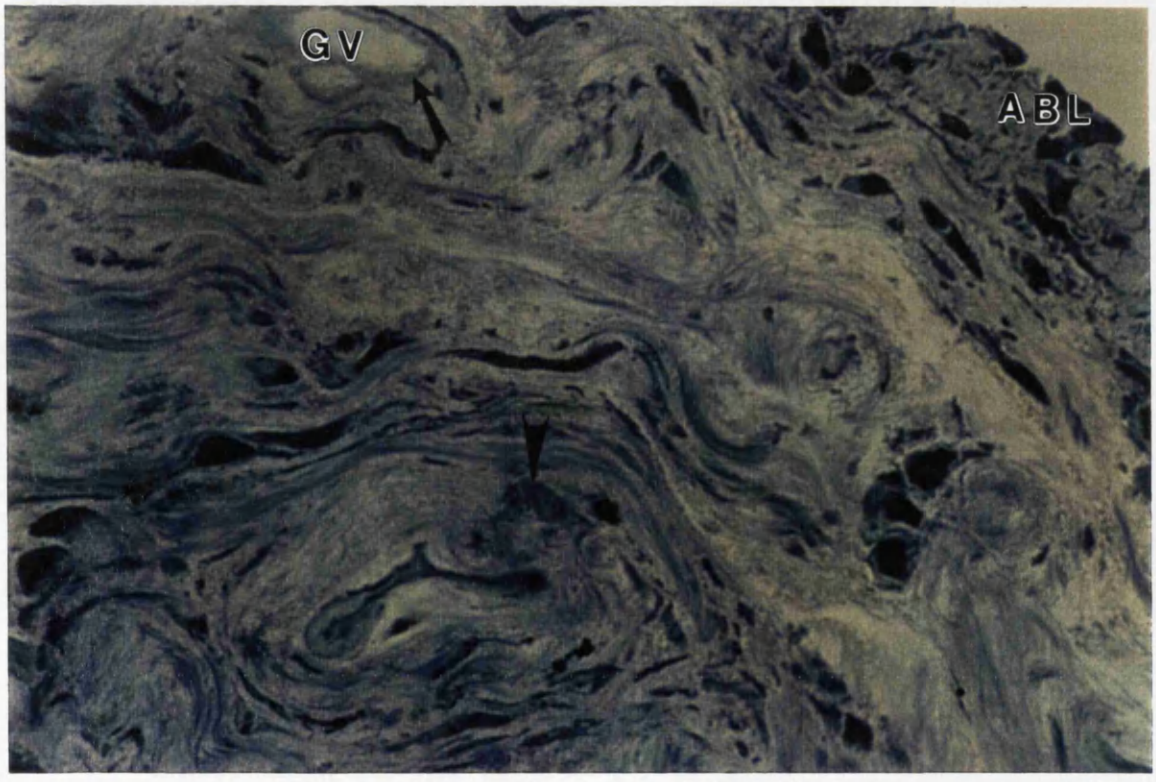


Fig 4.7: Higher magnification of Fig 4.6. an 'abnormal deposit' (arrowhead) in the upper part of the perivascular matrix was subsequently shown by TEM to be exfoliation material. Note ghost vessel (GV), (arrow) with prominent lumen. ABL:anterior border layer. Araldite, LM.

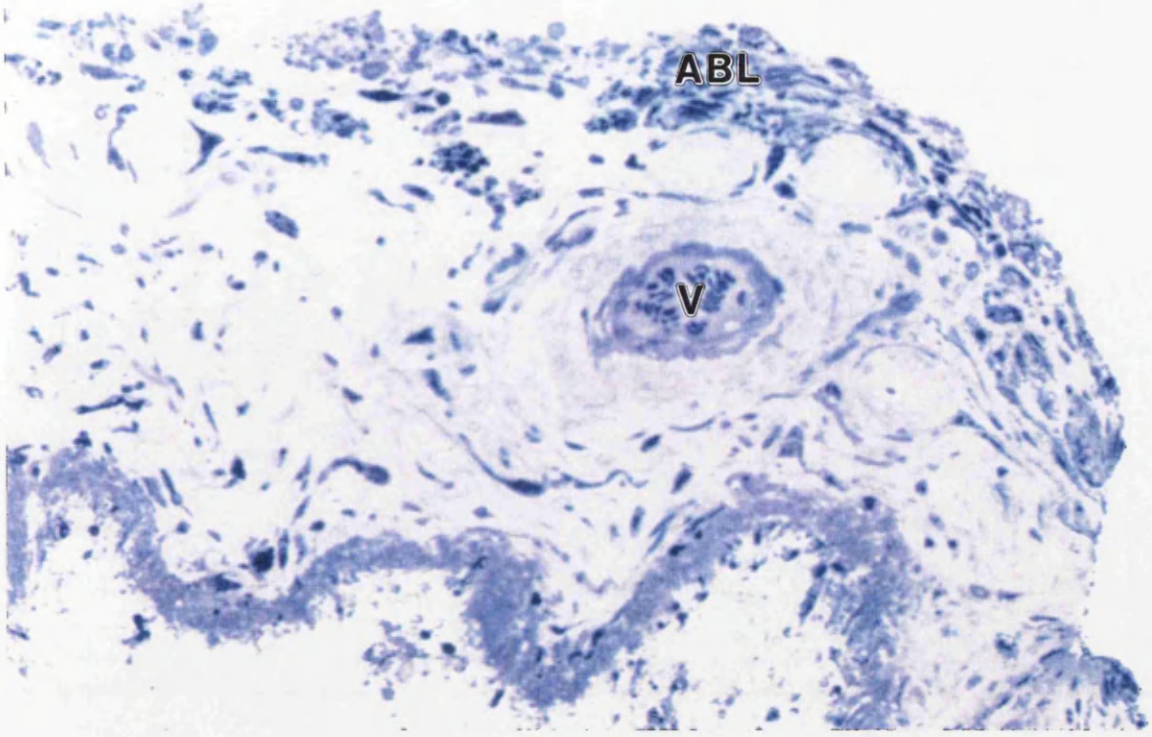


Fig 4.8: LR white semi-thin section of POAG control iris tissue has a less compact appearance with reduced staining contrast. The large iris vessel (V) is of normal appearance and is surrounded by a lightly stained zone. ABL:anterior border layer. LM.

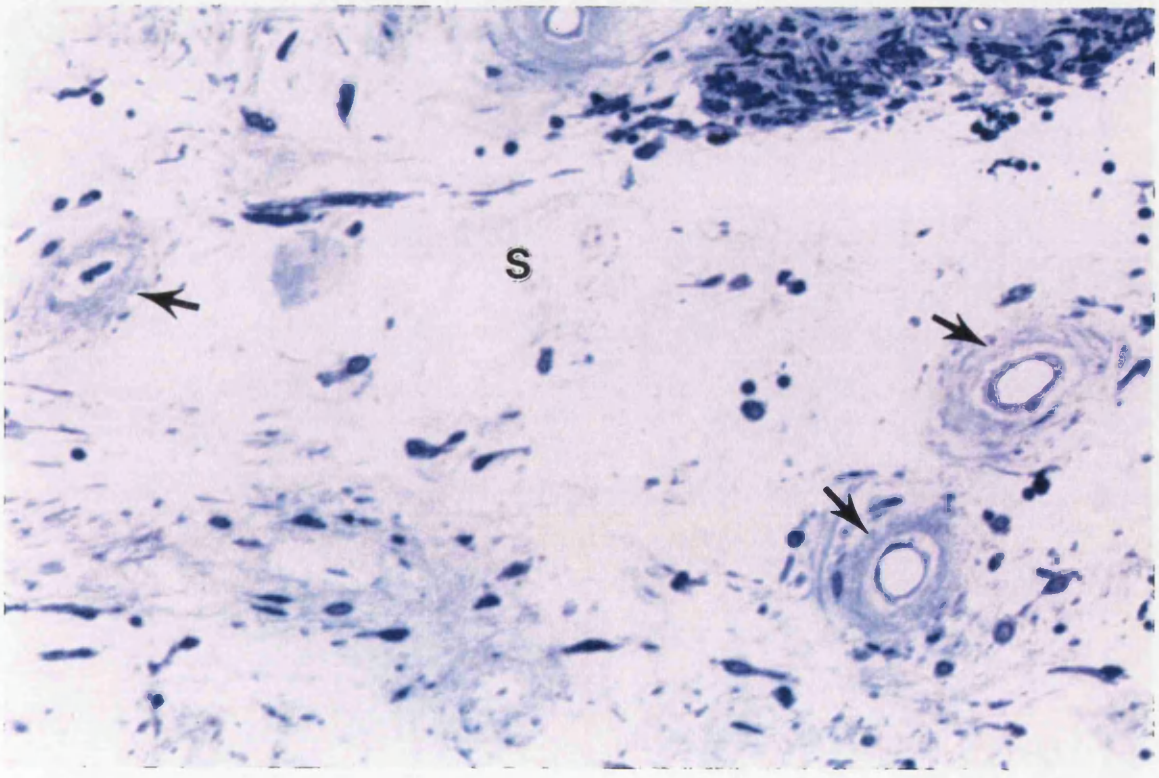


Fig 4.9: LR white section of exfoliative iris specimen in which exfoliation material is seen as increased staining of the perivascular matrix (arrows). S:iris stroma. LM.

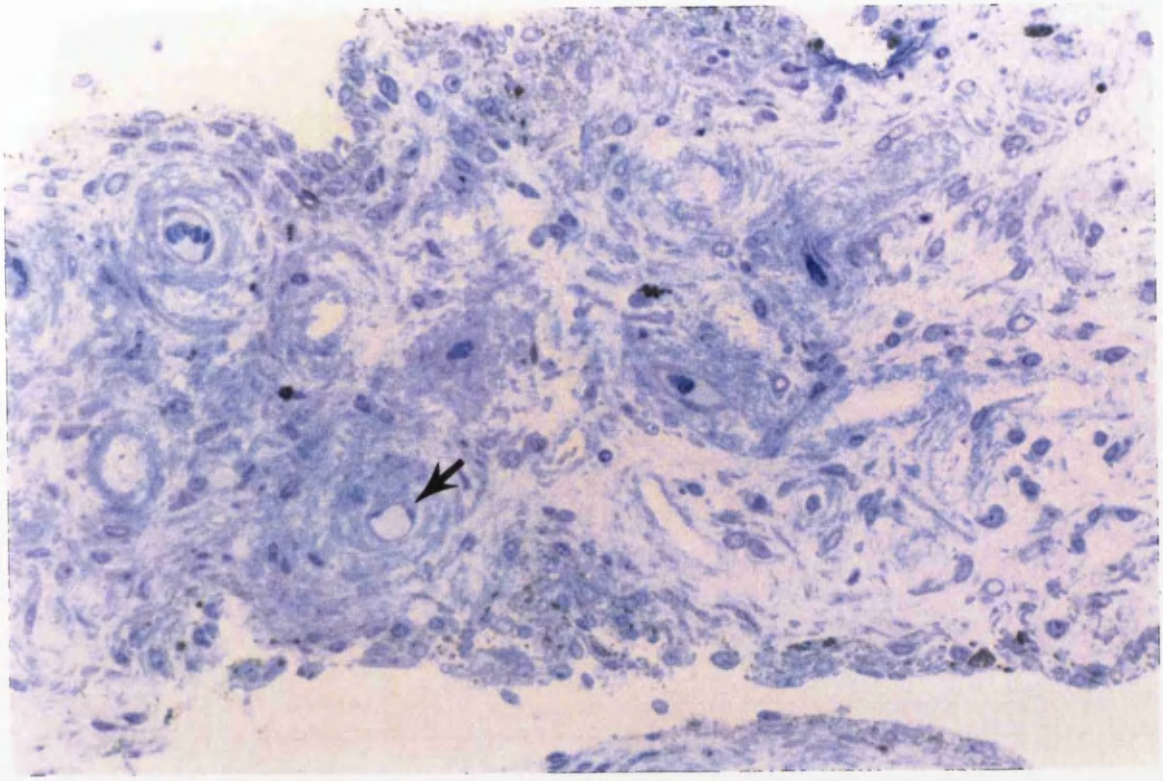


Fig 4.10: Control iris tissue which exhibits a vessel suspected of being associated with exfoliation material (arrow). LR white, LM.

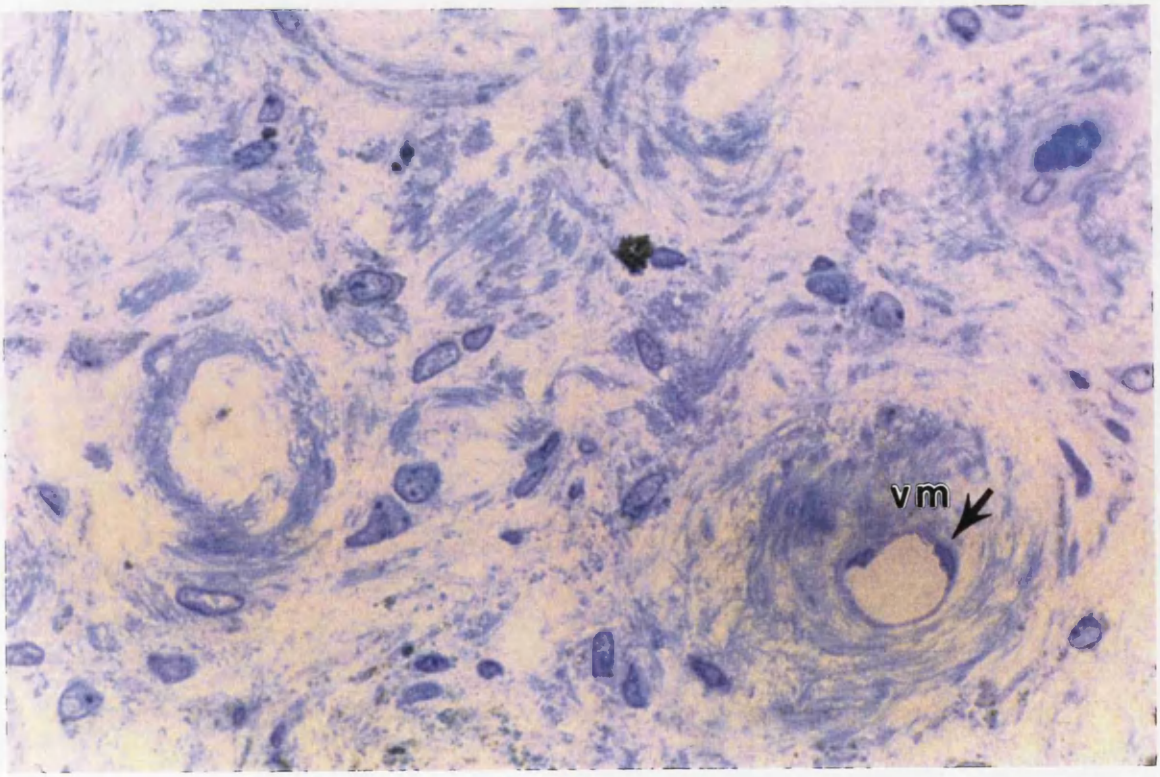


Fig 4.11: Higher magnification of suspected vessel in Fig 4.10, reveals a regular circular orientation of the perivascular matrix (vm). Absence of exfoliation material was subsequently confirmed by TEM. LR white, LM.

border layer, the iris stroma and the anterior pigmented epithelium were generally well preserved (Figs 4.5 & 4.7). Thus, in this investigation no systematic study could be carried out on the morphological changes of the posterior pigment epithelium. Since all specimens reported herein were taken from the periphery of the iris the sphincter muscle was absent.

All 52 surgical specimens contained iris vessels and in some specimens there were more than 20 vessels. The iris vessels were easy to identify due to their circular perivascular collagenous zone (Fig 4.5). In the iris vessels of the POAG controls an unstained, or lightly stained, region separated the supporting cells from the identifiable fibres of the inner collagenous zone (Figs 4.8 & 4.9). In exfoliative iris tissue there were ostensibly normal vessels and vessels which contained blue fibrillo-granular clumps, 'abnormal deposits' (Figs 4.4, 4.5 & 4.7). These 'deposits' were found to lie adjacent to the cellular layer of the wall of some iris vessels. The frequency and density of these 'deposits' varied considerably between specimens and within individual specimens. In some cases the whole circumference of the blood vessel had been replaced by this fibrillo-granular 'abnormal material' (Fig 4.5). Approximately one third of the iris vessels in the 26 exfoliation glaucoma cases were affected. It is noteworthy that it was not possible to identify 'abnormal deposits' in the extravascular stroma with any degree of confidence.

Ultrastructural features

The 'abnormal deposits' observed by LM were confirmed by TEM to be exfoliation material in every case. In the exfoliation glaucoma specimens a few ostensibly normal vessels by LM, contained small nodular aggregates of exfoliation material by TEM. In 3 POAG specimens the presence of exfoliation material was suspected but this was not subsequently confirmed by TEM (Figs 4.10 & 4.11). However, in no case where the suspicion for the presence of exfoliation material was not raised by LM, was exfoliation material detected by TEM. Conversely, when the POAG specimens were examined in no case was exfoliation material identified.

The typical TEM appearance of an exfoliative iris specimen is shown in Figure 4.12. The specific ultrastructural features are described in anatomical regions:

Anterior border layer

In both POAG and exfoliative specimens a fine fibrillo-granular matrix, different in appearance from exfoliation material, was observed between adjacent fibrocytes in the anterior border layer. In the diseased tissue exfoliation material was not a common occurence, but when present in the anterior border layer two types of exfoliation material aggregates were noted. The first pattern observed in 3 cases, comprised loosely arranged exfoliation fibres dispersed in the intercellular spaces in the anterior border layer of the iris (Fig 4.13). The second pattern noted in 2 cases, consisted of densely packed exfoliation material, clearly separated from the surrounding tissue. In one case a

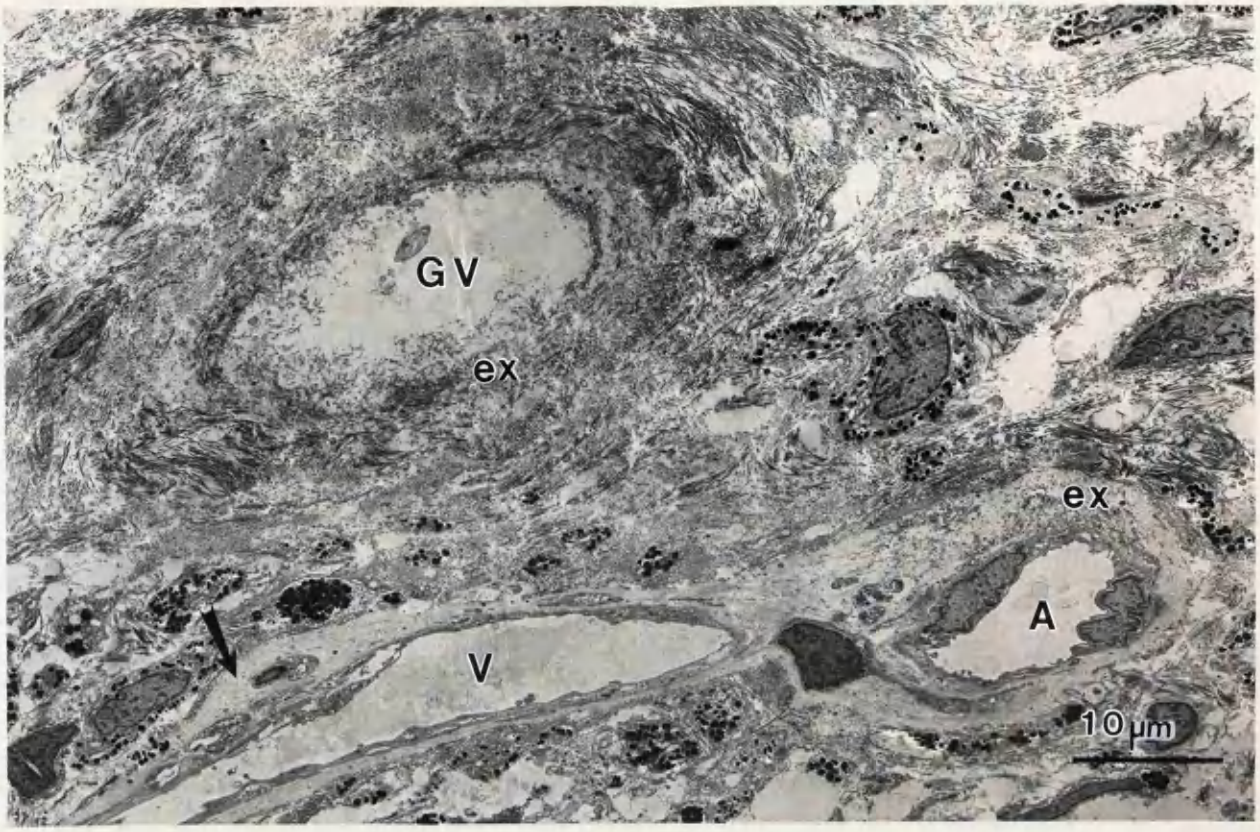


Fig 4.12: Exfoliative iris. an arteriole (A) has a small exfoliation material aggregate (ex). Venule (V) possesses few supporting cells and lamina densa is reduplicated (arrow). Note ghost vessel (GV). TEM.

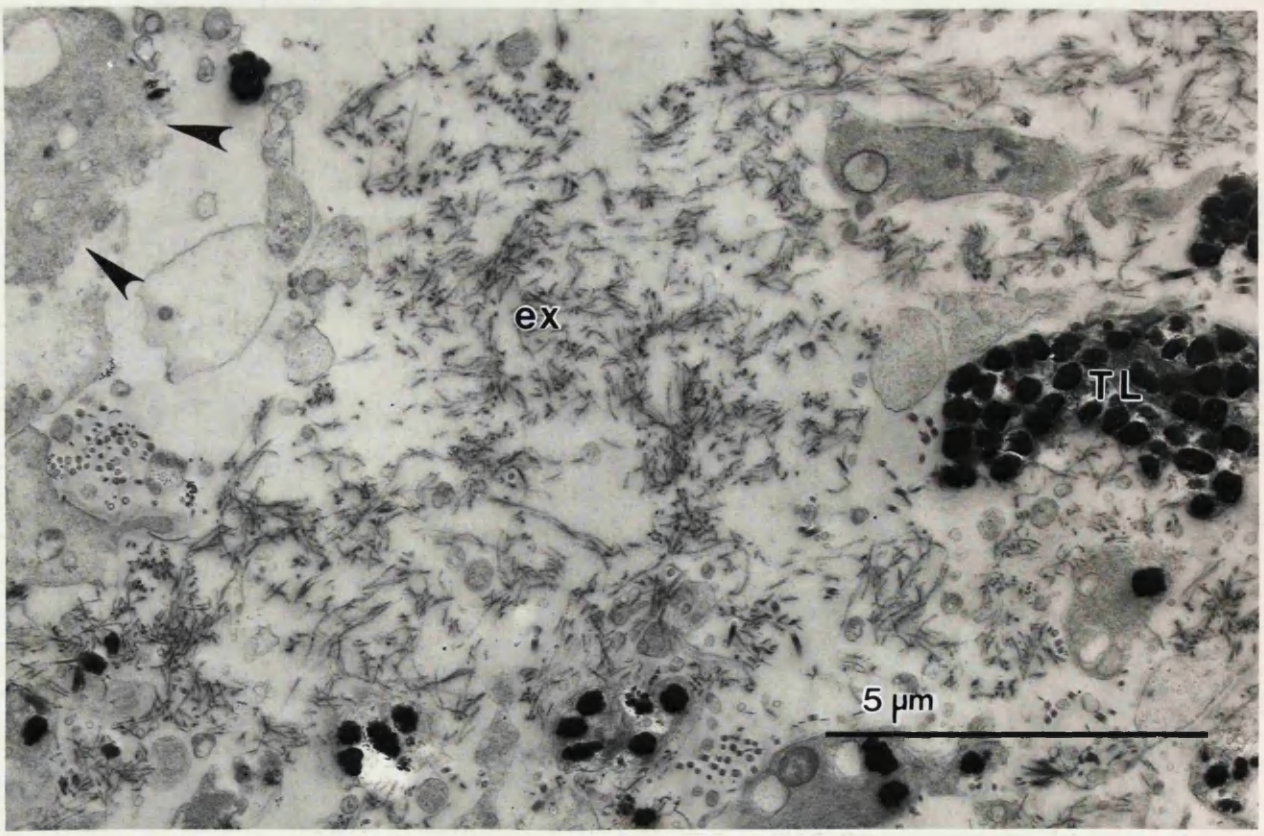


Fig 4.13: Loose exfoliation material (ex) in the anterior border layer of an exfoliative iris specimen. Note absence of cell membranes (arrowheads) and tertiary lysosome (TL). TEM.

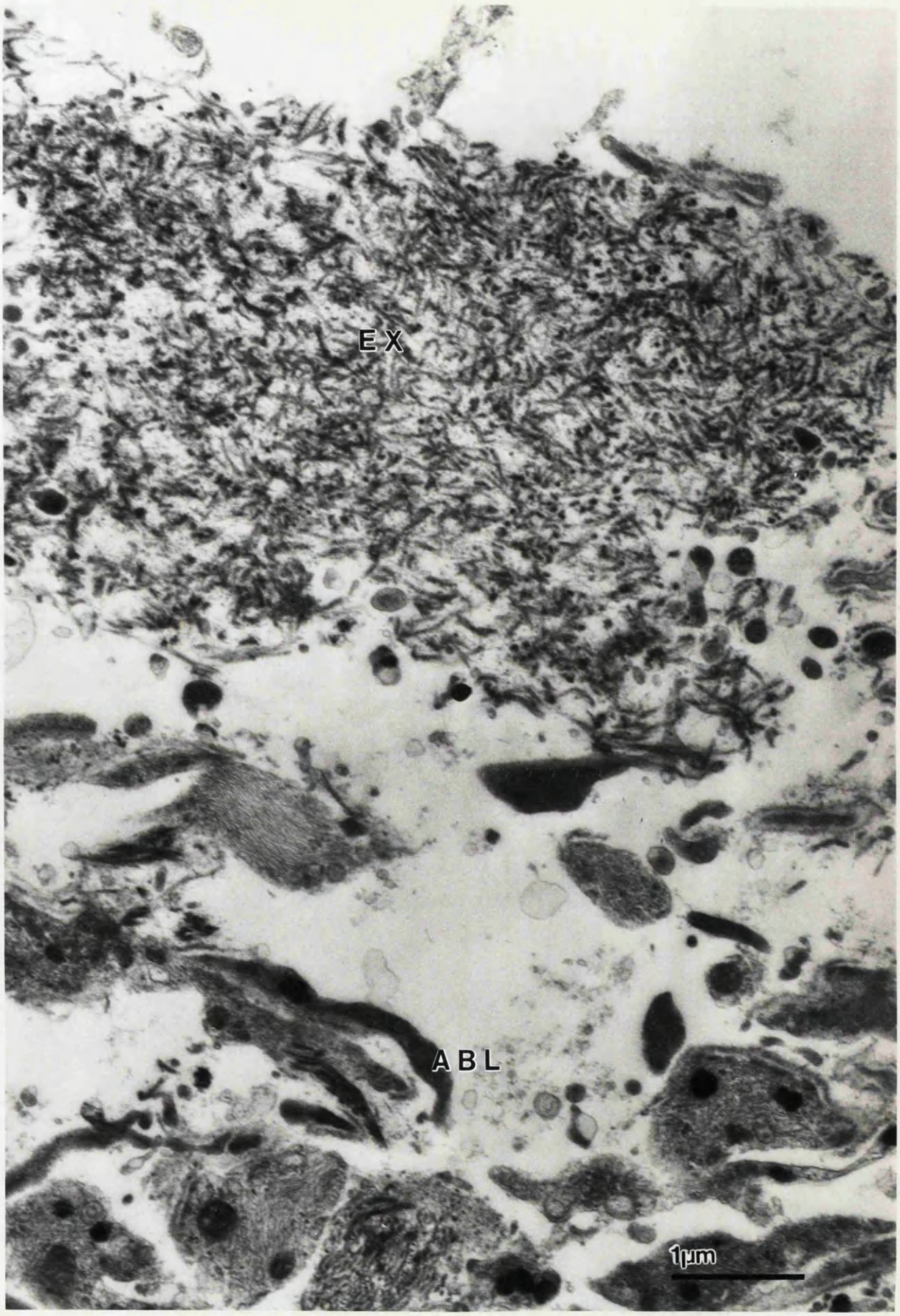


Fig 4.14: Large aggregate of exfoliation material (EX) outwith the anterior border layer (ABL). TEM.

nodular aggregate of exfoliation material was present outwith the anterior border layer, (Fig 4.14). Exfoliation material in the anterior border layer was always associated with advanced exfoliation vasculopathy.

Iris stroma

In the iris stroma of the control and exfoliative cases collagen fibrils were distributed in a random pattern. Some exfoliation material aggregates initially thought to represent stromal deposits, were identified as 'ghost vessels' when the original contour, remnants of the vascular matrix, and fragments of supporting cells were noted within these aggregates (see section on iris vessels). Exfoliation material in the majority of cases was identified in the iris stroma in association with iris vessels.

Iris pigment epithelium

In all control and exfoliative samples a thin basement membrane surrounded the muscular processes of the anterior myoepithelium. A fibrillo-granular matrix, (basement membrane material), often fused with this basement membrane and filled the extracellular spaces. In only a few exfoliative samples exfoliation material was seen to infiltrate this matrix, or the blood vessels close to the muscular processes (Fig 4.15).

In a few exfoliation glaucoma cases where the posterior pigmented epithelium was well preserved exfoliation material aggregates were observed in association with the basement membrane of neighbouring pigmented cells (Figs 4.16 to 4.18).

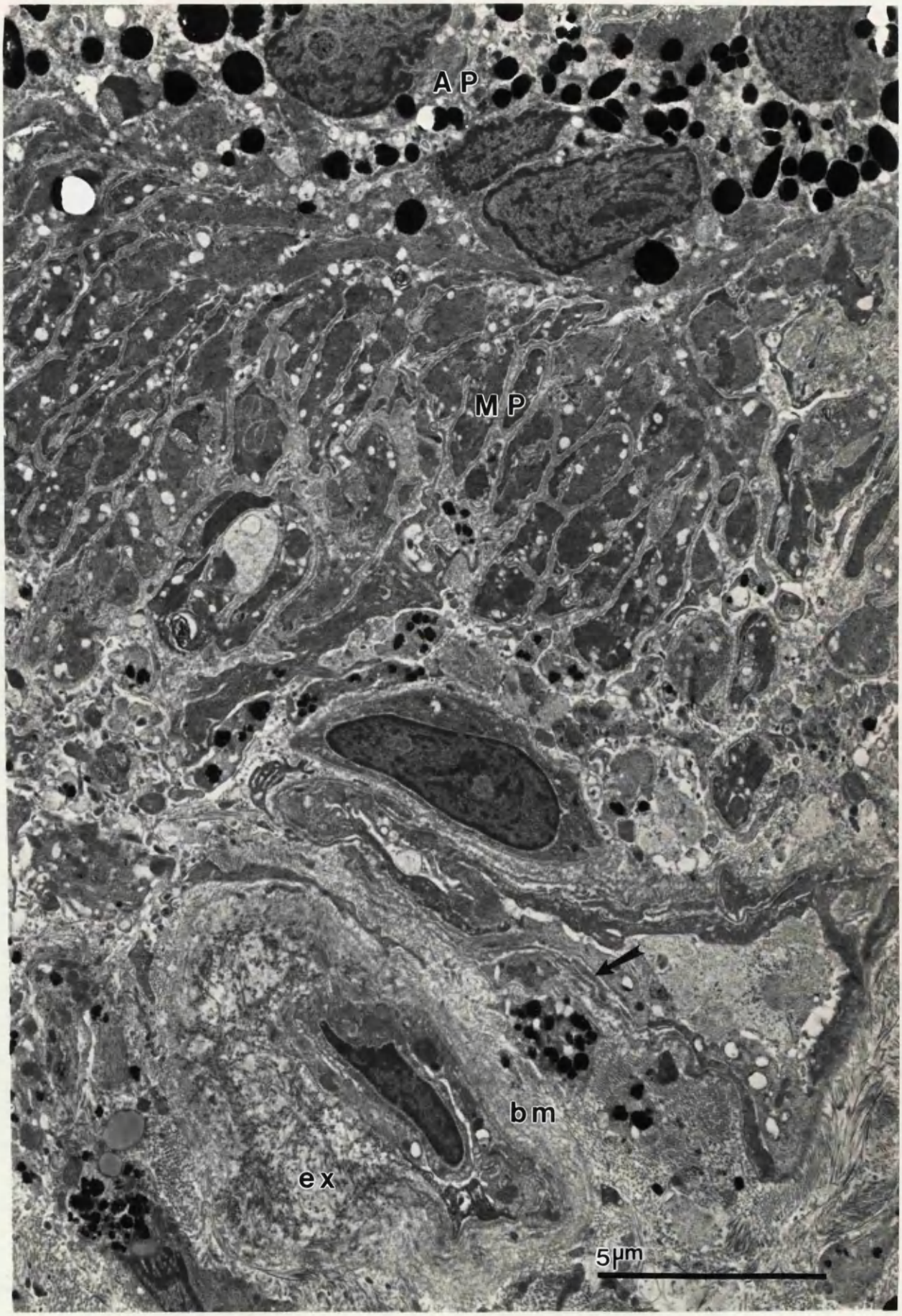


Fig 4.15: Apical pigmented epithelial portion (AP) and basal myo portion (MP) of anterior pigmented epithelium in an exfoliative iris specimen. Reduplication of lamina densa (arrow) is present in an iris vessel. An adjacent vessel exhibits a grossly thickened basement membrane (bm) in association with a large aggregate of exfoliation material (ex). The lumen is obliterated. TEM.

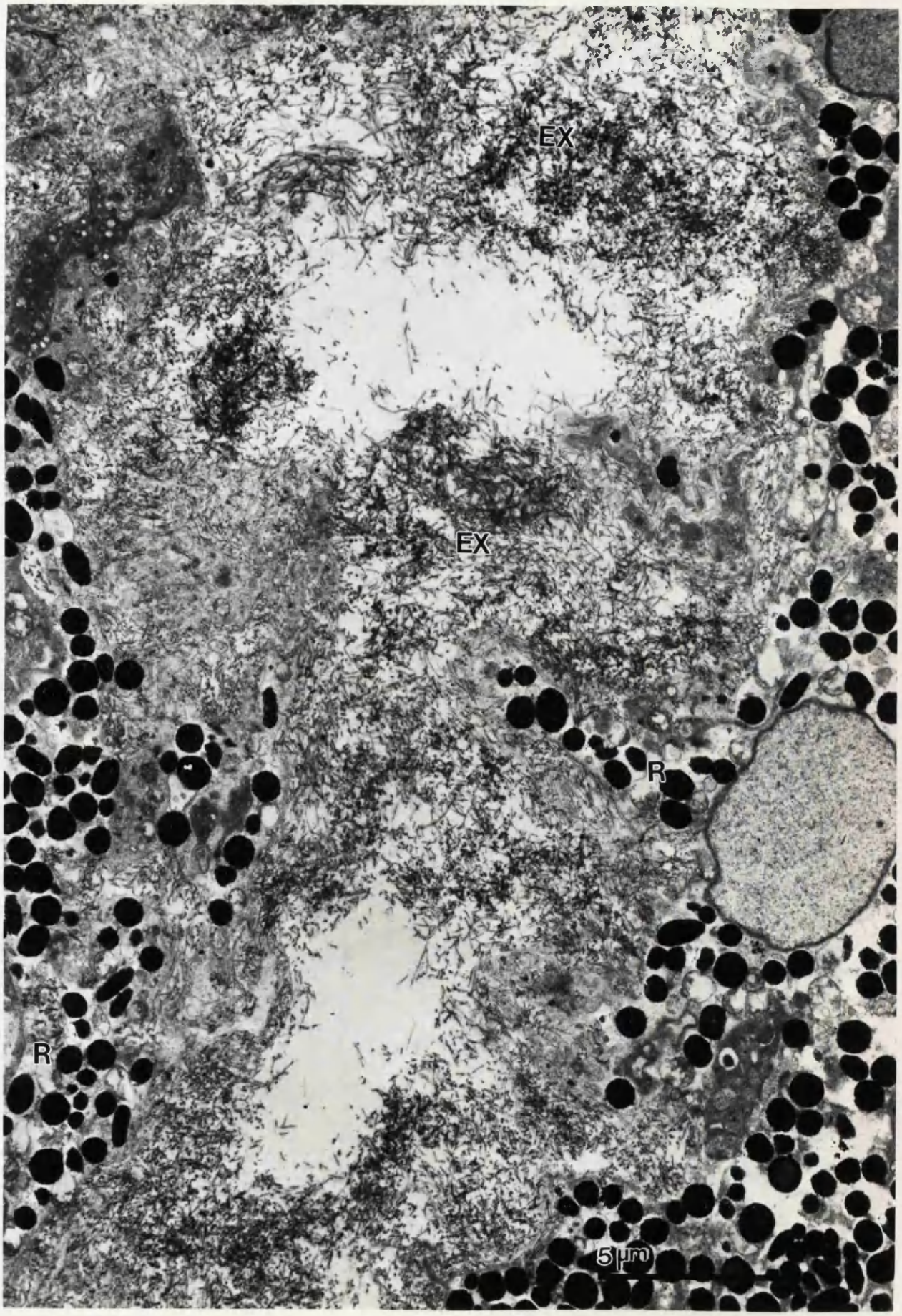


Fig 4.16: Massive deposition of exfoliation material over basal cell membrane of posterior pigmented epithelium. Note cytoplasmic rarefaction (R). TEM.

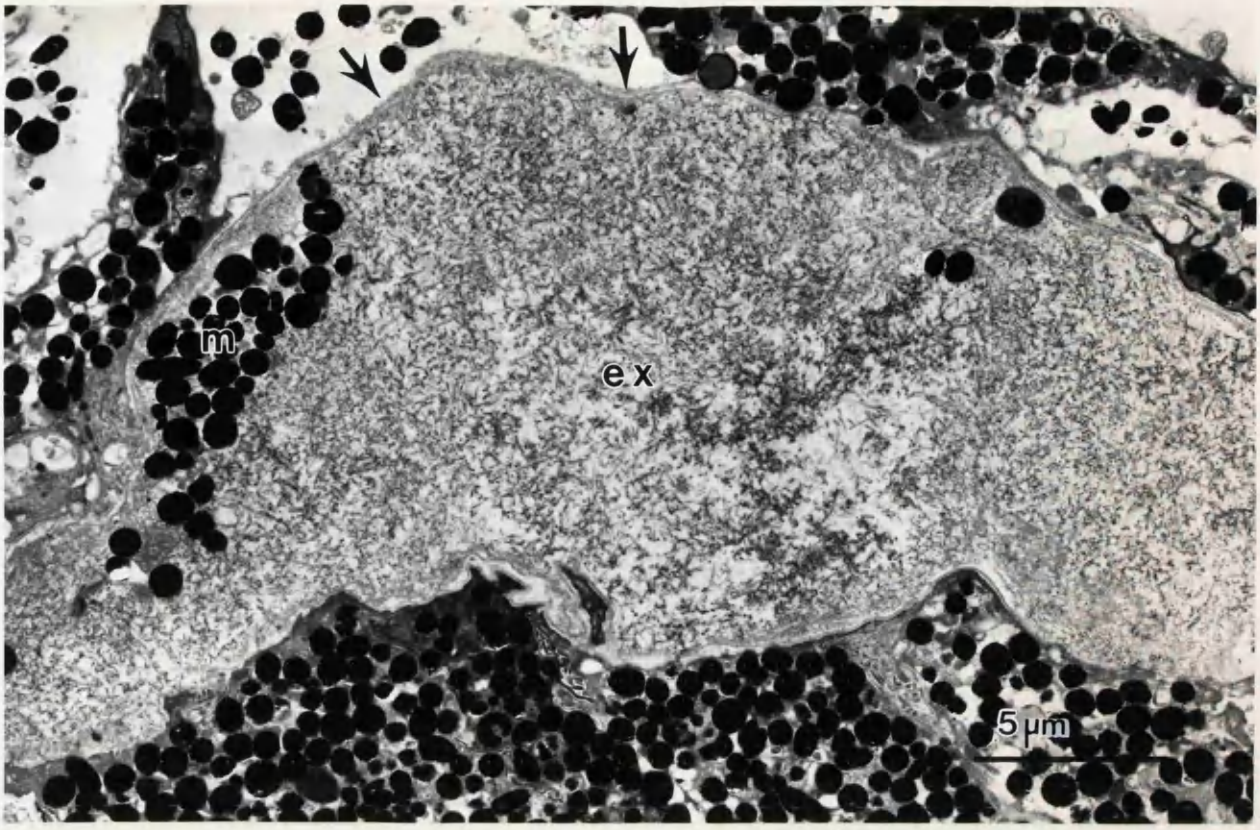


Fig 4.17: Exfoliation material aggregate (EX) bounded by basement membrane in the region of the posterior pigmented epithelium. Note free melinin granules (m) and basement membrane bounding exfoliation deposits (arrows). TEM.

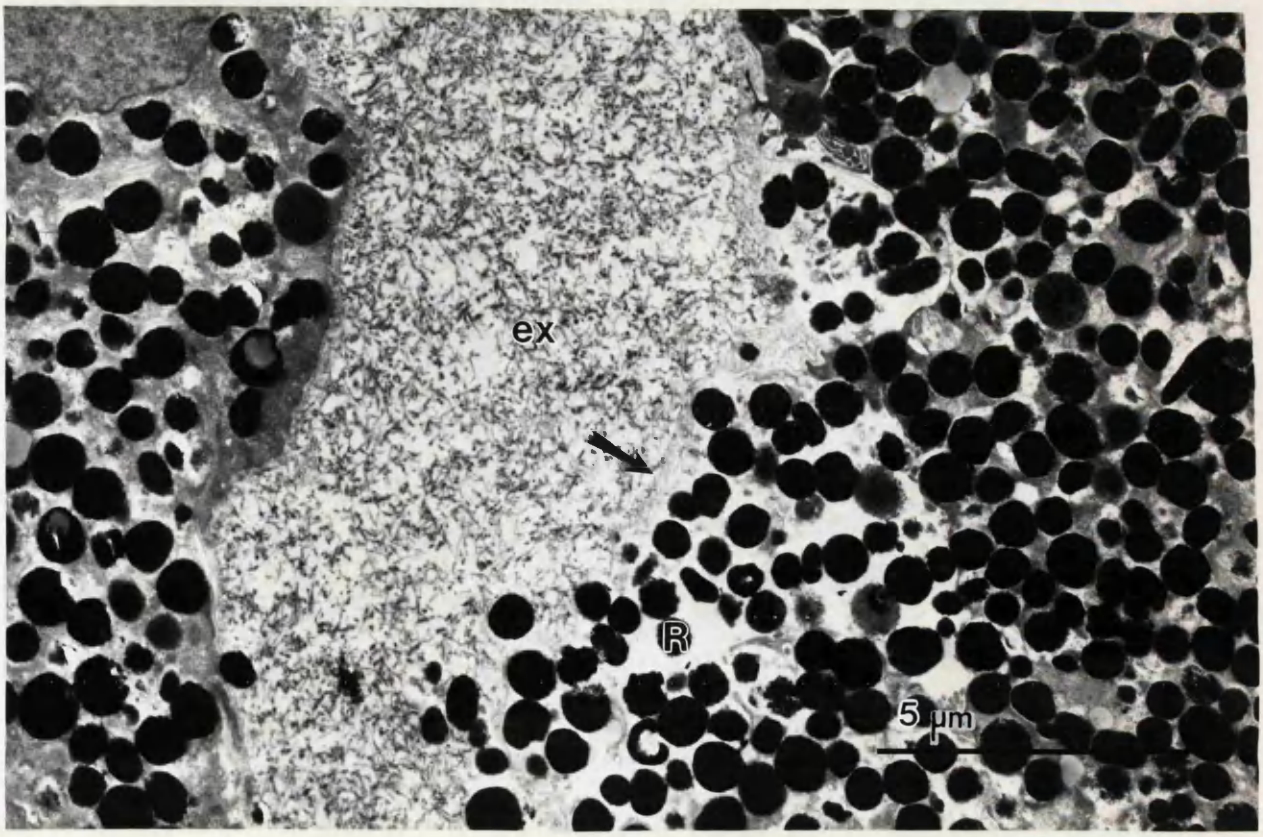


Fig 4.18: A more homogeneous aggregate of exfoliation material (ex) in the region of the posterior pigmented epithelium. Note rarefaction of cytoplasm (R) and concomitant compromise of cell integrity (arrow). TEM.

Reduplication and disruption of the basement membrane of the posterior layer of the pigmented epithelium was sometimes noted (Fig 4.15) in these cases. In one case exfoliation material aggregates were bounded by 'new' basement membrane (Fig 4.17).

Iris vessels

The normal ultrastructure of iris vessels is considered in section 4.2 and illustrated in Figs 4.19 & 4.20. The vascular endothelium of both control and exfoliative iris samples was thicker in some vessels with tall endothelial cells protruding into the lumen. These were thought to represent arterioles (Fig 4.19). On the other hand, some vessels showed a thin endothelium with a higher ratio of wall thickness to lumen diameter and fewer supporting cells. These vessels were considered to be venules (Fig 4.20). In some sections zonulae occludentes were prominent (Fig 4.19). The perivascular collagenous zone surrounding the vessel wall consisted of two layers: an inner layer of fine collagen fibrils and an outer layer of thicker fibrils (Fig 4.21).

The most interesting ultrastructural features were observed in the iris vessels of exfoliative specimens. All 26 exfoliative specimens demonstrated vascular involvement but this was patchy and diseased tissue often contained ostensibly normal vessels. In exfoliative specimens arterioles appeared to be affected more frequently and more severely than venules, but this was not uniform in all specimens.

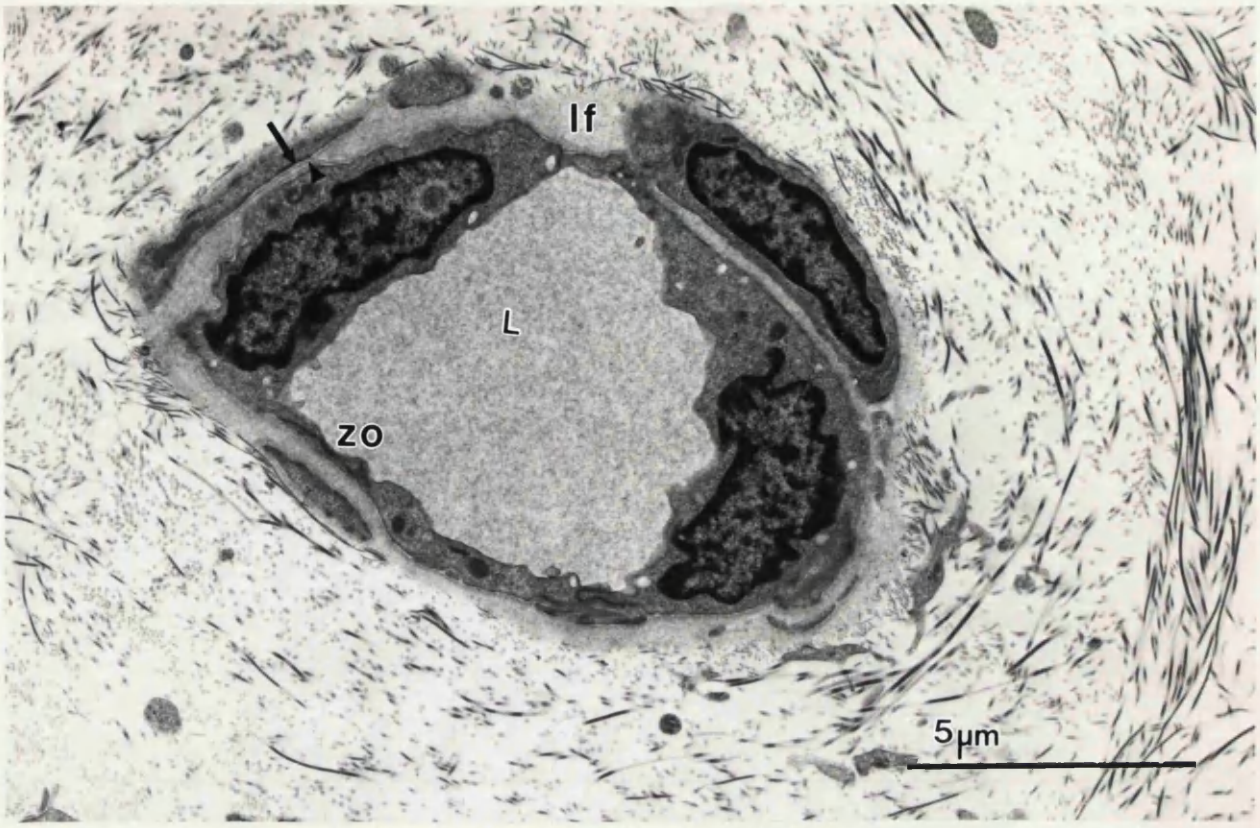


Fig 4.19: Normal arteriole with lamina lucida (arrow), lamina densa (arrowhead) and lamina fibroreticularis (lf). Collagen fibrils are inserted into the basement membrane. Note zonulae occludentes (zo). L:lumen. TEM.

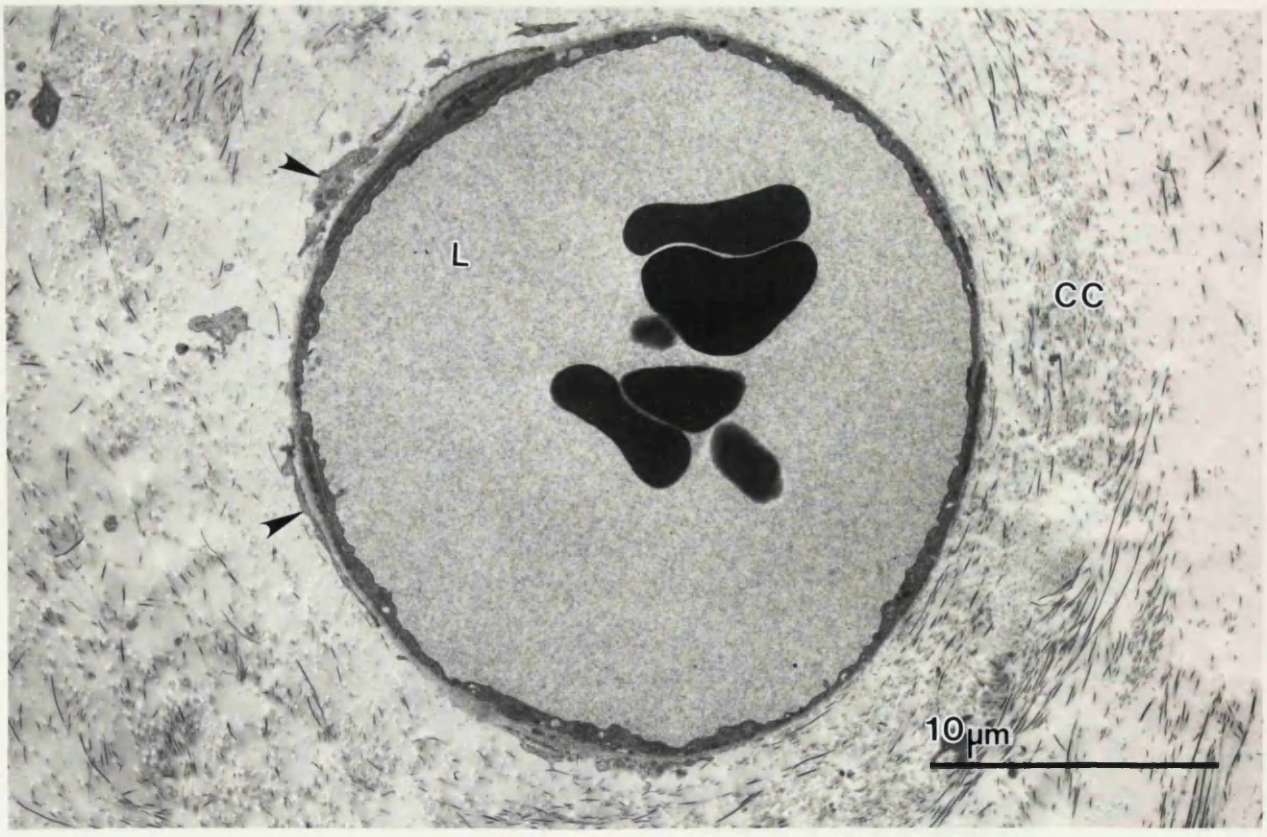


Fig 4.20: Normal venule. The wall thickness ratio to lumen diameter is considerably greater than that arterioles. Supporting cells arrowheaded. CC:perivascular collagenous coat.
TEM.

Exfoliation vasculopathy (Figs 4.21-4.33)

All 26 exfoliation glaucoma specimens demonstrated vascular involvement with exfoliation aggregates accumulating around and within the vascular wall of iris vessels. Exfoliation fibres showed a variable degree of staining: some exfoliation fibres displayed a distinct outline and some a more indistinct contour. The spectrum of vascular pathology observed in these exfoliation glaucoma cases was divided in early and advanced vasculopathy on the basis of analysis of the specific ultrastructural changes occuring in all cases. Early vasculopathy corresponded to exfoliation material accumulation, with, or without evidence of degeneration of supporting and endothelial cells. Advanced vasculopathy corresponded to the presence of non-functioning 'ghost vessels'.

In exfoliative specimens a zone of finer collagen fibrils was often seen close to the supporting cells (Fig 4.25). It was noteworthy that even in advanced exfoliation vasculopathy the outer perivascular collagenous zone appeared 'free' from exfoliation aggregates (Fig 4.30). In most specimens exfoliation aggregates were localised in the space between the endothelial lining of vessels and the inner collagenous zone (Fig 4.24). Rarely, small aggregates of exfoliation material infiltrated the inner collagenous zone.

The thickness of the vascular matrix in exfoliative vessels was more diverse than in POAG vessels. It varied from 0.2-0.5 microns in small capillaries to 6 microns in larger vessels depending on the degree of exfoliation vasculopathy

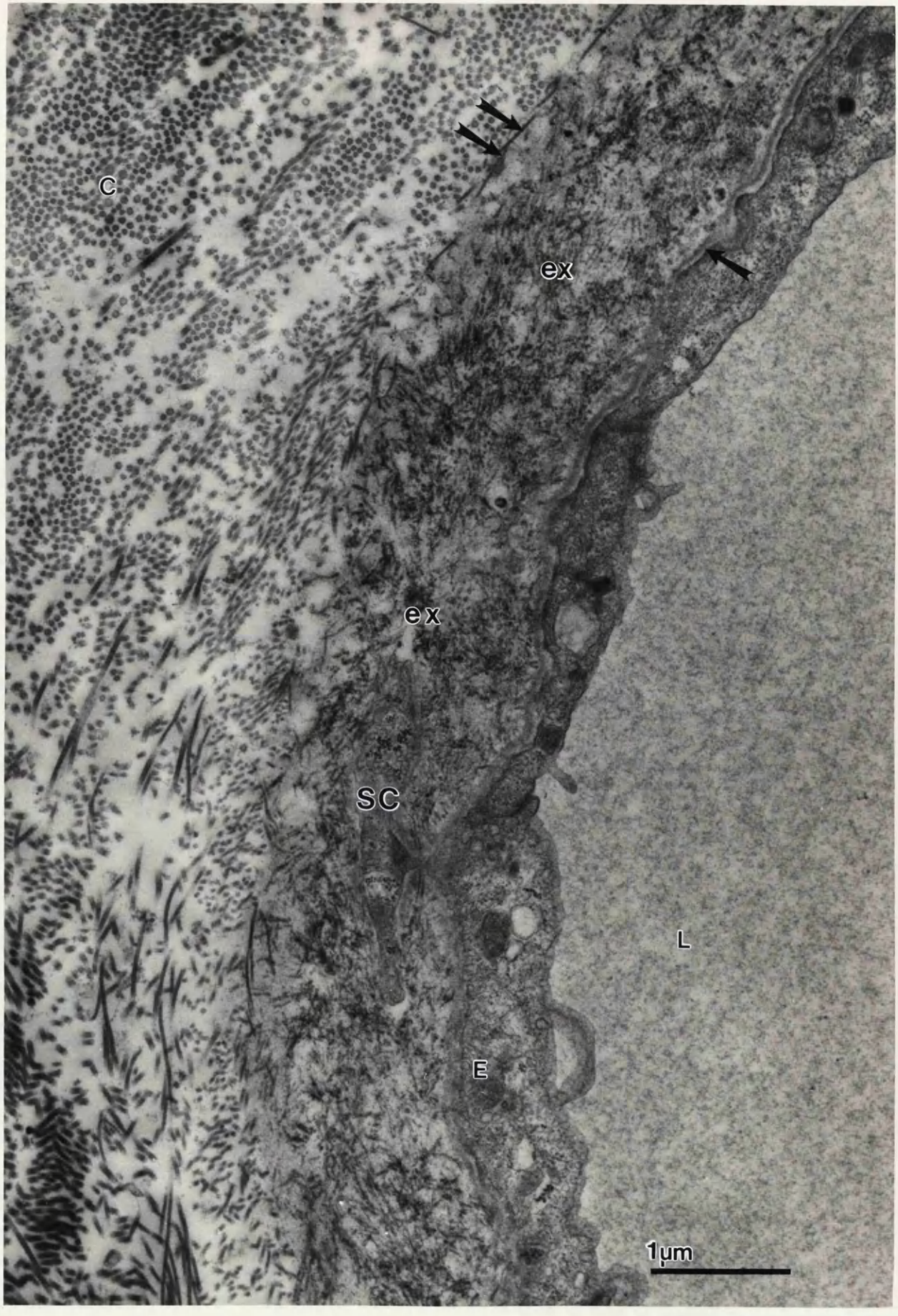


Fig 4.21: Degenerate supporting cell (SC) associated with band of exfoliation material (ex). Vascular matrix between the lamina densa of the endothelial cells (single arrow) and the boundary of the perivascular collagenous coat (double arrows) is thickened. L:lumen; E:endothelium. C:collagenous coat. TEM.

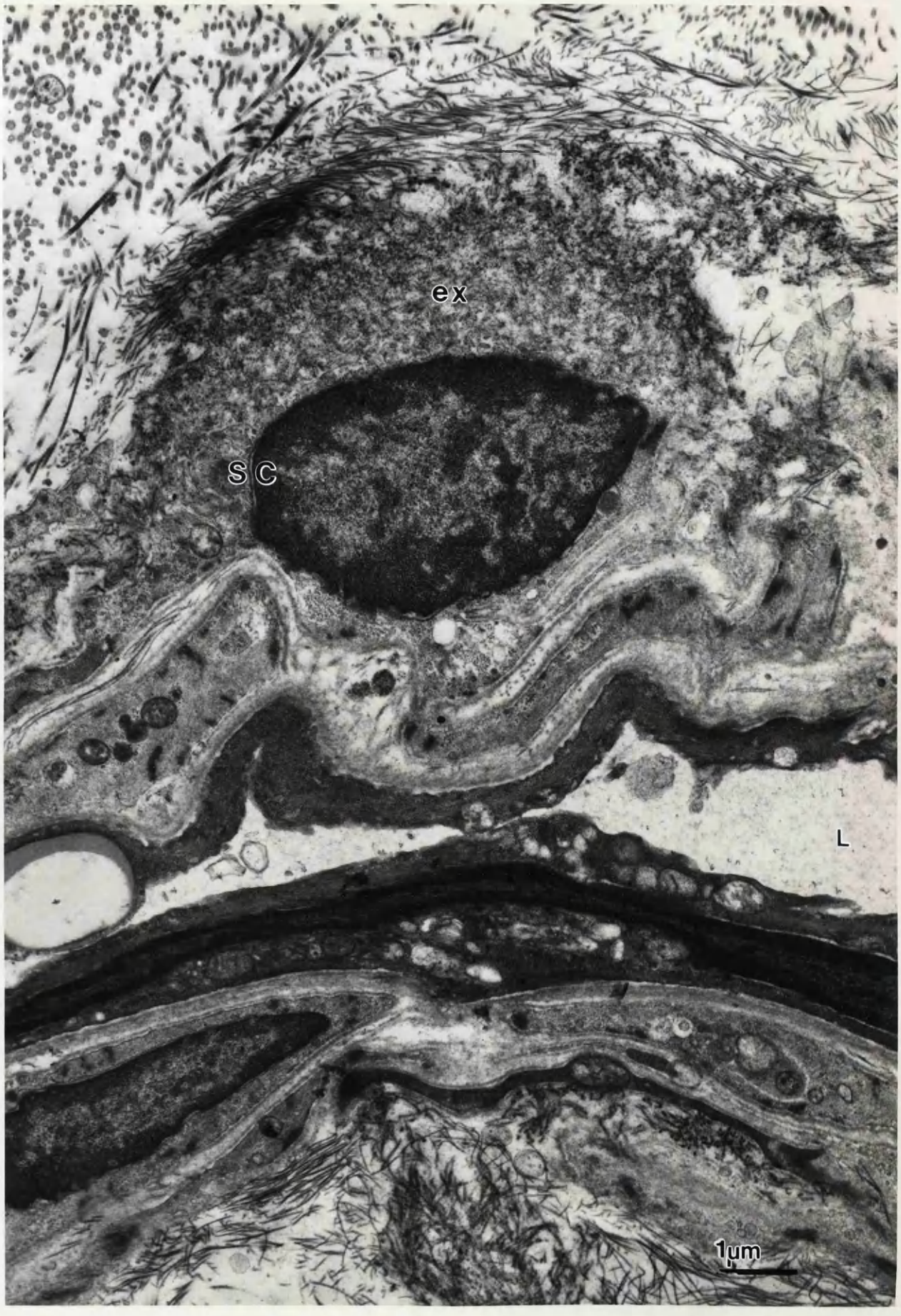


Fig 4.22: Transmission electron micrograph to demonstrate the close relationship between exfoliation material (ex) aggregation and supporting cell (SC) in an exfoliative iris vessel. L:lumen.

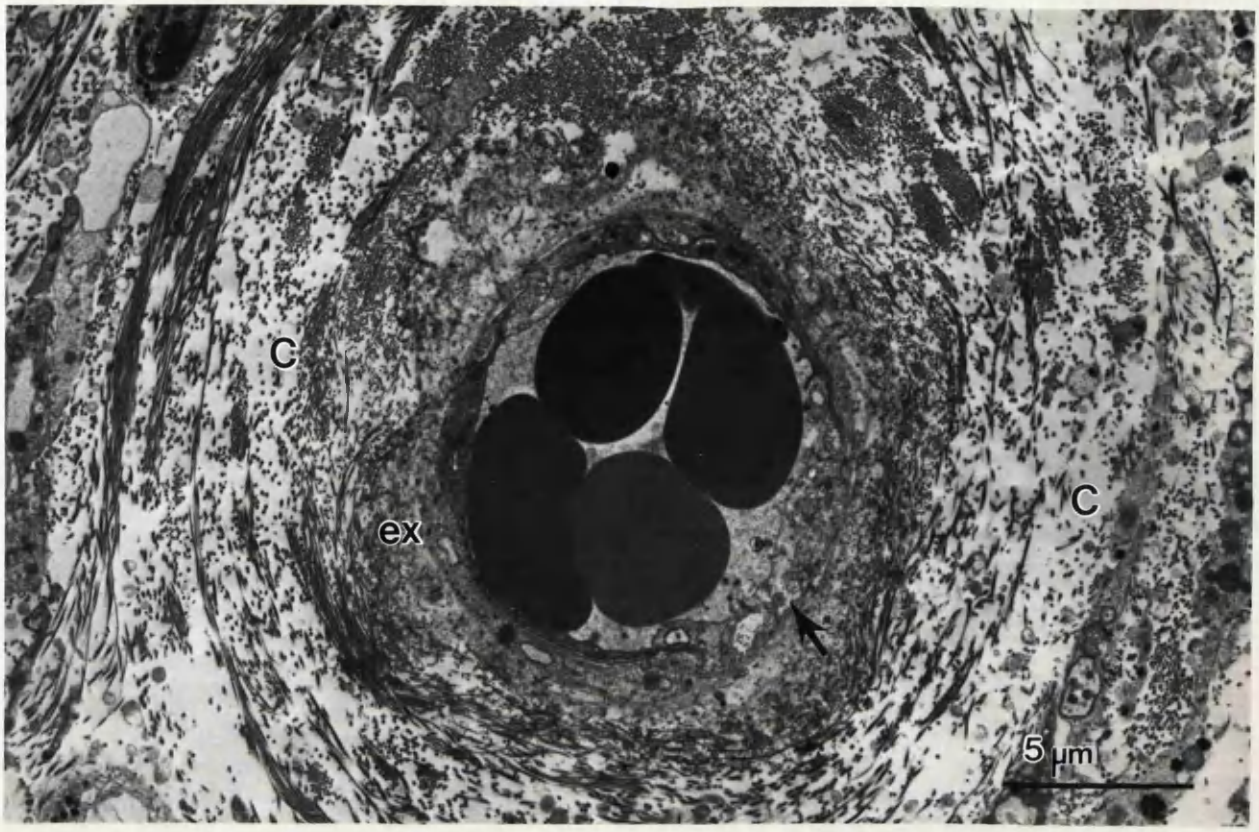


Fig 4.23: Exfoliative iris vessel (arteriole). Degeneration of endothelial cell (arrow) is accompanied by disruption of its basement membrane. The perivascular collagenous coat (CC) is free of exfoliation material (ex).

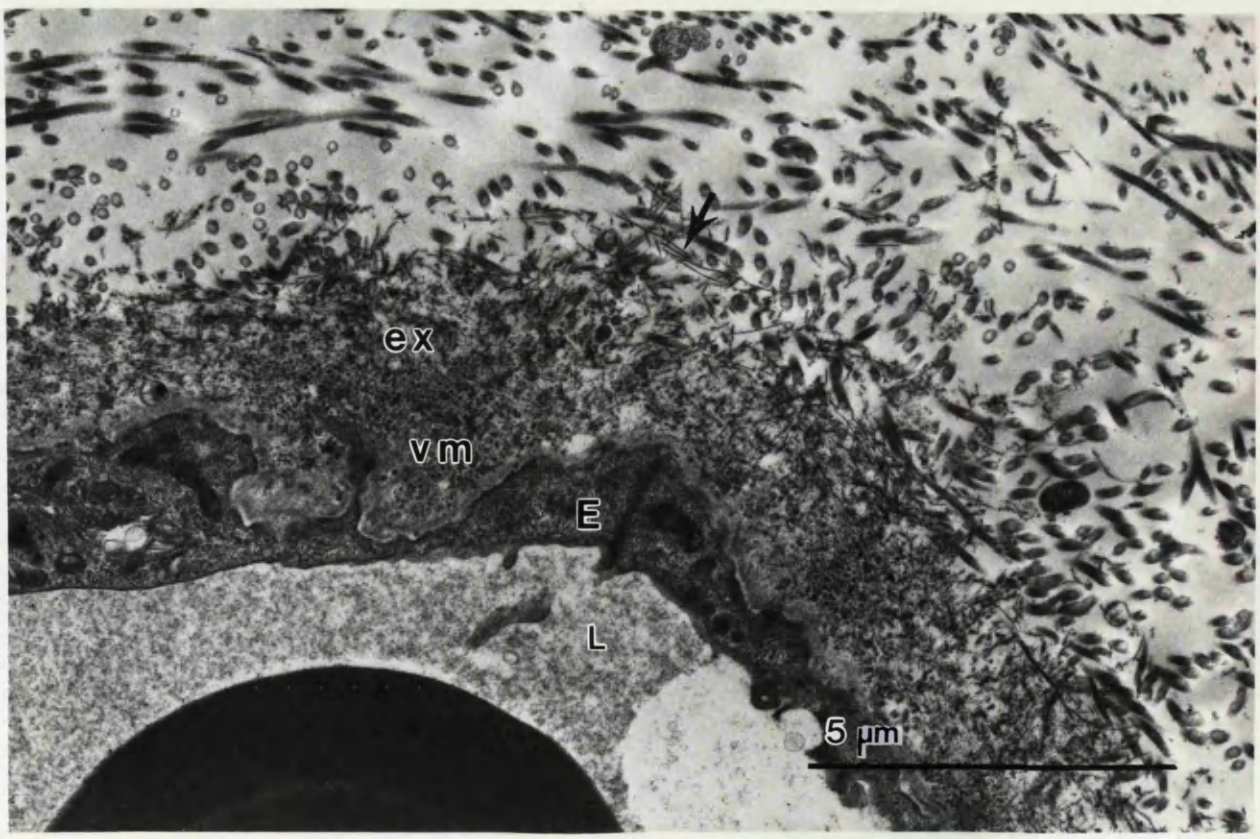


Fig 4.24: Exfoliation material (ex) intermingled with filaments (arrow), which are more apparent outwith an abnormal granular vascular matrix (VM). TEM.

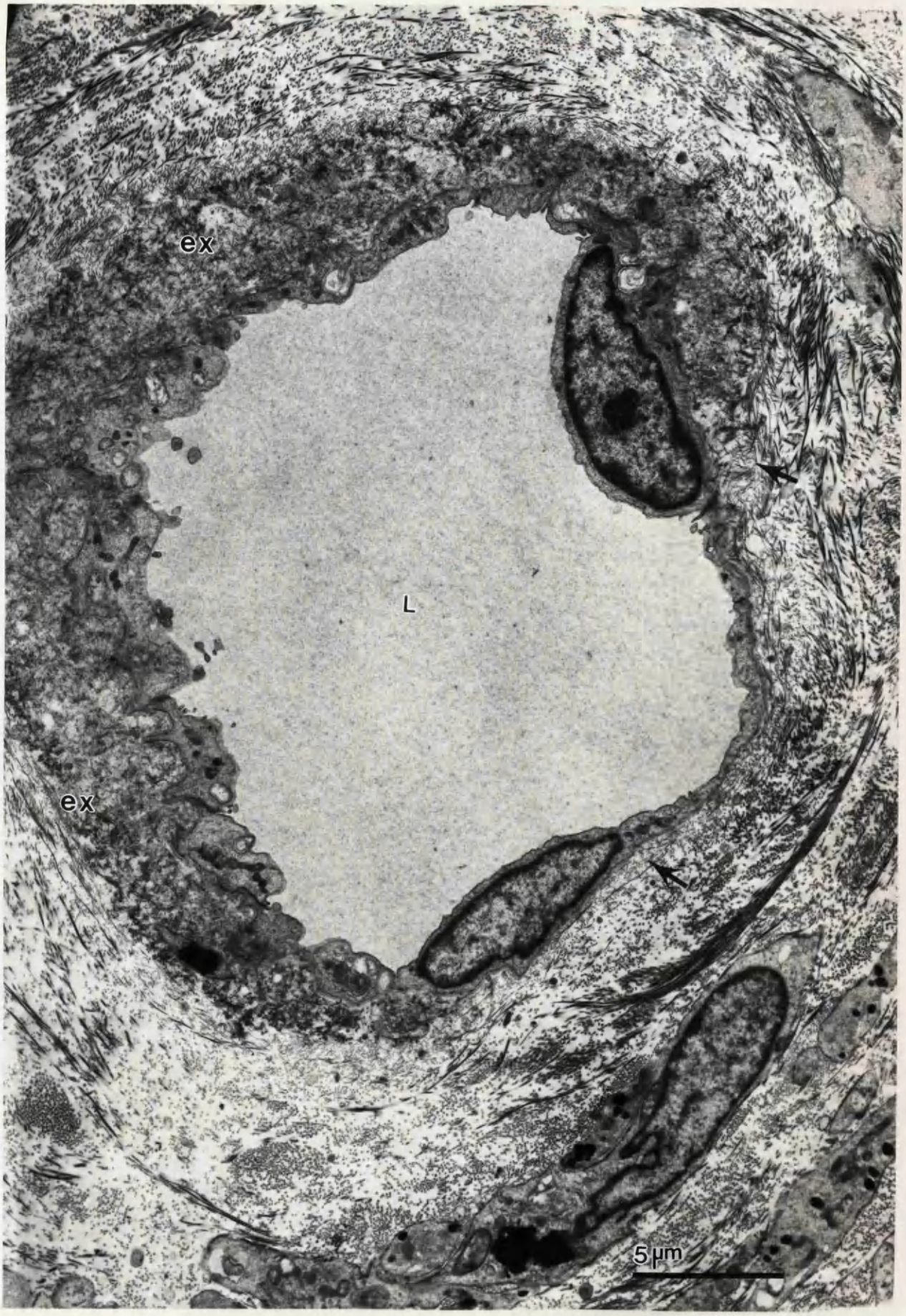


Fig 4.25: Exfoliative iris vessel showing extensive perivascular aggregation of exfoliation material (ex) with concomitant thickening of the vascular matrix. The remainder of the vessel is mormal. Note absence of supporting cells and fine collagen fibrils (arrows).L:lumen. TEM.

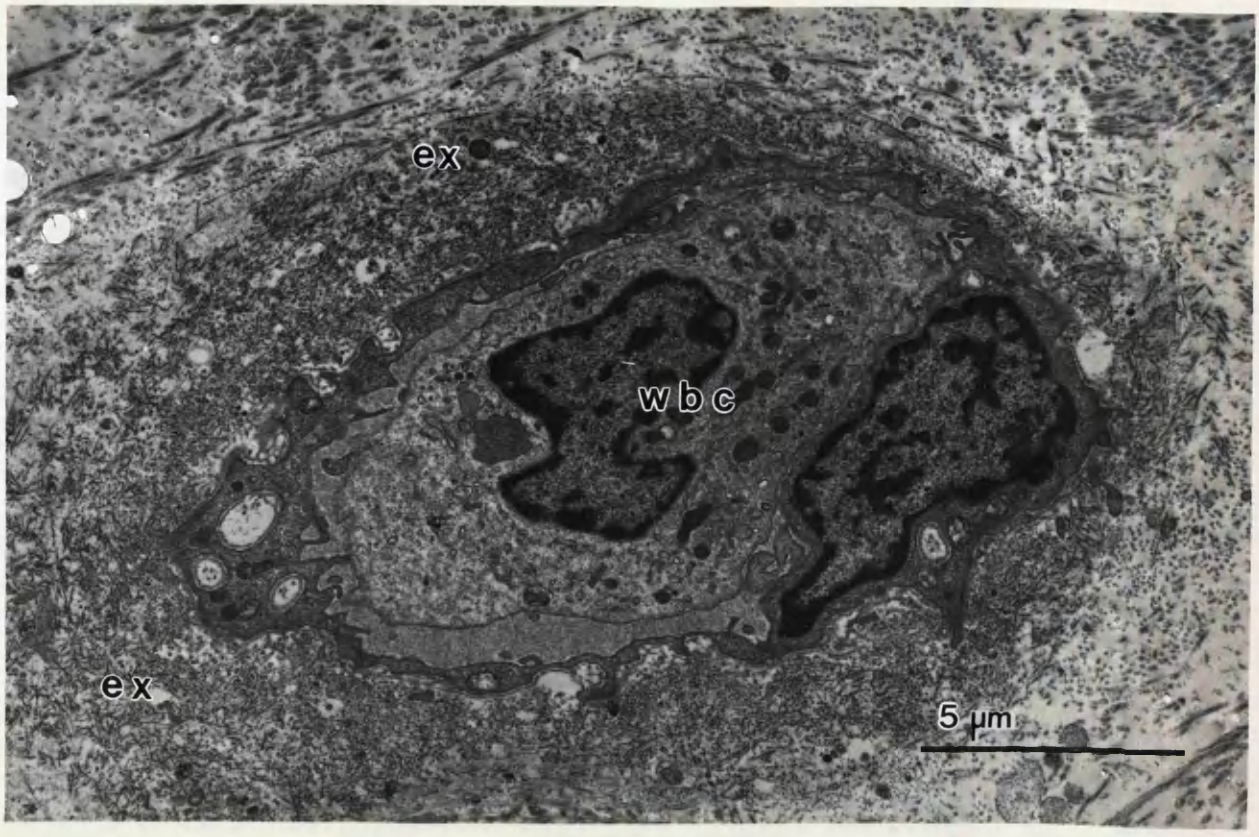


Fig 4.26: Exfoliation material (ex) aggregation within granular matrix completely surrounds an arteriole. The lumen is completely filled with a white blood cell (wbc). Supporting cells are absent. TEM.

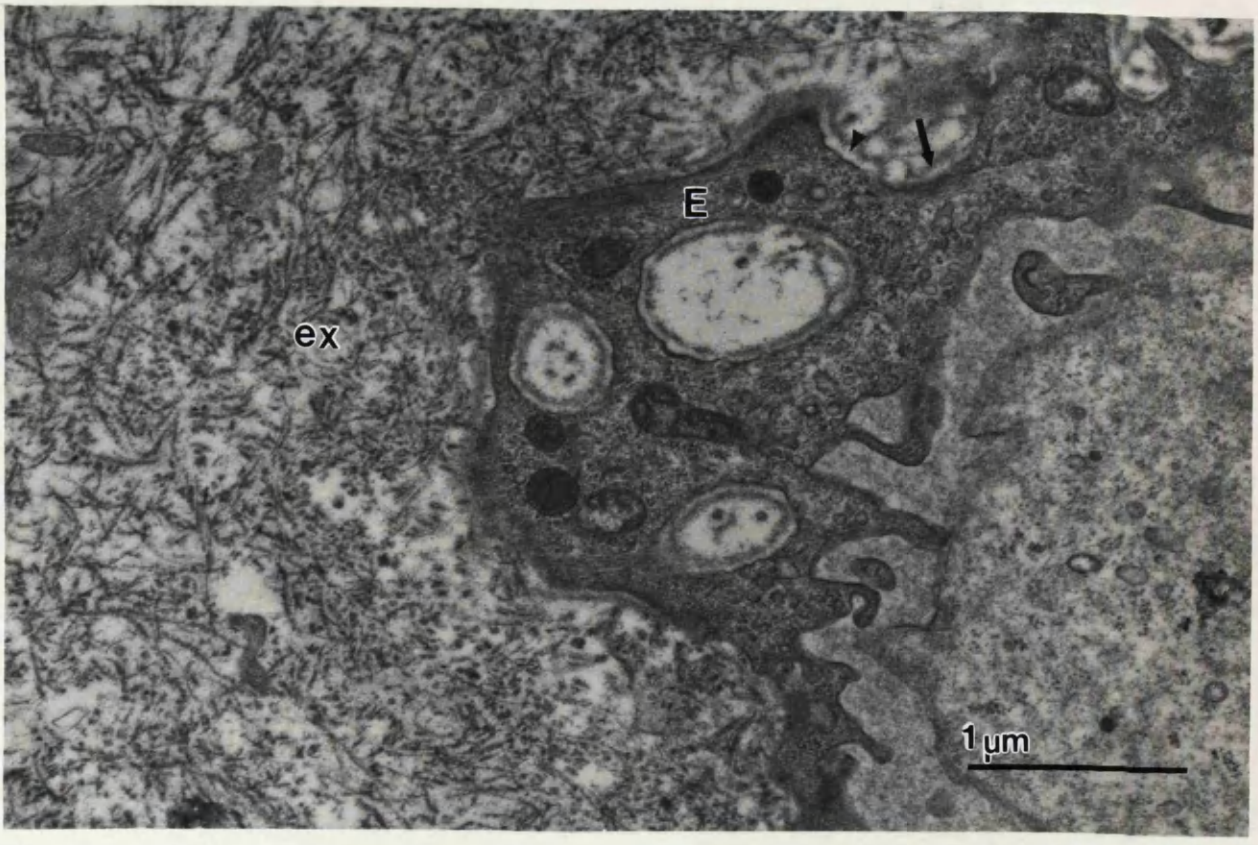


Fig 4.27: Higher magnification of the vascular wall in Fig 4.26. Endothelial cell (E) is rich in organelles and appears to be normal. Note distinctive lamina lucida (arrowhead) and lamina densa (arrows); ex:exfoliation material. TEM.

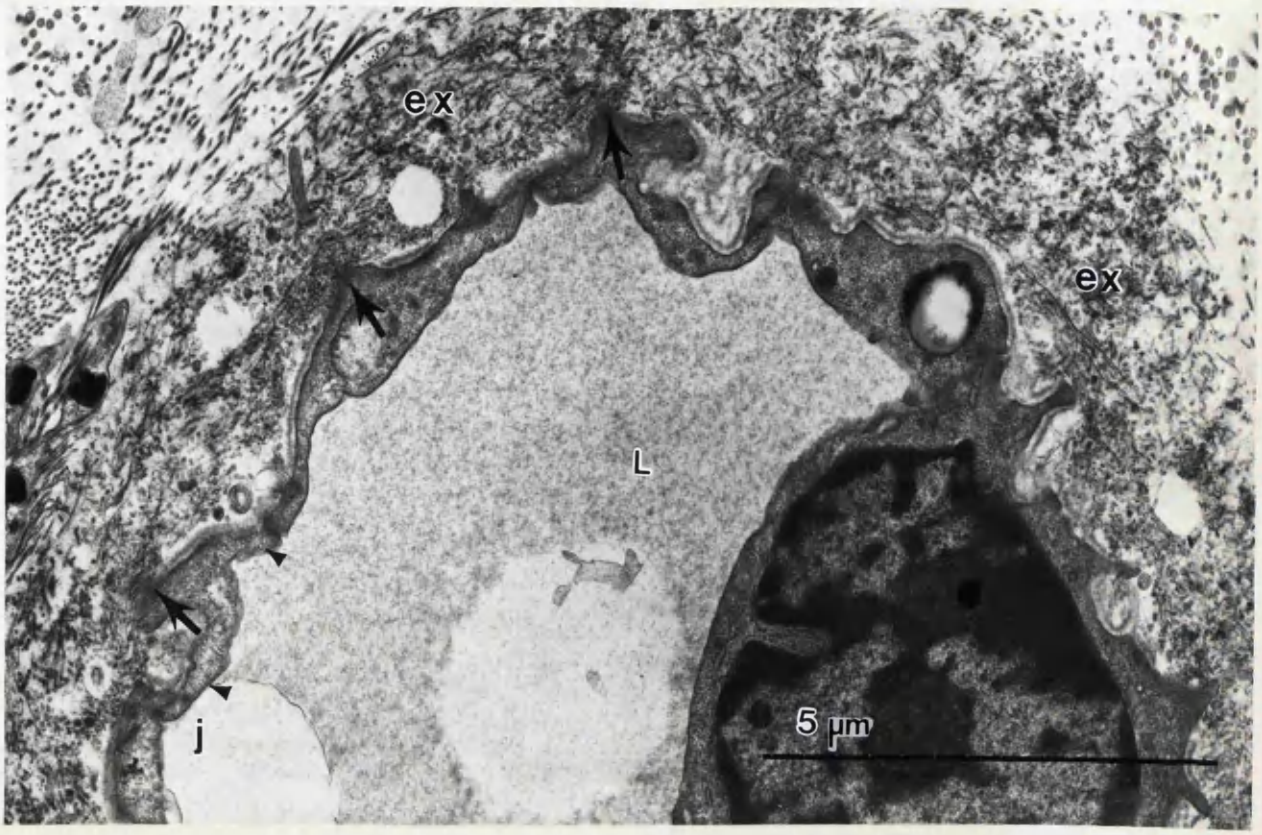


Fig 4.28: Absence of lamina densa (arrows) associated with infiltration of exfoliation material (ex) surrounding an arteriole. Cell junction:j; pinocytotic vesicles:arrowheads. TEM.

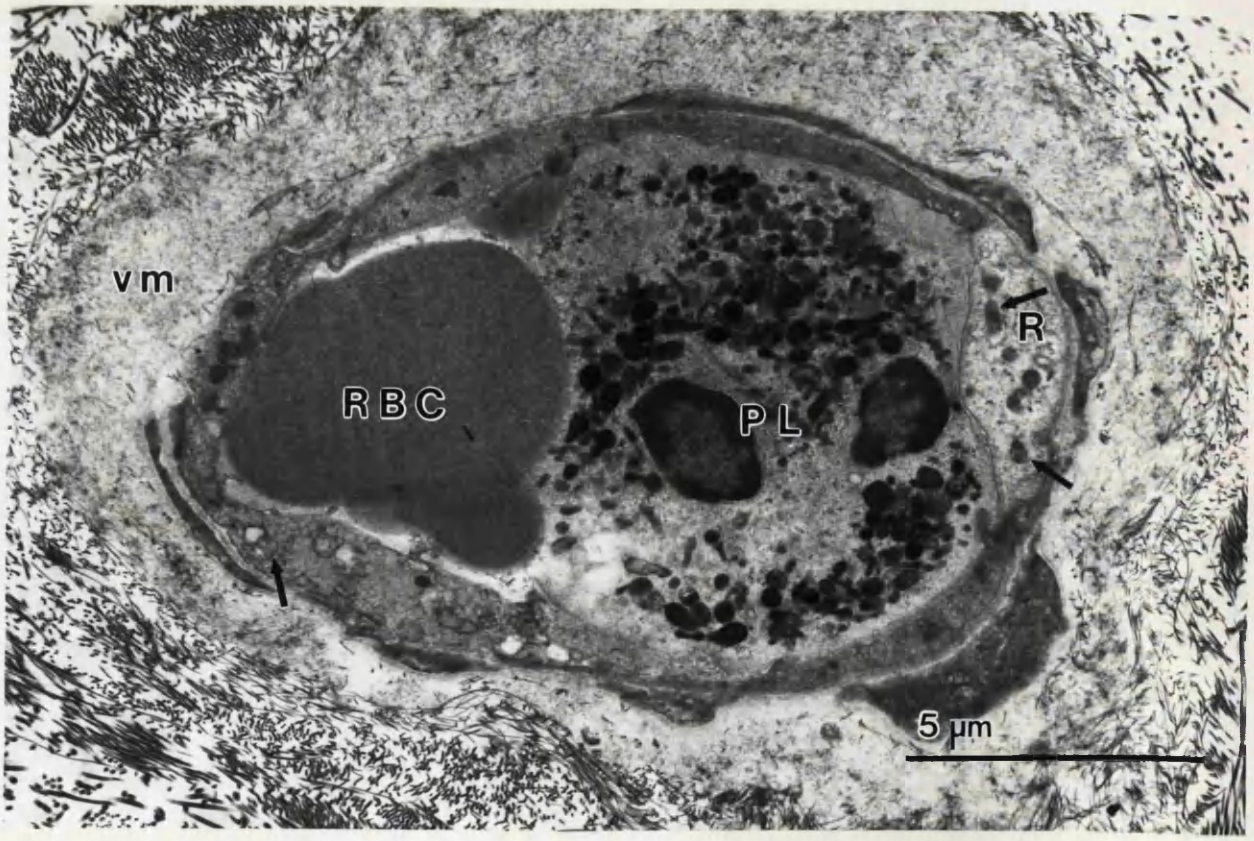


Fig 4.29: Arteriole with grossly thickened vascular matrix (vm) exhibits moderate to advanced degeneration of its endothelial lining (rarefication of cytoplasm:R; degeneration of organelles:arrows). Lumen is filled by a polymorphonuclear leucocyte (PL) and a red blood cell (RBC). TEM.

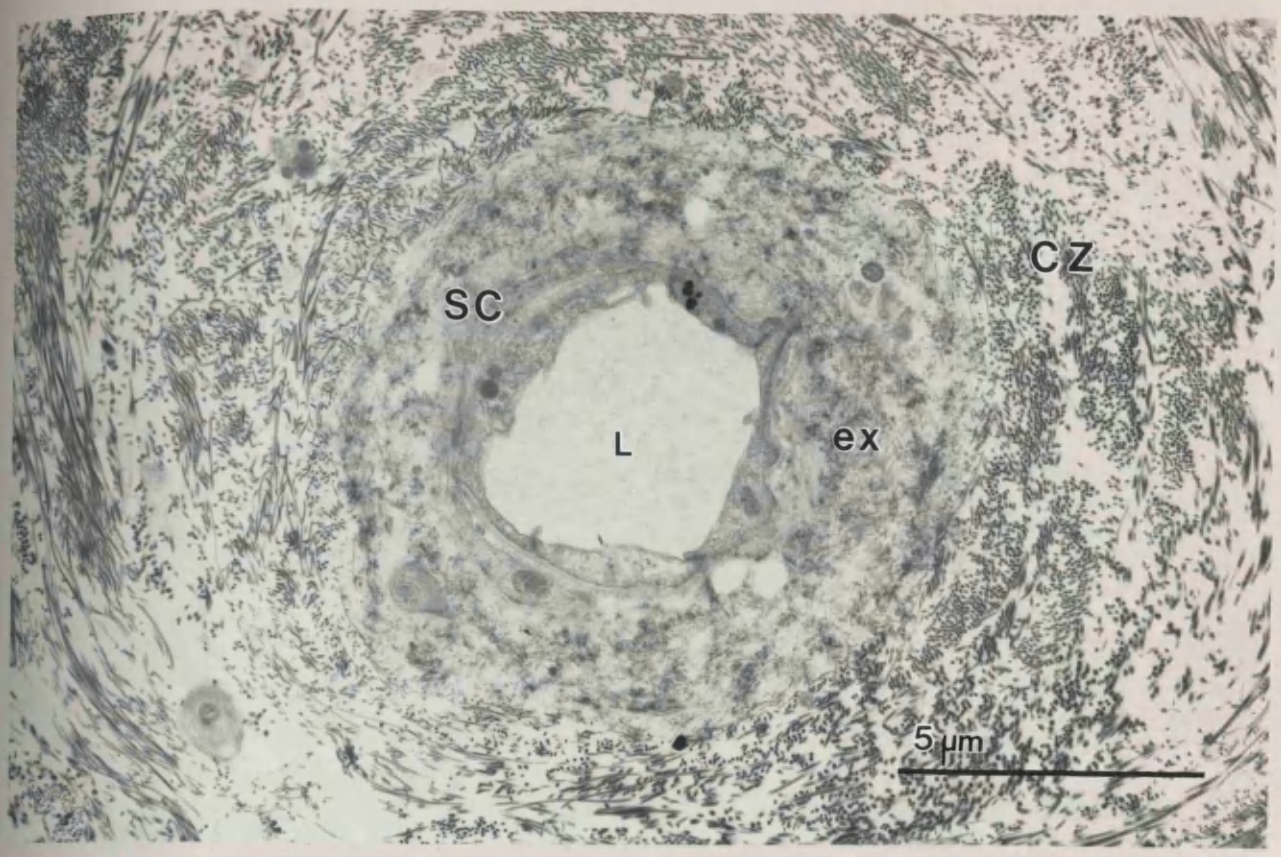


Fig 4.30: Advanced exfoliation vasculopathy. Exfoliation material (ex) aggregation throughout the circumference of the vessel is associated with degeneration of supporting cells (SC) and is absent from the collagenous zone (CZ). Vascular integrity is maintained. L:lumen. TEM.

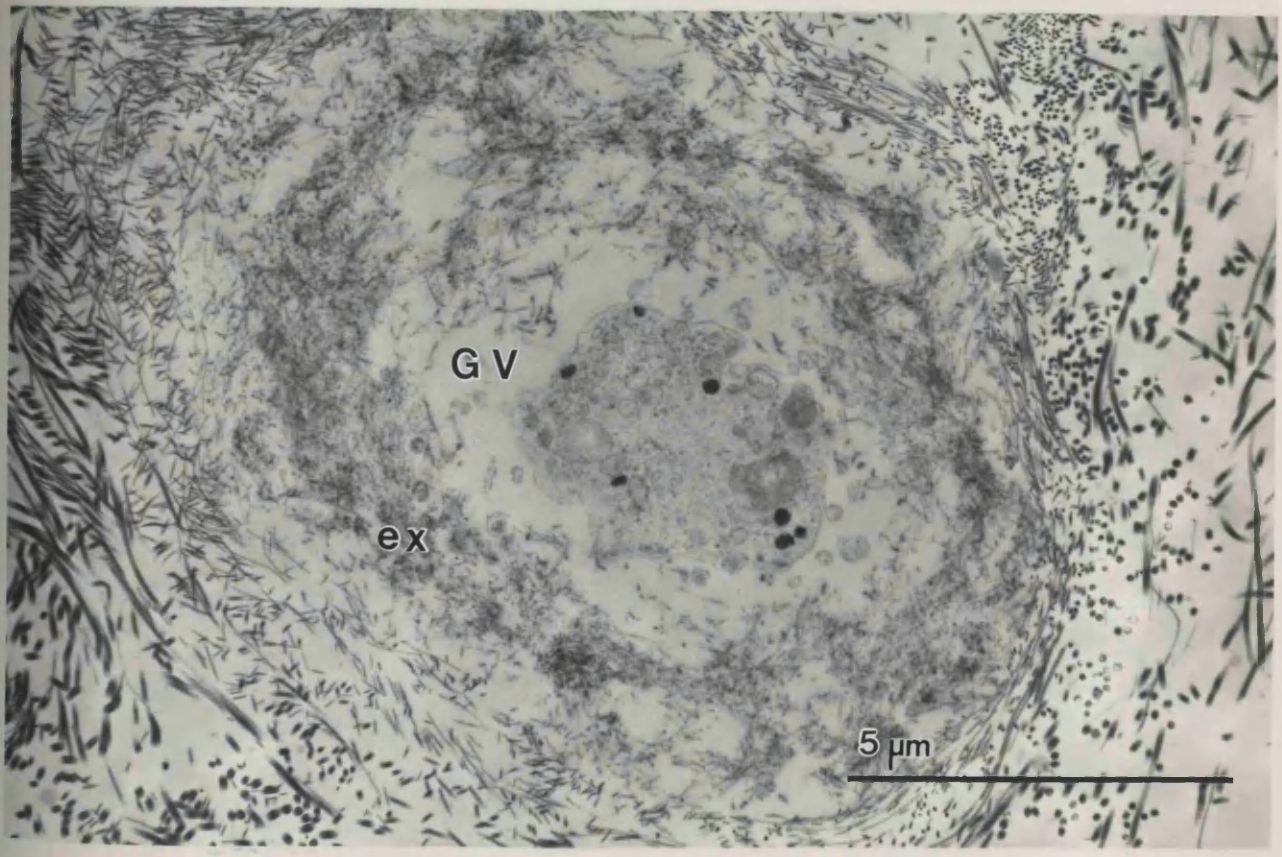


Fig 4.31: End stage exfoliation vasculopathy; ghost vessel (GV). Vascular matrix has been replaced by exfoliation material (ex). Identity of cell within lumen unknown. TEM.

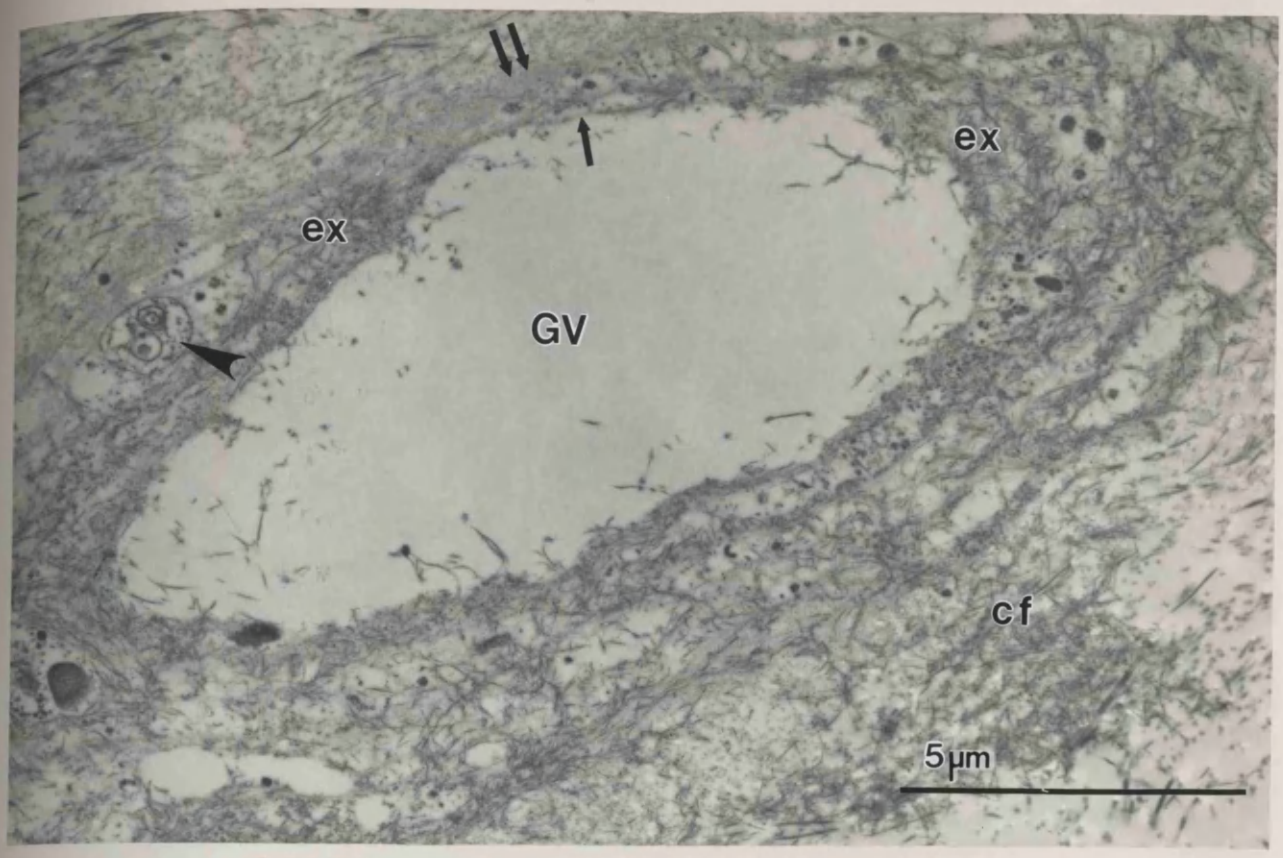


Fig 4.32: Degenerate exfoliative vessel (ghost vessel, GV). Exfoliation material (ex) is intermingled with remnants of lamina densa (single arrow), basement membrane material (double arrow), cell debris (arrowheads) and collagen fibrils (cf). TEM.

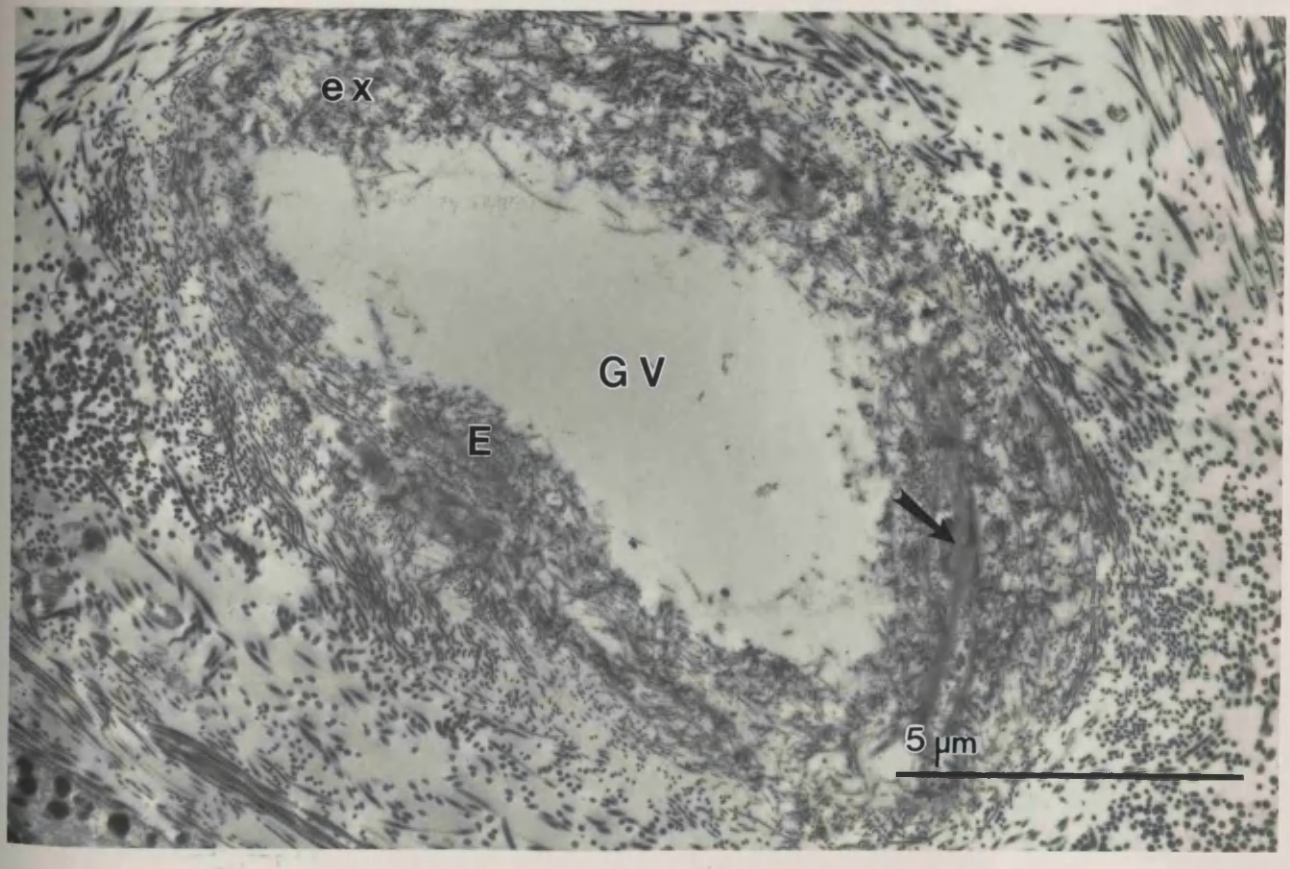


Fig 4.33: Ghost vessel (GV). Exfoliation material (ex), intermingled with basement membrane (arrow) has replaced endothelial lining. Note degenerate endothelial cell (E). TEM.

cells was typically associated with a halo of exfoliation material, whilst supporting cells within the same vessel free of exfoliation material were relatively healthier. Globules of lipid and myeloid bodies were sometimes present within the diseased vascular matrix. As a rule advanced degeneration of supporting cells was associated with large amounts of exfoliation material surrounding most of the circumference of the vessel. The more prominent features of cell distress were swelling of organelles, rarefaction of cytoplasm and the appearance of vacuoles on the luminal side of the affected vessels. Despite these changes convincing evidence of breakdown of the endothelial barrier (separation of cell junctions) was seldom encountered.

Nearly all specimens contained one or more 'ghost vessels' i.e. vessels with advanced vasculopathy. The entire circumference of such ghost vessels contained exfoliation material. In only two cases exfoliation material did not surround a ghost vessel entirely and where the material was absent, a portion of a basement membrane remained. In advanced vasculopathy the endothelial lining was severely disrupted and discontinuous, with remnants of the lamina densa intermingled with cell debris and exfoliation material (Figs 4.31-4.33). In these specimens exfoliation material aggregation appeared to 'replace' the original outline of the vessel. Only in ghost vessels was exfoliation material observed inside the lumen of the vessel. Membranous debris were often observed within the wall of ghost vessels. Electron dense granules possibly tertiary lysosomes were also seen. In some ghost vessels, several layers of basement

membrane material surrounded a portion of the vascular circumference. In a more 'advanced stage' no fragments of basement membrane, membranous debris, or cellular fragments were observed.

It was of interest to note that in the exfoliation glaucoma cases exfoliation material aggregates initially thought to represent stromal deposits, were subsequently identified as 'ghost vessels' i.e. end stage exfoliation vasculopathy. This observation was made when the original contour, remnants of the vascular matrix, and fragments of supporting cells were noted within these aggregates. Indeed, exfoliation material was rarely identified in the iris stroma when it was not in association with iris vessels.

4.5 Discussion

Serial sectioning of iris tissue from each of the exfoliation glaucoma specimens revealed the presence of exfoliation material around some vessels in every case. This supports the concept that exfoliation material aggregates, are morphologically similar to those seen in other ocular tissues and occur in the affected iris vasculature. Therefore this represent a pathognomonic feature of the disorder. The present study is in agreement with Ghosh & Speakman (1974) who studied 4 iris specimens with exfoliation glaucoma and found some 'abnormal iris vessels' in every case. It was of interest to compare the proportion of affected vessels in the present study with that recorded by Ringvold (1970). In that study which included 12 iris

specimens from patients with exfoliation syndrome approximately 20% of iris vessels showed exfoliation material. In the present study with 26 specimens exclusively from exfoliation glaucoma patients the rate of involvement of the iris vasculature was considerably higher.

The relative paucity of exfoliation material on the anterior border layer observed in the present study has been noted by Ghosh & Speakman (1974). However, Ringvold (1970) stated that he found 'abundant exfoliation material in the area measuring 20-30 microns from the iris surface'. Other authors, for example Layden & Shaffer (1973), only comment that 'exfoliation material was present in the anterior border layer'. On the basis of the evidence of the present study it is suggested that exfoliation material aggregates are not synthesised in that subcompartment and most likely originate from the aqueous. This study is therefore in agreement with Ghosh & Speakman (1974) who considered that the anterior border layer is not a likely source of exfoliation material.

By LM it was difficult to identify isolated aggregates of exfoliation material within the iris stroma, but foci of exfoliation material were identified with certainty by TEM. In many cases serial sections showed that these isolated foci were continous with the walls of diseased vessels. This is in agreement with the elegant LM reconstructions of diseased vessels by Shimizu (1985). This author has also found that deposits of exfoliation material could frequently be traced back to the vascular supporting cells of exfoliative vessels.

From the present investigation no conclusion can be drawn on the role of the iris pigmented epithelium in the synthesis of exfoliation material in the diseased iris. This is because the posterior layer of the pigmented epithelium was severely traumatised in many cases. However, the close association between exfoliation material aggregates and the basement membrane of the posterior layer of the pigmented epithelium in the few cases, where the pigmented epithelium was preserved, suggests a synthetic role for this anatomic structure. Ghosh & Speakman (1974) have found fragmentation, redublication and 'shedding' of the basement membrane of the posterior layer of the pigmented epithelium in areas where there was accumulation of exfoliation material. These investigators have interpreted these findings as evidence that the pigmented epithelium is a source of exfoliation material. In a recent communication Ritch and coworkers (1992) on the basis of cell culture studies have suggested that posterior pigmented epithelial cells can synthesise exfoliation material *in vitro*. The deposition of exfoliation material within the anterior layer of the pigmented epithelium was better studied in the material processed for immunocytochemistry and is more appropriately discussed in in chapter 6.

Significantly, aggregation of exfoliation material was found around some, but not all, iris vessels. Ringvold (1969) was the first to document the presence of normal iris vessels close to vessels with exfoliation aggregates. This study therefore confirms previous suggestions (Ghosh & Speakman 1974, Shimizu 1985) that passive deposition of exfoliation

material via the aqueous circulation is not the mechanism for the presence of exfoliation material within the iris stroma. The present study could not confirm the observation by Anastasi and coworkers (1974) that in exfoliative iris, blood vessels are very rare. In all specimens there were at least 4-5 vessels and some specimens exhibited up to 20 iris vessels. Similarly, it was not possible to corroborate the observation of Ringvold & Davanger (1981) that in some diseased vessels there is extreme reduction of the vascular lumen due to increased volume of the endothelial cells. This may be due to the fact that 2 out of the 3 eyes investigated by these authors were examples of painful end stage exfoliation glaucoma.

The consistent ultrastructural involvement of the iris vasculature in all 26 specimens studied suggests that in the iris this anatomical subcompartment is involved in the synthesis of exfoliation material. In accordance with Shimizu (1985) and Ringvold (1969) it was found in the present study that exfoliation material aggregates frequently do not surround the entire circumference of diseased vessels. The observed pattern of involvement suggests that vascular supporting cells and endothelial cells have a role in the synthesis of exfoliation material. Thus the present study contradicts the view expressed by Sugar and coworkers (1976) that in the iris only the pigment epithelium is a likely source of exfoliation material.

Vascular supporting cells appear to be the first cells involved in the synthesis of exfoliation material since

changes in these cells were often not accompanied by changes in neighbouring endothelial cells. The present study does not agree with the general observation made by Shimizu (1985) that exfoliation aggregates are 'attached to vessels with degenerated walls'. The role of endothelial cells requires further elucidation because exfoliation material was rarely adherent to the basement membrane of endothelial cells and was only convincingly seen attached to endothelial cells in advanced vasculopathy.

In agreement with Layden & Shaffer (1973) there was generally no evidence of exfoliation material in the lumen of diseased vessels. Exfoliation material however, did appeared in the lumen of a few ghost vessels exhibiting advanced vasculopathy and disorganisation of the vascular wall. One of the most interesting observations in this study has been the presence of one or more 'ghost vessels' in each of the 26 specimens studied. These vessels which presumably indicate advanced vasculopathy have rarely been noted in the literature (Ghosh & Speakman 1974, Sugar et al 1976). Previous studies have often included iris tissue from exfoliation syndrome patients (Ringvold 1969; 1970) but even Shimizu (1985) who studied 8 specimens from patients with exfoliation glaucoma did not mention the presence of ghost vessels. It is likely that ghost vessels are more common in exfoliation glaucoma than in exfoliation syndrome. The functional significance of the presence of these ghost vessels has not been dealt with before in the literature. It is conceivable that their presence could lead to localised hypoxia which subsequently leads to new vessel formation. The latter feature has been

consistently documented in anterior segment fluorescein angiographic studies (Brooks et al 1987). It is also relevant to note that Ghosh & Speakman (1974) have documented marked atrophy of iridic cellular elements in a case with end stage exfoliation glaucoma and speculated that these may be associated with the obliteration of the lumen of these ghost vessels.

The problem of iris vasculopathy has not been properly addressed by previous morphological studies. For example, Shakib and coworkers (1965) did not find 'changes in the iris vasculature'. Spinelli and coworkers (1985) have only stated that all their iris specimens showed 'some vessels in different stages of pathological alteration'. From the present study it is possible to reconstruct the evolution of exfoliation vasculopathy. I would like to suggest the following morphological sequence. Initially, subtle changes develop in the vascular matrix and the supporting cells. Subsequently, exfoliation material synthesis by the vascular supporting cells is accompanied by more extensive accumulation of exfoliation material and attenuation, or degeneration of the synthesising cells. Endothelial cells begin to degenerate at a later stage. Finally, as an extreme manifestation of advanced disease, the iris vessel disappears and is replaced by a circular aggregate of exfoliation material (formation of a ghost vessel). The latter may account for the strong resemblance between stromal deposits of exfoliation material and ghost vessels.

The present study is in agreement with previous suggestions

that the histological manifestation of the disorder in the eye may exist in the absence of clinically obvious exfoliation material deposition (Prince et al 1987, Schlötzer-Screhardt et al 1991a). In those studies however, the definition of an exfoliation suspect patient relied upon a number of pigmentary signs which arise as a consequence of pigment liberation from the posterior layer of the iris. This suggests that in all these cases the iris was involved early in the disease process. The precise timing of appearance of exfoliation material in the iris in comparison with other ocular, orbital or systemic tissues is as yet unknown. It is likely that iris is only one of many tissues involved in the synthesis of exfoliation material (Morrison & Green 1988). Indeed recent investigations (Streeten et al 1990, Sugino 1990, Schlötzer-Screhardt et al 1991a) have focused on other ocular, orbital and systemic tissues for possible sources of exfoliation material.

It is well documented that in exfoliation syndrome it is sometimes difficult to establish a definitive diagnosis (sections 1.7 & 1.8). As discussed in section 10.2 the pathological examination of iris biopsies from open angle glaucoma patients can provide a definitive diagnosis. Furthermore, it was confirmed in the present study that that TEM examination of tissue biopsies can identify exfoliation material in cases where it is not detected by clinical examination. This suggests that exfoliation syndrome may be responsible for a greater proportion of glaucomas than previously thought.

The reliability of the electron microscopic localisation of exfoliation material in the iris was convincingly demonstrated in this study by finding exfoliation material in all 22 specimens with the clinical diagnosis of exfoliation glaucoma (chapter 10). Thus, iris biopsies appear more reliable with regard to the ultrastructural presence of exfoliation material than conjunctival biopsies (see section 1.2.3). Of course, unlike conjunctival biopsy, iris tissue can only be studied in surgical patients.

4.6 Summary

Iris tissue obtained from 26 consecutive patients operated upon for exfoliation glaucoma and control iris tissue from 26 age matched patients with POAG was used to investigate the involvement of the iris subcompartments and the vasculopathy associated with exfoliation glaucoma. By LM exfoliation material was discerned by the increased density of the perivascular matrix of affected vessels. By TEM exfoliation vasculopathy was divided into early and advanced. Early vasculopathy was associated with focal accumulation of exfoliation material with or without degeneration of intrinsic vascular cells. Degeneration of vascular supporting cells was sometimes accompanied by preservation of endothelial cells. In advanced exfoliation vasculopathy exfoliation material occupied an acellular vascular wall (ghost vessel). It is suggested that in iris vessels the synthesis of exfoliation material can be attributed primarily to the supporting cells.

CHAPTER 5 IMMUNOGOLD ULTRASTRUCTURAL LOCALISATION OF COLLAGENS IN THE NORMAL AND EXFOLIATIVE IRIS

5.1 Preface

This chapter deals with the distribution of collagens in the normal and exfoliative iris. An ambiguity was observed in the distribution of collagen type I in exfoliation material. To solve this problem a supplementary study was carried out on exfoliative trabecular meshwork and for convenience the results are included in this chapter (section 5.2.4).

5.1.1 Introduction: collagens in the normal iris

The eye is composed of highly specialised tissues possessing distinct collagenous structures that exhibit a great degree of biological diversity. Amongst the ocular structures, the iris is unique in possessing a barrier-free stroma, atypical blood vessels and an ability for contractile movement. Collagens are the major constituents of the extracellular matrix (ECM) and thus provide biomechanical support for this tissue, which undergoes constant movement. In addition, the ECM of the iris influences the exchange of macromolecules between the plasma of the iris vessels and the aqueous humour which bathes the anterior segment structures. This constant exchange is primarily dependent upon the presence and complex interaction of the ECM components present in the iris vascular basement membranes (vascular matrices). This is suggested by immunocytochemical studies in other tissues of both man and animals (Abrahamson 1986, Grant & Leblond 1988,

Morrison et al 1989, Das et al 1990b), which indicate that the fluid movement is dependent on the barrier and hydrophilic properties of laminin, heparan sulphate proteoglycan and the intrinsic basement membrane collagens.

To my knowledge, the precise distribution of the various collagen types has not been documented previously in either human or animal iris tissue. It is important to document the presence and distribution of the iris collagens in order to study potential collagen alterations in the exfoliation syndrome and other disorders. The fine structural localisation of collagen types I-V was investigated in the normal human aged iris.

5.1.2 Results

All 6 iris specimens studied (cases 1-6 in Appendix I) were ostensibly normal on LM examination of toluidine blue stained LR white semithin sections. An intense specific signal for collagen types I, III and IV was demonstrated in the aged human iris of all cases. Collagen types II and V did not demonstrate a signal fulfilling the criteria for positive localisation as described in section 3.3.6 and were therefore considered to be negative.

Collagen type I

Collagen type I was present in the vascular matrices of iris vessels (Figs 5.1, 5.2 & 5.3), the iris pigment epithelium, and within striated collagen fibrils of the iris stroma (Fig 5.4). Labelling intensity increased proportionally with the

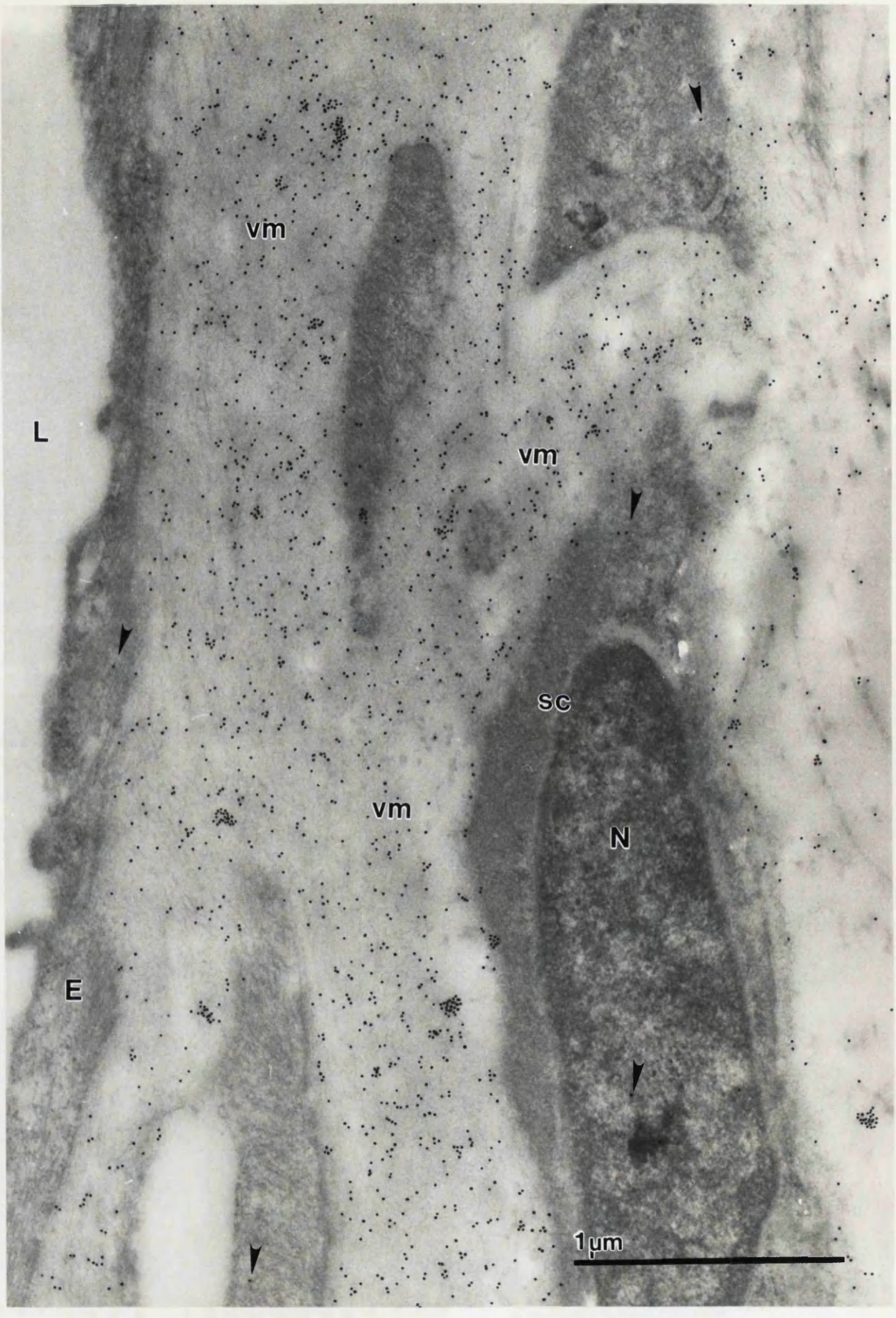


Figure 5.1: Distribution of collagen type I in the wall of an iris vessel. Intense labelling is observed in wall's vascular matrix (vm),which has a distinct fibrillar nature. Arrowheaded gold particles are considered non-specific. L:lumen; E:endothelium; sc:supporting cells; N:nucleus.

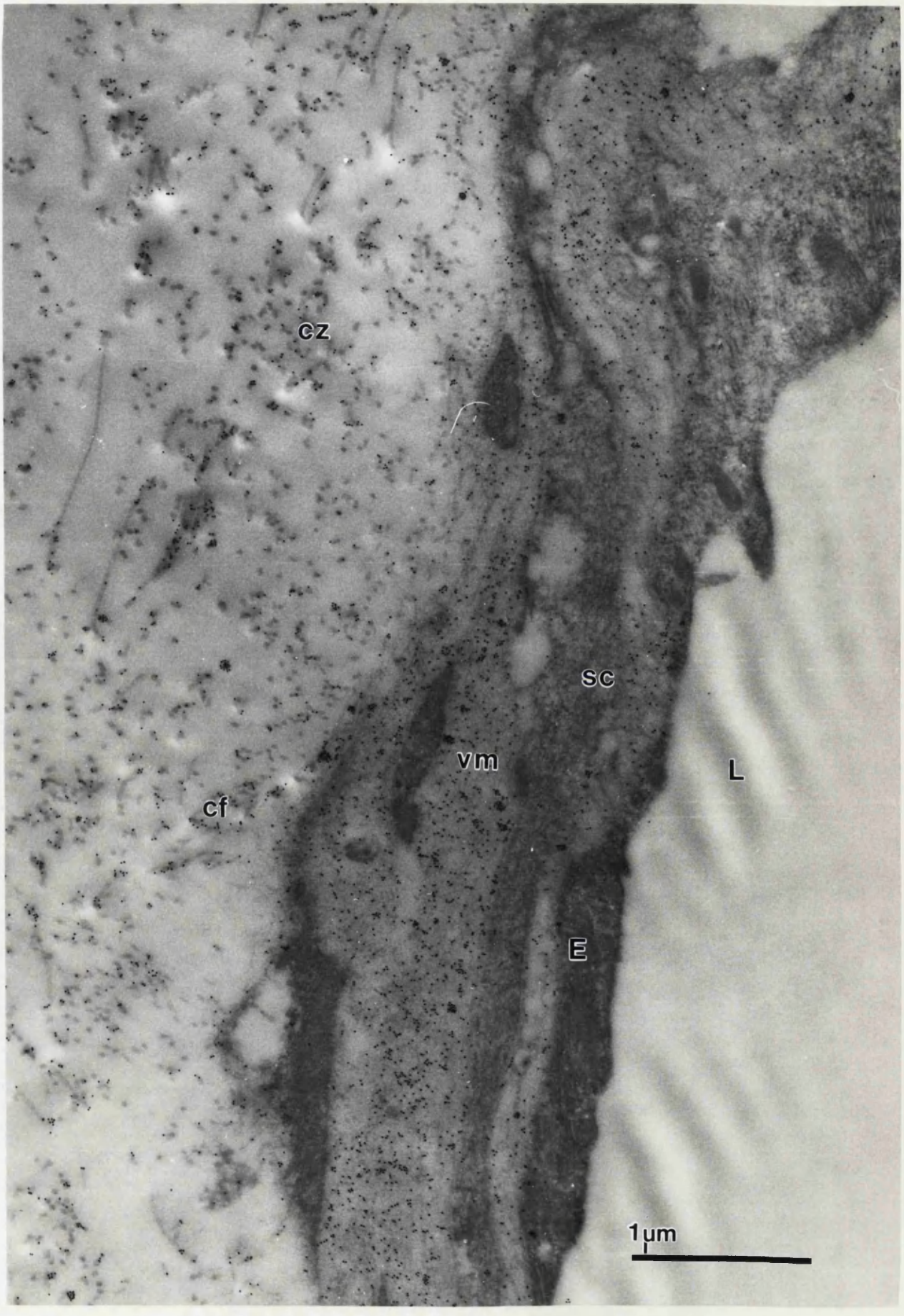


Figure 5.2: Collagen type I in an iris vessel and surrounding collagenous zone(cz). The fibrillar vascular matrix(vm) and striated collagen fibrils(cf) of the collagenous zone exhibit strong labelling. L:lumen E:endothelium; sc:supporting cells.

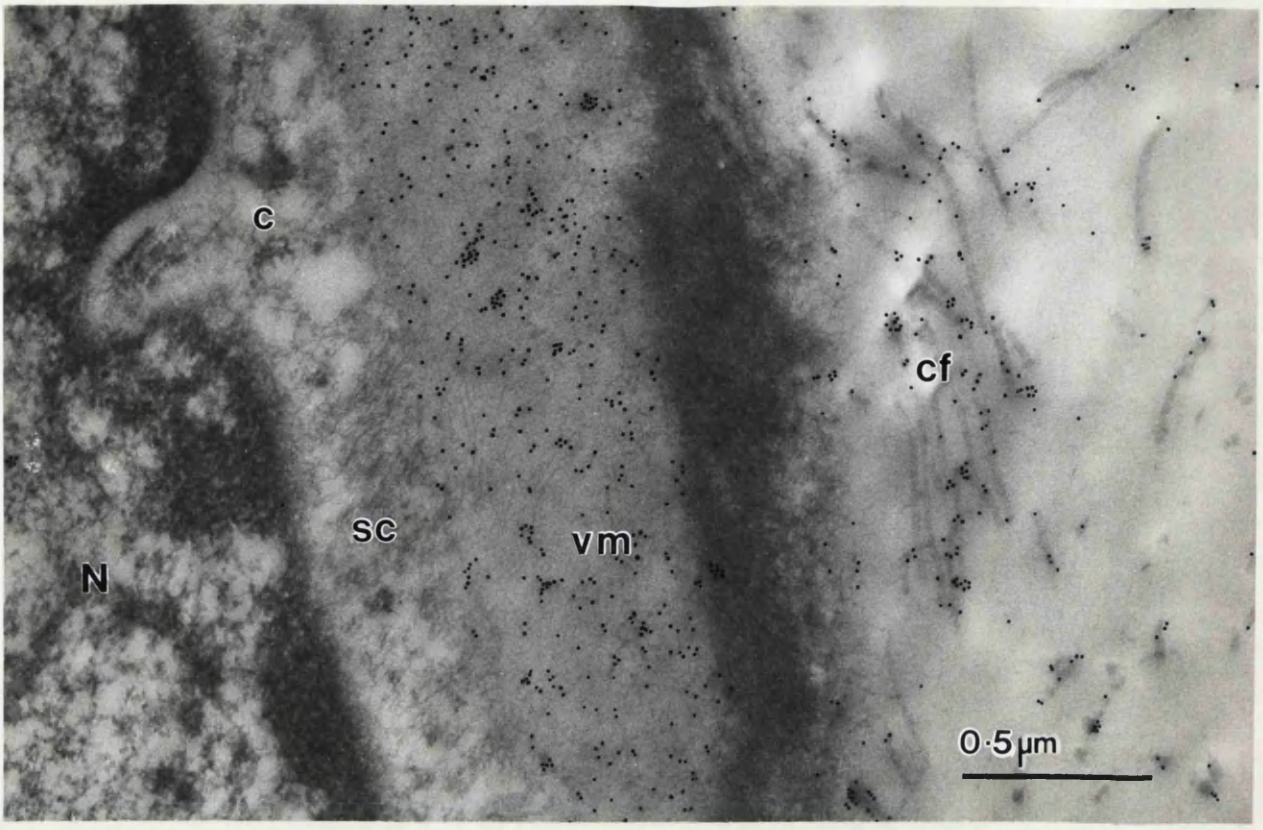


Figure 5.3: Collagen type I labelling over the fibrillar vascular matrix(vm) of a supporting cell(sc) and collagen fibrils(cf). Note absence of labelling over nucleus(N) and cytoplasm(c).

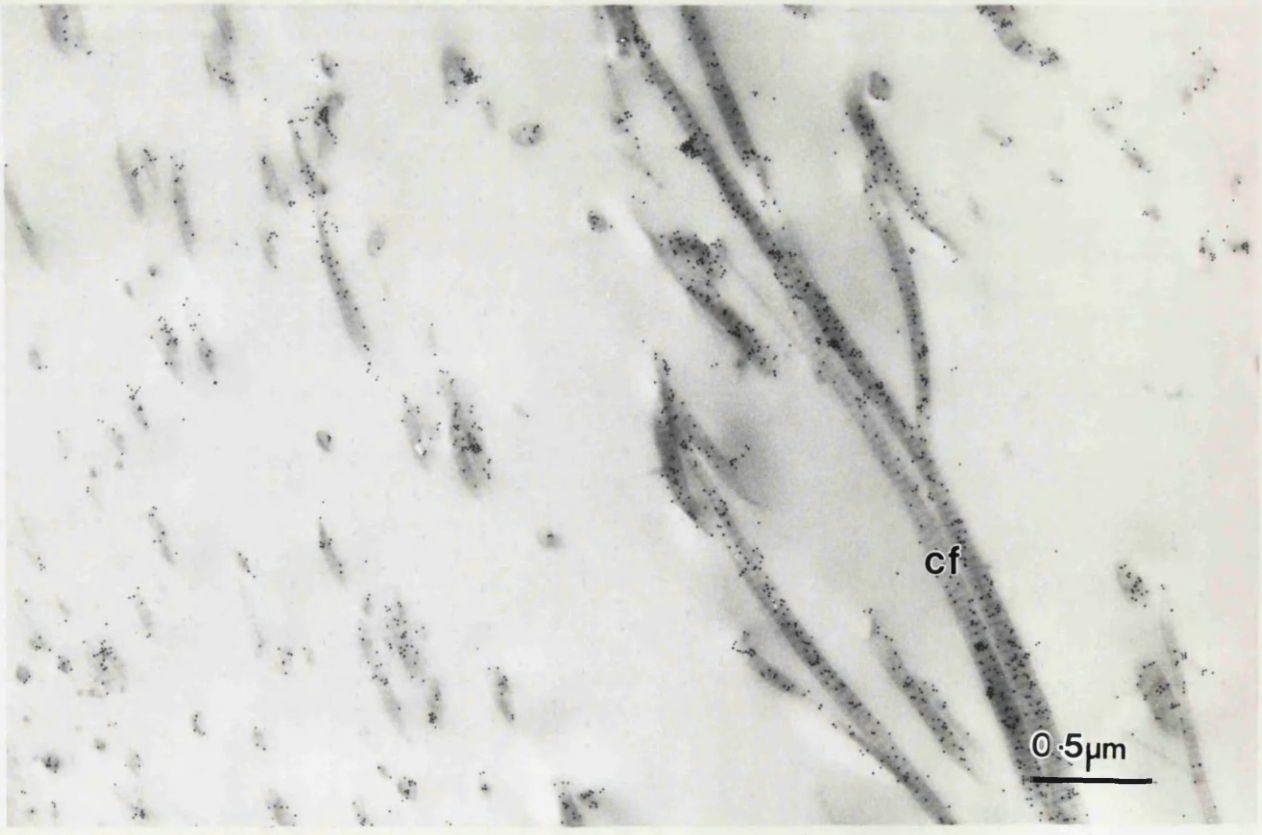


Figure 5.4: Collagen type I is present within the core of striated collagen fibrils(cf) in the iris stroma.

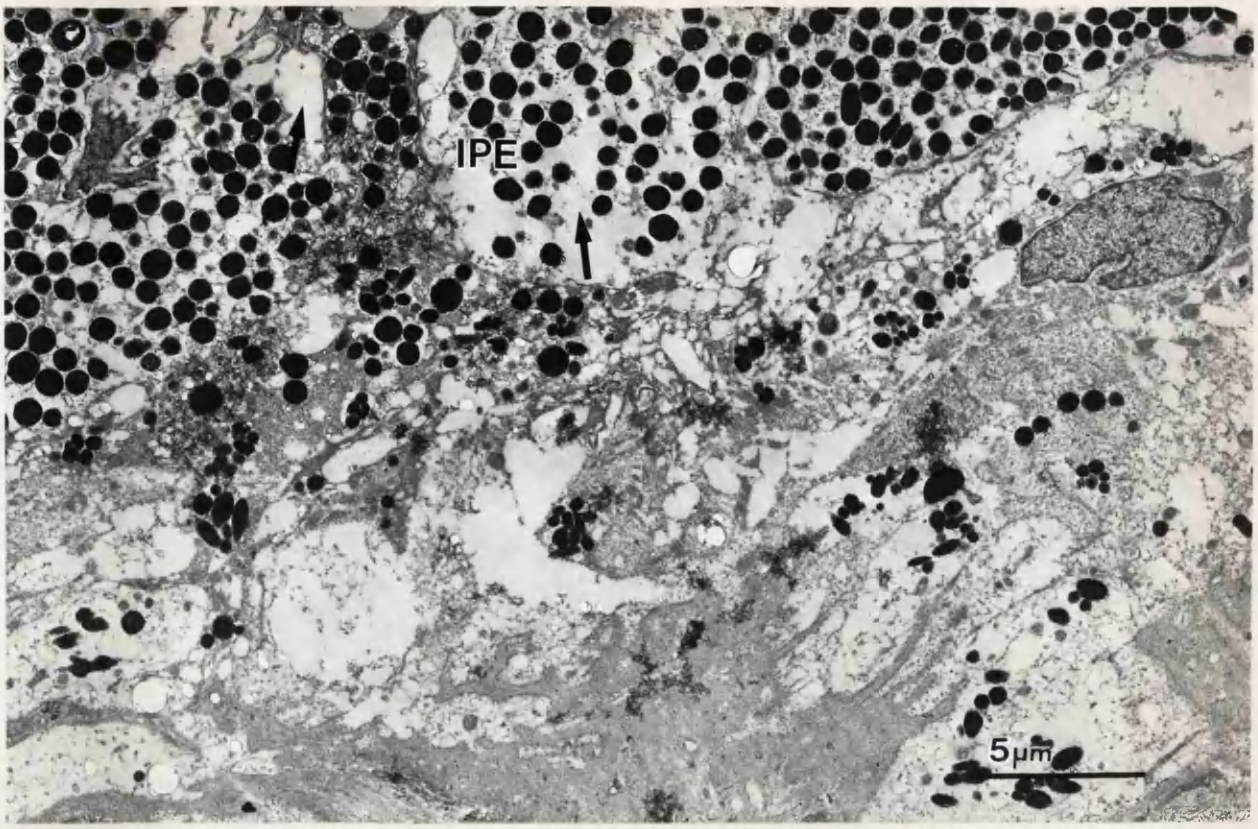


Figure 5.5: Example of tissue preservation obtained with LR white resin embedding. Ultrathin section of the iris pigment epithelium(IPE) shows cytoplasmic rarefaction(arrows).

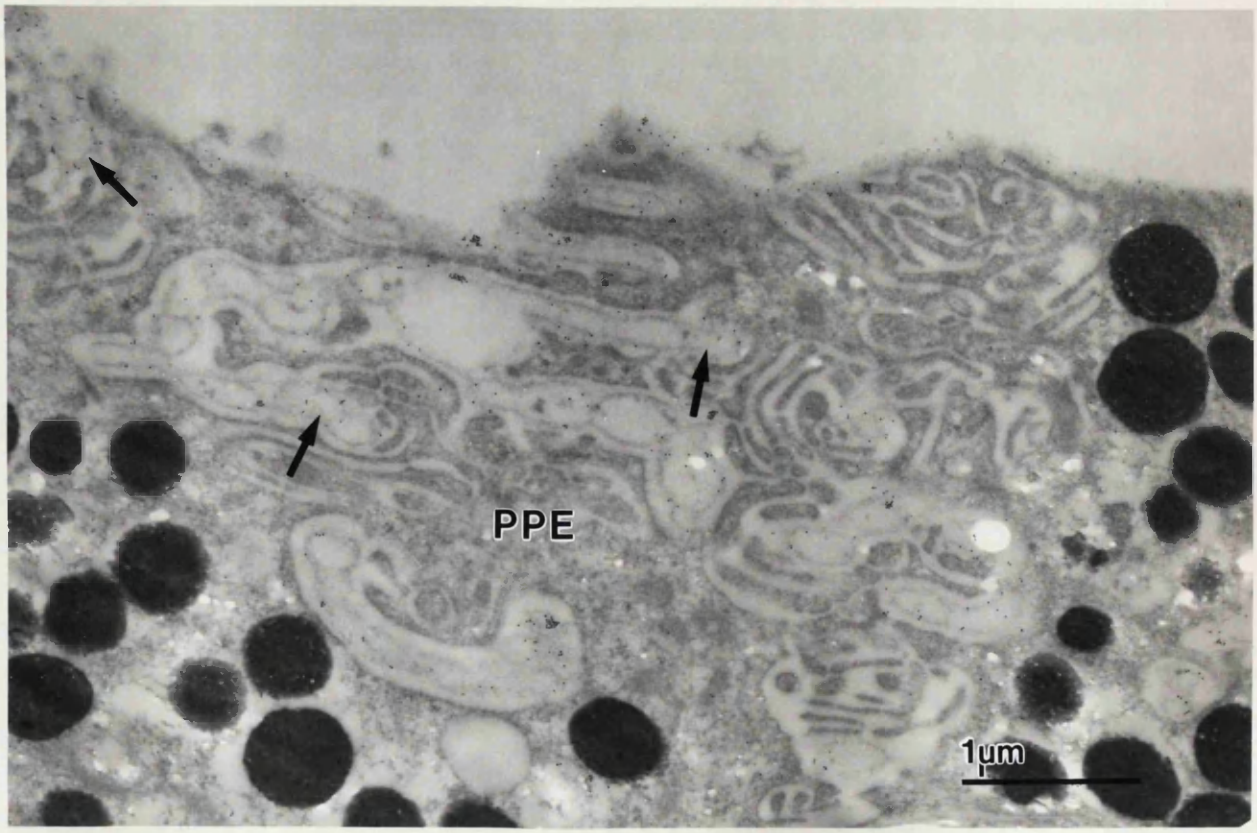


Figure 5.6: Type I collagen in the posterior pigmented epithelium(PPE). Immunogold labelling of the basement membrane follows the posterior infoldings (arrows).

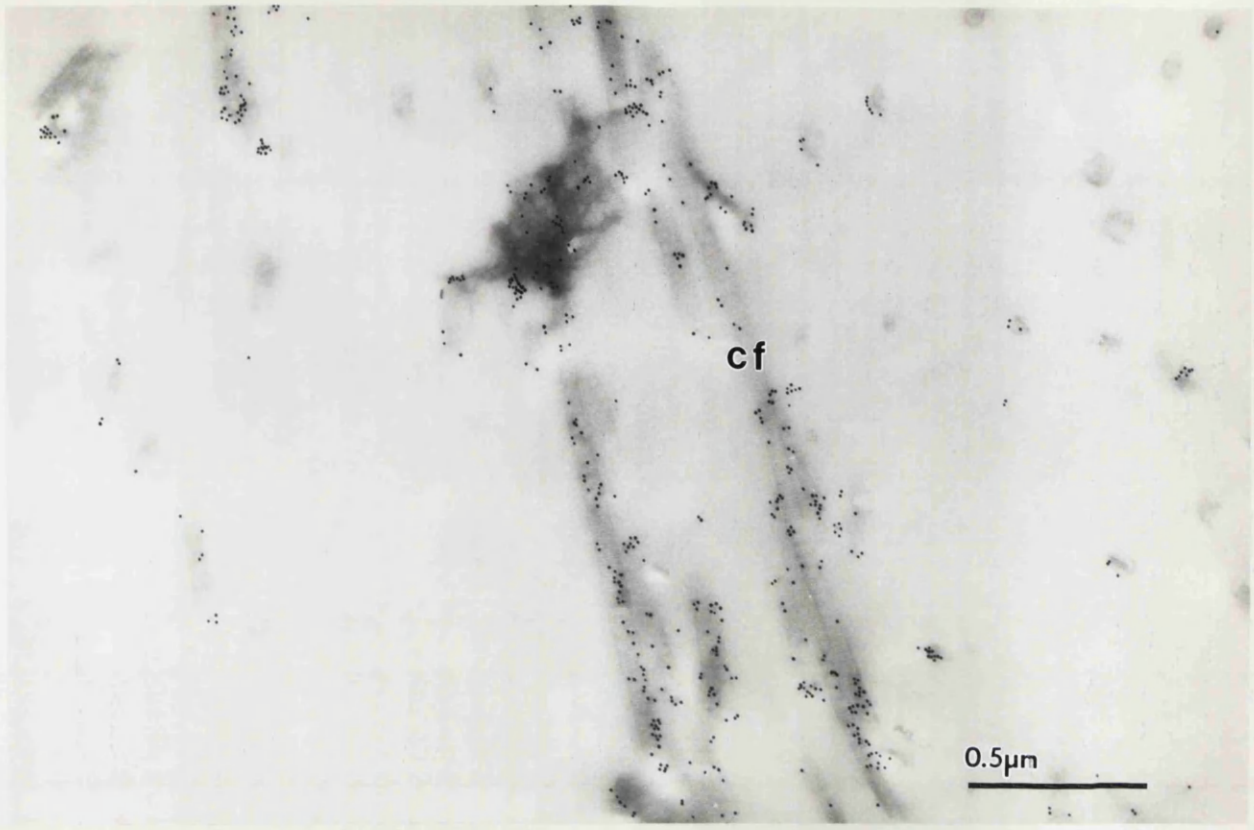


Figure 5.7: Type III collagen in the iris stroma is restricted to striated collagen fibrils(cf). Peripheral labelling is apparent in transverse sections of fibrils. TEM.

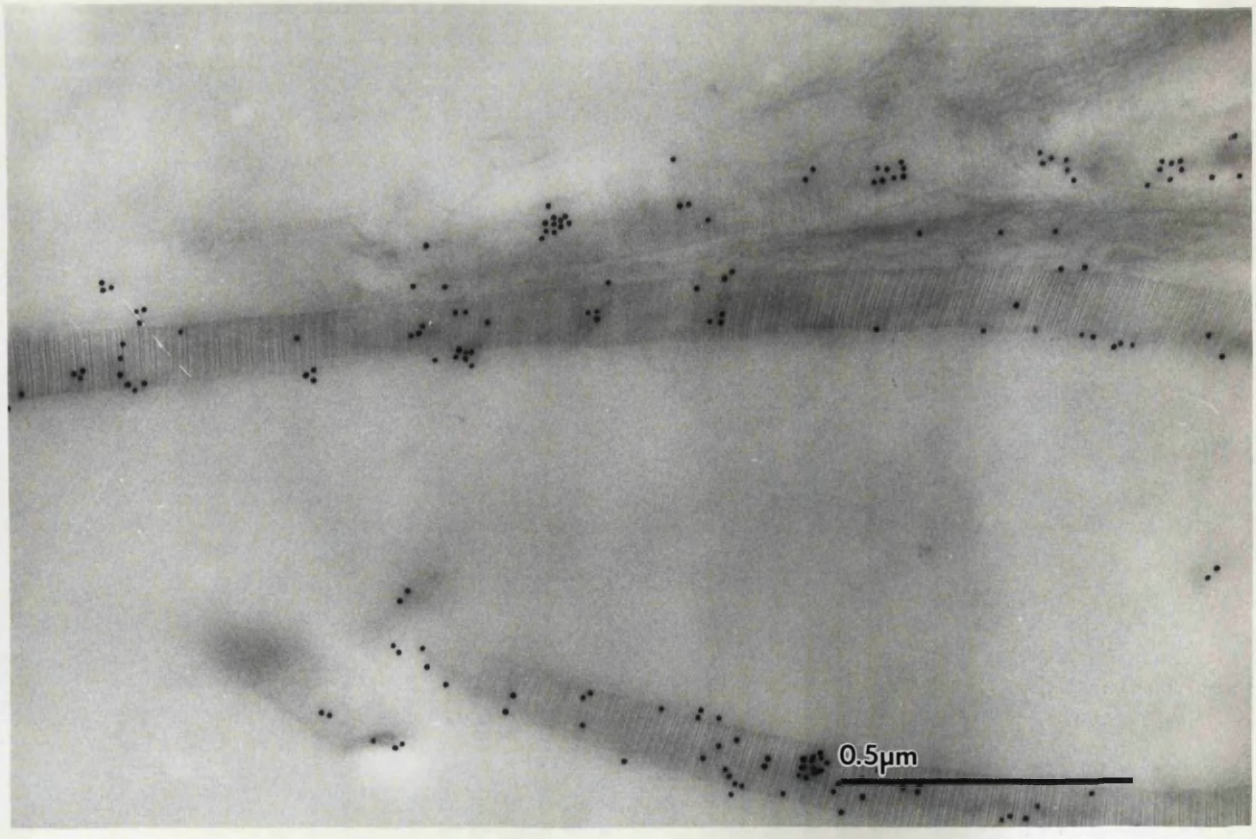


Figure 5.8: Higher magnification of type III labelling in striated collagen fibrils of the iris stroma.

size of the vessel, i.e. larger vessels exhibited more intense immunolabelling. Collagen type I was present within the basement membrane of the myoepithelial cells (dilator muscle) and the basement membrane of the posterior pigmented epithelium (Figs 5.5 & 5.6).

Collagen type III

In the case of collagen type III, immunolabelling was absent from epithelial, or vascular basement membrane in the iris. Intense labelling however, was noted on the striated collagen fibrils comprising the circular collagenous zone and present throughout the iris stroma (Figs 5.7 & 5.8). An interesting, but not uniform, finding was the more intense immunogold labelling present on large collagen fibrils.

Collagen type IV

Collagen type IV was strongly positive for all iridic basement membranes (Fig 5.9). The most intense signal was observed on the lamina densa and lamina fibroreticularis of the iris vessels (Fig 5.9). The lamina lucida appeared not to contain collagen type IV. It is of interest to note that labelling within the basement membrane (vascular matrix) of the iris vessels did not depend on the size of the vessel, but labelling intensity appeared to increase with increasing endothelial cell thickness. Positive immunolabelling was also observed in the loose ECM (basement membrane material) forming a supporting sheath around the processes of the myoepithelial cells and the basement membrane of the posterior pigmented epithelium.

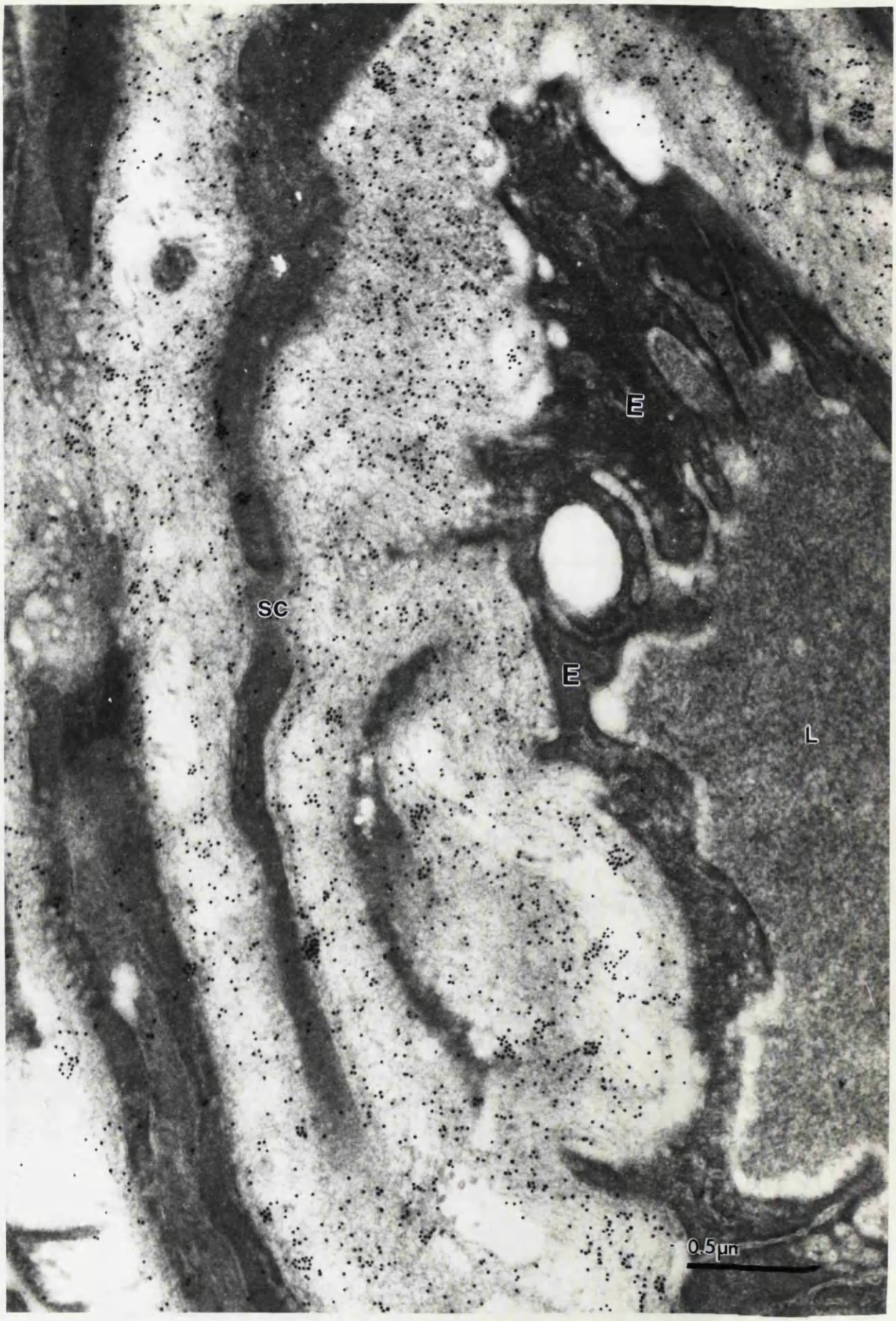


Figure 5.9: Type IV collagen distribution in an iris vessel. Labelling intensity between endothelium(E) and the first layer of supporting cells(sc) is more intense than that of subsequent layers. L:lumen.

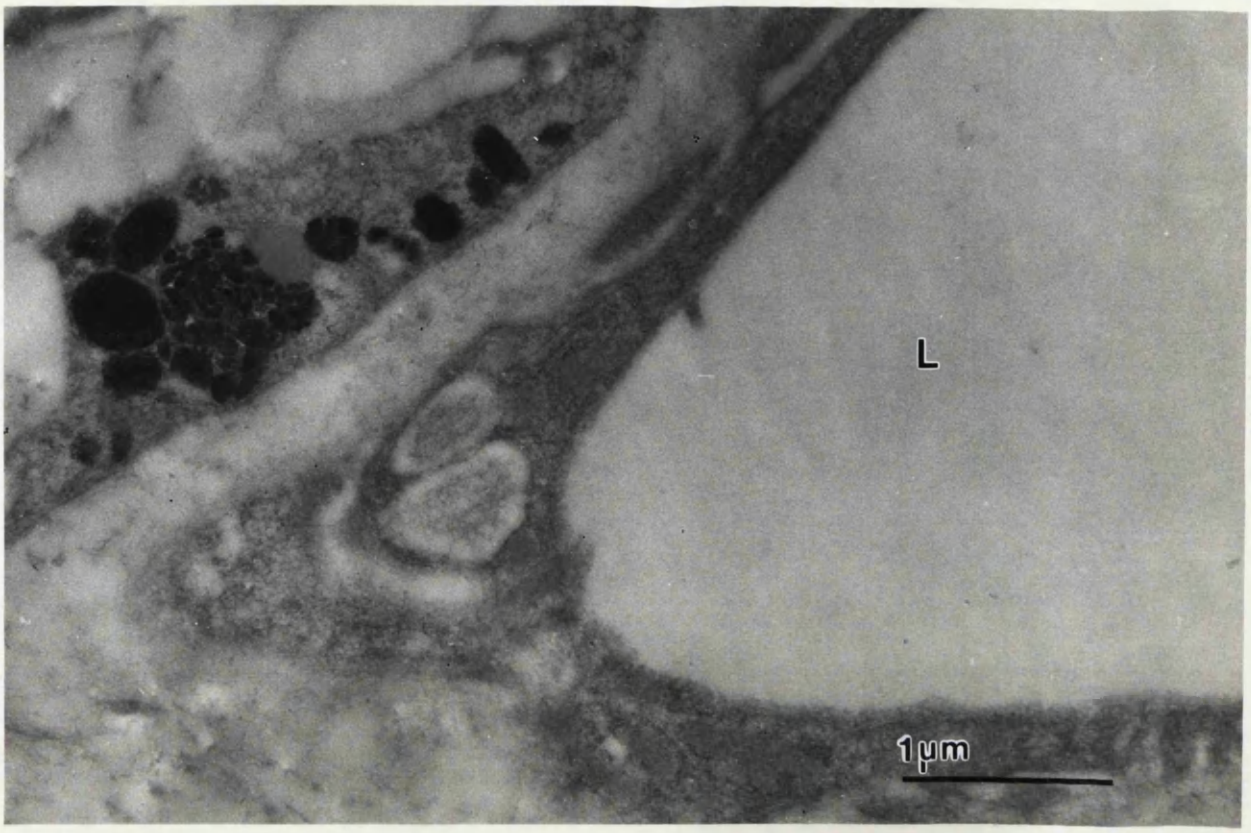


Figure 5.10: Collagen type II in an iris vessel. Labelling is sparse and non-specific. L:lumen.

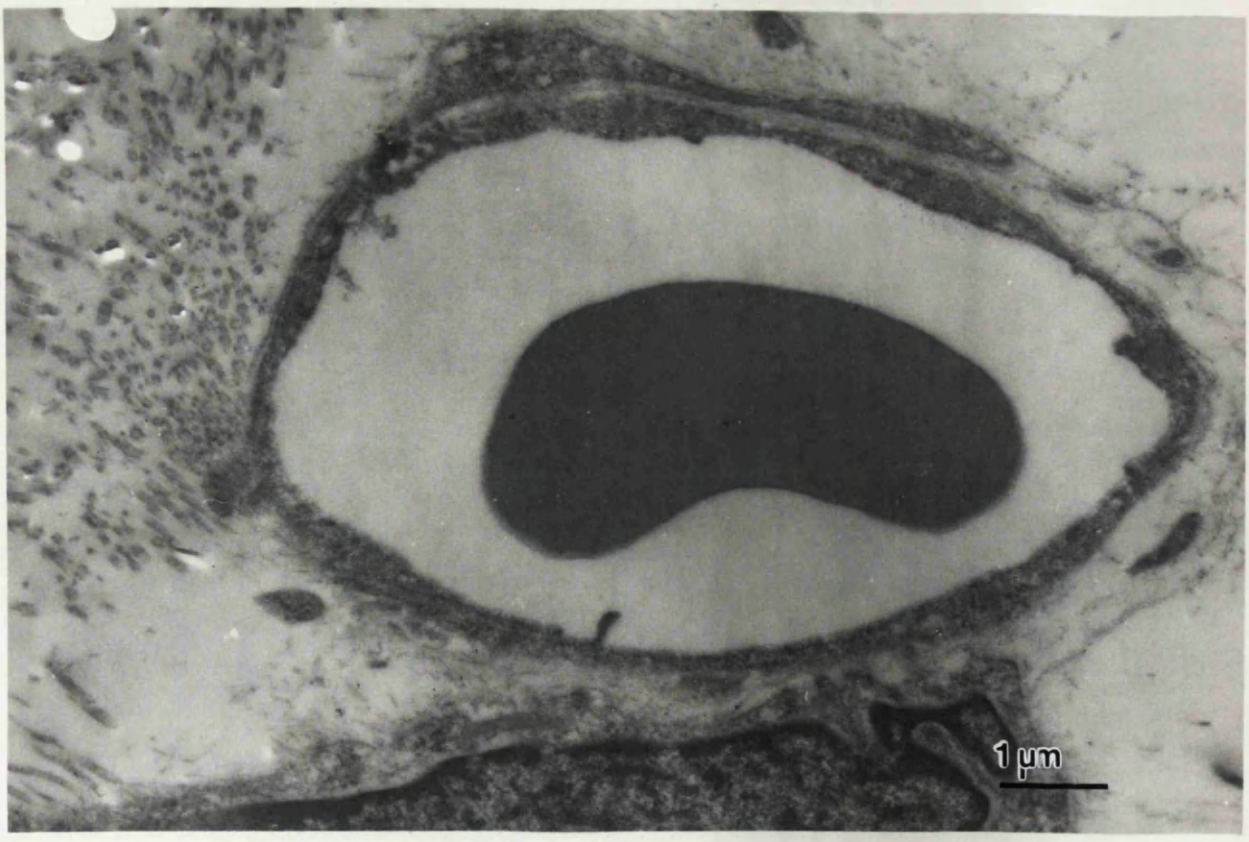


Figure 5.11: Absence of collagen type V in an iris capillary. Although some labelling is present in the vascular matrix and striated collagen fibrils it is also present over the red blood cell and was therefore regarded as non-specific.

5.1.3 Discussion

To my knowledge, there have been no immunohistochemical reports published on the presence, or distribution of collagen types in the human or animal iris, at either the EM, or the LM level. In all cases the iris stroma and the pigment epithelium was normal and qualitatively there was no apparent age-related change. The results of the present study have indicated that type I is the most prevalent collagen in iris tissue. This is in agreement with biochemical reports (Bailey 1987) and immunocytochemical studies (Das et al 1990a, Marshall et al 1991a) in other ocular tissues. A similar distribution for collagen type I has been reported in other body tissues (Burgeson 1988, Fleischmajer et al 1990).

However, type I collagen, an interstitial collagen, has not been shown previously to be present in the vascular matrix of ocular blood vessels. Therefore, the identification of type I collagen at this site was unexpected. Nevertheless, two recent immunogold studies on human retinal vessels revealed the presence of type I in the outer basement membrane of retinal arterioles (Das et al 1990) and in the whole extent of the retinal vascular basement membrane (Marshall et al 1990a). When the basement membrane of the iris vessels was examined carefully at high magnification, it became evident that the immunogold delineated fine strands within the lamina densa and the signal was stronger in the lamina densa of supporting cells. The fact that small vessels in the iris exhibited weaker signal may have been due to the smaller

concentration of accompanying supporting cells (pericytes and myocytes). Das et al (1990a) could not demonstrate type I collagen in retinal capillaries, but this may have been due to autolysis and diminished antigenicity as their work was performed on autopsy material.

The basement membrane material around the myoepithelial cells in the iris showed immunolabelling, which suggests that these contractile cells synthesise type I collagen. The basement membrane of the posterior pigmented epithelium of the aged iris also exhibited immunogold labelling within the basal infoldings of the posterior pigmented epithelial cells. This also implies a synthetic capability of this monolayer. In the iris stroma, the signal intensity for type I collagen did not increase with the diameter of the collagen fibrils, which is in contrast to the results obtained with type III collagen. This would indicate that in the iris an increase in fibril diameter is due to the deposition of type III rather than type I collagen.

Collagen type II was not detected in the iris and the controls (cornea, vitreous and trabeculum) with the technique employed in this study. To date collagen type II has not been demonstrated in any of the ocular tissues studied by the immunogold technique (LR white plastic embedding technique, or cryoultramicrotomy), (reviewed by Marshall 1991). Thus it was not possible to confirm biochemical findings in the vitreous concerning type II collagen (Bailey 1987). This might be explained in terms of antigenic sensitivity of type II collagen to fixation. The question whether type II

collagen is present in the iris must therefore remain open.

Collagen type III was localised within the striated collagen fibrils throughout the iris, but was lacking from the basement membranes of the cellular constituents (supporting cells). It is likely that type III collagen co-exists with type I (Keene et al 1987). This would be in agreement with previous evidence that uniform banded collagen fibres often contain more than one collagen type. (Kühn 1987, Birk et al 1988, Burgeson 1988, Linsenmayer et al 1990). Considerable effort is currently being directed towards the elucidation of the role of the different fibrous collagens in the process of fibrillogenesis (Birk et al 1990, Fleischmajer et al 1990, Linsenmayer et al 1990). It is suggested that the particular codistribution of specific collagens within a 'heterotypic' collagen fibril determines the biomechanical properties of the ensuing tissue matrix.

The main difference in distribution between type I and type III in the aged iris was the absence of type III collagen from the iridic basement membranes. It is of interest to note that two immunogold studies employing LR white embedding have localised type I collagen in the basement membrane of normal and hyalinised retinal vessels (Das et al 1990a, Marshall et al 1990a). This implies a certain degree of biological diversity in the ECM composition of ocular vascular basement membranes.

Although the function of type III collagen is as yet ill defined, it has been suggested that it may increase the

plasticity of tissues; whenever the collagen ratio shifts in favour of type III, the tissue becomes more pliable (Lindberg & Pinnell 1982, Birk et al 1986, Bailey 1987, Deluca et al 1990, Fleischmajer et al 1990). It is possible that the proportion of type III collagen changes throughout life as maturation of the collagen ensues and this change may occur more rapidly, or markedly in ageing and disease. There are many ocular disorders in which a specific defect of collagen metabolism is either known or suspected. These include the Ehlers-Danlos syndrome, keratoconus, osteogenesis imperfecta, Marfan's syndrome and pseudoxanthoma elasticum (reviewed by Lindberg & Pinnell 1982). Abnormal regulation of type-specific collagen has been indicated with biochemical studies in patients with osteogenesis imperfecta and Marfan's syndrome; type III collagen was found to be increased relative to type I collagen (Lindberg & Pinnell 1982).

Collagen type IV has been identified in a series of basement membranes of various animal tissues (Laurie et al 1982, Kennedy et al 1986, Abrahamson 1986, Grant & Leblond 1988, Charonis & Tsilibary 1990) and has been shown to be a major component in the basement membrane of retinal vessels in cats (Essner & Lin 1988) and human aged retinal vessels (Marshall et al 1990a, Das et al 1990a). In this study, an intense signal was obtained in the lamina densa of the iris vessels. Labelling was also observed in the loose non-fibrous matrix around the myoepithelium and the basement membrane of the posterior pigmented epithelium. As expected, type IV was absent from the stroma of the iris. There was increased labelling in association with the perinuclear zone of the

endothelial cells. This indicates that type IV, like other ECM components present in basement membranes, may be secreted by endothelial cells (Mecham 1986, Glaser 1988).

The absence of type V collagen from the iris could not be definitively established because a low intensity label was observed on striated fibrils. It was thought unlikely that the epitopes of this fibrous collagen were not available since in postembedding immunolabelling the fibrils are opened by ultrathin sectioning, thereby potentially exposing the type V collagen epitopes. Moreover, a positive signal was obtained in the corneal controls. It would therefore appear that type V collagen does not play a significant role in the normal aged human iris.

A high content of type V collagen within a collagen fibril has been reported to result in a fibril of small diameter (Linsenmayer et al 1990). This explains the presence of type V collagen in the corneal controls. Corneal transparency requires that the collagen fibrils within the corneal stroma are thin and of uniform diameter. Nevertheless, future technical refinements are required to produce a definitive answer to the question of presence or absence of this fibrous collagen in the iris.

5.1.4 Summary

Immunogold labelling has been applied at the fine structural level for the identification and precise distribution of collagen types I, II, III, IV, and V in the normal human aged

iris. Tissue was obtained from 6 surgically enucleated globes. LR white plastic embedding was employed. Collagen types II and V were not identified. Types I and III collagen were localized to the striated collagen fibrils of the iris stroma. Collagen types I and IV were present in all iridic basement membranes.

5.2 Collagen types I-V in the exfoliative iris

5.2.1 Introduction

Current knowledge concerning the distribution and role of collagens in the exfoliation syndrome is limited. Initially, exfoliation material was considered to be similar to collagen (Ringvold 1970a; 1970b), but histochemical (Dvorak-Theobald 1954, Arnesen et al 1963, Bertelsen & Ehlers 1969), immunohistochemical (Harnisch et al 1981) and biochemical (Ringvold 1973b) studies have indicated that collagens contribute little to the composition of exfoliation material. However, Harnisch and coworkers (1981) have suggested that type IV collagen may be an integral component of the exfoliation material. Besides, preliminary biochemical, or immunohistochemical data on the collagen profile of tissues has often conflicted with subsequent electron microscopic immunocytochemical data. This conflict was best illustrated in the attempts to document the distribution of collagen types I-IV in the animal and human cornea. For example collagen type I has been reported as both present in (Ben-Zvi et al 1986) and absent from (Bailey 1987), Bowman's layer of the human cornea. In addition, biochemical analysis yields

little information as to the precise localization of collagens. As far as I am aware, no other immunohistochemical (LM), or immunocytochemical (EM) study has been performed on the presence and precise distribution of the various collagen types in exfoliative tissues. It was thus decided to apply the immunogold technique to elucidate the fine structural distribution of five collagen types (I-V) in the exfoliative iris. When specific and unexpected features of interest in the pattern of immunolabelling of types I and IV collagen in exfoliative iris vessels were found, these were further investigated (chapter 7). Furthermore, in the course of this study it became evident that in order to provide a definitive answer as to the presence of type I collagen in the exfoliation material it was deemed necessary to also study exfoliative meshwork (see section 5.2.4). The results of the latter investigation are reported in the second part of this section.

5.2.2 Results

Material for this study was provided from 2 patients with exfoliation syndrome and cataract and 4 patients with exfoliation glaucoma. In all 6 patients the diagnosis was confirmed by clinical examination. The exfoliative iridectomy specimens used in this study exhibited exfoliation material on LM examination of toluidine blue stained LR white semithin sections. These findings were confirmed by TEM assessment of ultrathin LR white embedded tissue sections.

Type I collagen

Type I collagen was identified in the vascular matrix of exfoliative vessels. Immunolabelling was detected within the thickened vascular matrix, whether or not the vessel contained clumps of exfoliation material (Fig 5.12). Labelling for type I was associated with the exfoliation material present within the thickened vascular matrix. Immunogold particles were also present in exfoliation deposits in the iris stroma but the labelling density there was significantly lower (Fig 5.13). Sparse labelling was observed in ghost vessels where exfoliation material had replaced the normal vascular wall and in exfoliation deposits in the anterior border layer. It was not possible to draw definitive conclusions as to the presence or absence of type I in the exfoliation material in the iris because of the affinity of the label for the underlying collagenous matrix.

Type II collagen

Type II collagen was not detected in the exfoliation material present within the exfoliative iris. A weak immunolabelling for type II observed in all regions of the exfoliative iris was considered non-specific (for specificity criteria see section 3.3.6). As in the studies of the normal iris, no labelling was demonstrated on the corneal controls.

Type III collagen

Collagen type III localised, almost exclusively, to striated collagen fibrils in the iris stroma. Exfoliation deposits within the iris stroma were labelled with collagen type III antibodies. However, the labelling pattern indicated that

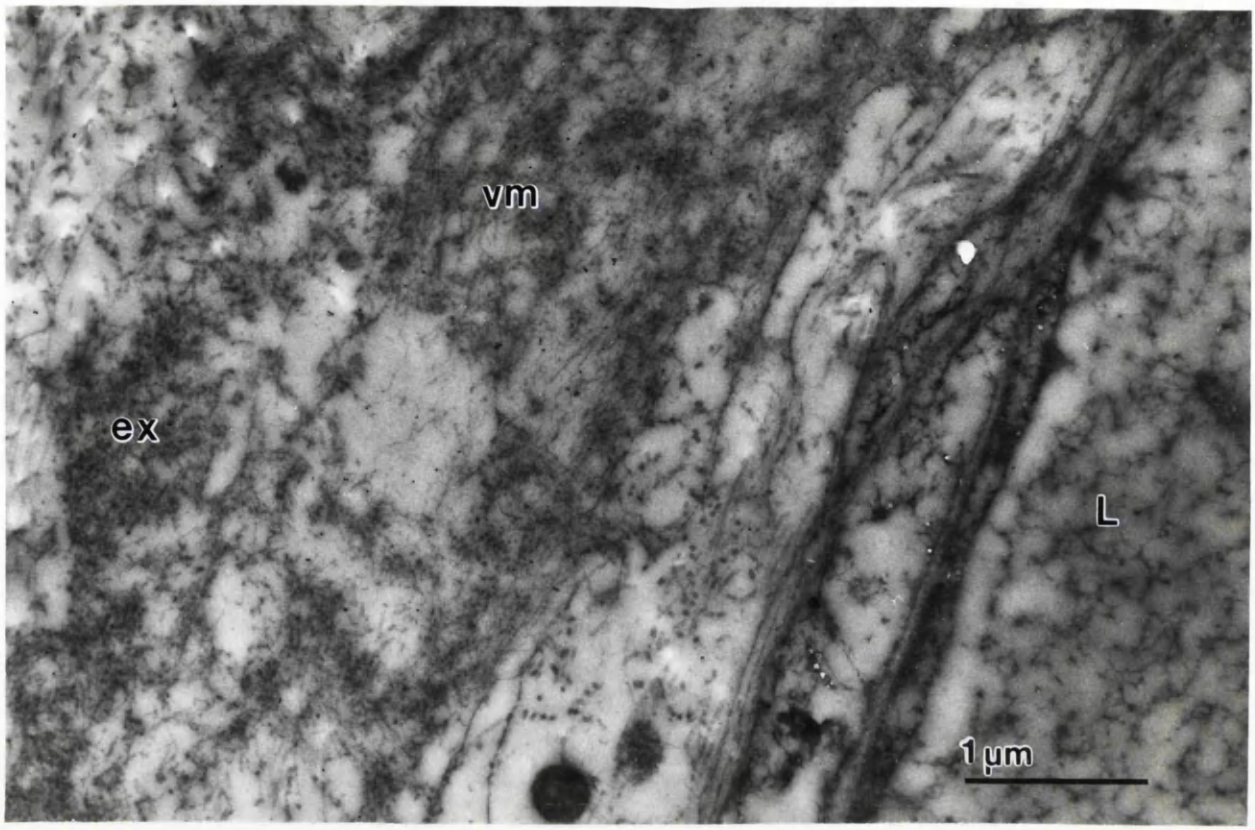


Figure 5.12: Collagen type I in exfoliative iris vessel. Immunolabelling is largely restricted to the vascular matrix(vm) which is infiltrated with exfoliation material(ex). L:lumen.

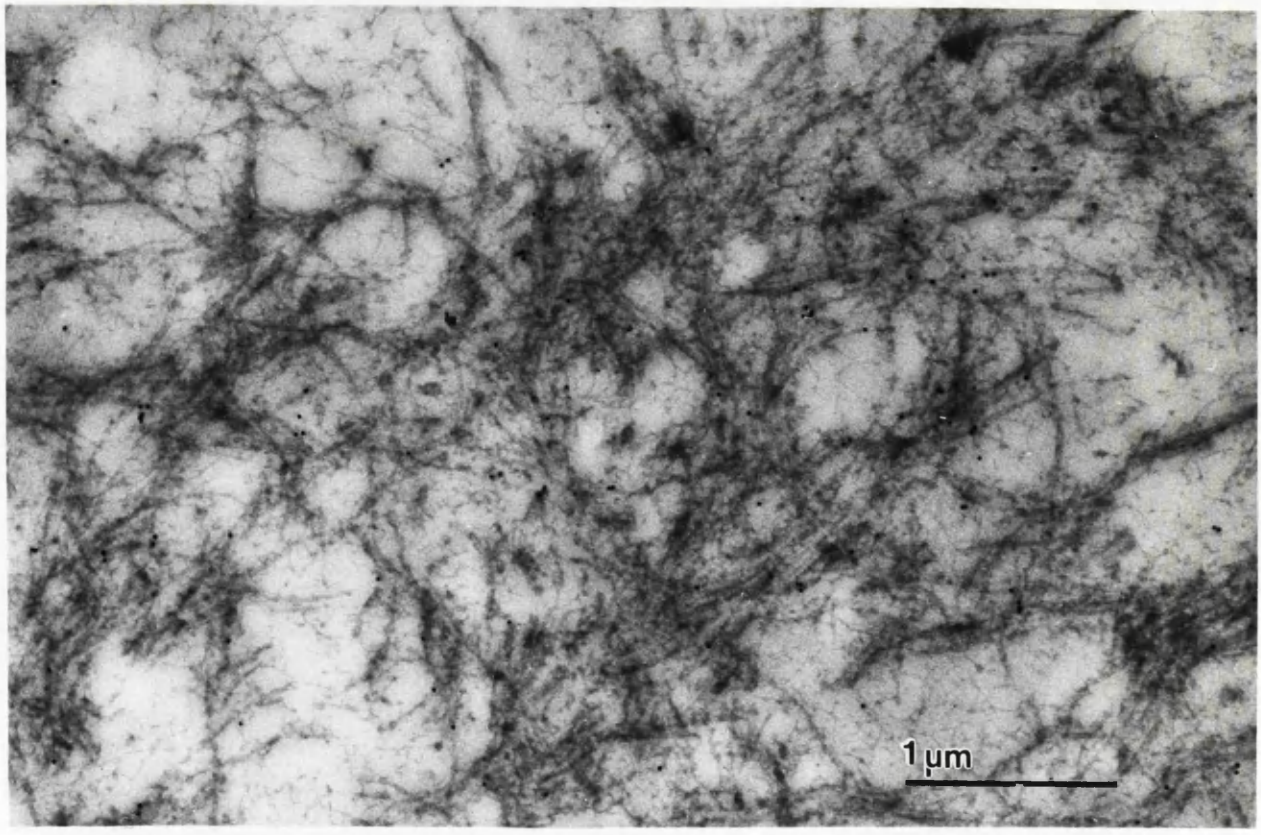


Figure 5.13: Collagen type I in exfoliation material within iris stroma. Immunogold particles are largely localised to 'fibres'.

type III was not a component of exfoliative fibres, because immunogold particles were mainly associated with the interfibrillar matrix of both exfoliation material and normal collagen. Significantly, collagen type III labelling was absent from exfoliation aggregates present within the vascular matrix and the anterior border layer (Fig 5.14).

Type IV collagen

Collagen type IV was detected in the exfoliative specimens exclusively in association with the vascular matrix. The distribution of type IV collagen in the vascular matrix of healthy iris vessels of exfoliative iris specimens without evidence of exfoliation material was similar to that observed in the control normal iris vessels. Labelling for type IV collagen in the diseased iris vessels exhibiting exfoliation material deposits was diverse. Collagen type IV was found in abundance in the thickened multilayered vascular matrix of those exfoliative vessels, which were infiltrated by exfoliation material. In contrast, the density of labelling was considerably reduced in thin degenerate vascular matrices (see chapter 7). The majority of immunogold particles were not located on the coarser individual exfoliative fibres, but on the finer filamentous material of the vascular matrix. Immunolabelling for type IV collagen was not detected in exfoliation material deposits outside the vascular matrix: in the iris stroma and in exfoliation material aggregates in the anterior border layer (Fig 5.15).

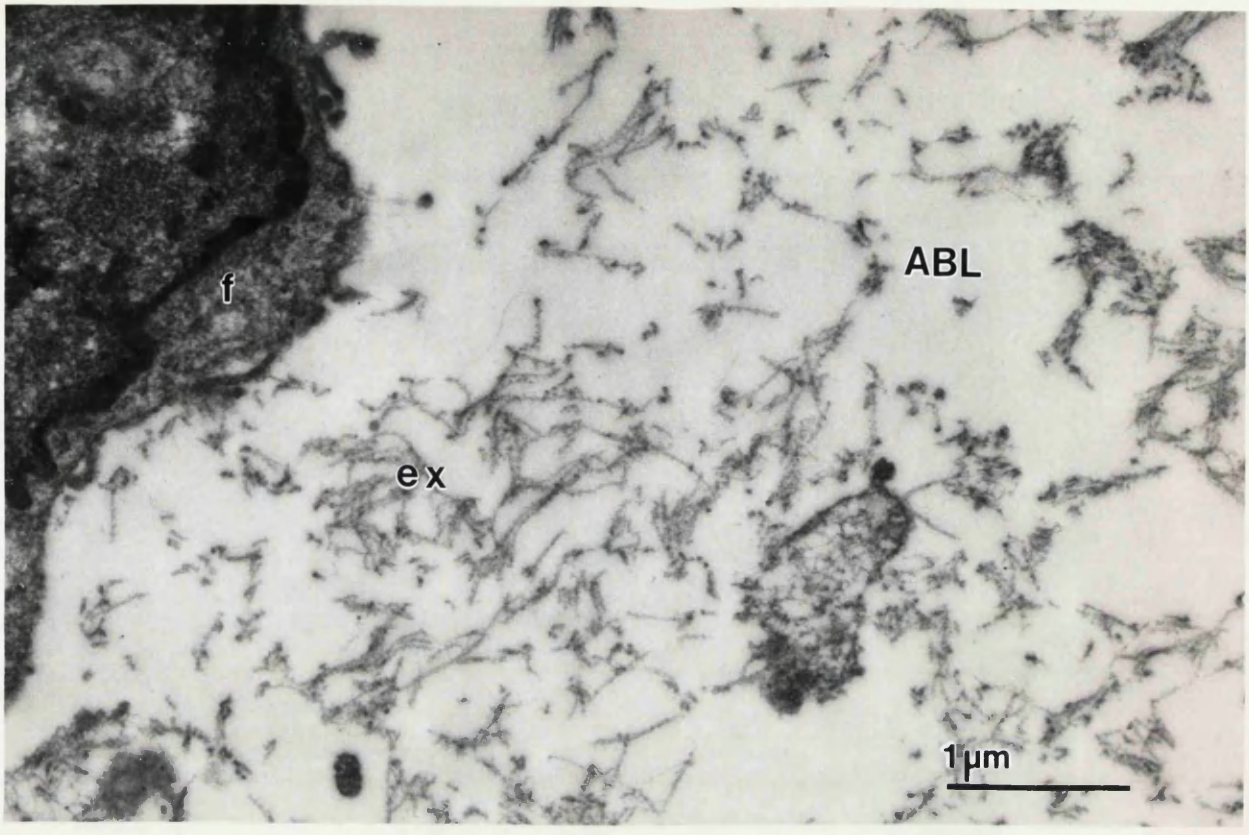


Figure 5.14: Absence of collagen type III from a loose aggregate of exfoliation fibres(ex) in the anterior border layer (ABL)of the exfoliative iris. f:fibrocyte.

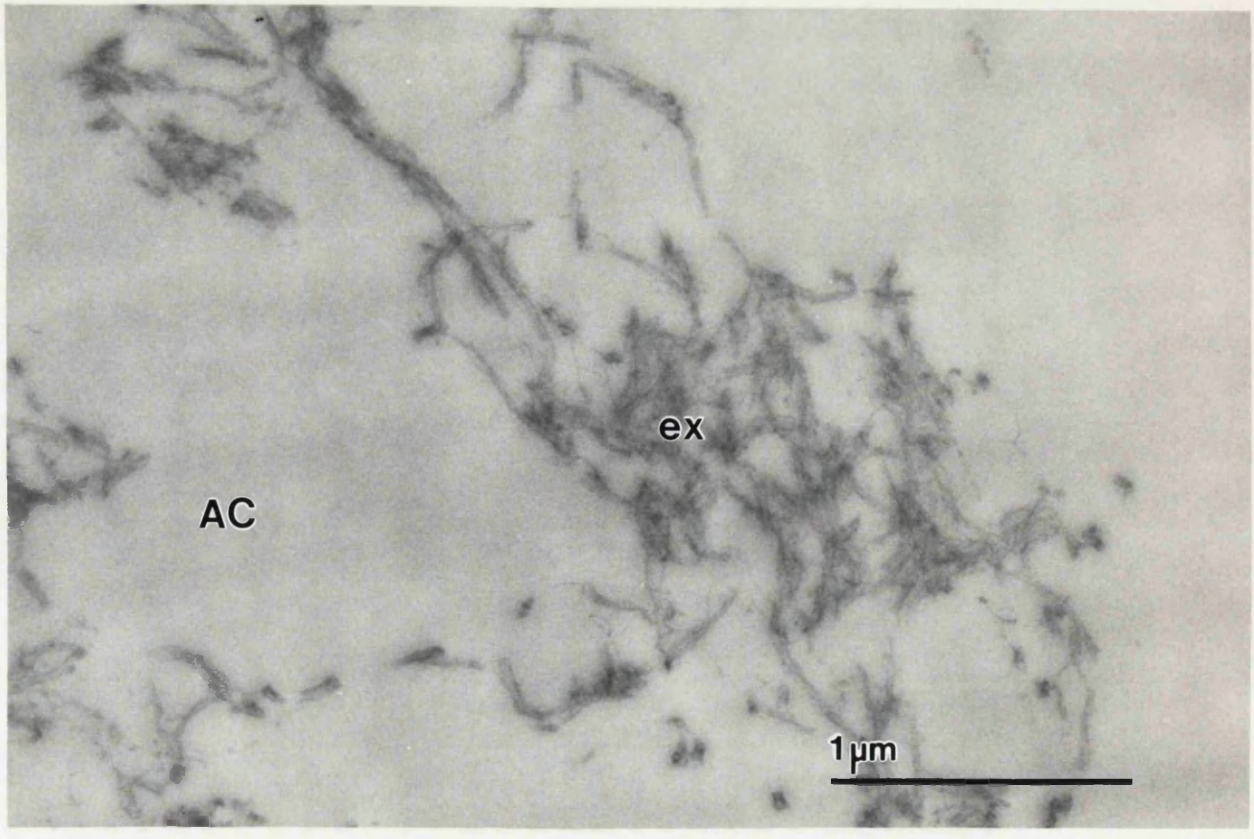


Figure 5.15: Type IV collagen is absent from an exfoliation material(ex) vegetation projecting from the anterior border layer to the anterior chamber(AC).

5.2.3 Discussion

It should be noted that the morphological appearance of exfoliation aggregates in LR white sections differs somewhat from that of the conventionally fixed and processed iris tissue; firstly, because of different fixation (see section 3.3.5) and more importantly because this method leads to retention of larger amounts of ECM (Marshall et al 1990b; 1991c; 1992a). This is due to the hydrophilic nature of this resin. This feature is advantageous in the study of the interfibrillar matrix of the exfoliation material. Still, the qualitative ultrastructural features observed in this study were comparable to those reported in the conventional morphological studies of chapter 4.

In this investigation it was not possible to detect collagen types II, III and V in exfoliation material aggregates in the iris. With regard to type II collagen this negative finding is in agreement with the only immunohistochemical study conducted on collagens in the exfoliation syndrome (Harnisch et al 1981). This study reported the absence of collagen types I and II from the exfoliation material. However, this study employed the LM immunoperoxidase technique, which is limited in resolution and specificity due to the diffusion of the stain (see chapter 3). The absence of type III collagen from exfoliation material was as expected. The potential effects of exfoliation material synthesis and deposition on the normal synthetic functions of the native cells are discussed in chapters 6 and 7.

Collagen types V was absent throughout the exfoliative iris. Collagen type V is known, from animal studies, to occur in association with basement membranes (Mayne & Burgeson 1987) and, separately, in fibrous stroma such as cornea (Marshall et al 1991b). As in the normal aged iris, it is most likely that this collagen is absent from the stroma of the exfoliative iris. Experiments in the chick cornea, at low temperature employing double immunogold labelling, have indicated that collagen type V co-assembles in the same collagen fibril with type I collagen in order to control the diameter of the fibril (Linsenmayer et al 1990). This controlling infuence however, may not be required in the iris. The detection of collagen type V may also be facilitated by the use of cryoultramicrotomy (Marshall et al 1991b; 1991c), which was not employed in this study. Therefore, a possibility exists that a small amount of this collagen may be present in the exfoliative iris.

Collagen type IV was not found to be an integral component of exfoliation material. This conclusion was reached on the absence of labelling in exfoliation aggregates outside the iris vascular matrix. The subject of the quantitative alterations of collagen type IV in the exfoliative vascular matrix will be dealt with in a separate investigation (chapter 7). The present study has indicated that the biosynthesis of the exfoliation material is associated with alterations of intrinsic vascular matrix components, such as collagen type IV. This lends weight to the hypothesis advanced by some investigators (Ringvold 1969; 1988, Eagle et al 1979, Sugar 1984, Seland 1985, Streeten et al 1986,

Yannoff & Fine 1989) that local basement membrane-producing cells, are involved in the synthesis of exfoliation material.

The significance of the positive identification of type I collagen in exfoliation material aggregates was uncertain. It was impossible to decide with conviction whether or not this collagen comprises a true component of exfoliation material, or simply results from labelling of collagen of the indigenous iris tissue. As type I collagen was found to be the main collagen of the normal iris vascular matrix and stroma (section 5.1.2), the label between exfoliative fibrils could easily be attributed to a residue of intrinsic basement membrane, or stromal collagen. Alternatively, exfoliation material may have entrapped collagen type I within its structural framework. To address the contribution of type I collagen to the formation of exfoliation material, it was considered appropriate to study the ocular tissue spaces from which intrinsic collagens are absent.

5.2.4 Further studies. Collagen type I in the exfoliative meshwork

Introduction

Having established the fine structural localisation of collagens types II-V in the exfoliative material by studying exfoliative iris the question as to the role of type I collagen remained unresolved. Ocular tissue spaces where intrinsic collagens are absent can be found in the aged outflow system (Marshall et al 1991c). Exfoliative trabecular tissue from patients with exfoliation glaucoma

undergoing filtration surgery have been collected, fixed suitably and processed routinely by the author (see section 3.3.3). It was decided to study further the distribution of collagen type I in the exfoliation material employing exfoliative meshwork.

Results

Most exfoliative specimens exhibited a significant degree of surgical trauma. The ultrastructural distortion prevented investigation of morphologically distinct zones. One out of 8 specimens exhibited considerable surgical trauma and was not considered appropriate for detailed study. In 5 out of 7 specimens exfoliation material deposition was noted in the inter-trabecular spaces (3 cases), within the cribriform layer (1 case) and in both (1 case). Exfoliation material was not found in 2 specimens. In the intertrabecular spaces exfoliation material was arranged in the form of isolated deposits often intermingled with pigment. The endothelial cell covering of the trabecular beams was sometimes disorganised and not infrequently endothelial cells contained pigment granules. Exfoliation material within the cribriform layer had a much more confluent appearance: masses of randomly distributed exfoliation fibres were located underneath the endothelial lining of Sclemm's canal. Pigment granules were not present there.

Collagen type I

Collagen type I was absent from exfoliation material deposits within the cribriform layer (Fig 5.16) and lying in the intertrabecular spaces (Fig 5.17). Weak immunolabelling was

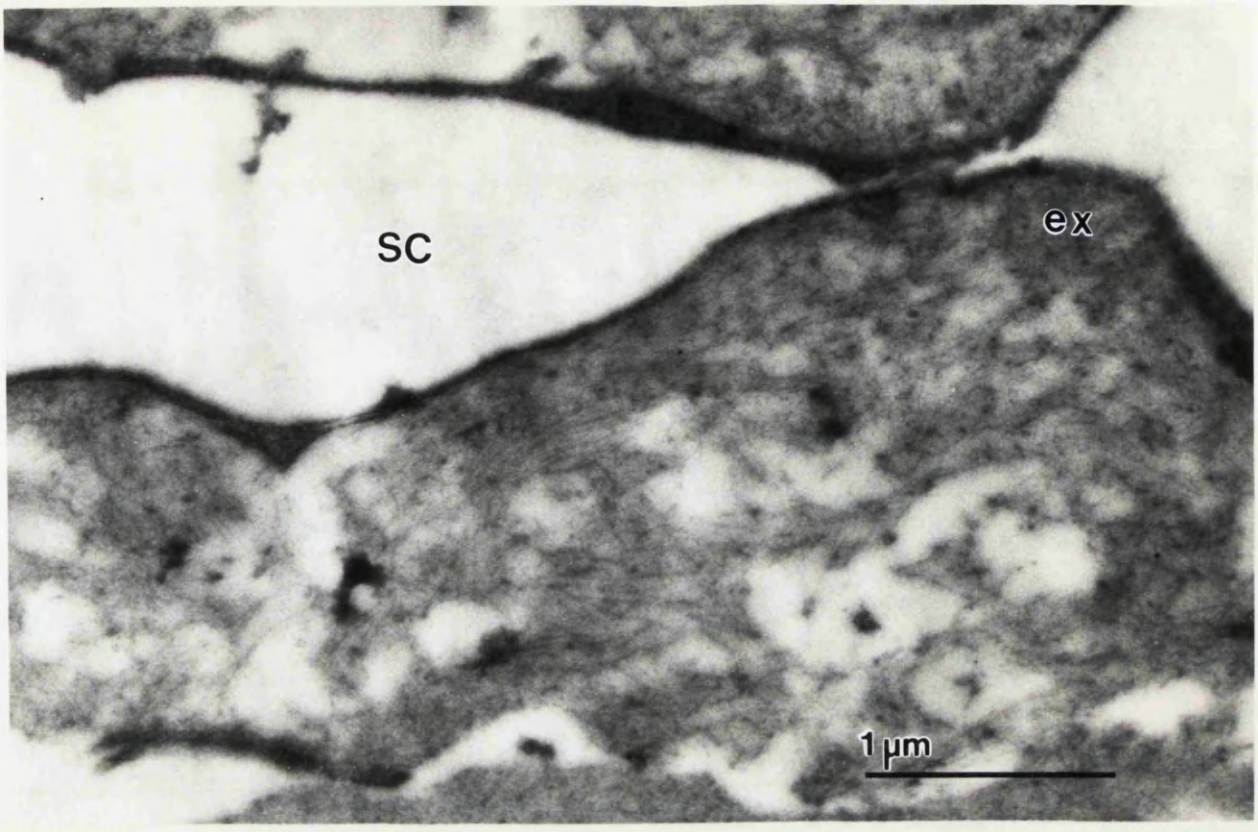


Figure 5.16: Collagen type I in the exfoliative outflow system. Exfoliation material(ex) in the cribriform layer is devoid of gold labelling. SC: Schlemm's canal.

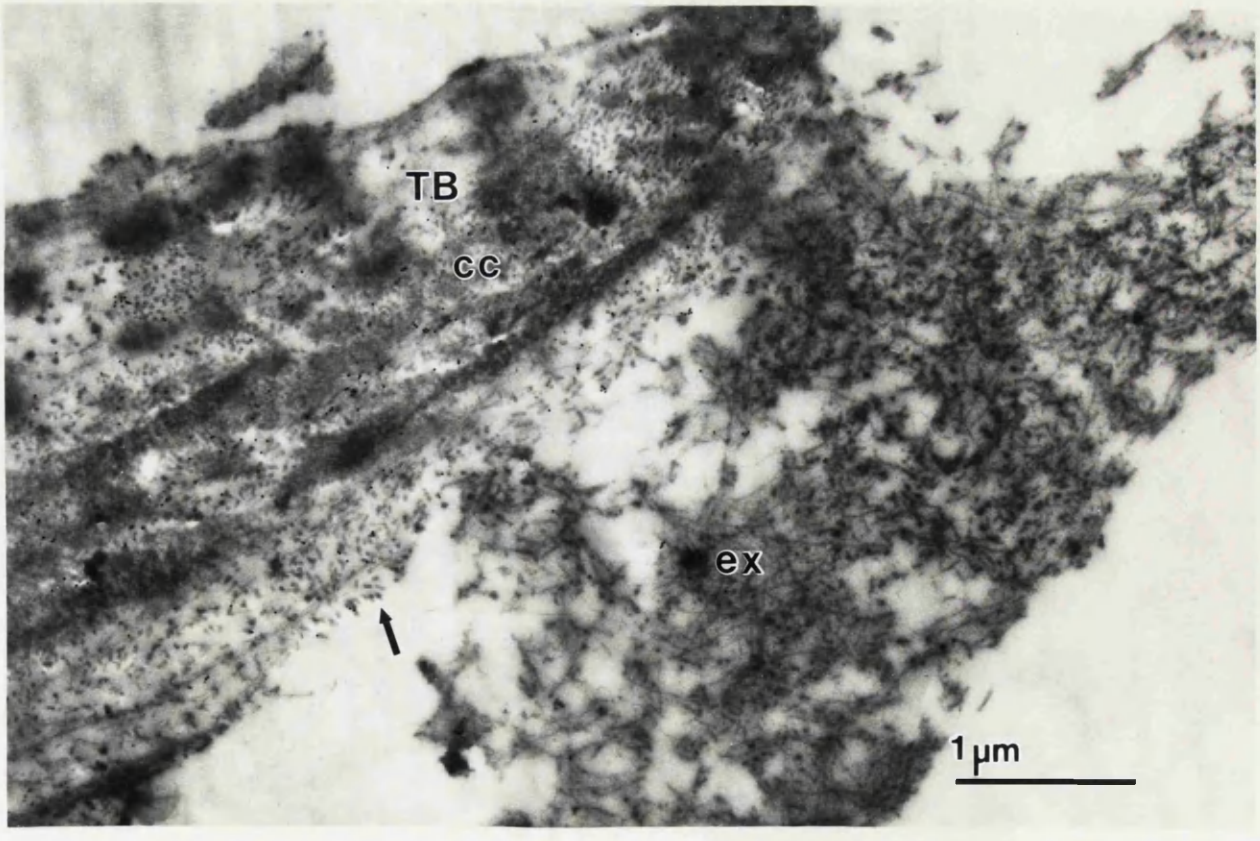


Figure 5.17: Collagen type I in the exfoliative trabecular meshwork. Labelling is present over the collagenous core(cc) of the trabecular beam(TB), but is absent from exfoliation material(ex) in the intertrabecular space. The endothelial cover of the trabecular beam is missing(arrow).

noted in one case where a clump of exfoliation material was attached to a trabecular beam denuded of endothelial cover. Sparse labelling was present in exfoliation material lying underneath the endothelial lining of Sclemm's canal in two specimens. The label however, was not located specifically on the exfoliative fibres but was randomly distributed and was thought to be associated with striated collagen fibrils. Collagen type I was convincingly demonstrated in the collagenous core of the trabecular beams (Fig 5.17). More specifically, the immunogold particles were restricted to collagen fibrils comprising the collagenous cores. Labelling of moderate intensity was also observed in the periphery of the trabecular beams. Collagen type I was present in the iris controls; the distribution of collagen type I there was identical to that described in section 5.1.2.

Discussion

Collagen type I was shown not to be an intrinsic constituent of the exfoliation material in the outflow system. By contrast, exfoliation aggregates observed within basement membranes and other iris tissue subcompartments label with antibodies against collagen type I. The question arises whether this phenomenon is due to the presence of collagen type I within the exfoliation compound, or is simply due to the presence of intrinsic collagen intermingled with collagen. The lack of immunolabelling for type I collagen from exfoliation deposits located in the empty spaces between trabecular beams provides evidence that the latter hypothesis is correct. The combined results of the present study and section 5.2.2 suggest that exfoliation syndrome may affect

the biosynthesis of collagen type I, but that this collagen is not an integral component of exfoliation material.

The qualitative distribution of collagen type I in the exfoliative meshwork was similar to that reported by Marshall et al (1991c) in the normal meshwork. No attempt in was made in the present study to analyse quantitative changes specific to the exfoliation outflow system for collagen type I. This because the normal aged outflow system does not provide suitable control data for the exfoliation glaucoma cases. Only the outflow system of primary open angle glaucoma patients can provide suitable control data for exfoliation glaucoma.

5.2.5 Summary

The immunogold technique combined with LR white embedding was employed for the study of the fine structural distribution of types I-V collagen in exfoliative iris tissue. Collagen types II, III and V were not identified as constituents of the exfoliation material in any region of the iris. Type I collagen was present within the exfoliation material aggregates in the vascular matrix (basement membrane) of exfoliative vessels and in stromal deposits. Subsequent studies on exfoliative meshwork confirmed that collagen type I is not an intrinsic constituent of the exfoliation material. Collagen type IV was present in the interfibrillar matrix of exfoliation material in the vascular matrix of exfoliative vessels, but was absent from stromal deposits of exfoliation material.

CHAPTER 6 IMMUNOGOLD ULTRASTRUCTURAL LOCALISATION OF LAMININ IN NORMAL AND EXFOLIATIVE IRIS

6.1 Laminin in the normal iris

6.1.1 Introduction

Little is known about the fine structural distribution and the functional role of laminin in the normal human ocular tissues (see section 2.5). To date, there have been three published studies on the subject (Das et al 1990a, Marshall et al 1990b; 1991a). The electron microscopic (EM) localisation of this macromolecule in the various ophthalmic tissues is important for understanding their normal function, as well as their alteration in disease (Morrison et al 1989, Das et al 1990a, Marshall 1991).

Laminin has not been previously studied in the normal iris. The investigation of the fine structural distribution of laminin in the normal aged iris serves two purposes. To evaluate the effect of the exfoliation syndrome on normal tissues it is necessary to know the normal distribution of laminin. Added to this, as iris is commonly involved in many ophthalmic and systemic disorders, this study would provide baseline information for future investigations.

6.1.2 Results

Seven normal iris specimens were studied (cases 6-12 in Appendix I). For details of the source of material used for

this study see section 3.3.3; the techniques applied and the control tissues are described in section 3.3.4.

Laminin was specifically localized to the basement membranes (vascular matrices) within the walls of blood vessels and to the extracellular matrix (ECM) in the dilator muscle region. No difference in the qualitative pattern of laminin distribution was noted between specimens. A summary of the positive immunogold localisation of laminin and the iris collagens (chapter 5) is shown diagrammatically in Fig 6.1. The specific features were as follows:

Vasculature

The label was most intense on the lamina densa of the vascular matrix surrounding the supporting cells of the larger iris vessels (Figs 6.2 & 6.3). Labelling for laminin was prominent in large and medium sized vessels (Fig 6.3), and greatly reduced, or absent in small vessels. When present in small vessels the label was more intense over the vascular matrix of the outer side of supporting cells. Of the 7 cases studied the highest intensity of labelling around small vessels was observed in the youngest individual (52 years old). In this particular specimen laminin was demonstrated throughout the whole of the iris vasculature. In contrast, laminin was markedly reduced in iris vessels from the oldest patient (81 years old).

Iris pigment epithelium

Laminin was localised to the ECM (basement membrane material) in which the basal muscular portion of the anterior iris

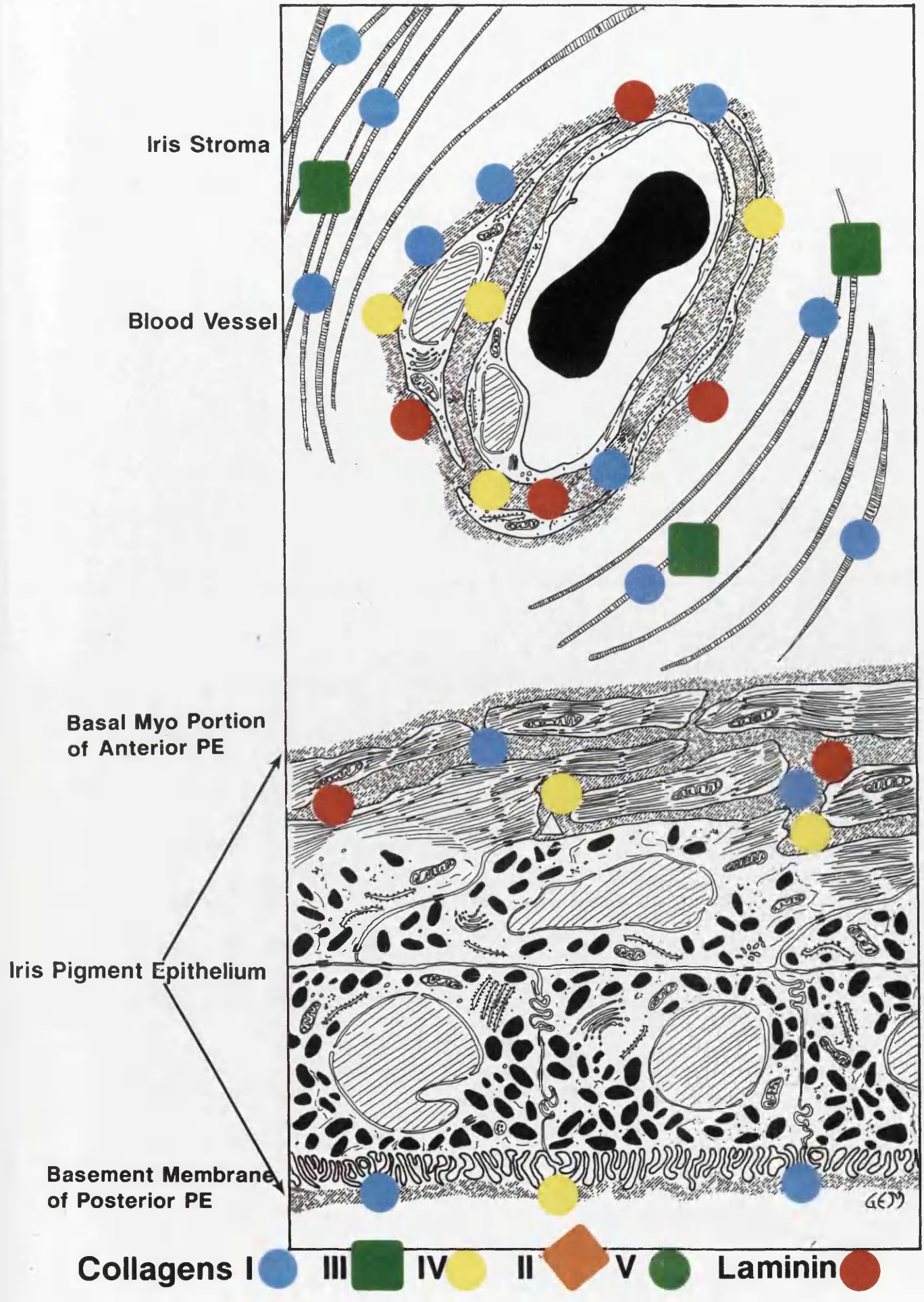


Figure 6.1: Summary diagram of positive localisation of laminin and collagens types I, III and IV in the normal aged iris. PE:pigmented epithelium.

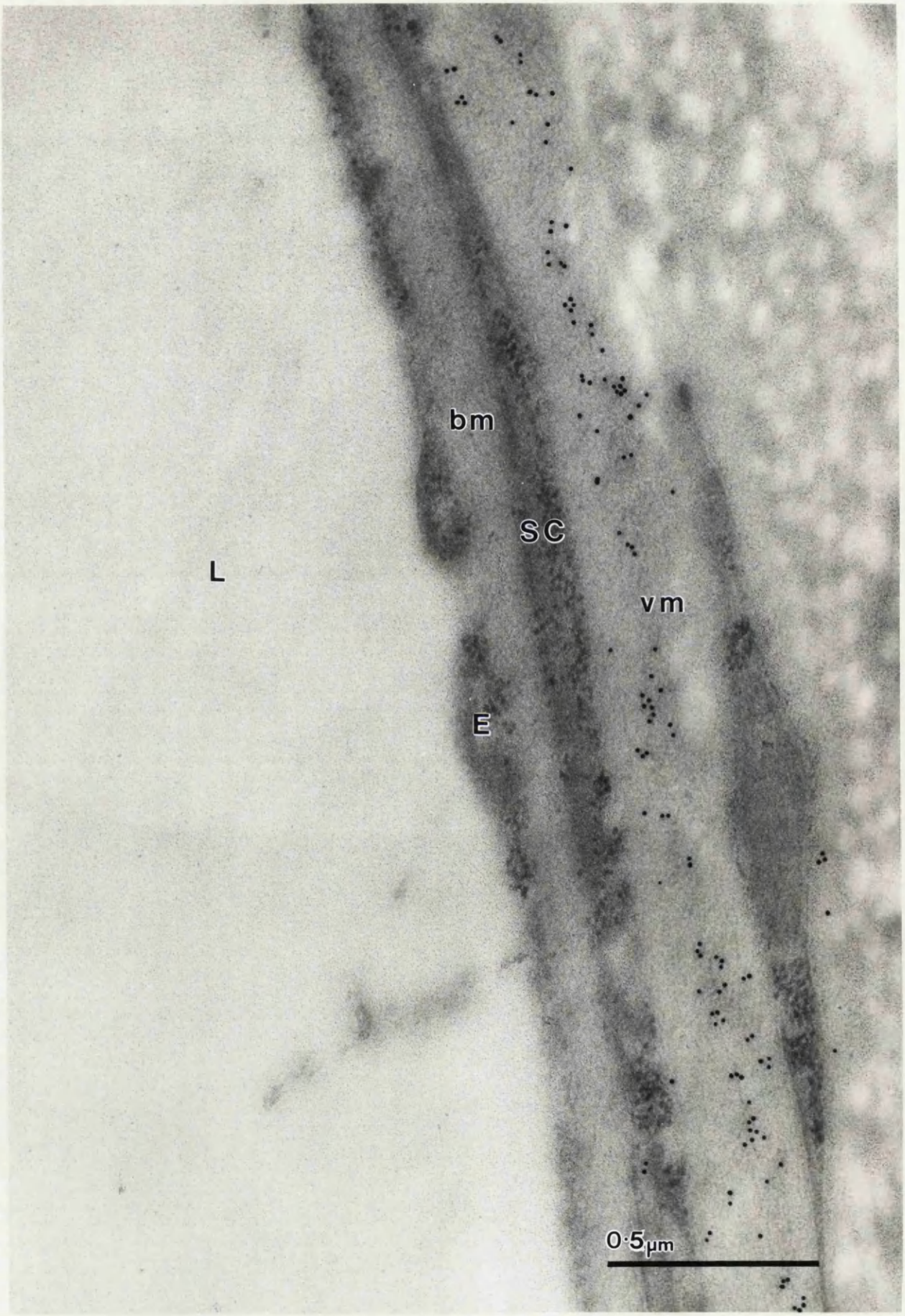


Figure 6.2: Labelling for laminin in the vascular matrix (vm) of the normal aged iris Immunogold particles are confined to the vascular matrix of supporting cells (SC). Labelling is absent from the basement membrane (bm)of the endothelium (E). L:lumen.

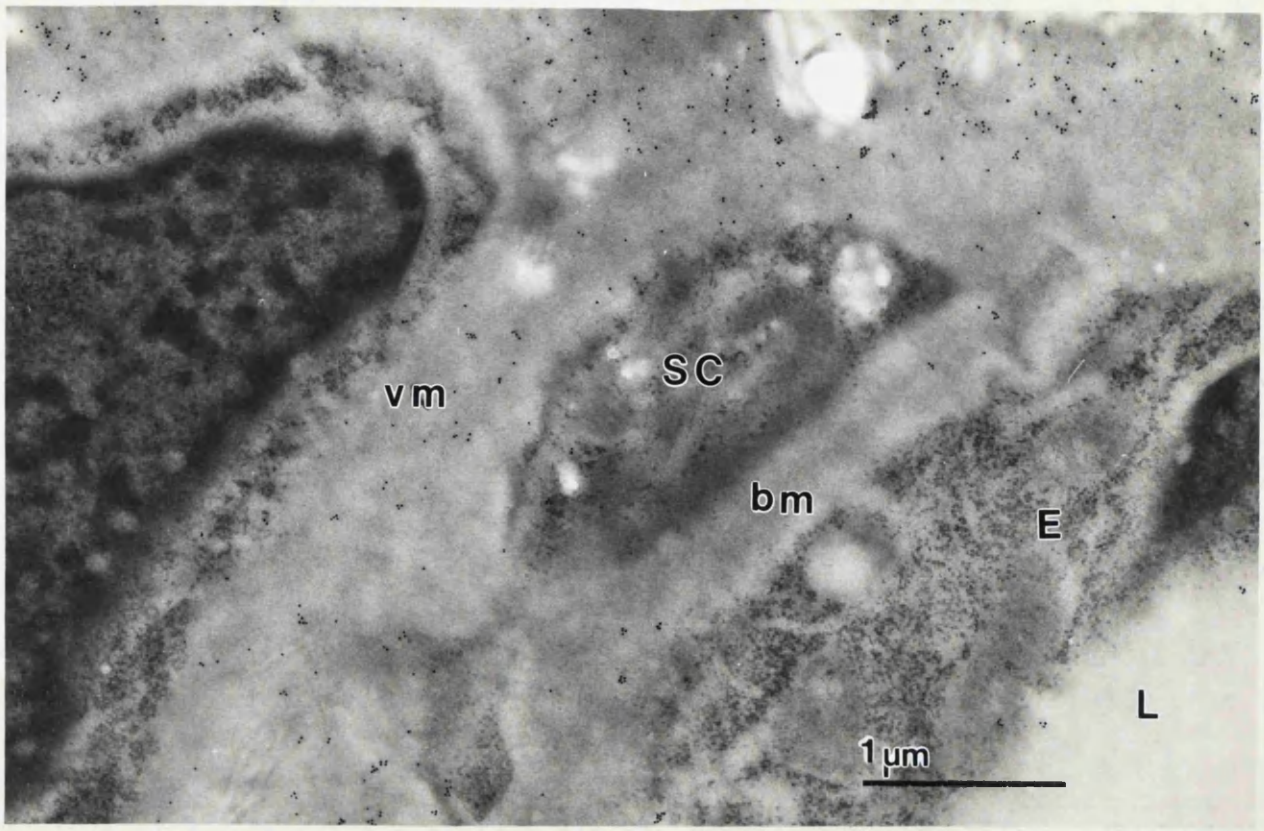


Figure 6.3: Laminin distribution in a large iris vessel. Labelling is most intense over the vascular matrix (vm) surrounding supporting cells (SC) and reduced the endothelial basement membrane (bm). L:lumen; E:endothelium.

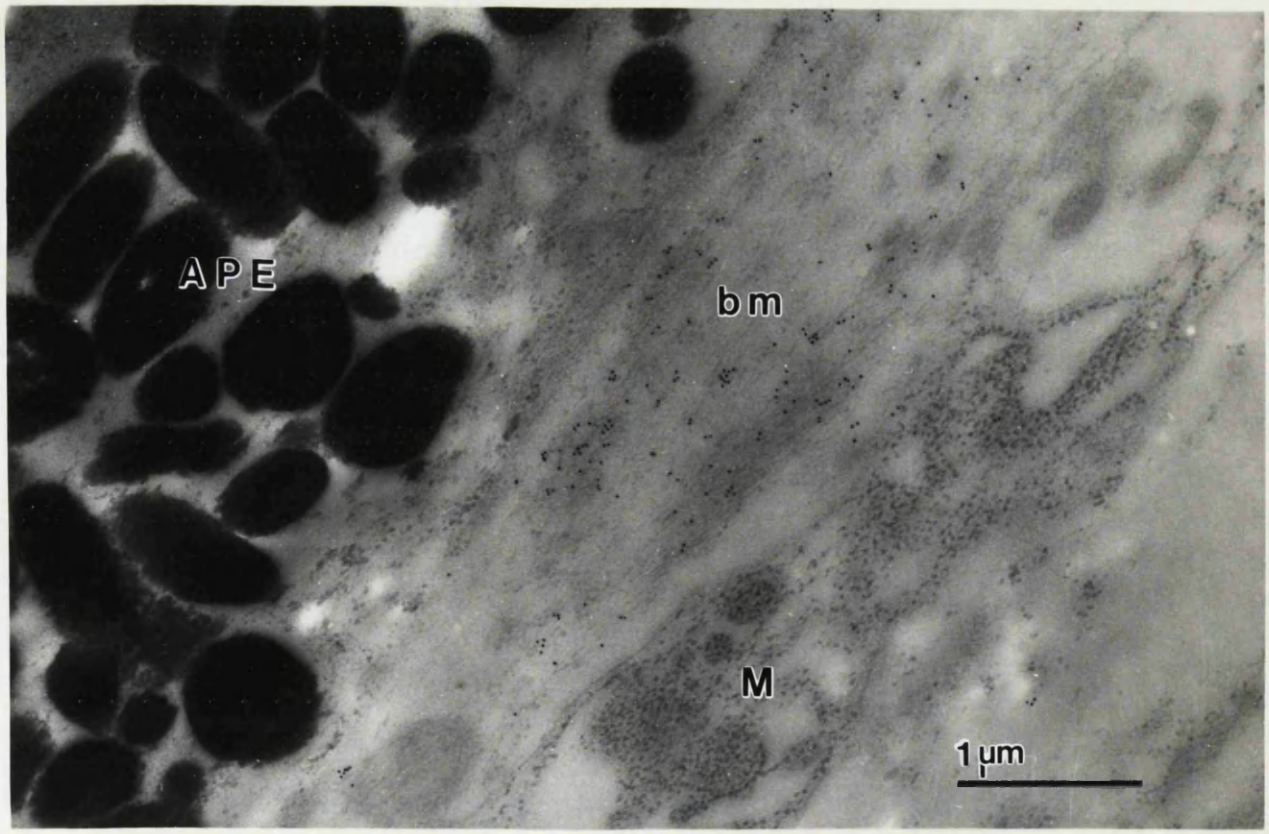


Figure 6.4: Laminin in anterior pigmented epithelium of the iris (APE). Gold particles are localised to clumps of filamentous electron dense basement membrane material (bm). Note basal myo process of anterior pigmented epithelium (M).

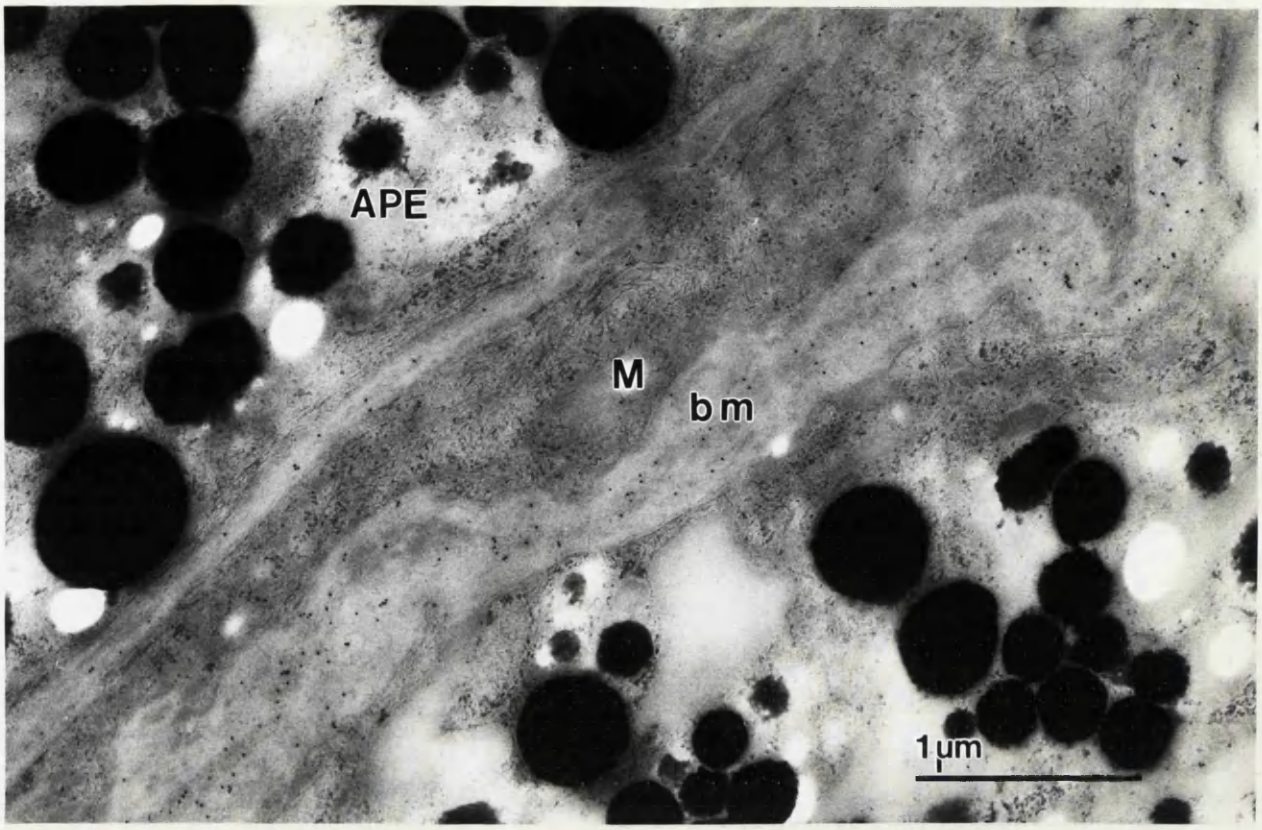


Figure 6.5: Laminin labelling of the electron dense core of the basement membrane (bm) surrounding macular portion (M) of anterior pigmented epithelium (APE).

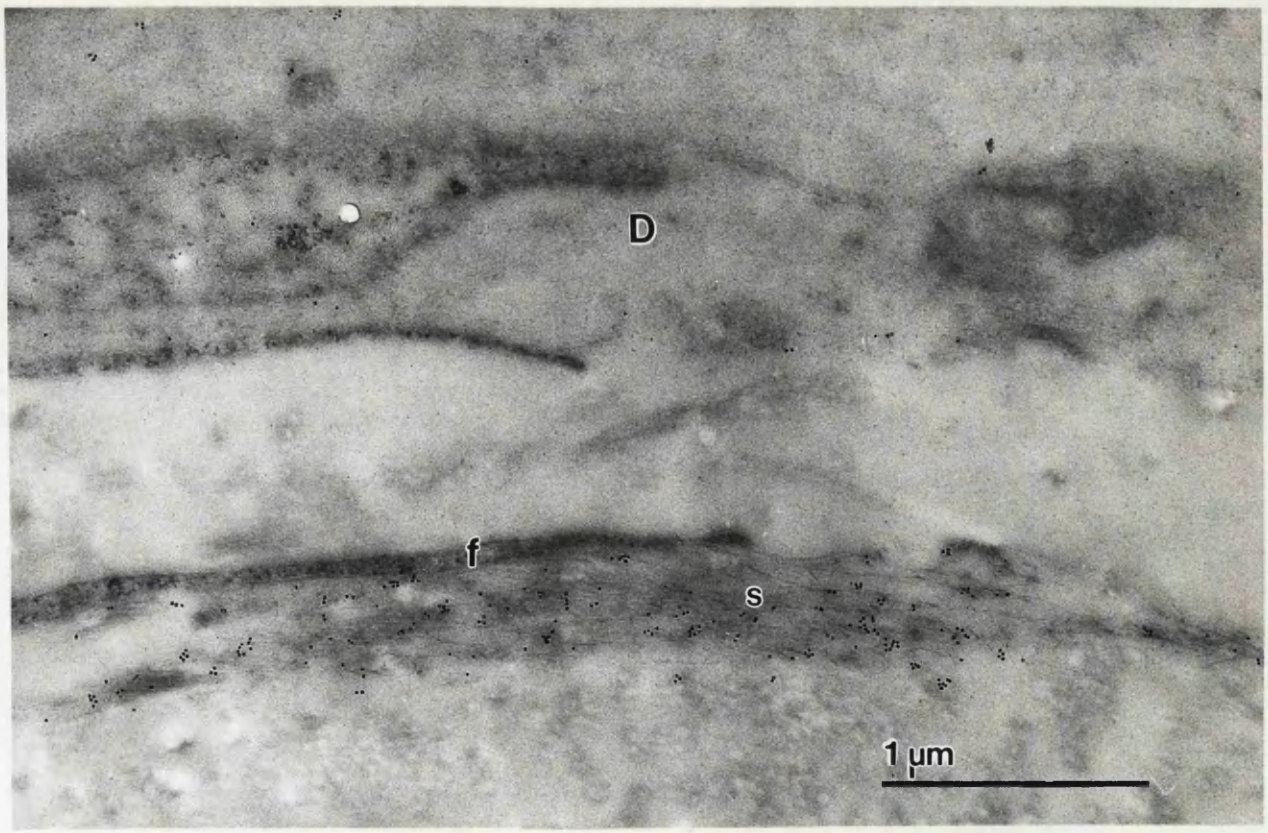


Figure 6.6: Intense labelling for laminin over bundles of parallel filaments adjacent to fibrocyte process (f). D:dilator muscle region.

pigmented epithelium was embedded, (Figs 6.4 & 6.5). Immunolabelling was mainly concentrated on dense bands of filaments within an amorphous granular substance filling the intercellular spaces of the muscular portion of the anterior pigmented epithelium. Labelling intensity increased in proportion to the quantity and density of the filamentous bands and was largely restricted to these electron dense areas in all of the normal iris specimens studied. Laminin was also demonstrated on the basement membrane of the anterior iris pigmented epithelium. The thin basement membrane of the posterior pigmented epithelium of the iris exhibited weak, but specific labelling. Intense labelling was noted in association with iris fibrocytes which form the boundary between the dilator muscle region and the iris stroma. Labelling was concentrated over filamentous bodies adjacent to these fibrocytes (Fig 6.6).

Stroma and anterior border layer

Gold particles were absent from the iris stroma and associated cells (fibrocytes, melanocytes, and mast cells). However, a discrete localized signal was present on small clusters of filaments associated with fibrocytes of the anterior border layer. Such labelling was generally absent from the outermost layer of fibrocytes.

6.1.3 Discussion

The biological functions of laminin have been reviewed in section 2.5. The following brief description serves as a basis for this discussion. Laminin is a large multidomain

glycoprotein which together with collagen type IV and heparan sulphate proteoglycan comprises most of the protein found in basement membranes (Timpl et al 1979, Abrahamson 1986, Martin & Timpl 1987, Charonis & Tsilibary 1990). Basement membranes are specialized sheets of extracellular matrix directly involved in a number of important biological processes (Vracko 1974, Timpl & Dziadek 1986, Martin & Timpl 1987). It is thought that individual components within basement membranes are responsible for particular functions.

It was of interest to note the distribution of laminin in the aged normal iris vasculature. Labelling for laminin was restricted principally to the ECM enclosing the supporting cells within the walls of the larger iris vessels. A similar pattern was also observed in smaller vessels where labelling was observed around supporting cells, but not over the lamina densa of the vascular endothelium. Essner & Lin (1988) also noted stronger labelling for laminin around the pericytes of cat retinal vessels. This pattern of laminin distribution may indicate that within the iris vasculature the synthesis of laminin is a responsibility of the supporting cells (myocytes and pericytes) rather than the endothelial cells. This conclusion is based on the general assumption that cells attached to basement membranes secrete most, if not all, of the ECM components present within that basement membrane (Ruggeri & Motta 1984, Martin & Timpl 1987, Trinkaus-Randall et al 1988, Tawara et al 1989).

By contrast, collagen type IV, (see section 5.1.2), exhibited more intense labelling in the subendothelial basement membrane of aged iris vessels than in the matrix around supporting cells. Consequently, it appears that in iris vessels endothelial cells secrete primarily collagen type IV, whilst laminin is produced mainly by supporting cells (myoepithelial cells and pericytes). This is important as differences in the production and utilization of laminin may contribute to variations in cell behaviour (Pratt et al 1985, Abrahamson 1986, Trinkaus-Randall et al 1988). It is relevant that a recent in vitro study demonstrated that aortic endothelial cell adhesion is preferentially promoted by collagen type IV when compared to laminin (Herbst et al 1988).

The observation that in the aged iris, laminin seems to be mainly present in the walls of thicker vessels indicates heterogeneity in the components of thick and thin vascular matrices within the iris. These findings are in accord with an immunogold quantitative study performed on a variety of animal basement membranes (Grant & Leblond 1988), and with results obtained in other ocular tissues (Das et al 1990a, Marshall et al 1990a; 1991e; 1992b). Abrahamson (1986) has emphasized that the chemical composition of basement membranes may vary according to the demands of particular tissues.

The significance of the presence of laminin in the matrix (basement membrane material) in which the dilator muscle is embedded is not completely understood. It is possible that

in this region laminin is acting as an adhesive factor between individual myocytes and between myocytes and the laminin rich posterior stroma region (Fig 6.6). The adhesive properties of laminin have been well documented (Abrahamson 1986, Martin & Timpl 1987, Adair & Mecham 1990). Its presence in the matrix may be instrumental for the orderly contraction of the dilator muscle. Therefore, changes in its distribution within this matrix may well interfere with the dilatation of the normal iris. Likewise, the significance of the label associated with the filamentous bundles bordering iris stromal fibrocytes is also difficult to assess, as the functional role of these cells in the iris remains unclear. These iris fibrocytes may separate the dilator muscle from the iris stroma and synthesise the matrix in which the dilator muscle is embedded. An intriguing possibility is that this layer anchors the stroma to the muscle.

The ECM possesses important regulatory properties governing cell behaviour (Abbot 1988, Getzenberg et al 1990, Streuli & Bissell 1990, Adair & Mecham 1990). Alterations in the interaction between cells and their matrices can precede and may eventually result in ocular tissue malfunction (Ben-Zvi et al 1986, Glaser 1988, Caldwell 1989, Das et al 1990b, Marshall 1991). It is conceivable that during the ageing process quantitative alterations of specific ECM components occur within ocular tissues and may result in functional impairment. Such a process could account for the reduction of laminin associated with ageing in the material of this study. Nevertheless, the effect of ageing upon the quantity of laminin can only be confirmed with the use of an

immunogold quantitation technique similar to that employed by Grant & Leblond (1988), or Das et al (1990b). However, quantitation of immunogold particles has many serious drawbacks.

The results of the present study could not be correlated with any previous human or animal iris study, as a literature search failed to retrieve published reports on the subject at the EM, or LM level. It is surprising that so little attention has been paid to the distribution of laminin in the normal iris considering that iris has many unique structural and functional properties. This work provides information in order to better comprehend the functional role of laminin in the normal aged iris.

6.2 Laminin in the exfoliative iris

6.2.1 Introduction

As stated before (section 1.5.1), it has long been speculated that exfoliation material may be abnormal basement membrane material synthesized at multiple sites by defective ageing cells. Since basement membranes contain collagenous and non-collagenous components, disordered synthesis, or degradation of collagenous and/or non-collagenous components may be a significant feature of the disorder. In normal tissue one non-collagenous component of basement membranes, namely the glycoprotein laminin, has been identified by established immunocytochemical techniques (Schittny et al 1988, Das et al 1990a, Marshall et al 1990a; 1990b). Therefore an attempt was made to identify laminin in the exfoliation material.

6.2.2 Results

Material for this study was provided by iridectomies performed in 13 patients with the clinical diagnosis of exfoliation syndrome (2 cases) and exfoliation glaucoma (11 cases), (see Appendix II). The specimens were fixed and processed according to the methods given in section 3.3. In all of the exfoliative iris specimens studied, exfoliation material was identified in the iris by LM examination of LR white semithin sections and subsequent TEM examination of ultrathin sections. Exfoliation aggregates were observed mainly around and within the vascular matrices of iris

vessels. The ultrastructural distribution of exfoliation material was similar to that seen in section 4.3. The specific immunocytochemical results are as follows:

Exfoliation material

Recognition of the exfoliation material was relatively easy in LR White processed tissue (Figs 6.7, 6.8 & 6.9). This was due to a difference in tissue contrast in comparison with conventionally processed tissue and it resulted in greater detail in the ECM components. In addition, larger amounts of ECM material were retained with the LR white embedding procedure. This often resulted in more prominent interfibrillar spaces (Fig 6.7). These spaces which contain the interfibrillar matrix of exfoliation material, did not appear to contain other ECM components, with the exception of iris granules (alternatively referred to as dense bodies). In only a few cases were collagen fibrils intermingled with exfoliation fibres in the boundary of exfoliation aggregates.

Intense labelling for laminin was observed over areas where exfoliation material was present within the iris (Figs 6.7 to 6.12). This finding was consistent in all exfoliative iris cases. The signal obtained was regarded as extremely specific on the basis of the absence of gold particles from the nuclei, cell cytoplasm and lumina of vessels (the criteria of specificity are explained in section 3.4.7). Better antigenic preservation was achieved in iris tissue fixed with paraformaldehyde 4% only, but this was at some expense of ultrastructural preservation (Fig 6.12). In tissue fixed with paraformaldehyde and glutaraldehyde (0.1-

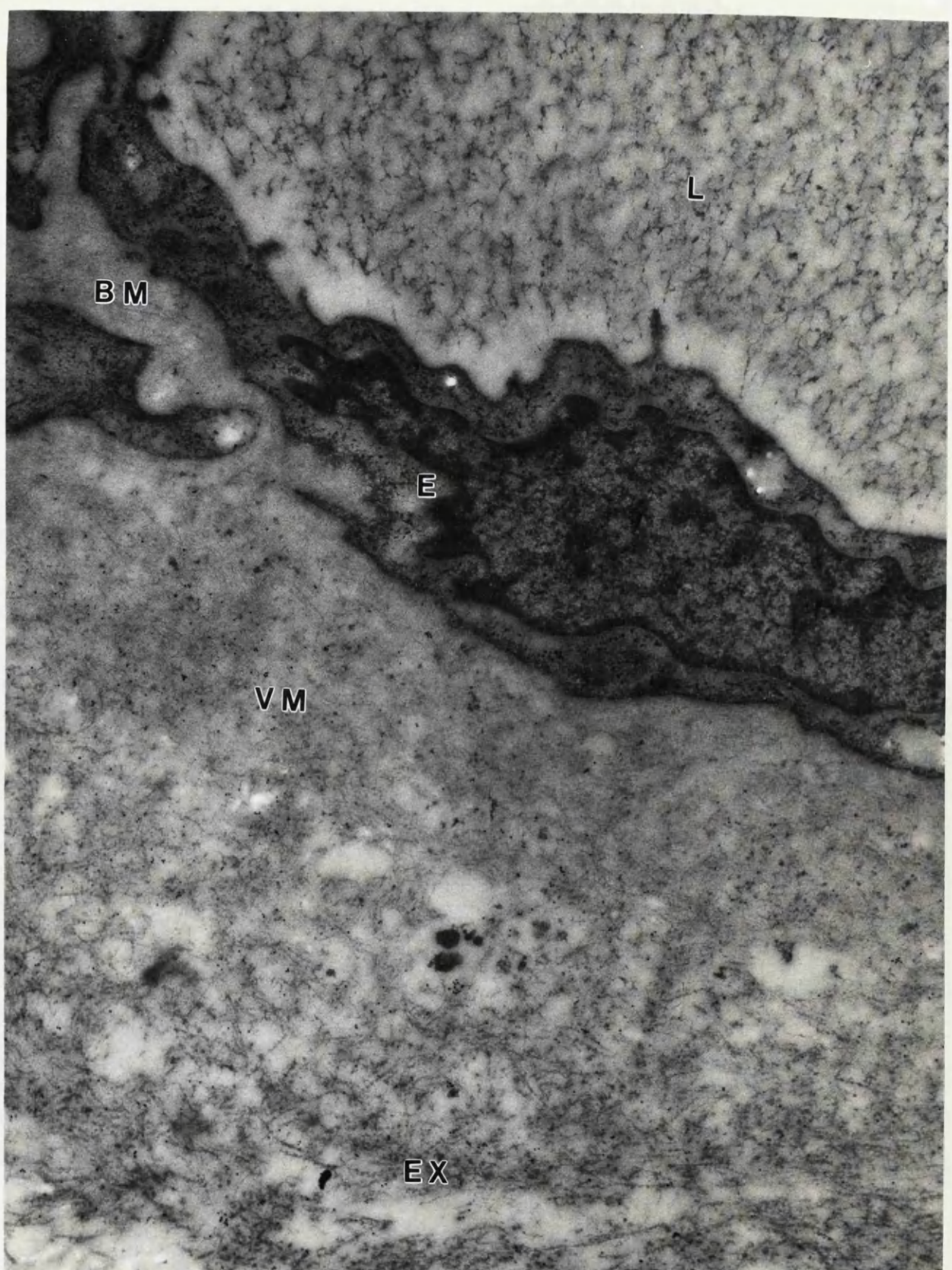


Figure 6.7: Exfoliative iris. Intense labelling for laminin is restricted to the vascular matrix (VM) infiltrated with exfoliation material and to exfoliation aggregates (EX) outwith the vascular matrix. The endothelial basement membrane exhibits a signal free zone (BM). L:lumen, E:endothelium.

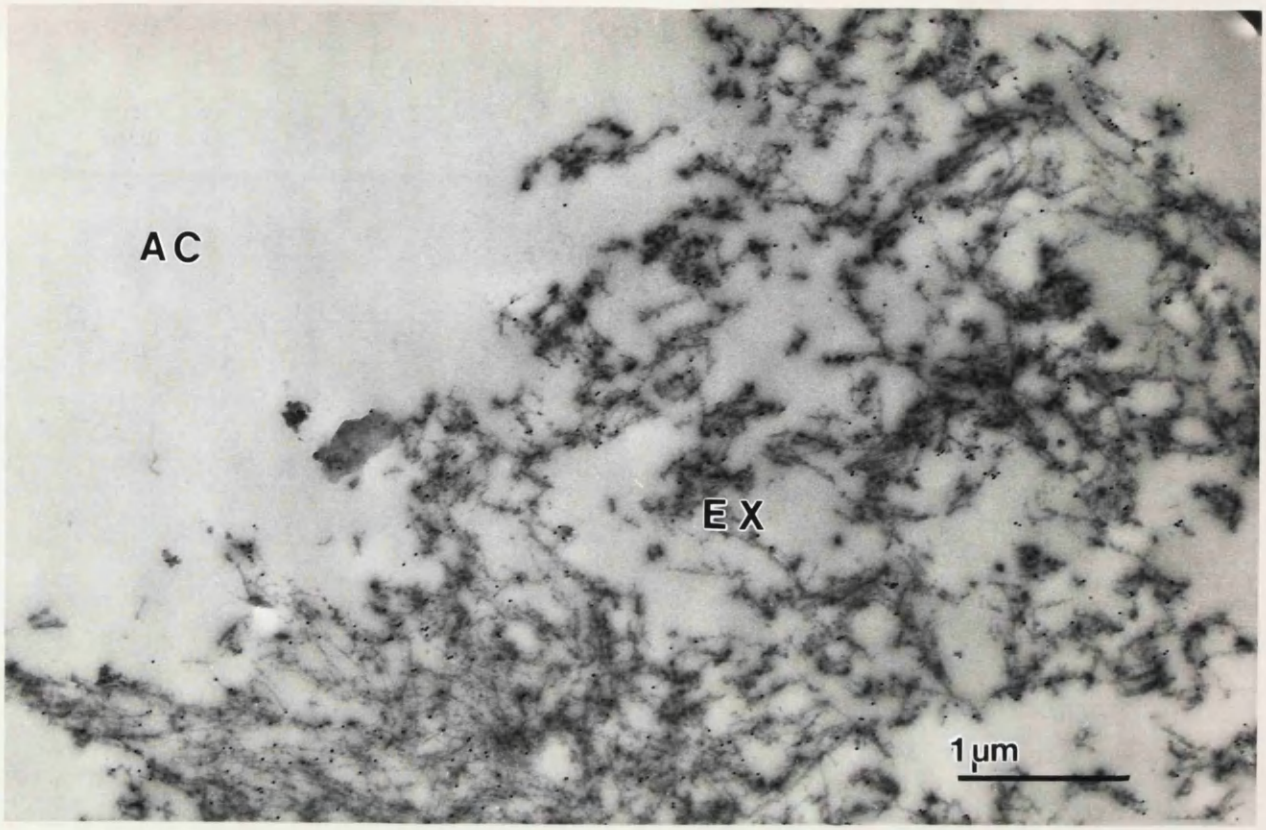


Figure 6.8: Loosely arranged exfoliation material (EX) excrescence on the anterior iris surface. Laminin is localised to the fibrillar component. Interfibrillar matrix is absent. AC:anterior chamber.

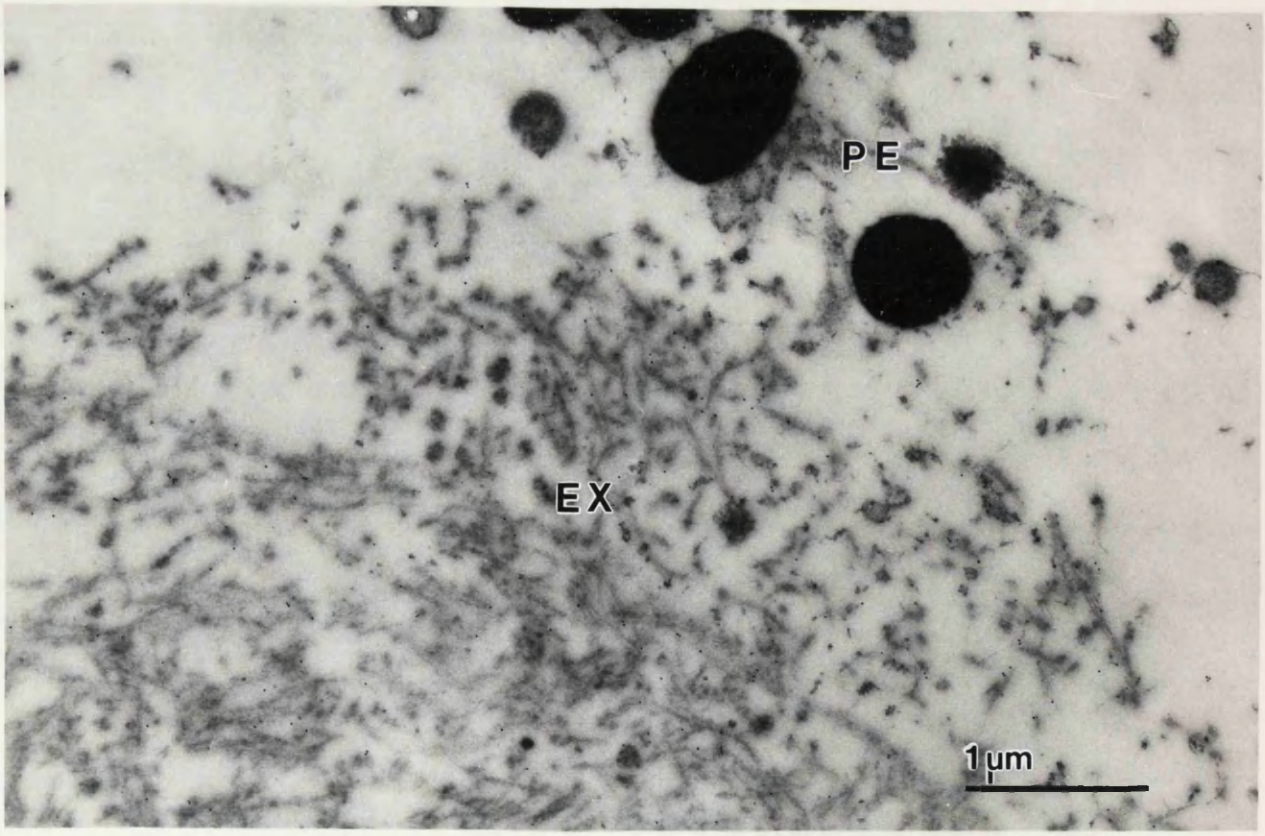


Figure 6.9: Exfoliation material (EX) labelled with laminin in the basal region of the posterior pigmented epithelium (PE), whose cellular integrity is absent.

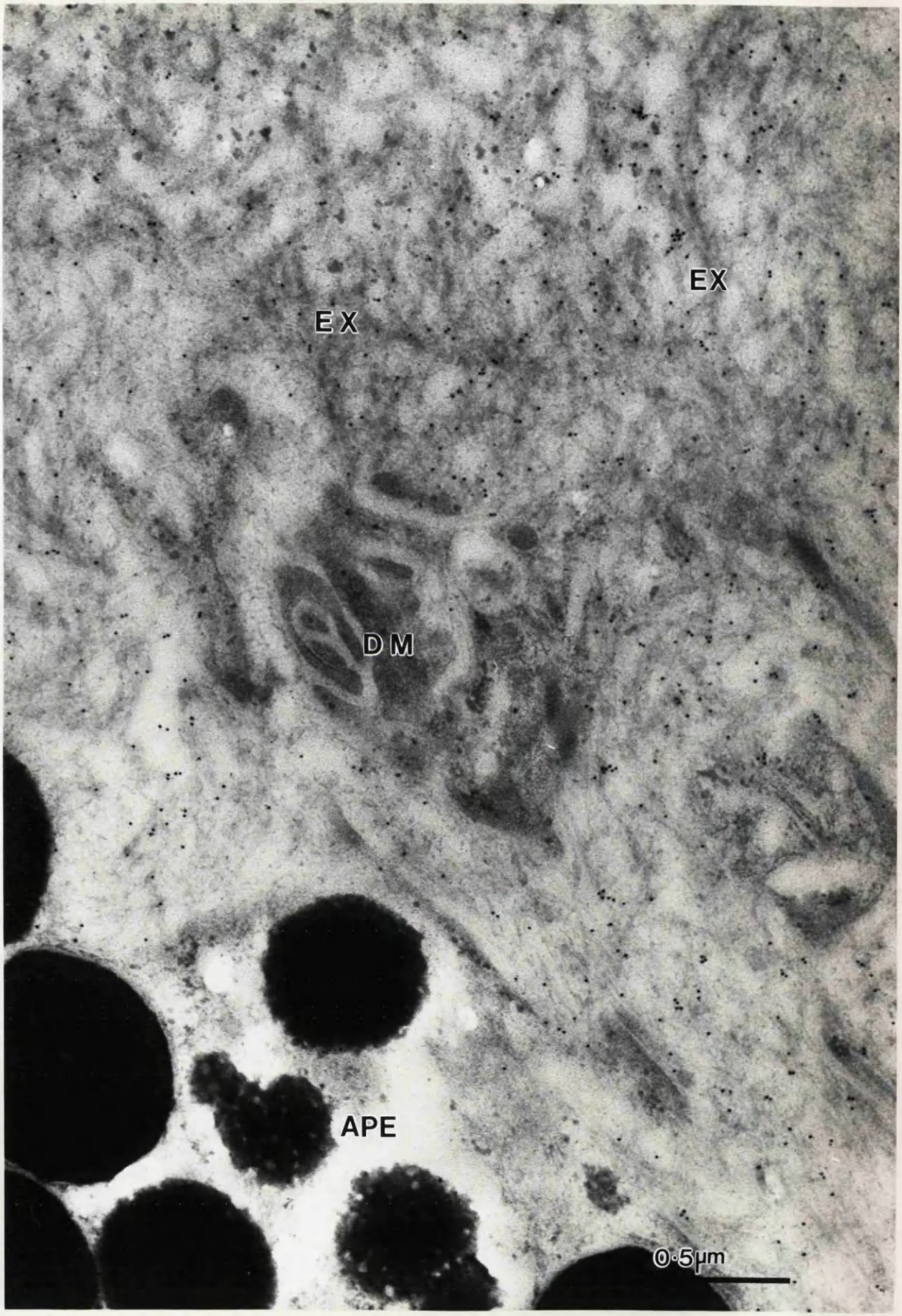


Figure 6.10: Laminin located in exfoliation material (EX) within the muscular portion of the anterior pigmented epithelium. Exfoliation material is packed in the vicinity of the dilator muscle processes (DM).

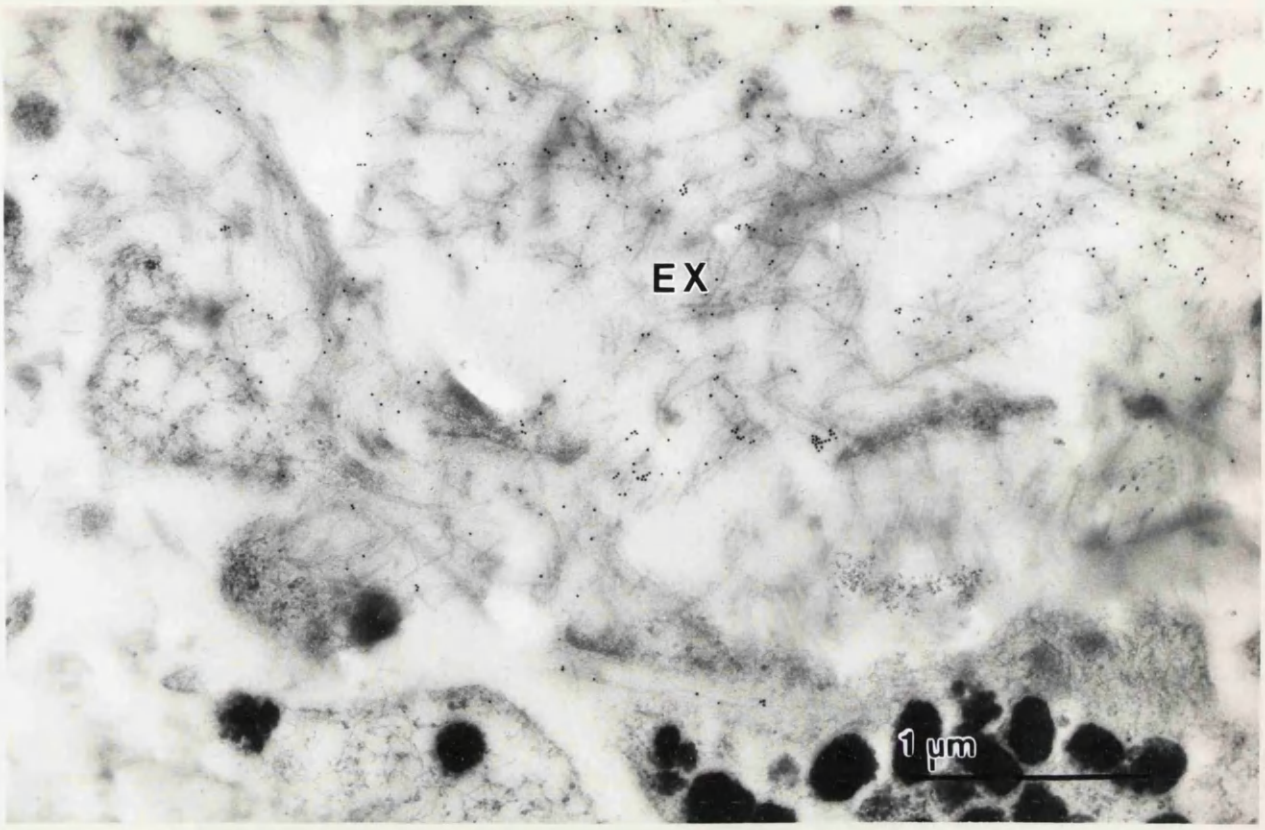


Figure 6.11: Laminin labelled exfoliation material (EX) in anterior border layer of exfoliative iris. As in the previous figures, glutaraldehyde fixation has highlighted the fibrillar nature of exfoliation material.

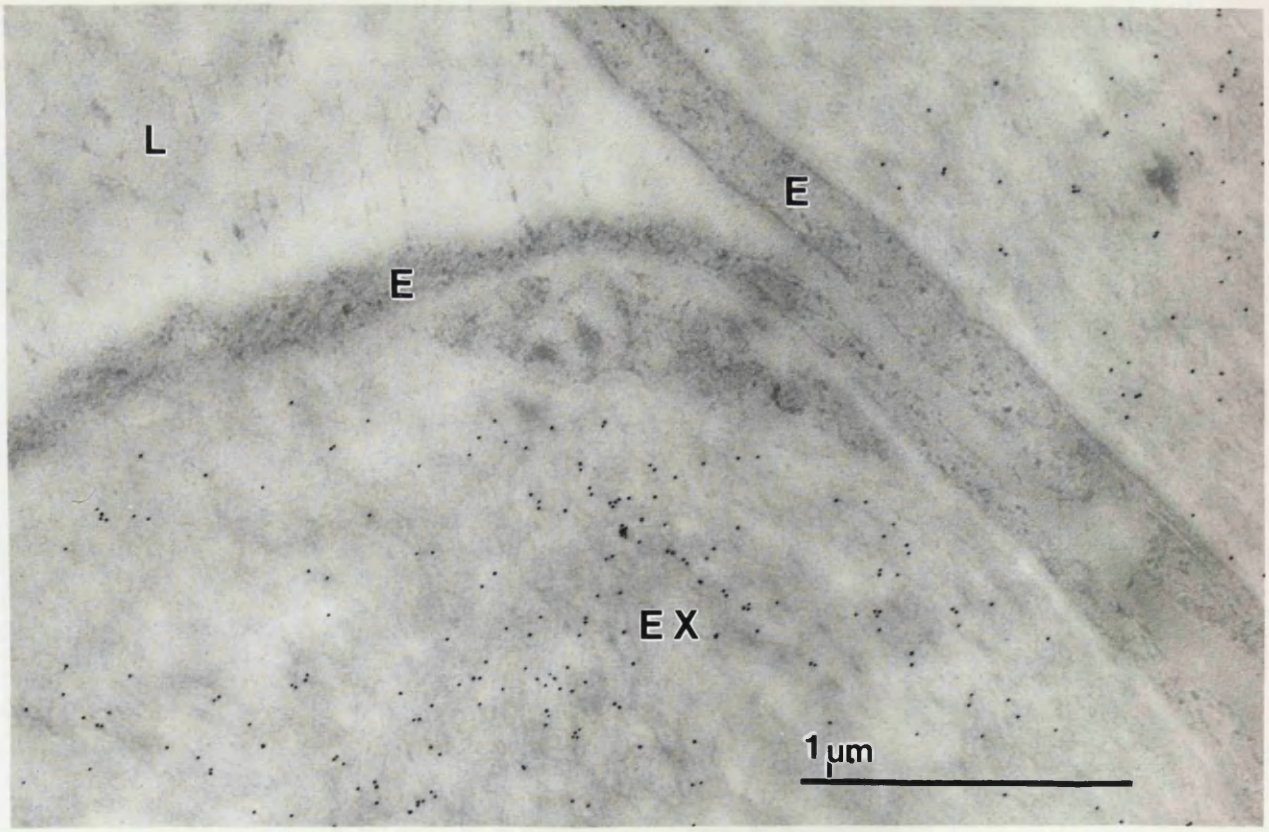


Figure 6.12: Exfoliation material labelled with laminin in wall of degenerate iris vesse The endothelium (E) is atrophic. This figure illustrates the poor preservation of the fibrillar nature of exfoliation material (EX) with the omission of glutaraldehyde from the fixative. L:lumen.

0.75%) labelling was less intense, but still highly specific.

Laminin was present predominantly in the exfoliation fibres but weak labelling was also observed on the electron-lucent interfibrillar matrix (Figs 6.7 to 6.11). In four of the specimens, vegetations consisting of laminin labelled exfoliation material were seen to project into the anterior chamber from the anterior border layer (Fig 6.8). Within an individual case the intensity of the signal was proportional to the density of the exfoliative deposits. Intense immunolabelling was observed on closely packed fibres of exfoliation material around large iris vessels in all cases (Figs 6.7 & 6.12) and in the dilator muscle region in two of the cases (Fig 6.10).

Iris vasculature

In exfoliation positive vessels laminin was almost exclusively associated with the exfoliation material (Fig 6.7 & 6.12). In vessels where exfoliation material was not identified (exfoliation free vessels), vascular matrix labelling was reduced or absent in comparison with normal iris vessels in control specimens. This phenomenon was considered in more depth in chapter 7. In all exfoliation positive vessels a thin signal-free zone was observed corresponding to the lamina lucida of endothelial basement membranes. The label for laminin closely followed the configuration of the ribbon-like exfoliative deposits focally distributed around some iris vessels. Some of the exfoliative vessels exhibited attenuation of the endothelial cells and atrophy of the myocytes. This was particularly

evident in advanced cases of exfoliation glaucoma.

Iris pigment epithelium

The association between exfoliation material and the dilator muscle was intriguing. In two of the specimens, exfoliation material had infiltrated the basal muscular portion of the anterior iris pigment epithelium causing a marked disturbance in the architecture of this layer (Fig 6.10). Laminin was again mainly localised to the exfoliation fibres (Fig 6.10). In these cases exfoliation material surrounded the muscular processes of the anterior iris pigmented epithelium. It was observed between the intact walls of adjacent epithelial cells, but did not penetrate the cell membrane. In every specimen exfoliation material was not noted between the two layers of the pigment epithelium. In only one case laminin labelled exfoliation material was seen on the basal surface of the posterior pigmented epithelium of the iris (Fig 6.9).

Controls for normal and diseased iris

Background labelling of negative serum controls was virtually absent. In the corneal positive controls employed in this study laminin was demonstrated in the basement membrane complex of the corneal epithelium (chapter 7, Fig 7.15). The fine structural distribution of laminin in the human aged cornea were in agreement with previously described immunohistochemical results for human cornea at the LM (Ben-Zvi et al 1986) and EM level (Marshall et al 1991a). In the exfoliative meshwork tissue controls, intense immunolabelling for laminin was observed in clumps of exfoliation material lying in the intertrabecular spaces and in the cribriform

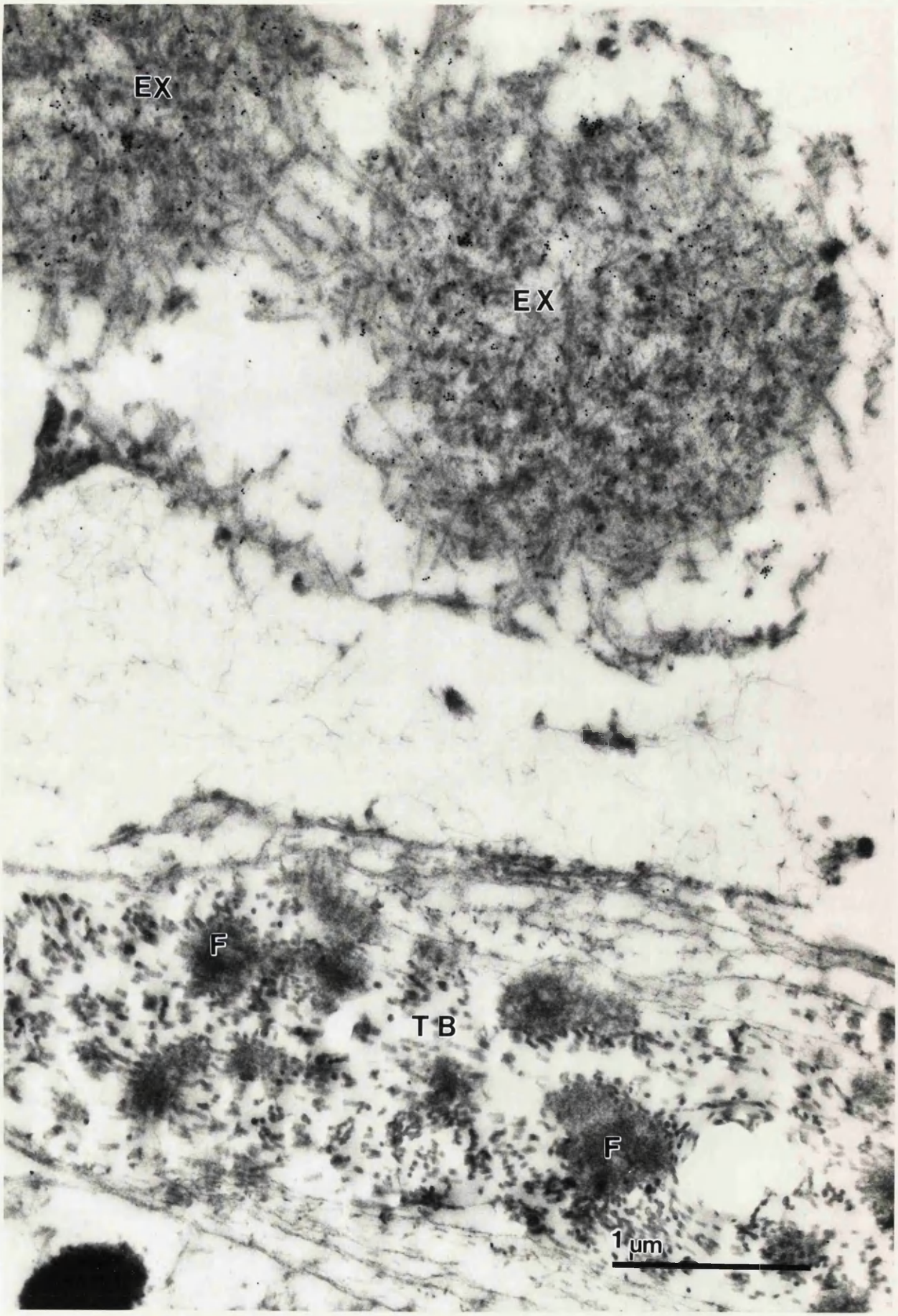


Figure 6.13: Exfoliative meshwork. Intense immunolabelling for laminin in an aggregate of exfoliation material (EX) lying within the intertrabecular space. The trabecular beam (TB), including elastic like fibres (F) is devoid of immunogold particles.

layer (Fig 6.13, see also controls in chapter 7).

6.2.3 Discussion

This is the first study that has shown a molecule with the antigenic characteristics of laminin to be an integral component of exfoliation material. Whether this molecule is identical to laminin found in normal ECM, or an aberrant form of laminin synthesised only in the exfoliation syndrome remains uncertain. However, since a signal for laminin was present in exfoliation free vessels within the same specimen and in the positive controls it is justified to assume that laminin is an important constituent of exfoliation material.

Intense specific immunolabelling associated with exfoliation aggregates was obtained in all specimens. Labelling was principally present on the exfoliation fibres, with a low density of immunogold label associated with the interfibrillar matrix. At high magnification immunolabelling was shown to be evenly distributed over the exfoliation fibres. Moreover, the labelling density increased with increasing packing of exfoliation fibres. Therefore, these results demonstrate that laminin constitutes an integral component of the exfoliation fibres and to a lesser extent to that of the interfibrillar matrix.

LR white processing resulted in retention of a larger amount of interfibrillar matrix. There is histochemical and morphological evidence that this electron lucent matrix may contain proteoglycans and it has been suggested that it

excludes other macromolecules and particles from penetrating into the exfoliation aggregates (Davanger & Pedersen 1975, Davanger 1977; 1978; 1980). The latter suggestion is supported by the present study. The presence of iris granules (dense bodies) within the exfoliation material aggregates is in agreement with previous investigations (Ringvold 1970a; 1988, Dark et al 1977, Dark & Streeten 1990). The significance of these granules remains unclear.

In diseased iris vessels, laminin was principally localised in exfoliation material and considerably reduced in exfoliation free vascular matrix (basement membrane). This reduction of laminin in exfoliation free vascular matrix may reflect an abnormal pattern of laminin utilization (see also chapter 7). This suggests that laminin, which normally is incorporated into the basement membrane by basement membrane-producing cells, is being diverted to the formation of exfoliation material. This is not surprising, as the cells that produce the matrix components for the ocular basement membranes are the same as those proposed for the synthesis of exfoliation material (Ringvold 1969; 1988, Ghosh & Speakman 1974, Eagle et al 1979, Seland 1985) The triggering mechanism behind this functional conversion awaits clarification. In the past, a 'metabolic mechanism' has been suggested for the pathogenesis of exfoliation syndrome (Bertelsen et al 1964, Dickson & Ramsey 1979, Mizuno et al 1980, Dark & Streeten 1982, Morrison & Green 1988).

The reduction of laminin from the iris vascular matrices surrounding supporting cells and the ensuing alterations of

its binding with the other basement membrane constituents, like heparan sulphate proteoglycan and collagen type IV, could compromise the structural integrity and the filtration properties of iris vascular basement membranes. This underlying mechanism may account for the increased permeability of exfoliative iris vessels visualized by fluorescein angiography (Vannas 1969, Laatikainen 1971, Friedburg & Bischof 1982, Brooks & Gillies 1987).

Another point of clinical interest was the relationship between exfoliation material and the iris dilator muscle. In two exfoliative specimens there was extensive infiltration of the dilator muscle ECM with laminin labelled exfoliative fibres and this was associated with atrophy of the muscle cells. This phenomenon together with a potential alteration of the laminin distribution in the embedding matrix of the dilator muscle could account for the impaired dilatation commonly seen in patients with exfoliation syndrome and glaucoma (Kristensen 1965, Bartholomew 1970a, Carpel 1988, Hovding 1988, Olivius et al 1989, Metge et al 1989, Pedersen 1990). The adhesive properties of laminin may be instrumental in the adherence of exfoliation aggregates to ocular structures, such as the anterior hyaloid face (Malling 1938, Sunde 1956, Petersen 1958) and the corneal endothelium (Sugar et al 1976, Brooks et al 1988). It is also conceivable that laminin 'adheres' to the exfoliation aggregates.

Despite the fact that most investigators in the field have favoured the involvement of basement membrane-producing cells

in the production of exfoliation material (Ringvold 1969; 1988, Layden & Shaffer 1973, Sugar et al 1976, Layden 1982, Seland 1985, Yanoff & Fine 1989), there has been less conformity on whether or not exfoliation material contains basement membrane components (see section 1.5.1). Histochemical evidence has already been advanced to indicate that exfoliation material may contain glycoproteins (Streeten et al 1986a, Morrison & Green 1988) and proteoglycans (Davanger 1980, Harnisch et al 1981), which are major components of basement membranes. However, a group of investigators has repeatedly shown an association between exfoliation material in the conjunctiva and skin of patients with exfoliation syndrome and components of the elastic system-elastin and amyloid P- (section 1.3.4). These components, with the possible exception of amyloid P (Li et al 1989), are not thought to be intrinsic constituents of normal basement membranes. Nevertheless, on the grounds of the variations seen in the histochemical staining properties, it is clear that exfoliation material is a composite of more than one substance (Davanger & Pedersen 1975, Baba 1983, Streeten et al 1984, Jerndal 1986, Tarkkanen 1986, Ringvold 1988, Streeten et al 1990). Furthermore, it has even been suggested that the composition of exfoliation material may vary in different tissues (Streeten et al 1987; 1990).

Although exfoliation fibres does not appear ultrastructurally similar to elastic fibres, it is possible that elastin and other constituents of the elastic system are produced as part of an aberrant ECM synthesis, a process which is probably orchestrated by basement membrane-producing cells.

The results of the present investigation suggest that within the iris vasculature, exfoliation material is synthesized by the vascular supporting cells. The presence of laminin in the basement membrane of the iris pigment epithelium suggests that these epithelial cells may also be involved in the synthesis of exfoliation material. The inconsistent presence of exfoliation material in this anatomical region however is difficult to explain. This can only be confirmed by the immunocytochemical investigation of whole eyes, as surgical iridectomy specimens represent only a small fraction of the whole iris tissue. Moreover, such specimens are unsuitable for the detailed study of the posterior pigmented epithelium of the exfoliative iris.

In conclusion, it is postulated that in the exfoliative iris the synthesis of exfoliation material is initiated at the molecular level by aberrant basement membrane biosynthesis, which also results in laminin being incorporated in the exfoliation material. In that context, it is reasonable to assume that the triggering factor underlying exfoliation syndrome is a disturbance of a specific factor which controls the orderly synthesis and remodelling of ECM. The main challenge however, remains the identification of this aberration as a first step towards prevention of this common blinding disorder.

6.2.4 Summary

Immunogold labelling was applied at the ultrastructural level to the identification of the glycoprotein laminin in the normal and exfoliative iris. In the normal iris, laminin was localised in iris basement membranes and in the extracellular matrix (basement membrane material) in which the dilator muscle is embedded. In the normal vascular basement membranes (vascular matrices) laminin was identified in the matrix surrounding vascular supporting cells, but not beneath the endothelium.

Immunoelectron microscopic studies of exfoliative iris tissue revealed the presence of laminin in the fibrillar component of exfoliation material. The immunogold label was uniformly distributed on the exfoliation fibres and increased with increased density of exfoliation aggregates. Deposition of laminin labelled exfoliation material in the dilator muscle in two cases was a noteworthy feature, as was an apparent depletion of laminin in the vascular matrix of ostensibly unaffected vessels.

CHAPTER 7 IRIS VASCULOPATHY IN THE EXFOLIATION SYNDROME: AN IMMUNOCYTOCHEMICAL STUDY

7.1 Introduction

Iris vasculopathy is a well recognized but poorly understood pathological feature of the exfoliation syndrome (Ringvold 1969; 1970a; 1970c, Vannas 1969, Anastasi et al 1974, Brooks & Gillies 1987). Conventional LM and TEM investigations have revealed abnormal accumulations of, as yet, unknown extracellular matrix (ECM) components within the iridic vascular wall, often in conjunction with deposition of exfoliation aggregates (Ringvold 1969, Ghosh & Speakman 1974, Harnisch 1977, Shimizu 1985 and section 4.4). In advanced disease the deposition, or infiltration of exfoliation material in the iridic vascular matrix is frequently accompanied with a varied degree of endothelial degeneration and, ultimately, the obliteration of the lumen in some vessels (Ringvold 1970a, Ghosh & Speakman 1974, Ringvold & Davanger 1981, Spinelli et al 1985).

The conventional morphological study of the exfoliative iris (chapter 4) and the immunogold studies of collagen types I and IV (chapter 5) and laminin (chapter 6) in the normal and exfoliative iris provided the background to the present chapter. The specific vascular matrix alterations that account for the ultrastructural features seen in exfoliative vasculopathy have not been investigated previously. Laminin, a major basement membrane constituent, has been shown to be an integral component of the exfoliation material (section

6.2.2) and collagen types I and IV have been identified in the normal iris vascular matrix (section 5.1.2). In this chapter the changes of these three ECM components in the various stages of exfoliation vasculopathy will be considered. Collagen type III was absent from the vascular matrix of normal and exfoliative vessels (section 5.1.2) and therefore was not considered here. The study of the vascular matrix of the affected and unaffected iris vessels merits further investigation. It was hoped that the immunogold study of vascular matrix changes could furnish important clues as to the aetiology and pathogenesis of the disorder.

7.2 Results

Fifteen exfoliative iris specimens (cases 1-15 in Appendix II) and 12 age matched control iris specimens from enucleations (cases 1-12 in Appendix I) were used. Details of the source of material used for this study and the techniques applied are described in sections 3.3.3 & 3.3.4. All the control specimens were examined by LM to exclude the possibility of unsuspected secondary pathological changes. Exfoliative specimens had the diagnosis confirmed clinically and morphologically. The morphological findings (LM and TEM) were similar to those described in sections 4.3 & 5.2.2. The immunocytochemical findings of this study are as follows:

Laminin

In all normal control iris specimens immunolabelling was localised predominantly in the ECM of vascular supporting cells (cf Figs 6.1 & 6.2). In all of the 15 exfoliative

specimens studied, every exfoliation aggregate was uniformly labelled with anti-laminin antibodies. Immunolabelling was almost exclusively located to the exfoliation fibrils (cf Fig 6.7 & Fig 7.1). Within the iris vasculature of each individual specimen a wide spectrum of additional pathological alterations was noted. Discrete clumps of exfoliation material, labelled with laminin, often appeared to form a bridge between the bulk of the exfoliative deposits and the outer surface of supporting cells (Fig 7.1). Iris granules, presumably originating from degenerate melanin containing cells, were occasionally present within the exfoliative deposits. In all of the specimens, the intensity of immunolabelling was directly proportional to the quantity and density of the exfoliation deposits around the vessel wall. Immunogold labelling assisted in the detection and recognition of discrete exfoliative deposits in other iris sites. In a few specimens such isolated aggregates of exfoliation material were only detected as a consequence of their gold labelling.

By comparison with the normal iris controls, the label for laminin was markedly reduced, or completely absent in that part of the vascular wall that did not contain clumps of exfoliation material (see also section 6.2.2), (Figs 7.2 & 7.3). A similar depletion occured in ostensibly 'normal' vessels in exfoliative iris specimens, (exfoliation free vessels).

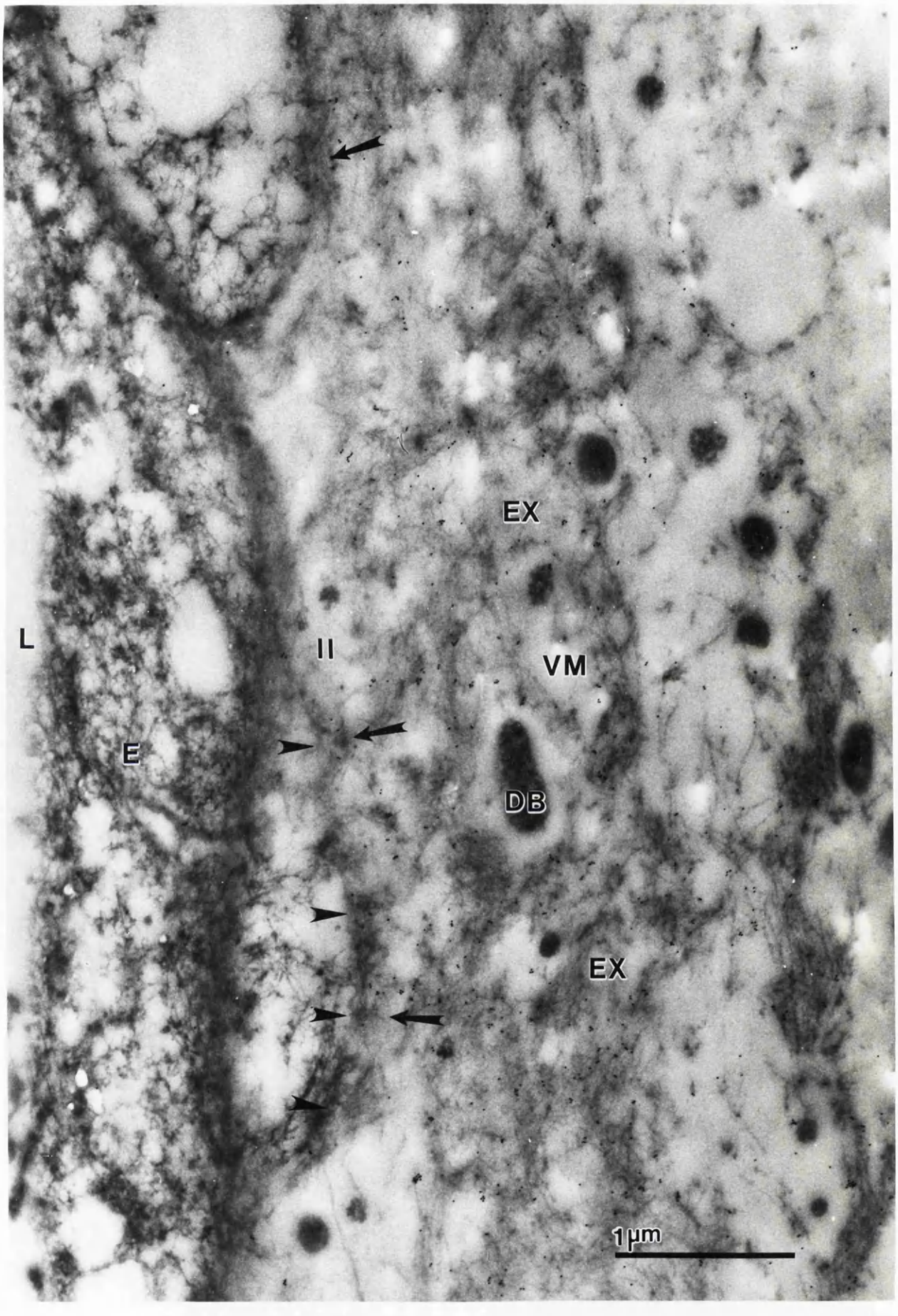


Figure 7.1: Laminin immunolabelling over a large aggregate of exfoliation materi (EX) in the vascular matrix(VM) of an exfoliative vessel. Exfoliation material is in contact(arrows) with the outer surface (arrowheads)of an endothelial cell(E); L:lumen; ll:lamina lucida; DB:dense bodies.

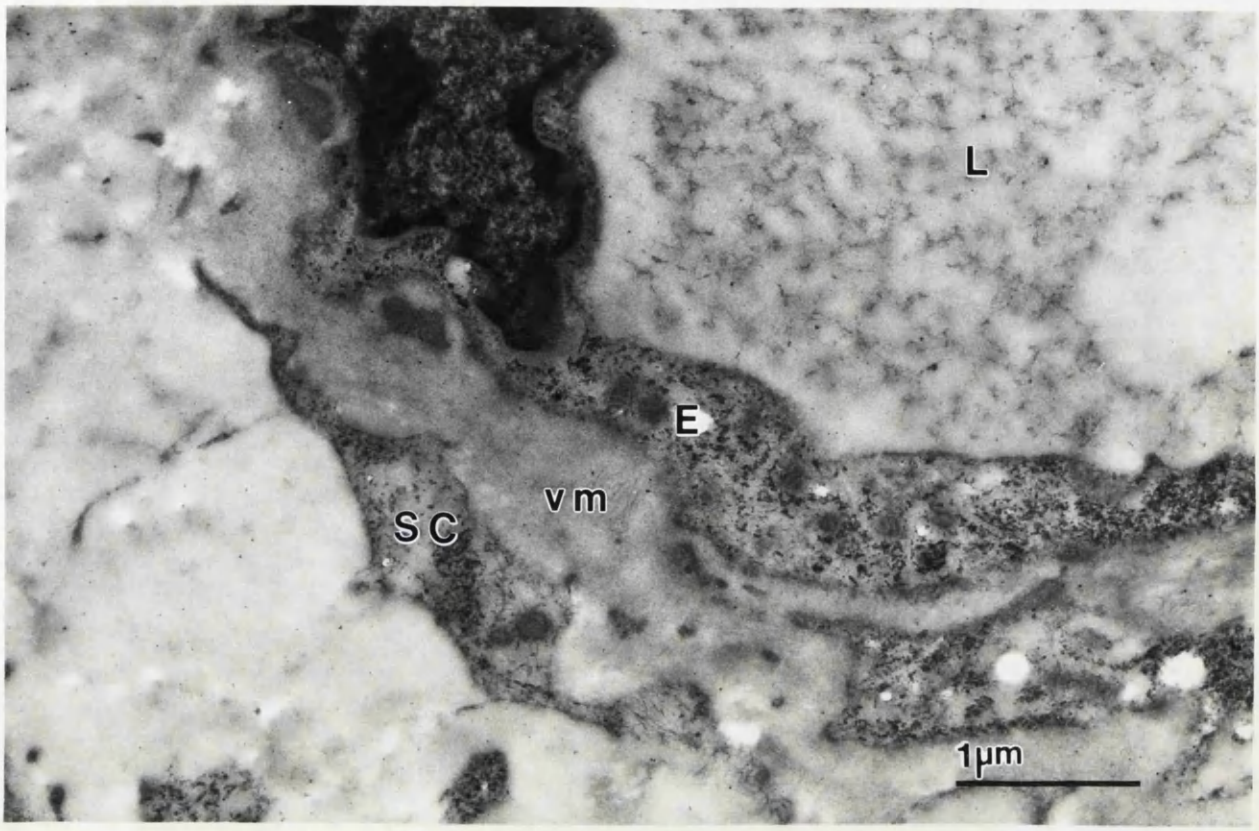


Figure 7.2: Laminin is greatly reduced in the vascular matrix(vm) of an unaffected vessel within an exfoliative iris specimen. L:lumen; E:endothelium; SC:supporting cell.

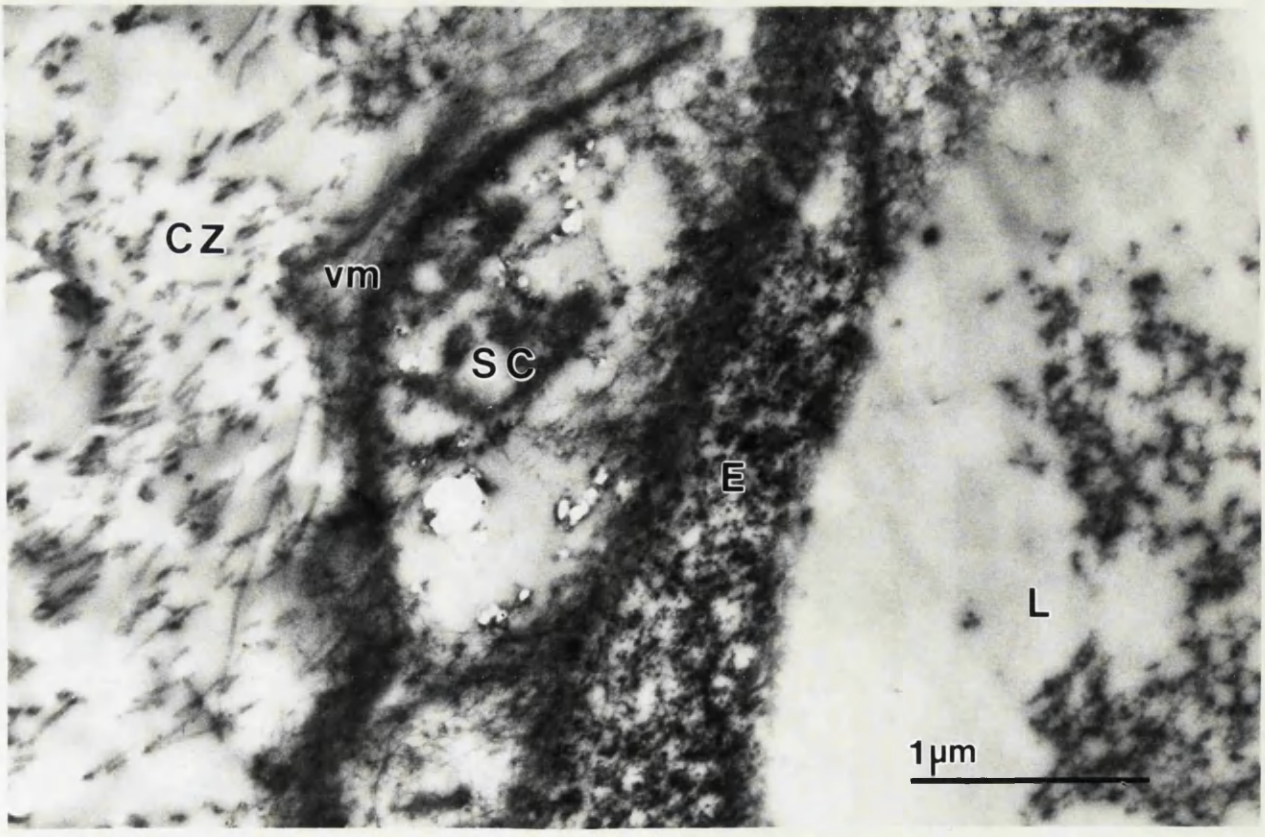


Figure 7.3: Absence of laminin from the vascular matrix(vm) of a supporting cell(SC) in an exfoliation free vessel. Failure of structural preservation highlights the drawback of PFA fixation. All other figures in this chapter are of tissue fixed with glutaraldehyde. L:lumen; E:endothelium; CZ:collagenous zone.

Type I collagen

Specific immunolabelling for type I collagen was present in the vascular matrix of normal (cf Fig 5.1) and exfoliative vessels (Fig 7.4) with the label evenly distributed throughout. In exfoliative vessels increased labelling for type I collagen was detected in the vascular matrix in the presence of exfoliation aggregates (Fig 7.5). In such vessels immunogold particles were present in exfoliation aggregates and 'appeared' to label them (Fig 7.5). Collagen type I was significantly reduced in the vascular matrices of exfoliation free vessels.

Type IV collagen

In the normal iris controls type IV collagen labelling was located in the basement membranes of endothelial cells and vascular supporting cells of all vessels (see section 5.1.2). In contrast to type I collagen and laminin, the label was most intense near the endothelium (cf Fig 5.9).

In all exfoliative specimens labelling for type IV collagen in iris vessels could be divided into four patterns, which related to the severity of the iris vasculopathy: (1) iris vessels with no evidence of exfoliation material and with a relatively healthy endothelium/supporting cells, (2) relatively healthy iris vessels with visible aggregates of exfoliation material, (3) those without evidence of exfoliation material deposition, but with a degenerate endothelium and atrophic, or fragmented supporting cells and (4) ghost vessels where exfoliation material had replaced the vascular wall and advanced degeneration or absence of

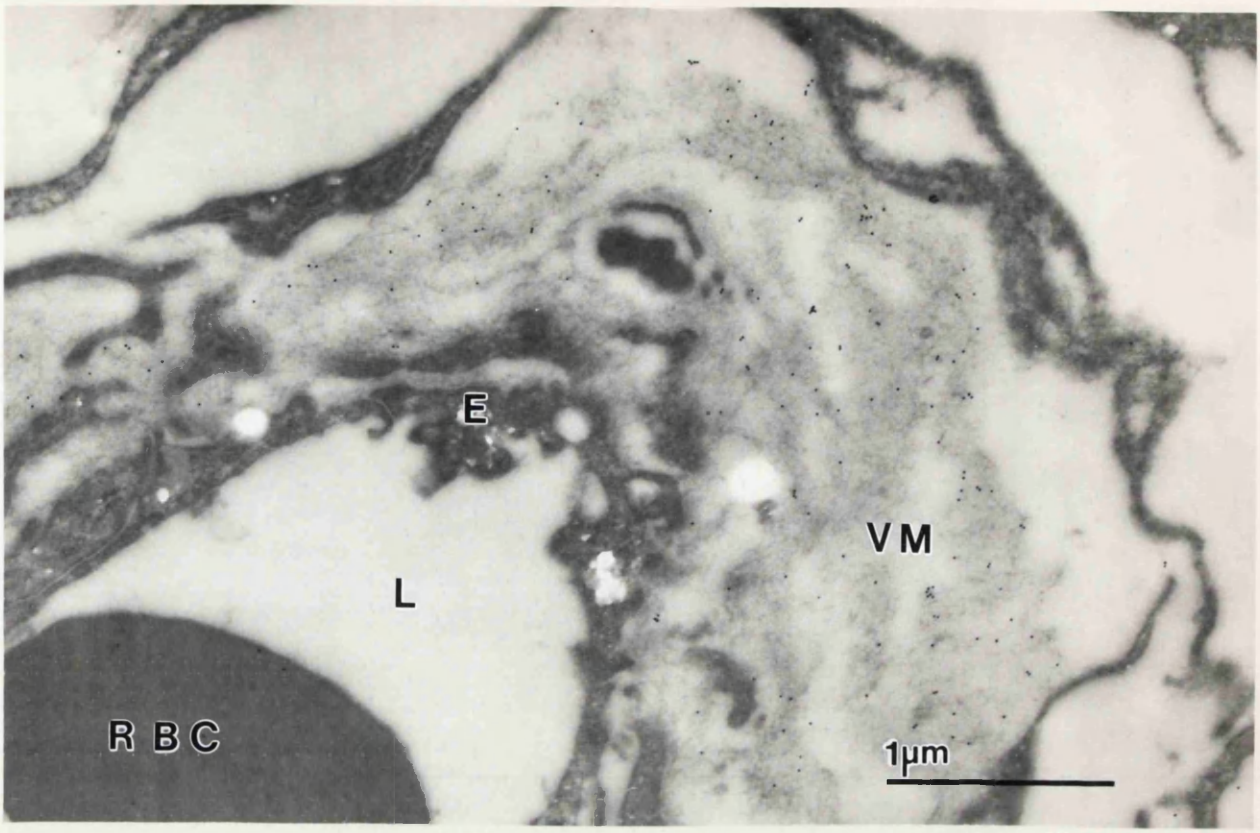


Figure 7.4: Collagen type I labelling in exfoliative iris. The vascular matrix (VM) of this unaffected vessel is diffusely thickened, but free from exfoliation material. L:lumen; RBC:red blood cell; E:endothelium.

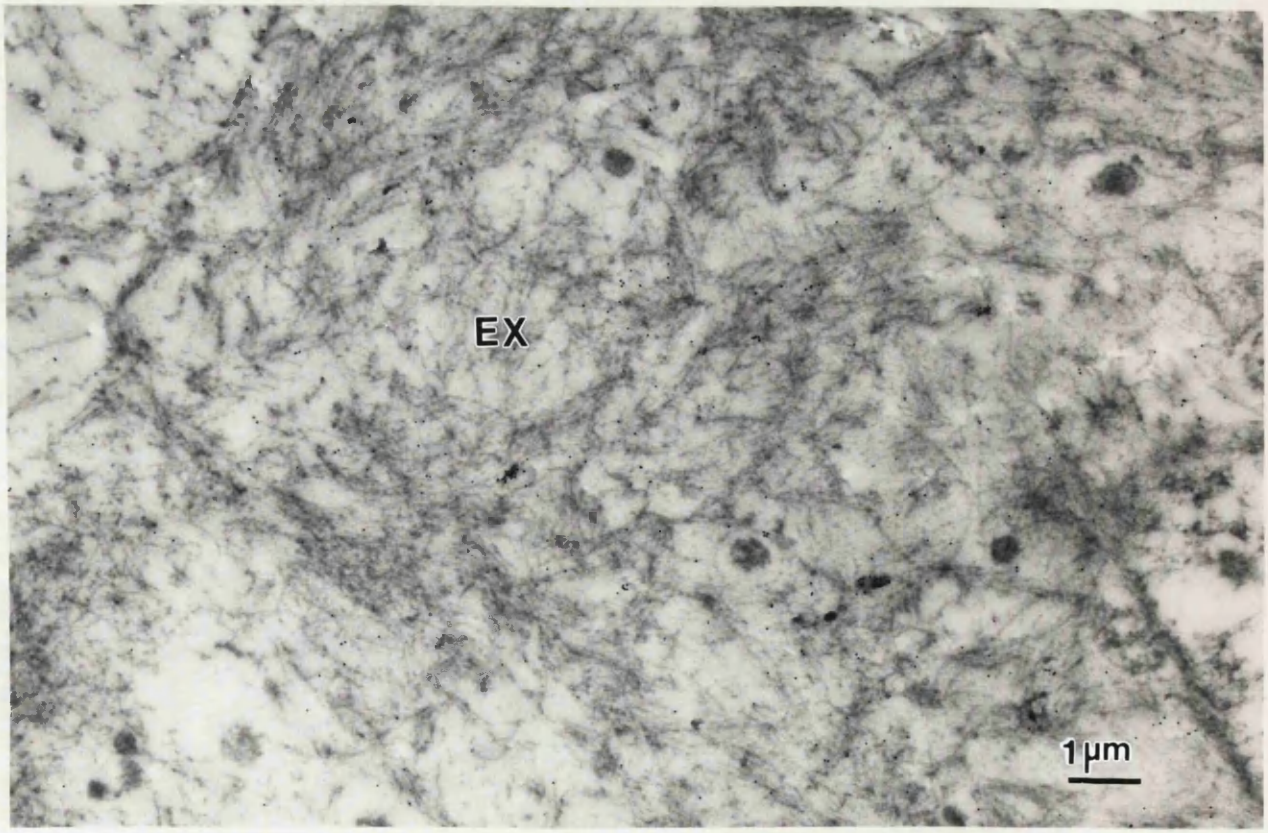


Figure 7.5: Vascular matrix of an exfoliative vessel infiltrated with exfoliation material(EX). Immunogold particles labelling for type I collagen are located over fibrils throughout the vascular matrix, which is of increased thickness.

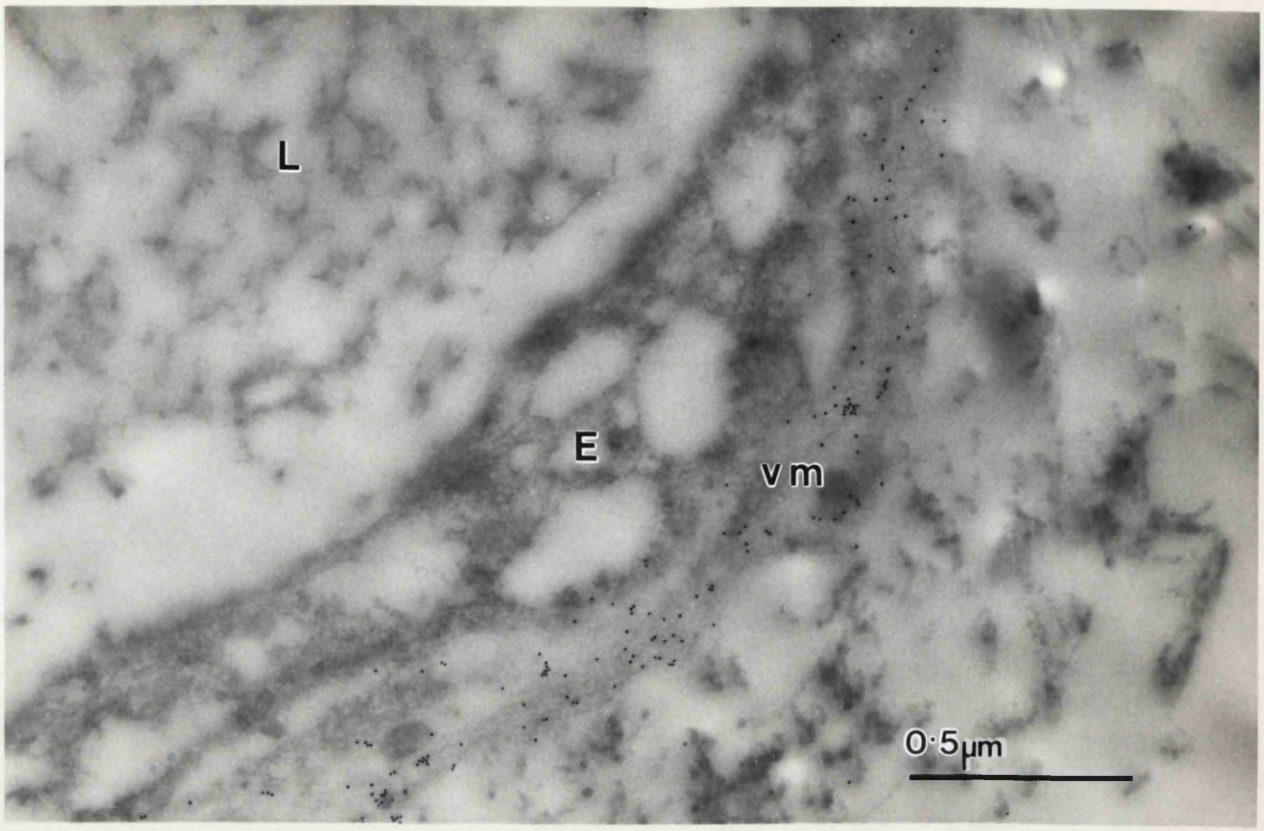


Figure 7.6 Collagen type IV localisation in the vascular matrix(vm) of a vessel devoid of exfoliation material. The vascular matrix(vm) is normal in appearance. L:lumen; E:endothelium.

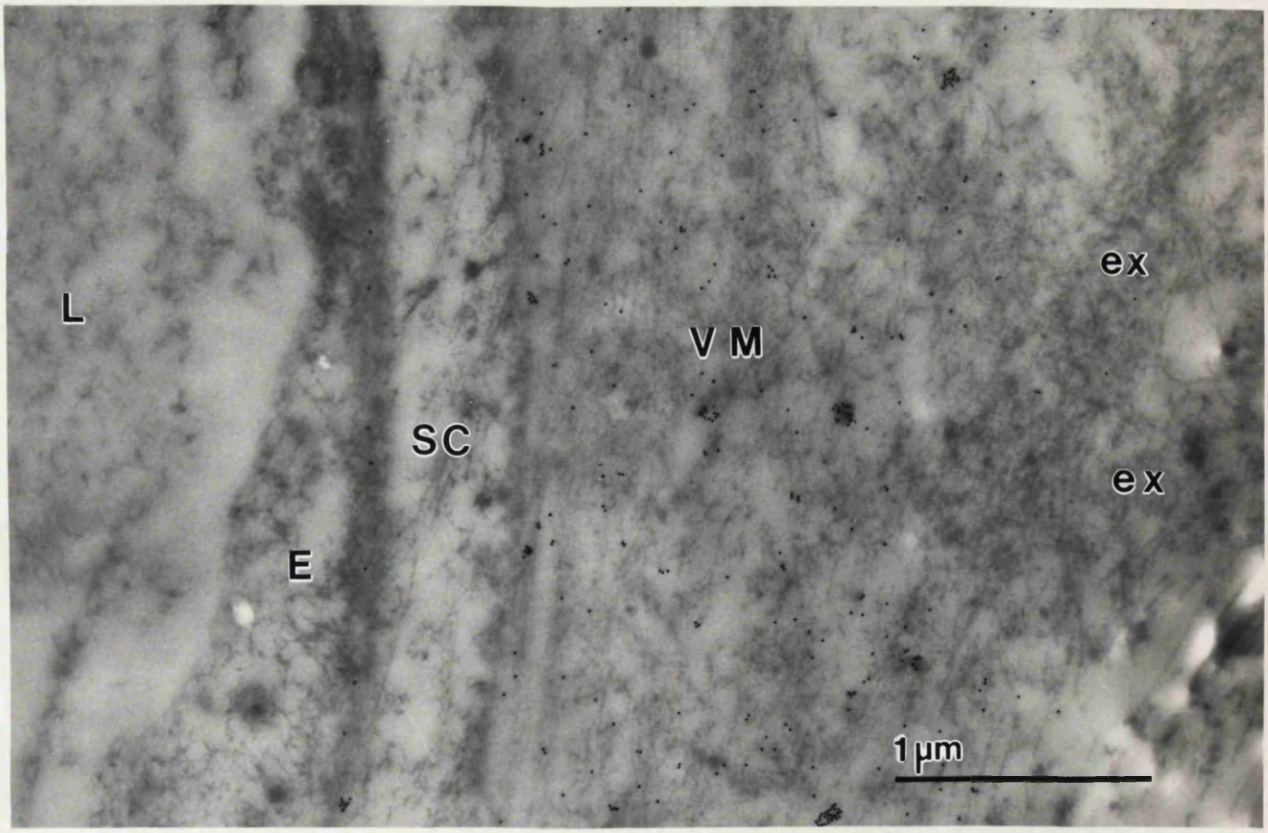


Figure 7.7: Immunolabelling for collagen type IV in early exfoliation vasculopathy. Exfoliation material is present in the thickened vascular matrix(VM). The distribution of collagen type IV is over a larger area than that of exfoliation free vessels, (cf 7.6);but is absent from exfoliation material (ex) outwith the abnormal vascular matrix. L:lumen; E:endothelium; SC:supporting cell.

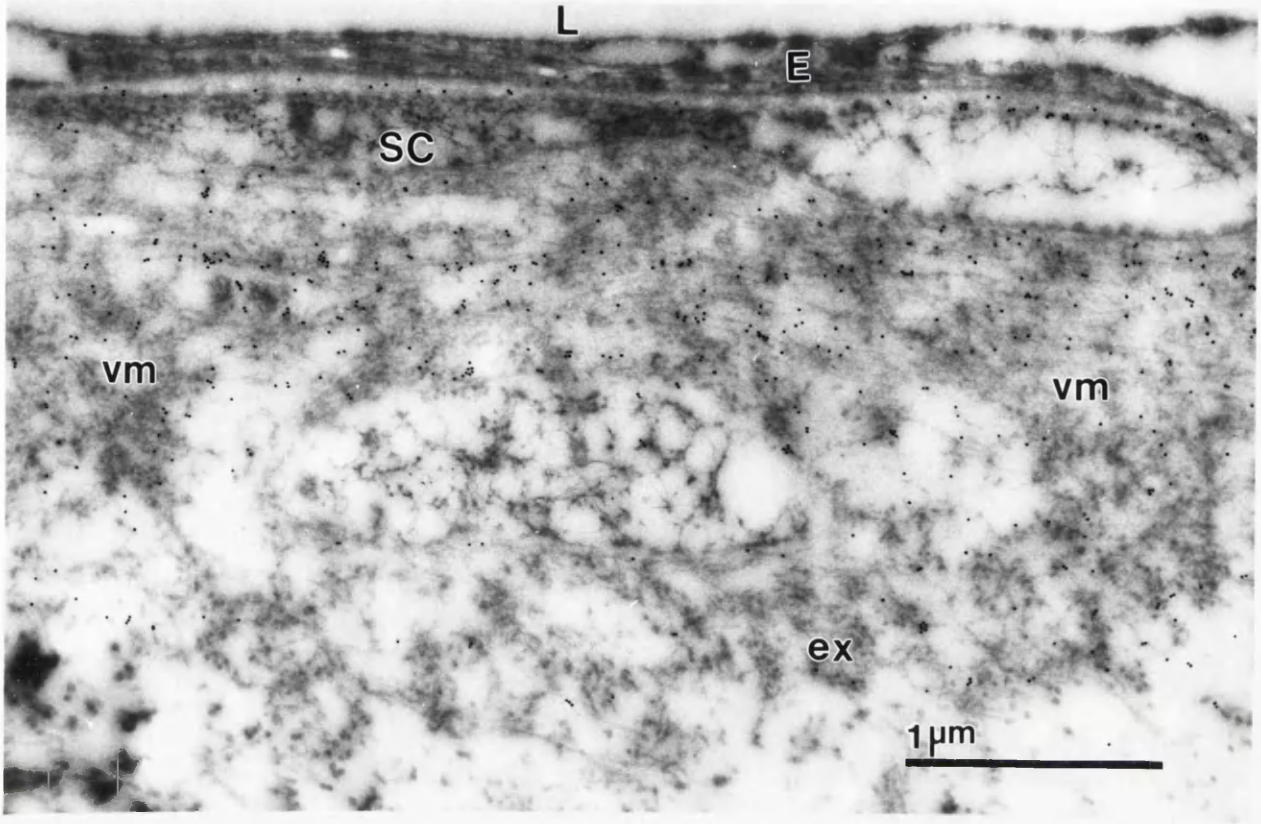


Figure 7.8: Collagen type IV in the thickened vascular matrix (vm) of an exfoliative vessel. Labelling is increased in comparison with exfoliation free vascular matrix. Note reduction of label in exfoliation material (ex) outwith the vascular matrix. L:lumen; E:endothelium; SC:supporting cell.

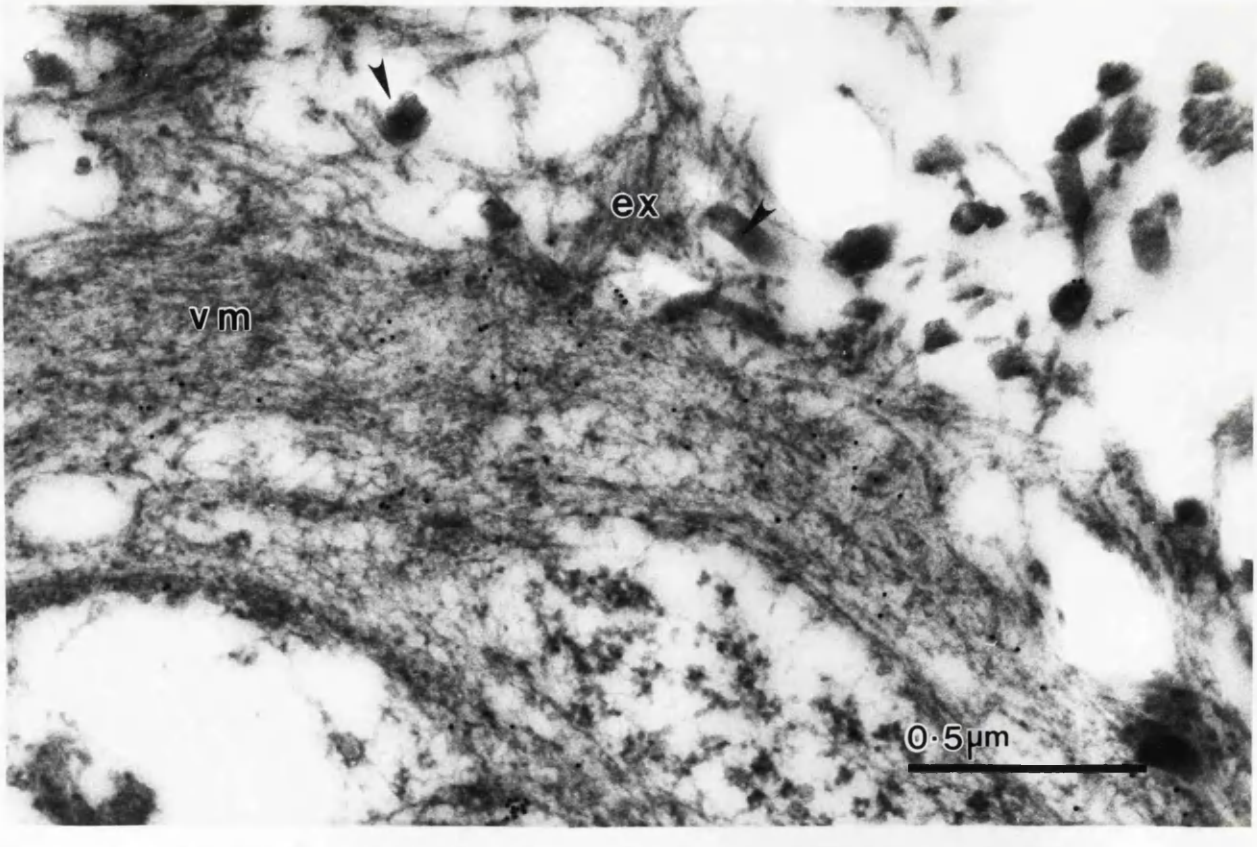


Figure 7.9: Absence of labelling for collagen type IV over exfoliation fibres(ex) outwith the thickened vascular matrix(vm).
In this micrograph some exfoliation fibres intermingle with thicker collagen fibrils (arrowheads).

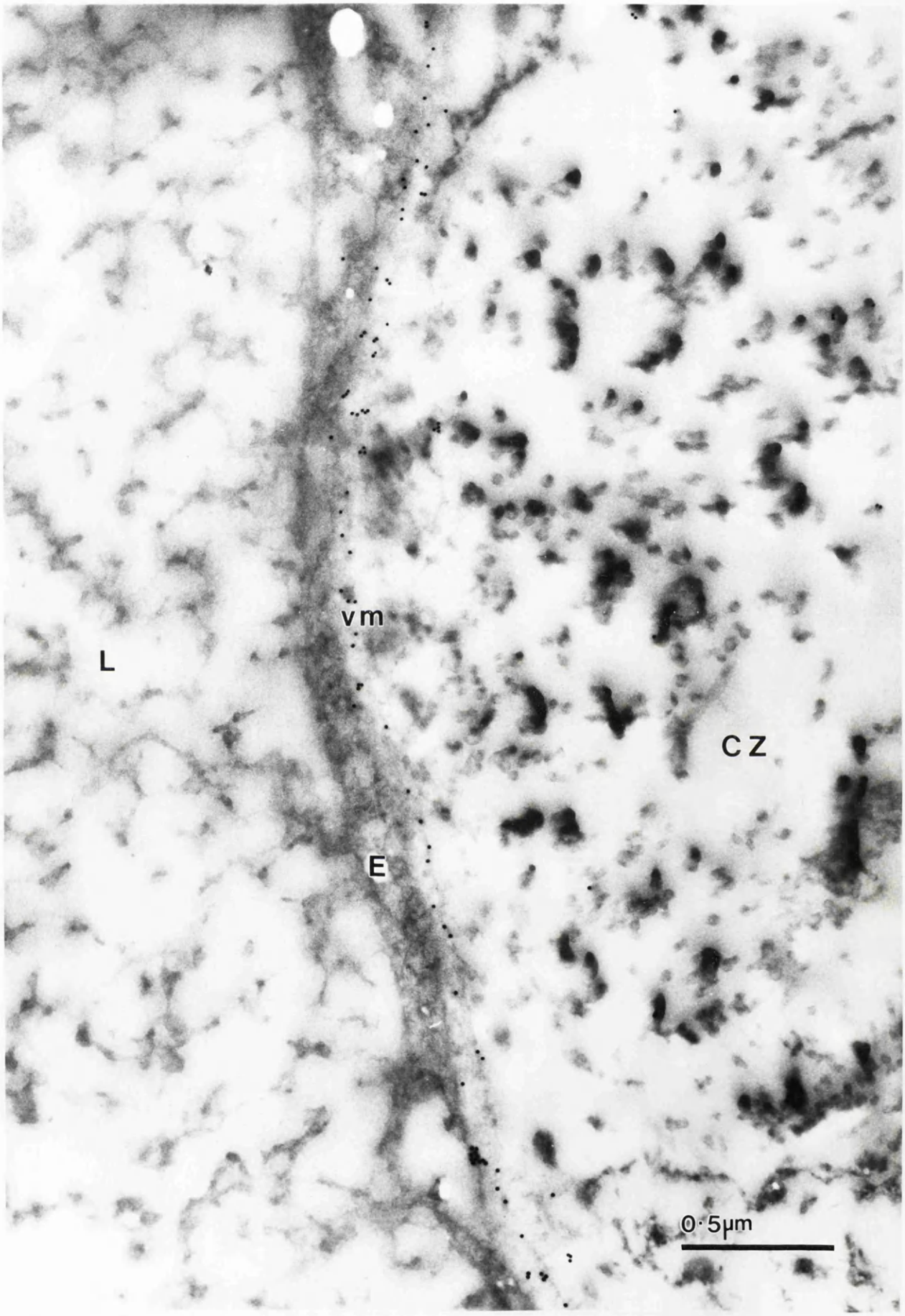


Figure 7.10: Collagen type IV localisation in vessel exhibiting advanced exfoliation vasculopathy. Labelling is restricted to a markedly thinned vascular matrix(vm); exfoliation material is absent. L:lumen; E:endothelium; CZ:collagenous zone.

endothelial and supporting cells was evident (for description of vascular changes see section 4.4).

In the first category (Fig 7.6) immunolabelling was similar to that seen in age matched normal iris controls. In the second category an increase of type IV collagen was noted in the thickened vascular matrix infiltrated by exfoliation material (Figs 7.7 & 7.8). Type IV collagen accumulation in the vascular matrix was uneven, and was more prominent in areas with densely packed exfoliation material. However, the immunogold label was not associated with the coarser individual exfoliative fibres, but was located on the finer filamentous material of the vascular matrix. Furthermore, exfoliation material outwith the vascular matrix (Figs 7.7 & 7.8) and in the iris stroma did not contain type IV collagen.

Few specimens contained vessels which belonged in the third category. In these vessels immunogold labelling for type IV collagen (Fig 7.10) was reduced in comparison with the normal age matched controls and exfoliative vessels of the first two categories. This reduction in collagen type IV was even more prominent with ghost vessels.

Exfoliation vasculopathy

At an early stage of exfoliation vasculopathy some of the vessels exhibited a varied degree of thickening of the vascular matrix. However, thickening of the vascular matrix was not accompanied by an anticipated increase in labelling for laminin. On the contrary, laminin labelling was sharply reduced (Fig 7.11). At an advanced stage of exfoliation

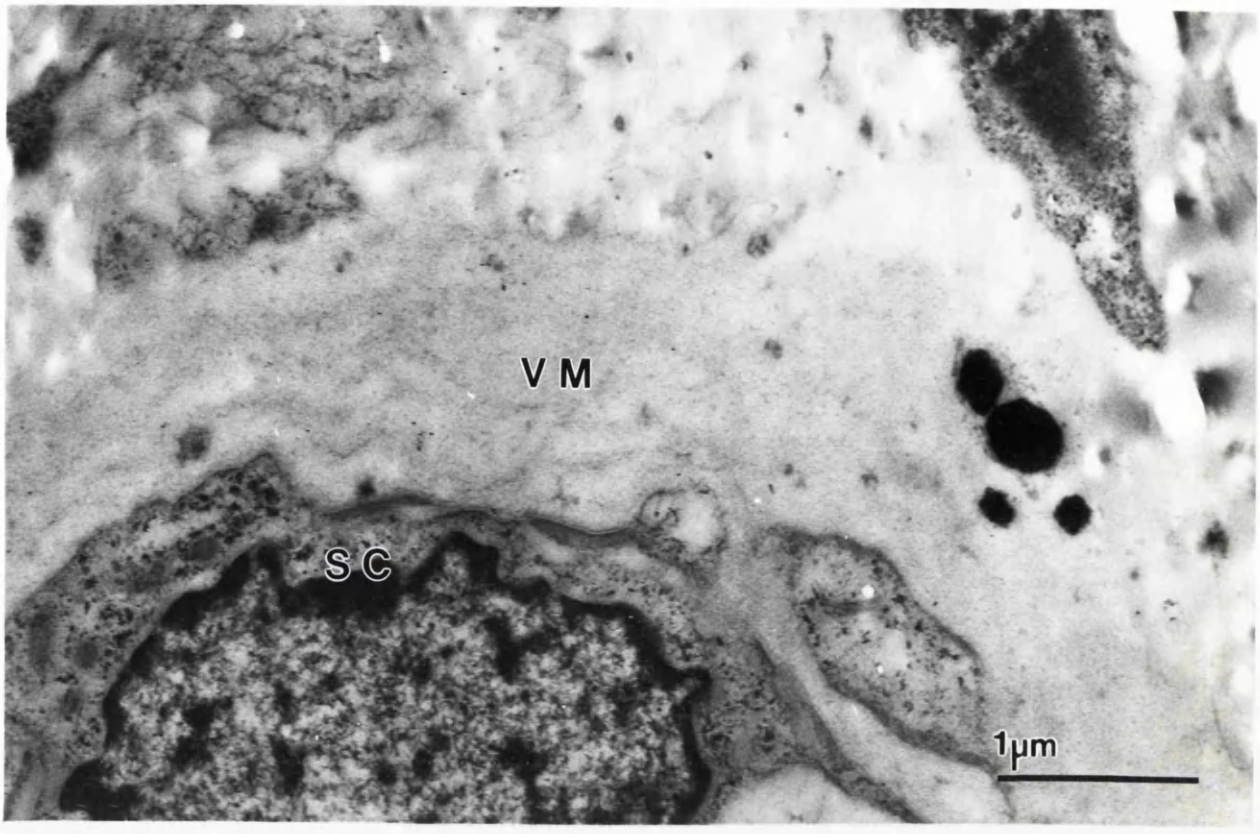


Figure 7.11: An exfoliation free vessel with thickening of the vascular matrix (VM), but sparse labelling for laminin. SC:supporting cell.

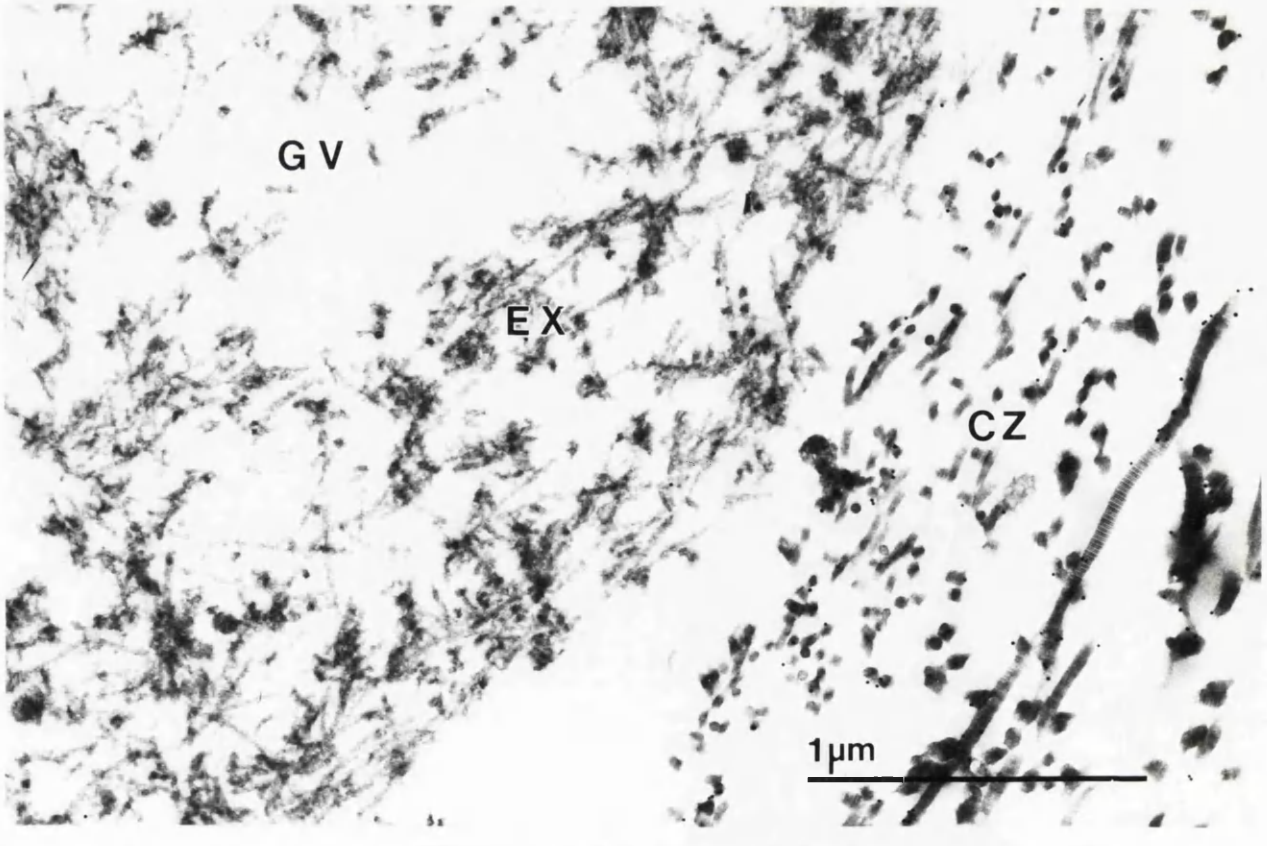


Figure 7.12: Ghost vessel(GV) in end stage exfoliation vasculopathy. Type I collagen is localised to the striated collagen fibrils of the perivascular collagenous zone (CZ), but is largely absent from exfoliation material(EX), which has replaced the vascular wall.

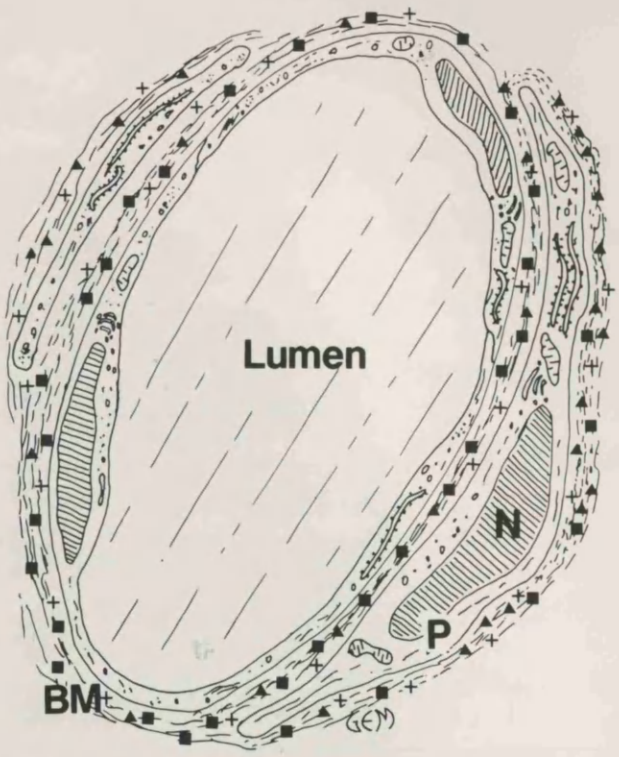


Figure 7.13a: Diagramatical description of the localisation of laminin (triangles), collagen type I (crosses) and collagen type IV (squares) in the vascular matrix-basement membrane complex(BM) of the normal aged iris. N:nucleus; P:pericyte (supporting cell).

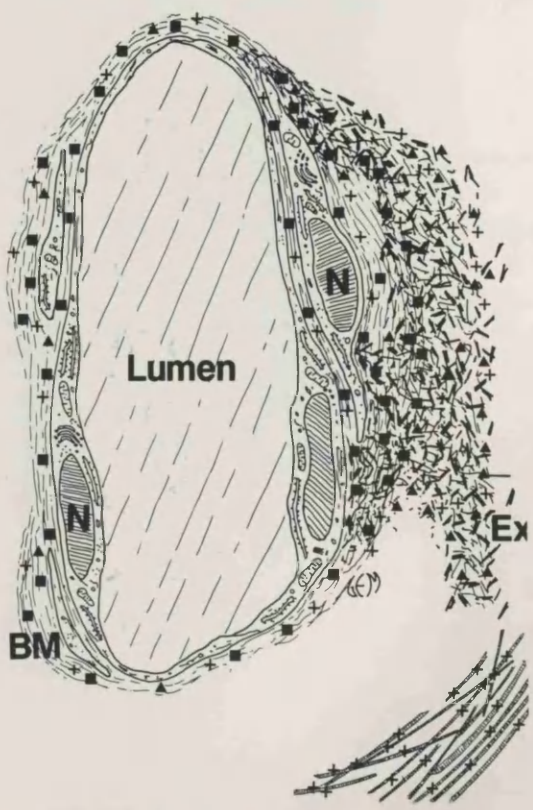


Figure 7.13b: Diagramatical representation of exfoliation vasculopathy in a relatively healthy vessel with visable exfoliation material(ex). Intense laminin labelling in exfoliation material aggregate is accompanied by a decrease in the label for laminin in the vascular matrix (BM), which is free from exfoliation material. Increased labelling for collagen types I and IV occurs in the affected matrix, whereas in the unaffected matrix labelling is comparable to that of normal vessels.

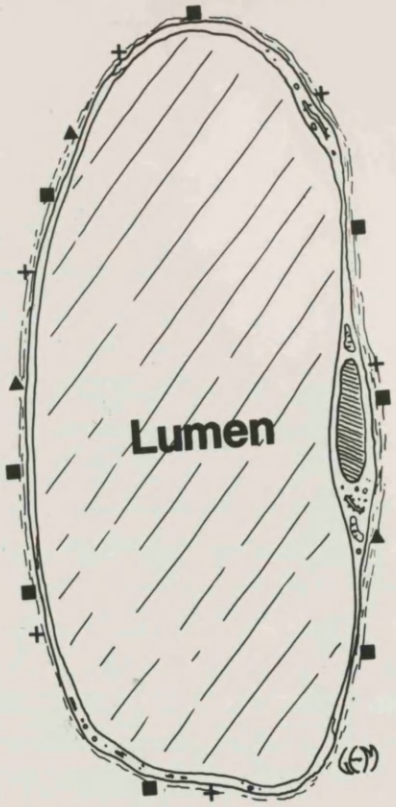


Figure 7.13c: Vascular matrix components in advanced exfoliation vasculopathy. Degenerate exfoliative vessel is ostensibly free from exfoliation material. Note reduction of laminin (triangles), collagen type I (crosses) and collagen type IV (squares) in association with visable thinning of the vascular matrix.

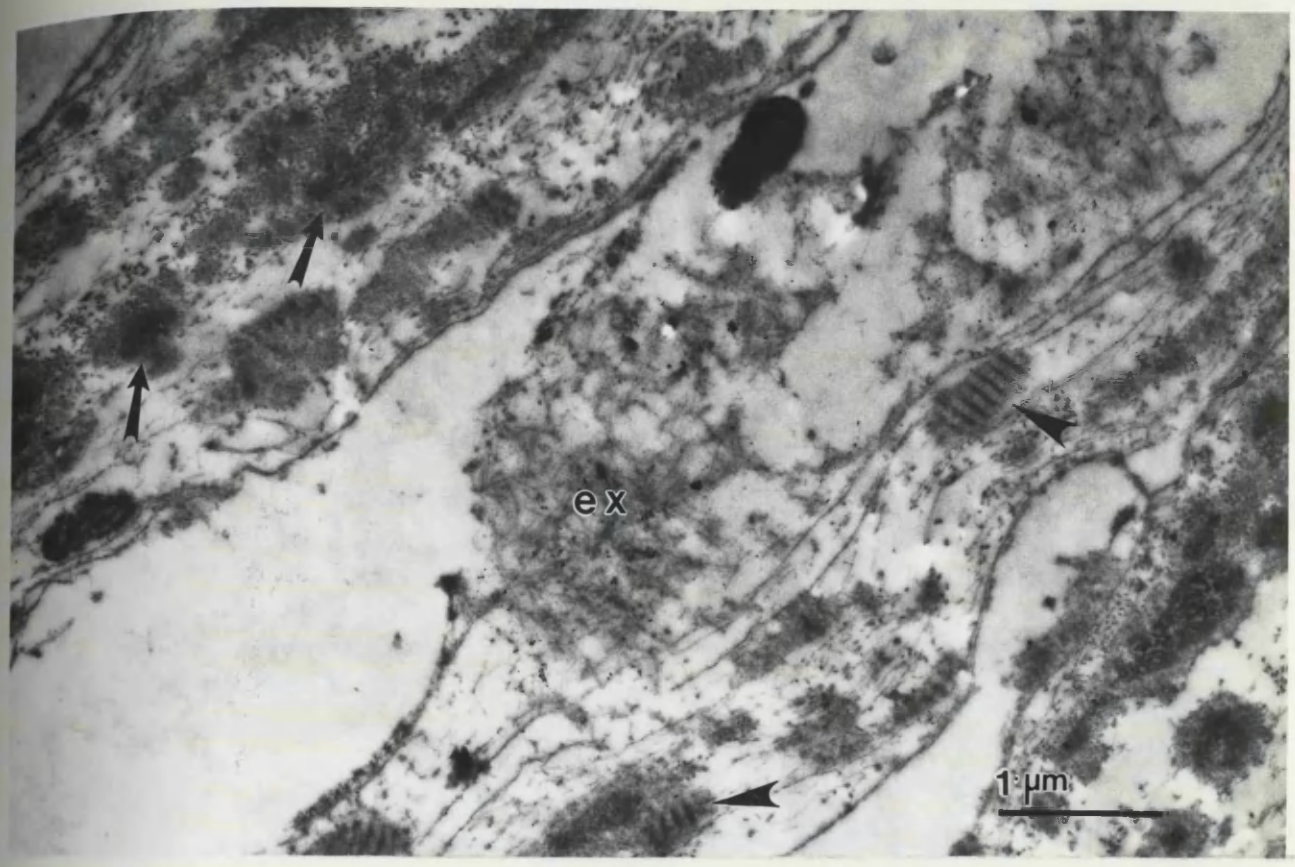


Figure 7.14: Exfoliative meshwork control. Laminin is present in an aggregate of exfoliation material(ex) within the intertrabecular space. Elastic-like fibres(arrows) and long spacing collagen(arrowheads) are devoid of label.

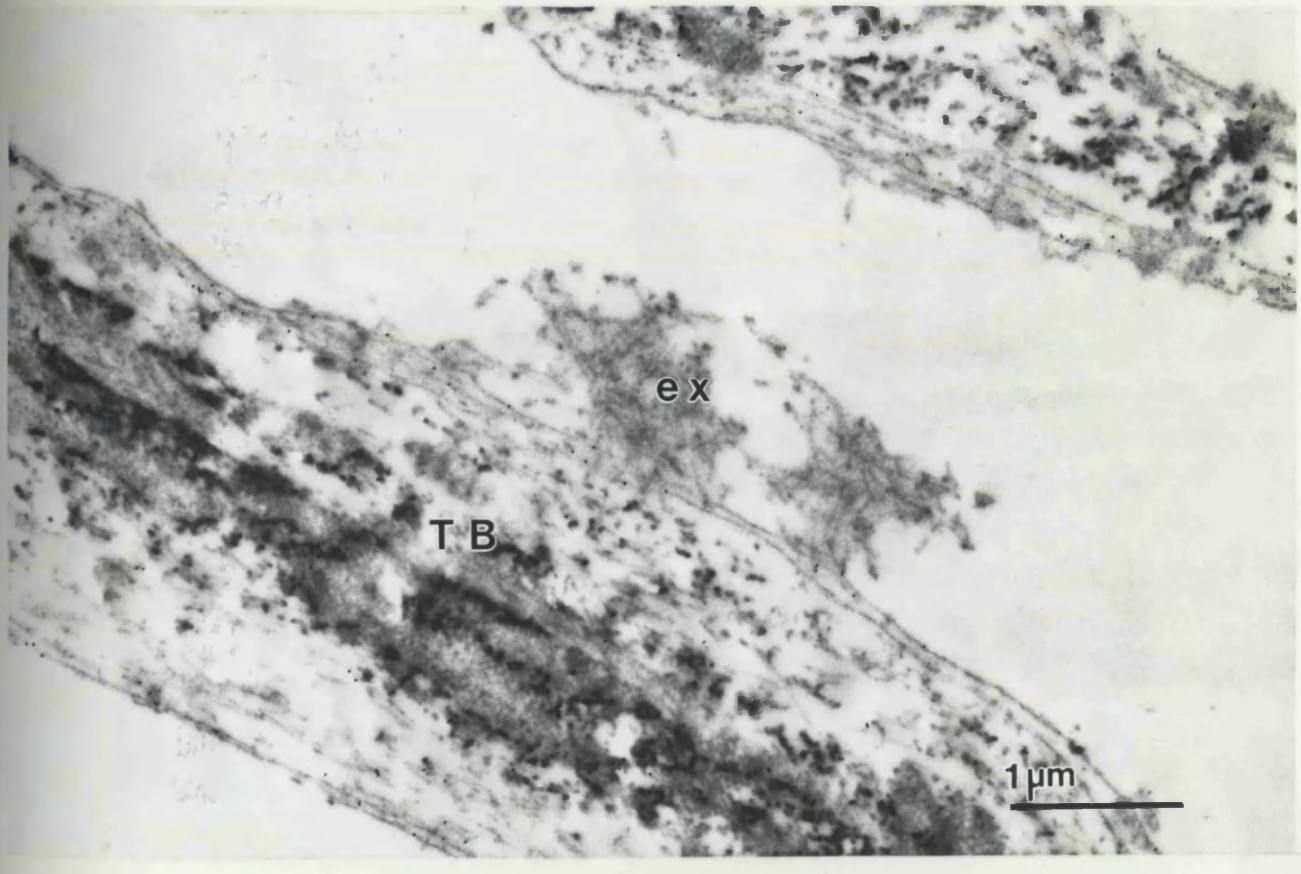


Figure 7.15: Exfoliative meshwork control. Collagen type IV is present in the basement membrane of trabecular beams (TB), but is absent from exfoliation material(ex). Endothelial cell covering is missing

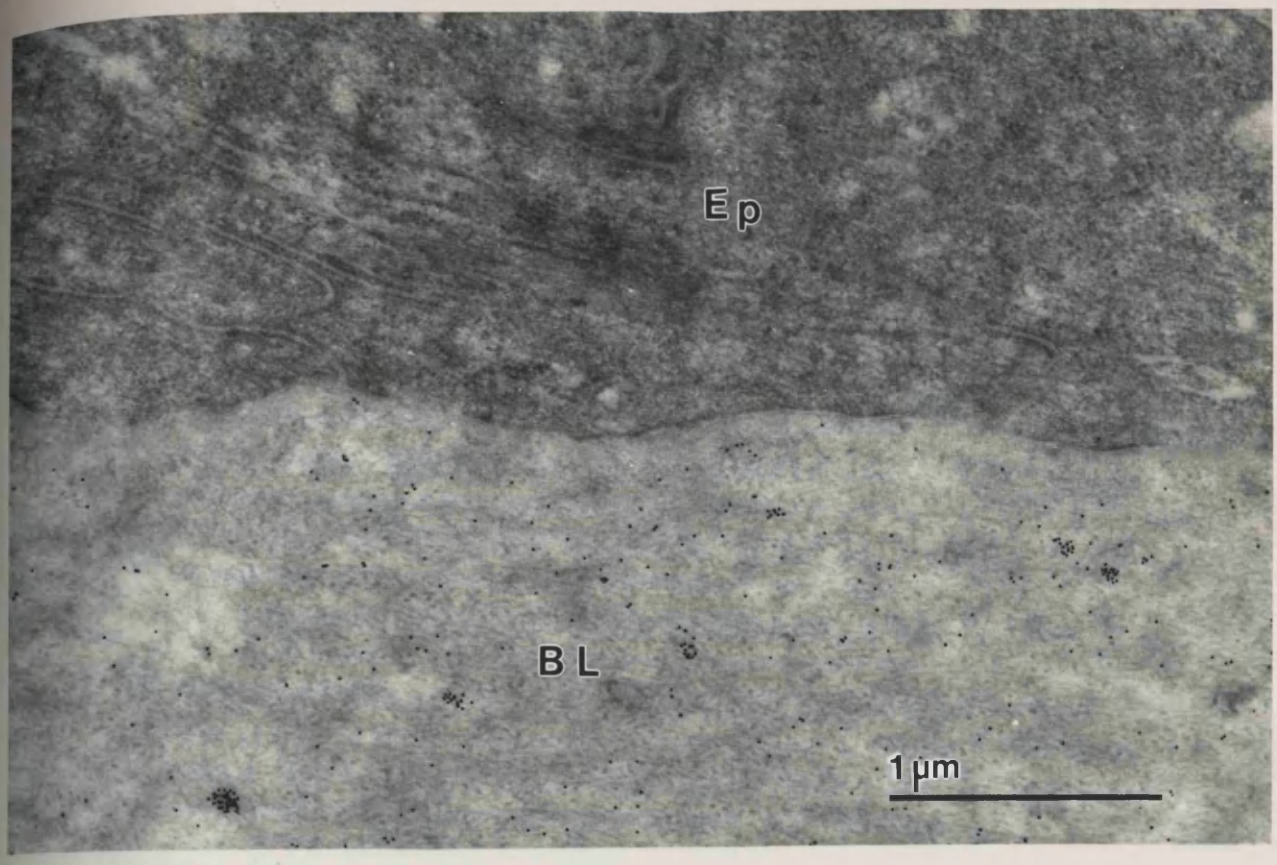


Figure 7.16: Positive corneal control for type I collagen with immunogold labelling of collagen fibrils in Bowmans layer (BL). The corneal epithelium (Ep) is devoid of immunogold particles.

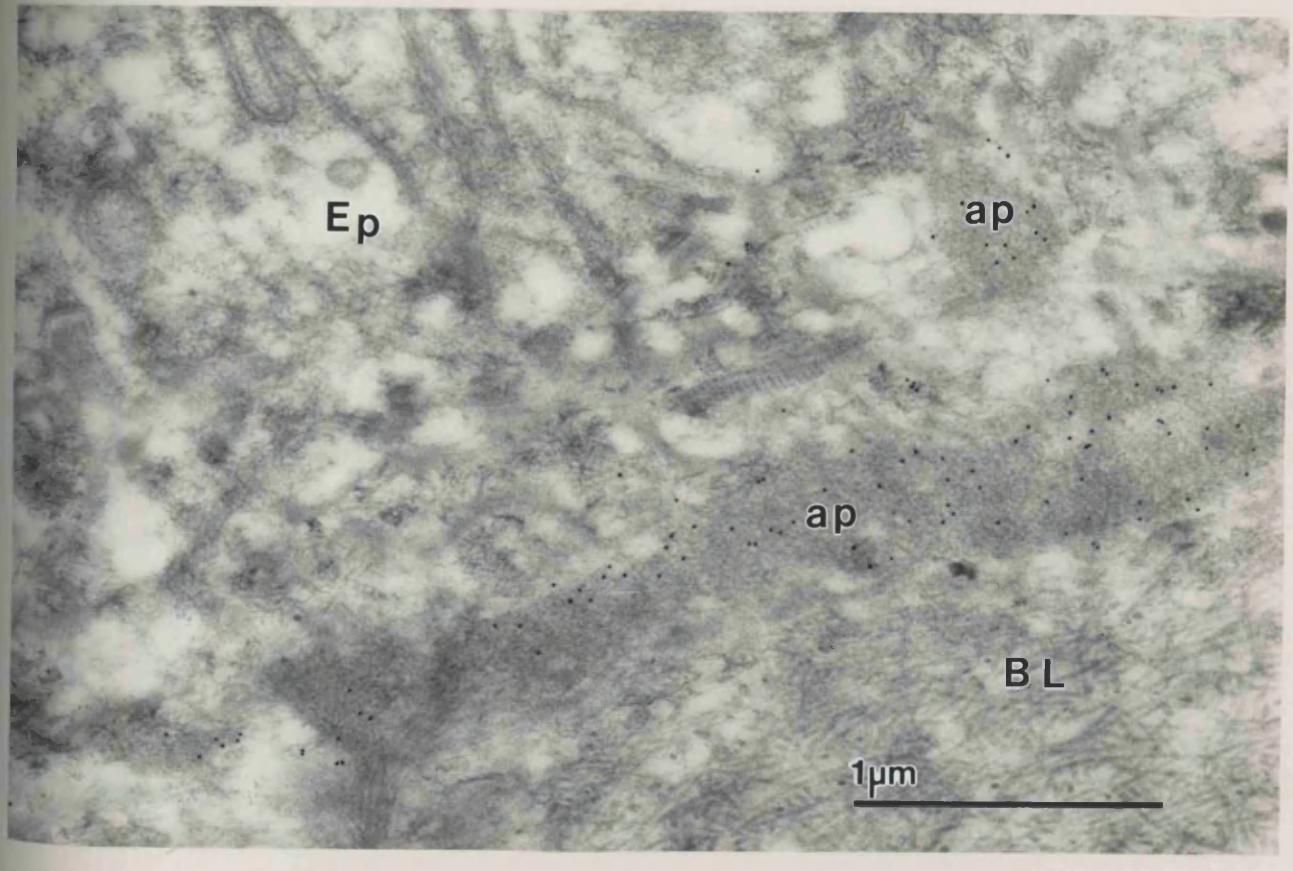


Figure 7.17: Positive corneal control for laminin. Laminin is localised to anchoring plaques (ap) of the epithelial basement membrane complex. Ep:epithelium; BL:bowmans layer.

vasculopathy laminin was absent from the residual vascular matrix (Fig 7.3).

Immunolabelling for type I collagen was significantly reduced in vessels which were at an advanced stage of exfoliation vasculopathy. These vessels exhibited thinning of their vascular matrices, atrophy and ultimately loss of all cellular elements. Sparse labelling for type I collagen was observed in ghost vessels in which exfoliation material had replaced the normal vascular wall (Fig 7.12). Overall, the pattern of type IV collagen labelling in vessels free of exfoliation material in exfoliative iris specimens, was dependent upon the degree of degenerative vasculopathy observed. Whenever the vessels exhibited significant degenerative changes i.e. atrophic supporting cells and marked degeneration, or absence of endothelial cells, type IV collagen was considerably reduced (Fig 7.10). This reduction was closely associated with thinning of the vascular matrix. The immunocytochemical findings of the present investigation are summarised diagramatically in Figures 7.13a,b,c.

Positive controls

In exfoliative meshwork control tissue, specific labelling for laminin was observed in exfoliation material aggregates within the intertrabecular spaces (Fig 7.14). Collagen types I and IV were not present in the exfoliation material (Fig 7.15). The qualitative distribution of collagen types I and IV in the exfoliative meshwork was similar to that reported by Marshall and coworkers (1991c). Corneal positive controls showed collagen type I in striated collagen fibrils in

Bowman's layer (Fig 7.16) and the corneal stroma. Laminin was localised to anchoring plaques in the corneal epithelial basement membrane complex (Fig 7.17).

7.3 Discussion

The range and characteristics of the exfoliation vasculopathy observed in the exfoliative iris vessels of the specimens was diverse, but similar to that reported previously using conventional TEM (chapter 4 and Ringvold 1969; 1970b, Anastasi et al 1974, Ghosh & Speakman 1974, Shimizu et al 1980, Spinelli et al 1985, Shimizu 1985). As previously stated, the morphological appearance of exfoliation deposits in LR white sections differs from osmicated/glutaraldehyde fixed tissue embedded in araldite. The main difference comprises the less 'dense' appearance of exfoliation aggregates. It is conceivable that conventional fixation removes a proportion of ECM present within the electron lucent amorphous structural framework of the exfoliation material (interfibrillar matrix).

With TEM the detection of the larger, electron-dense aggregates of exfoliation material was easy, but the identification of discrete clumps of exfoliation material, or isolated exfoliative fibres was more difficult. This difficulty was exacerbated by the morphological resemblance between isolated exfoliative fibres and collagen fibres. Immunogold labelling of exfoliative fibres with anti-laminin antibodies was thus, advantageous in the identification of exfoliation material. In agreement with a previous

conventional TEM study of exfoliative iris vessels by Shimizu (1985) the present study confirmed the intimate relationship between exfoliation aggregates and iris vessels. Exfoliation aggregates in the iris frequently extended to form a bridge which linked with vascular supporting cells.

The intimate relationship between exfoliation aggregates and the vessel wall observed in this study is important as it could mean that vascular cells, which normally remodel the vascular matrix, are the 'synthesisers' of exfoliation material. The cells in the iris vessels can be divided into supporting (smooth muscle cells and pericytes) and endothelial cells. The vascular extracellular matrix is thought to be synthesised by the cellular components of the vessel wall, but there may be a contribution to the overall structure from the plasma components (Garner 1982). The results of this study together with evidence cited in chapters 4 & 6 suggest that in the iris the vascular supporting cells are the principal source of exfoliation material. It is generally thought that these cells provide structural support to the underlying vasculature and possess contractile, phagocytic and synthetic properties (reviewed by Garner 1982). It is highly likely that these cells control aspects of vascular matrix metabolism and become the 'target cells' in the development of the exfoliation syndrome. If such a hypothesis is right, this disturbed synthetic activity leads to the deposition of exfoliation material.

The localisation of laminin in the normal vascular matrix surrounding supporting cells suggests that these cells

produce laminin, a principal basement membrane component (section 2.5). Chapter 6 has shown laminin to be an important constituent of exfoliation material. Moreover, laminin labelled exfoliation material frequently appeared to be preferentially located in proximity to supporting cells. Thus, it is possible that the same cells that under normal conditions incorporate this macromolecule into the ageing iris vascular matrix, may at some stage deviate and begin to incorporate laminin into the exfoliation material. The present study lends weight to the hypothesis that exfoliation material is abnormal basement membrane material produced at multiple sites by abnormal ageing cells (Eagle et al 1979).

One finding which was not anticipated was the reduction in the laminin content of the vessel wall in exfoliation free vessels, i.e. when there was no evidence of exfoliation material deposition in that particular level of section. This enigmatic depletion of laminin could reflect a widespread disturbance in matrix remodelling in exfoliation vasculopathy. This reduction could be attributable to an aberrant diversion of laminin macromolecules into exfoliation material synthesis. A physico-chemical attraction between laminin and exfoliation material is also possible.

If there is a sequence of abnormal utilisation of normal matrix components, the aberrant vascular cohort cells may be stimulated to synthesise other ECM molecules (which under normal conditions are not produced by vascular basement membrane cells). This abnormal ECM synthesis could explain the reported presence of components of the elastic system

within the exfoliation material structural framework (Streeten et al 1986; 1990, Li et al 1988).

In chapter 5 evidence was provided to show that collagen types I & IV are not constituents of the exfoliation material structure. Though the amount of type IV collagen increased in the vascular matrix in association with exfoliative aggregates, exfoliation deposits outside the vascular matrix in the iris stroma were completely negative for type IV collagen. The results of the present study are in accordance with preliminary biochemical data furnished by Ringvold (1973) which indicated that collagens do not comprise a significant proportion of the exfoliation material. The present investigation however, has indicated for the first time that the biosynthesis of the exfoliation material is combined with alterations of collagenous vascular matrix components, such as collagen types I and IV.

Increased accumulation of collagen types I and IV in the vascular matrices of exfoliative iris vessels probably accounts for the thickening of vascular matrices described in chapter 4. The lamination of vascular basement membranes in exfoliation syndrome is most likely due to the deposition of collagen type IV. Increased synthesis of collagen types I and IV appears to be an early feature of the disease. Enhanced collagen synthesis may either reflect a generalised disturbance of vascular matrix synthesis by distressed vascular supporting cells, or may represent a secondary attempt at repair in response to subtle ECM alterations, such as the reduction of laminin. Alternatively, it could

represent a 'failure' in the cycle of collagen turnover in which deficient enzymatic degradation results in accumulation of these two collagen types.

In advanced exfoliation vasculopathy in the material studied the loss of endothelial cells and the fragmentation of cohort cell was accompanied by a reduction of collagen types I and IV. This implies that while at an early stage of exfoliation vasculopathy the vascular cells are stimulated to synthesise increased amounts of collagen types I and IV, at a late stage of the disease the ailing cells lose the ability to maintain a healthy matrix. The reduction of collagen types I and IV and the corresponding reduction of the thickness of the vascular matrix do not support the theory of aberrant resorption of the vascular matrix, at least at the stage of advanced vasculopathy.

Alternatively, the findings in exfoliation vasculopathy may reflect secondary ECM alterations which occur as a result of the exfoliation material being transferred into the iris from the aqueous. Exfoliation material may circulate with the aqueous humour and enters the iris subcompartments and vessel wall by osmotic attraction. However, such a concept of passive exfoliation material deposition cannot account for all the morphological features and the preferential distribution of exfoliation material in the iris vessels (see section 4.5). Nevertheless, more studies are required to explore the possibility of passive exfoliation material deposition.

Degenerative changes of the vascular matrix seen by conventional morphology (chapter 4) have been confirmed in this study. It is interesting to relate the ECM changes observed in the iris vessels with two clinical features with, as yet, an obscure aetiology: 1)increased permeability of the affected iris vasculature and 2)iris neovascularisation. Fluorescein studies performed on patients with the condition have found profuse leakage of fluorescein from the iris vasculature (Vannas 1969, Brooks & Gillies 1987). Similarly, clinical and ultrastructural observations concerning the formation of new vessels in the disorder have puzzled investigators (Ringvold & Davanger 1977, Shimizu 1985). It is reasonable to assume that these findings result from remodelling of the vascular matrix in the course of exfoliation vasculopathy. A reduction of the laminin and collagen content in the vascular wall of the affected vessels would compromise the structural integrity and adhesion of endothelial cells (see section 4.4). The breakdown of healthy vascular matrices to ultimately form degenerative 'ghost vessels' would bring about iris tissue underperfusion, anoxia and atrophy which would stimulate neovascularisation.

It is relevant to note that in the walls of hyalinised retinal vessels the fibrous tissue which gradually replaces the cellular constituents was found to comprise an accumulation of collagen types I, III, IV and VI (Marshall et al 1990a) and this precedes neovascularisation. Information on the ECM content of the walls of ocular vessels in disease is of importance, because the location of the biochemical products help to identify the cells producing the protein.

Moreover, the ECM components produced during the various vasculopathies modify the behaviour of the vascular cells in the matrix.

The results of the present study support the hypothesis that in the exfoliative iris the pathogenesis of exfoliation syndrome is associated with disordered synthesis and/or assembly and turnover of the vascular ECM. It is tempting to speculate whether exfoliation material synthesis is associated with similar ECM changes in other ocular and extraocular tissues. Given that there is, as yet, no consensus on the primary site(s) of exfoliation material synthesis, immunocytochemical studies on other ocular tissues bathed by the aqueous humour and other ocular tissues not in contact with the aqueous humour may assist in the elucidation of exfoliation material synthesis.

In conclusion, the investigation of iris vasculopathy in exfoliation glaucoma and its precursor the exfoliation syndrome, is complicated by the extensive range of vascular abnormalities witnessed within single specimens. The results of the present study indicate that specific ECM alterations may occur in the iris vasculature during the evolution of the disease. These findings highlight the enigmatic nature and pathogenesis of the disorder.

7.4 Summary

Samples of control iris tissue obtained from 12 enucleated eyes and 15 exfoliative iridectomy specimens were prepared for an immunocytochemical study of the matrix of the walls of iris vessels. The distribution of collagen types I, IV and laminin was studied in normal and exfoliative vessels. Laminin was an integral component of exfoliation material and was present mainly in the matrix of the outer surface of normal vascular supporting cells. The laminin content in the latter location was reduced in vessels in which exfoliation aggregates were not visible. The iris vascular supporting cells, which produce laminin are implicated in the synthesis of exfoliation material. Exfoliation deposits appeared to stimulate the synthesis, or impair the resorption of collagen types I and IV at an early stage of the disease. In an advanced stage of exfoliation vasculopathy (thinning of vascular matrix, degeneration of cellular elements) a significant reduction of collagen types I and IV was noted.

PART II CLINICAL STUDIES ON THE EXFOLIATION SYNDROME AND OPEN ANGLE GLAUCOMA

CHAPTER 8 PLAN OF PROPOSED CLINICAL INVESTIGATION

8.1 Exfoliation glaucoma in a group of Scottish surgical patients

The first clinical study of part II is an attempt to determine the prevalence, diagnostic features and response to trabeculectomy in exfoliation glaucoma. The rationale for this investigation is highlighted in sections 1.12 & 10.1. One hundred consecutive Scottish patients requiring trabeculectomy for open angle glaucoma (exfoliation glaucoma and POAG) were prospectively investigated (selection of patients and methods are discussed in section 9.1). Thirty-one consecutive patients with closed angle glaucoma were used as controls to determine whether the presence of exfoliation material is linked to the development of open angle glaucoma or represents a coincidental feature (discussed in section 10.3). All patients were comprehensively examined (section 9.1.2) by the author for the presence of the well known exfoliation and pigmentary signs (described in sections 1.7, 1.8 & 1.9).

Following surgery all peripheral iridectomy specimens were collected, fixed in buffered glutaraldehyde and processed through araldite for light and electron microscopy (see sections 3.2.2 and 9.1.2). Examination of the iris biopsies were carried out to provide the most accurate prevalence

figure before delineating the clinical features of exfoliation glaucoma and POAG in the studied group (reasons outlined in section 10.3). In addition, iris biopsy allowed determination of the accuracy of clinical observations (section 10.2.5). The aims of the first clinical study are:

(1) To determine the prevalence of exfoliation glaucoma in a Scottish surgical population and assess the optimal diagnostic features in such patients.
(2) To highlight important clinical differences between exfoliation glaucoma and POAG.
(3) To compare and contrast the surgical complications and IOP response after trabeculectomy in patients with exfoliation glaucoma and POAG.

8.2 Modification of trabeculectomy to avoid postoperative hyphaema

The second clinical study of part II aims at determining the influence of the position of a trabeculectomy fistula on the rate of postoperative hyphaema in a group of open angle glaucoma (exfoliation glaucoma and POAG) patients undergoing trabeculectomy. The rationale for this investigation is discussed in sections 11.1 & 11.3. Seventy-eight patients with either exfoliation glaucoma or POAG were randomised into two groups and examined by the author in a manner outlined in section 9.2. The principal aims of this study are given below:

(1) To investigate whether varying the size and site of the fistula in a trabeculectomy affects the postoperative rate of hyphaema and the subsequent duration of hospitalisation.

(2) To establish whether exfoliation glaucoma patients exhibit a higher incidence of postoperative hyphaema in comparison with POAG patients.

(3) To determine whether the type of surgery performed influences the final surgical outcome.

CHAPTER 9 PATIENTS AND METHODS FOR PART II

9.1 Exfoliation glaucoma in a group of Scottish surgical patients

9.1.1 Patients and control subjects

Selection criteria

Consecutive phakic patients deemed to warrant trabeculectomy for open angle glaucoma and admitted to the Tennent Institute between September 1989 and February 1991 were recruited to this study, which investigated the prevalence and clinical attributes of exfoliation glaucoma. Patients were enrolled if they were white Caucasian and born and residing in the Greater Glasgow area. Data concerning one eye of each of 100 patients operated on for POAG, or exfoliation glaucoma were entered in this study. Patients undergoing bilateral trabeculectomy had their first eye to be operated upon selected (23 cases). All degrees of severity of glaucoma were included in the present study.

Patients with closed angle glaucoma, secondary glaucoma, previous ocular surgery, concurrent ocular inflammation, diabetes mellitus and eyes with previous trabeculectomy procedure were excluded. Three patients who underwent trabeculectomy for open angle glaucoma during the course of this study were also excluded for other reasons, such as loss of the peripheral iridectomy specimen (2 cases) and poor state of general health hindering proper assessment (1 case).

A second clinical study, which investigated the prevalence of postoperative hyphaema in patients with POAG and exfoliation glaucoma, following two methods of surgical dissection (section 9.2) was carried out. Seventy-two out of the 100 patients enrolled in the first study were also included in this investigation.

Clinical and morphological classification

Following detailed clinical examination, seeking the signs listed in section 1.7, patients were allocated to one of three groups:

Group I (definite exfoliation glaucoma)

A diagnosis of exfoliation glaucoma was made if exfoliation material was present upon the pupillary margin, or on the lens surface.

Group II (possible exfoliation glaucoma)

Patients were categorised into this group if they exhibited pigment related signs and/or exfoliation syndrome/glaucoma in the other eye, (in the absence of biomicroscopical evidence of exfoliation material). The inclusion criteria for this group are shown in Table 9.1.

Group III (POAG, no evidence for exfoliation)

This group of patients consisted of all open angle glaucoma patients clinically assessed as having POAG and no signs commensurate with possible exfoliation glaucoma.

TABLE 9.1 INCLUSION CRITERIA FOR GROUP II

Loss of the pupillary ruff (>90°)
Pigment deposits upon the iris and/or the corneal endothelium
Moderate to dense pigmentation in the angle (2+ plus)
Presence of Sampaolesi's line in the angle
Exfoliation syndrome/glaucoma in the other eye

Light microscopy (LM) and transmission electron microscopy (TEM) provided a definitive diagnosis of the presence or absence of exfoliation material in all cases (see also section 3.2.2). Patients from groups I, II and III were classified morphologically into two final groups. They comprised exfoliation glaucoma patients (group A) and POAG patients (group B).

The determination of the definitive diagnosis allowed: 1) calculation of the diagnostic sensitivity, specificity and predictive value of the classic clinical features. 2) accurate delineation of the overall clinical characteristics of exfoliation glaucoma patients, in order to compare them with those of POAG patients in whom the diagnosis has been established without doubt (group B).

Closed angle glaucoma controls

Controls for the presence and role of exfoliation in the open angle glaucoma population comprised 31 consecutive patients who underwent trabeculectomy for acute, or chronic primary closed angle glaucoma. These patients had been admitted to the Tennent Institute during the course of this study. Only patients with primary closed angle glaucoma secondary to pupillary block were enrolled in the control group. These patients underwent a detailed clinical examination for the presence of exfoliation material, in a comparable way with that for the open angle glaucoma group, but only underwent pupillary dilatation postoperatively in order to complete the clinical examination to exclude concomitant exfoliation.

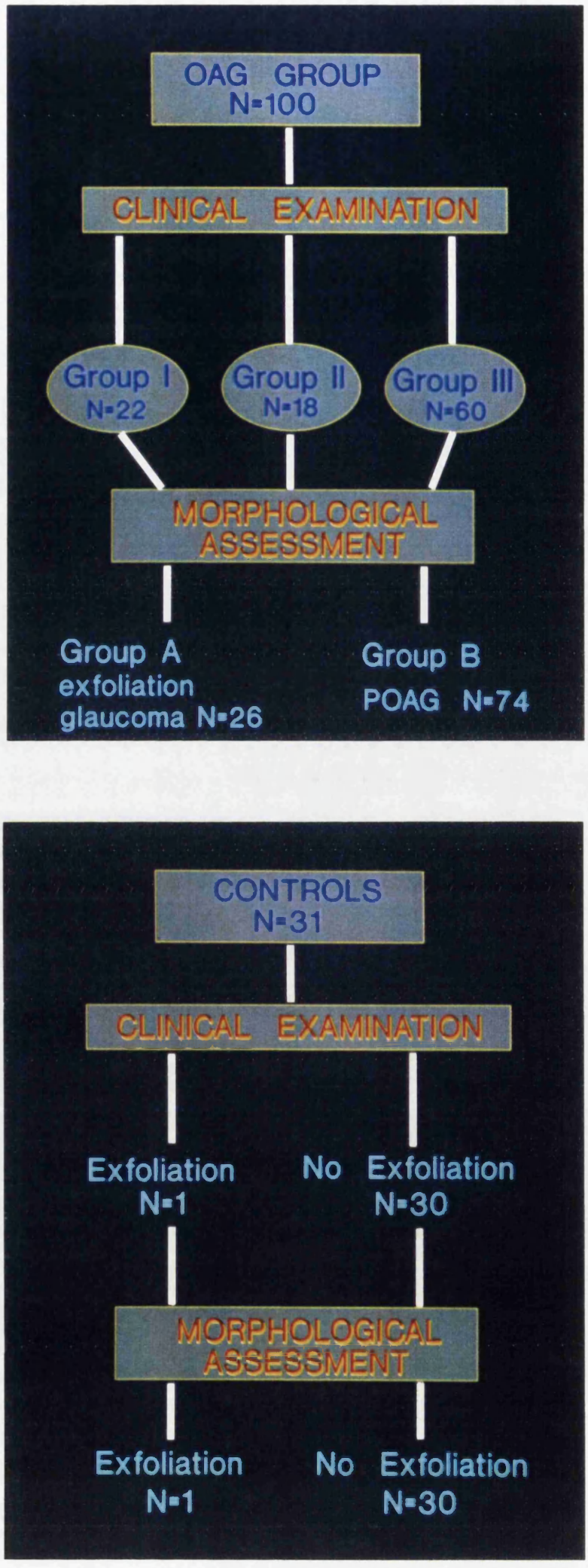


Figure 9.1: Flow chart indicating the research methodology followed in the exfoliation glaucoma study.

9.1.2 Methods

Preliminary data collection

Patient details were recorded on a study card, which was used to store and analyse the information obtained. All patients were given a serial code number preoperatively. The following information was recorded on the study card: sex, age, systemic disorders, other ophthalmic conditions, presenting clinical feature (screening, visual symptoms- vision/field loss- and pain) and details of glaucoma at time of diagnosis (IOP without treatment, cup to disc ratio and visual field). The same glaucoma details at the time of listing for surgery (including the IOP on medical therapy) were recorded together with the time from diagnosis to operation (in months). A detailed search of the case notes was conducted to identify whether the diagnosis 'exfoliation glaucoma' had been made prior to the admission of the patient to the hospital.

Indication for surgery

Patients were classified as follows: those who underwent primary trabeculectomy and individuals who were operated upon following failed medical therapy. For the purposes of the present study the term primary trabeculectomy refers to a decision for surgical intervention at the time of diagnosis of glaucoma. Any attempts to control the glaucoma medically, with one, or multiple medications, prior to filtration surgery were classified into the failed medical therapy group. The specific indication(s) for surgery in the group of patients who were operated upon because of failed medical

treatment were: 1) uncontrolled IOP, 2) progression of visual field loss, 3) uncontrolled IOP and progression of visual field loss, 4) intolerance/side effects to medications and 5) poor compliance. The failed medical regime at the time of listing for surgery was recorded for every patient.

Visual field grading

The visual field loss in this study was graded according to the classification by Jay and Murray (1988). Briefly, visual field changes were classified in 5 stages: early relative defects (stage 1); absolute defects outside 10 degrees of fixation in all quadrants (stage 2); encroaching between 10° and 5° from fixation in one to four quadrants (stage 3); within 5° of fixation 1-3 quadrants (stage 4); and within 5° of fixation in all quadrants (stage 5).

Ethnic origin

An attempt was made to identify the geographic region of the parents and grandparents of each patient. Particular attention was paid to recent Irish and English ancestry.

Examination for exfoliation

All patients with open angle glaucoma were examined by the author on a Haag-Streit 900 Slit Lamp in a dark room for the presence of exfoliation, before and after pupillary dilatation with two drops of tropicamide 1%. Control subjects with primary closed angle glaucoma were examined as above but in these patients pupillary dilatation was only employed after filtration surgery.

The biomicroscopic examination was conducted according to a standard protocol: first, the corneal endothelium was examined for the presence of pigment, exfoliation material and Sampaolesi's line. The anterior surface of the iris was inspected for the presence of pigmentary dust, the 6 o' clock pigmentary sign, new vessels and exfoliation material. The degree and localisation of pupillary and peripupillary iris atrophy was recorded in all cases. The presence, or absence of a relative afferent pupillary defect was noted together with the presence of iridodonesis, phakodonesis, or lens subluxation. All but twelve patients were gonioscopically examined (Goldmann gonioscope) for the presence of pigmentary signs consistent with exfoliation glaucoma. In these 12 unselected patients gonioscopy was not possible. Iris tissue from these 12 patients was submitted for LM and TEM assessment. Gonioscopic findings recorded in 88 patients included the presence of Sampaolesi's line, degree of pigmentation of the angle (1+ to 4+ according to Konstas & Dutton 1991), visible exfoliation material and angle depth.

Morphological examination

The protocol of morphological examination and the range of pathological changes is described in detail in sections 3.2 & 4.3. Following morphological assessment, exfoliation positive patients were categorised in Group A and exfoliation negative patients were included in group B.

Group I patients

Patients for whom the diagnosis of exfoliation glaucoma had been established underwent a detailed biomicroscopical

examination of the anterior segment to delineate the appearance and the distribution of exfoliation material and pigmentary signs in the anterior segment. Transillumination defects were drawn on their clinical cards. A drawing of the distribution of pigmentation and exfoliation material was made in every case. Particular attention was paid to the configuration of the exfoliation material deposited upon the lens (appearance of the central disc, intermediate zone, granular zone; peeling off; rolled off edge; bridges between the central disc and the granular zone) when pupillary dilatation was sufficient. The fellow eye in exfoliation glaucoma patients, was also examined. Any other ocular abnormalities were also recorded. Anterior segment photography was carried out when possible preoperatively. The majority of eyes were photographed postoperatively.

Group II

Possible exfoliation glaucoma patients were clinically identified according to the criteria described in Table 9.1. Suspicion of exfoliation was often raised because of the presence of pupillary ruff loss, subtle pigment deposits, or radial striations upon the lens surface. These features are consistent with the appearance of 'early disease' and have been described in sections 1.7 & 1.8. All iris specimens from these eyes were examined by LM and TEM to confirm, or disprove the presumed clinical diagnosis. To allow unbiased assessment all iridectomy specimens were coded.

Group III

Open angle glaucoma cases without evidence for exfoliation

glaucoma were categorised in group III. A flow chart of the classification and examination process is shown in Figure 9.1

Closed angle glaucoma controls

Following the clinical examination, morphology was used to confirm the absence of exfoliation material from the 31 control subjects with closed angle glaucoma (Fig 9.1).

Surgical technique

In the present study, each participating surgeon followed his own approach for the preparation of the conjunctival and scleral flap. The dissection of the inner block was randomised in 72 patients for the benefit of the second protocol (section 9.2). The first group of patients underwent a modification of the trabeculectomy method described by Cairns (1968) with the inner block located entirely in corneal tissue. The second group had a conventional inner block fashioned according to the method described by Watson (1970).

The operations were performed under local, or general anaesthesia depending on the choice of the surgeon and the patient. Approximately three quarters of the operations were performed under local anaesthesia. A facial nerve block with a mixture of Lignocaine 1% and bupivacaine 0.5% was used for lid akinesia during surgery. Subconjunctival injection of 0.5 cm^3 of Lignocaine 1% was employed in a number of cases to aid the dissection of the conjunctival flap. Retrobulbar or peribulbar anaesthesia was not used in most cases.

Following the insertion of a superior rectus suture, a fornix-based flap of conjunctiva and Tenon's capsule was reflected. A rectangular, or triangular outer scleral flap (half to two-thirds of the scleral thickness) was outlined measuring between 2.5x3 mm and 4x4 mm (depending on the surgeon). This flap was incised initially along the posterior incision which lies 2.5-4 mm posterior to the limbus. The edges of this flap were not extended into the cornea beyond the limbus. A small square measuring between 0.5x0.5 mm to 2.5x2 mm, (depending on the surgeon), was fashioned in the bed of the outer scleral flap. Anterior incision of the deep block was accomplished by two radial incisions at full thickness and the excision of the inner block was completed with straight Vannas scissors.

A peripheral iridectomy was performed in all cases by grasping the iris in its centre with a pair of Hoskin forceps and cutting it with DeWecker's scissors. The peripheral iridectomy specimen thus obtained was fixed immediately in buffered glutaraldehyde 2.5%. The superficial flap was sutured with one to three 10-0 nylon, or 8-0 vicryl sutures. The conjunctiva was closed with continuous, or interrupted sutures.

Postoperative assessment

Peroperative details and complications were recorded from the patients notes. During the immediate postoperative period, patients were treated with topical steroids and antibiotics 4-6 times a day and examined every day on the ward.

Postoperative complications sought during the hospitalisation period included: hyphaema, uveitis, fibrinous reaction in the anterior chamber, flat/shallow anterior chamber and raised IOP. Following the discharge of the patient the final follow up data were obtained from the patients notes approximately 6 months after surgery. Possible elevation of the IOP due to steroid reaction was recorded. All postoperative IOP values were recorded but only the untreated IOP, (a minimum of two weeks after cessation of drops), at approximately 6 months after surgery was used to determine the final outcome of surgery. Other complications including visual symptoms and surgical development of lens opacities were documented as were the indication for and nature of supplementary medical therapy.

Analysis of data

The prevalence of exfoliation in the open angle glaucoma group was compared with that of the closed angle glaucoma controls. All main features of groups A and B (exfoliation glaucoma and POAG group as determined by clinical and morphological assessment) were compared at diagnosis, time of listing for surgery, postoperatively and at the final follow up time (approximately 6 months after filtration surgery).

Parameters analysed for the two groups included age, sex, systemic diseases and cause of diagnosis (screening, visual loss). Glaucoma variables that were examined comprised: 1) the IOP at presentation (highest untreated value in mm Hg), the cup to disc ratio and visual field loss, 2) the duration of treatment before surgery, 3) the treated IOP at

the time of listing for surgery, 4) the cup to disc ratio and visual field loss 5) the indications for surgery, 6) the number of topical medications at time of listing and 7) the use of acetazolamide. Data for IOP was assigned into exposure levels. Three IOP gradings were analysed: 22-26 mm Hg, greater than 26 mm Hg and greater than 30 mm Hg.

Age and IOP matched subgroups

Two subgroups of patients were formed by exclusion of the higher and lower values for age in groups A and B in order to facilitate age-matched comparisons. These subgroups comprised 23 patients with exfoliation glaucoma and 57 patients with POAG matched for age: mean 71.9 and 70.9 years respectively (t-test p=0.54). In addition, comparisons were made between 20 exfoliation glaucoma patients and 20 POAG patients randomly selected and matched for age and untreated IOP at diagnosis. Finally, the 14 TEM exfoliation-negative patients from group II were compared and contrasted with glaucoma groups A and B (minus these 14 cases).

Sensitivity, specificity and predictive value

The accuracy of all clinical observations was estimated by calculating the sensitivity, specificity and predictive value for each clinical sign. The efficiency of any diagnostic sign may be assessed by determining the rates of true/false positive and true/negative results, which were defined by Chard (1988) as follows:

(1) True positive (TP): the result is positive, (exfoliation material was present morphologically), in the presence of the clinical sign in question.

(2) True negative (TN): the result is negative, (i.e. no exfoliation material detected on morphological examination) in the absence of the clinical sign in question.
(3) False positive (FP): the result is positive, (exfoliation material was present morphologically), in the absence of the clinical sign.
(4) False negative (FN): the result is negative (no exfoliation material detected morphologically), in the presence of the clinical abnormality.

For the purposes of this study, the sensitivity of each clinical sign was defined as an index of the ratio of all cases exhibiting exfoliation material morphologically (group A) correctly identified by the clinical sign. Specificity was defined as an index of the proportion of all patients in whom the absence of the exfoliation was accurately identified. Predictive value was defined as an index of the proportion of all cases who had exfoliation glaucoma identified by each clinical feature and those who actually have the condition (as determined by morphology). The formulae of calculation of these efficiency indices, as defined by Chard (1988), were as follows:

1) Sensitivity= TP/TP+FN;
2) Secificity= TN/TN+FP;
3) Predictive Value= TP/TP+FP

These values have been calculated for all clinical signs (exfoliation and pigmentary signs), which are relevant to the detection of patients with exfoliation glaucoma. Statistical analyses were performed with the Minitab software package. Differences between the groups were analysed by the two-

sample t-test, the chi-squared test, analysis of variance and non-parametric tests (Mann-Whitney U test and the Wilcoxon matched-pairs test) where appropriate. 'p' values of 0.05 or less were considered to be significant.

9.2 Modification of trabeculectomy to avoid postoperative hyphaema. The guarded anterior fistula operation

9.2.1 Patients

Selection criteria

A randomised prospective trial was set up to study first trabeculectomies for POAG and exfoliation glaucoma. One eye of 78 consecutive patients operated on between October 1989 and November 1990 was randomly allocated to either group A, or group B using a table of random numbers. Seventy-two patients for this group were also included in the first clinical study (exfoliation glaucoma in a Scottish surgical cohort). The allocation was identified from the code by the senior operating theatre nurse at the start of the operation and all 13 surgeons in the Tennent Institute of Ophthalmology participated.

Group A had the inner block fashioned and excised entirely anterior to the scleral spur so that only corneal tissue was excised (Fig 9.2). Group B had a conventional inner block of corneoscleral tissue extending posterior to the scleral spur with the trabecular meshwork near the centre of the excised tissue (Fig 9.2), (Watson 1970). Nine additional patients with POAG who underwent a trabeculectomy during the course of

the trial were excluded. The reasons for this were failure to allocate a randomisation in 2 cases, reluctance of the surgeon to take part (4 cases), other research requirements (3 cases). All degrees of severity of glaucoma were included in the present study, irrespective of level of IOP, visual field loss, optic disc cupping or duration of medical treatment. Patients with closed angle glaucoma, secondary glaucomas, previous ocular surgery and concurrent ocular inflammation were excluded. Patients undergoing combined extracapsular cataract extraction and trabeculectomy (18 cases) were also excluded from this study.

9.2.2 Methods

Data recorded

All patients were examined preoperatively by the author according to a predetermined protocol. The following information was recorded: sex, age, diabetes mellitus, arterial hypertension, haematological disorders, reason for diagnosis and details of glaucoma at time of diagnosis (IOP without treatment, cup to disc ratio and visual field). The same glaucoma details as described in section 9.1.2 were recorded together with the time from diagnosis to operation. The indication for surgery (uncontrolled IOP, progression of visual field loss, both, intolerance to medications and poor compliance) was also noted. The visual field loss was graded according to the classification by Jay and Murray (1988).

Surgical technique

The operations were performed under local, or general anaesthesia. Following the insertion of a superior rectus suture, a flap of conjunctiva and Tenon's capsule was reflected as shown in Fig 9.2. A rectangular or triangular outer scleral flap was fashioned as described in section 9.1.2. The edges of this flap were not extended into the cornea beyond the limbus. The inner block was dissected as described above according to the random allocation. A peripheral iridectomy was performed in all cases and the superficial flap was sutured with one to three sutures. The conjunctiva was closed with continuous or interrupted sutures. Viscoelastic substances were not used.

To ascertain the accuracy of dissection with respect to the scleral spur each surgical specimen was fixed immediately after excision in buffered glutaraldehyde (2.5%). The dimensions of the excised tissue were recorded and all specimens were examined with a dissecting microscope at a x50 magnification. Where doubt persisted as to the location of the dissected block, paraffin histology was used for confirmation (courtesy of Prof W.R. Lee). Similar histological confirmation was carried out in a random 25% sample of all specimens. If any part of the scleral spur, or a portion of trabecular meshwork was found in the specimens from group A, the case was reclassified as group B (posterior dissection). Similarly, inability to demonstrate anatomical structures consistent with the excision of a posterior block (scleral spur, part of the outflow system) in a specimen from group B led to its transfer to group A. The name of the

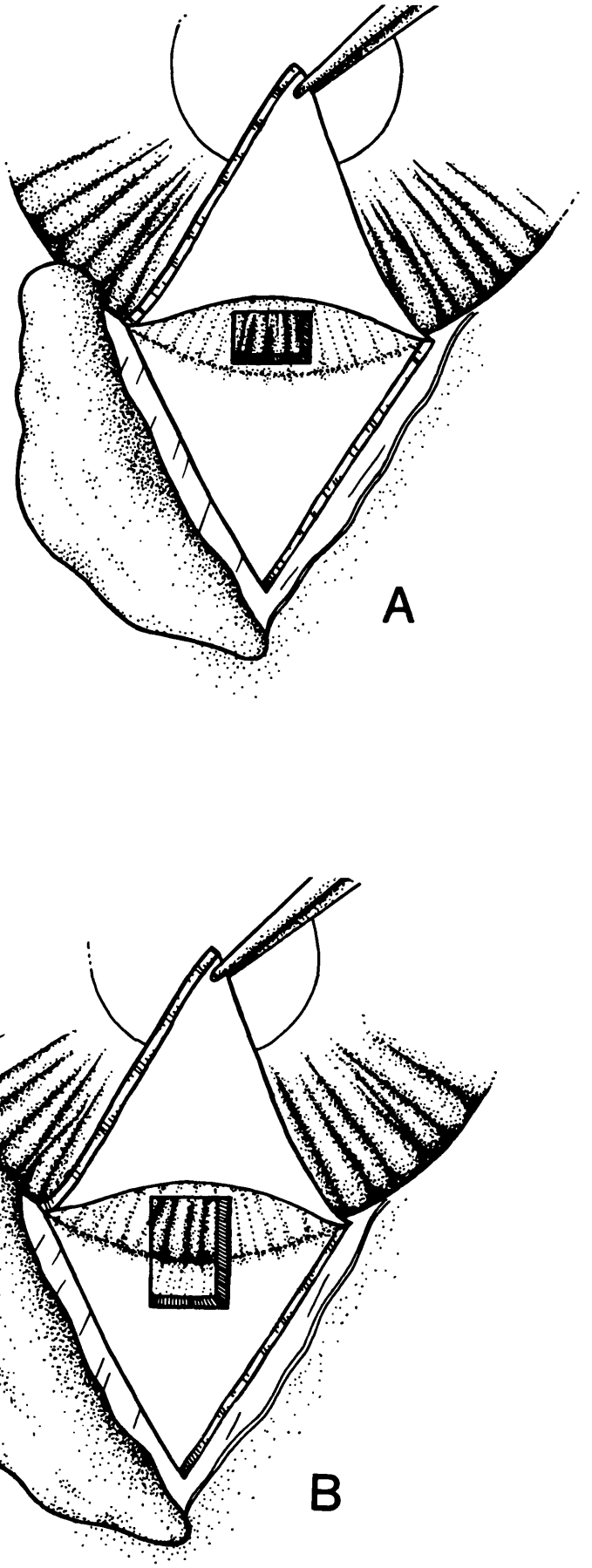


Figure 9.2: Schematic drawing of the area of tissue excised with the two methods of surgical dissection in the second study. In group A the dissection was anterior to the scleral spur and included only corneal tissue. Group B had excision of corneal and scleral tissue incorporating scleral spur and trabecular meshwork.

surgeon was recorded together with his comments as to whether there had been bleeding into the anterior chamber.

Postoperative follow-up

The following postoperative information was recorded: depth of the anterior chamber; hyphaema (grade 1-diffuse without formation of blood level; grade 2-up to 1 mm; grade 3-more than 1 mm), blood clot and other problems. The duration of hyphaema and hospitalisation were noted. Patient details were recorded three to seven days after discharge and again about six to eight weeks later. The final assessment was made approximately four months after the operation. The IOP was recorded after exclusion of possible steroid response at least two weeks after discontinuation of steroid eye drops (Thomas & Jay 1988) and without supplementary medical treatment. The reason for commencing any supplementary medical treatment after the operation was also recorded.

The results for the whole population were analysed first; subsequent separate analysis for the results for those patients with exfoliation glaucoma was also undertaken. Differences between the groups were analysed in a similar fashion to the exfoliation glaucoma study (9.1.2).

CHAPTER 10 EXFOLIATION GLAUCOMA IN A GROUP OF SCOTTISH SURGICAL PATIENTS. RESULTS AND DISCUSSION

10.1 Introduction

There are many unresolved questions concerning the clinical features of exfoliation glaucoma and its relationship to POAG. Most authors, interpreting clinical (Gillies 1972, Olivius & Polland 1980, Sugar 1984, Bertelsen 1985) and morphological (Rohen & Witmer 1972, Rohen 1983, Lutjen-Drecoll et al 1986) evidence believe that exfoliation glaucoma is a secondary open angle glaucoma, which differs from POAG. Others disagree believing that the entity 'exfoliation glaucoma' is merely a form of POAG (Tarkkanen 1984, Wollensak et al 1992).

The reported prevalence of exfoliation glaucoma within an open angle glaucoma population has varied from 0% to 79% (Leydhecker 1960, Aasved et al 1969, Blika & Ringvold 1987, Forsius 1988, Ringvold et al 1988, Konstas 1989) and remains a topic of controversy (for example Aasved 1969, Cashwell & Shields 1988; see also section 1.6). In addition, some authors have indicated that exfoliation glaucoma carries a worse prognosis than POAG, irrespective of treatment (Aasved 1971, Olivius & Thorburn 1978, Pohjanpelto 1985, Gloor & Robert 1989). These studies, however, are retrospective in nature and often inconclusive. Much of the controversy on the prevalence, attributes and prognosis of the disorder arises from the difficulty in establishing an unequivocal clinical diagnosis (Mizuno & Muroi 1979, Jerndal 1986).

On cursory examination of the eye the diagnosis may be missed (Tarkkanen 1984, Jerndal 1985, Prince & Ritch 1986 and section 1.7) and several reports suggest that the prevalence of the disorder is underestimated (Layden & Shaffer 1974, Layden 1982, Forsius 1988).

Electron microscopic examination of tissue biopsies can identify exfoliation material in cases where it is not detectable by clinical examination (Speakman & Ghosh 1976, Prince et al 1987, Sugino 1990). It has also been shown that a significant number of 'suspect' cases can be confirmed if electron microscopic examination of conjunctival biopsies is employed (Prince et al 1987). A number of clinical signs of disordered pigmentation in the anterior segment of the eye (see section 1.7.3) may assist in establishing the diagnosis in 'suspect' cases (Layden & Shaffer 1973, Jerndal 1986, Konstas & Dutton 1991) but, the specificity and sensitivity of these features is unknown.

To date, all comparative information about exfoliation glaucoma and POAG in surgical patients is retrospective (Jerndal & Kriisa 1974, Jerndal & Lundstrom 1977, Sziklai & Suveges 1988, Konstas & Allan 1989). Therefore, a prospective comparative study of Scottish patients with disease severe enough to require trabeculectomy was designed.

10.2 Results

10.2.1 Prevalence

Open angle glaucoma

One hundred consecutive patients with open angle glaucoma were prospectively examined by the author in this study.

Group I

Prior to this investigation, 19 out of these 100 surgical patients (19%) had been diagnosed as suffering from exfoliation glaucoma, whereas the remaining 81 patients had been considered to suffer from POAG. Clinical examination performed by the author upon the total glaucoma population on the day of admission confirmed the diagnosis in all 19 patients previously considered to suffer from exfoliation glaucoma. In addition, 3 patients, in whom exfoliation glaucoma had previously been undiagnosed, were detected. In one of these three cases the central disc of exfoliation material deposit on the lens was not visible on slit-lamp examination, but the granular zone was. Therefore the corrected clinical prevalence of exfoliation glaucoma in the total open angle glaucoma population under investigation was 22%. Light and electron microscopic assessment (section 9.1) confirmed the clinical diagnosis by identifying exfoliation material in the iris of these patients.

Group II

This group (see Table 10.1) consisted of 18 patients who demonstrated pigmentary signs on biomicroscopic, or

gonioscopic examination (16 cases), and/or exfoliation glaucoma in the other eye (2 cases). Ultrastructural assessment (LM and TEM) of iris tissue from all 18 possible exfoliation patients identified exfoliation material in the iris of 4 patients (22%).

Group III

Clinical examination identified 60 POAG patients without biomicroscopic, or gonioscopic evidence of exfoliation glaucoma. In none of these 60 iris biopsies was exfoliation material present on pathological examination.

Closed angle glaucoma (control subjects)

There were 31 control patients, 15 with acute closed angle glaucoma and 16 with chronic closed angle glaucoma. Only one control subject exhibited clinical and morphological evidence of exfoliation (3.2%). Exfoliation material was significantly more common in the open angle glaucoma group than in the closed angle glaucoma group (x^2 test, $0.01>p>0.001$).

10.2.2 Clinical features

The mean age of the exfoliation glaucoma group, 73.3 years (S.D., 7.3), was significantly higher than the mean age of the POAG group, mean 66.7 years (S.D., 10.3), (t-test, $p<0.001$). To ensure that the differences reported are not due to age, comparisons between exfoliation glaucoma and POAG patients were made after age matching (section 9.1.2). Where clinically relevant, groups A and B were also compared.

TABLE 10.1 SPECIFIC DIAGNOSTIC CRITERIA FOR GROUP II

No	AGE	SEX	PRL	OTHER CLINICAL SIGNS	GONIOSCOPY	MORPHOLOGY
1	64	M	>180°	iris pigm	pigm 2+ SPL	exf +
2	70	F	>180°	corneal pigm; exf other eye	pigm 3+ SPL	exf +
3	71	F	360°	iris pigm	pigm 3+	exf +
4	69	M	360°	exf other eye	pigm 3+ SPL	exf +
5	83	F	>180°	-	pigm 2+	exf -
6	80	M	>180°	-	pigm 2+	exf -
7	73	F	360°	-	pigm 1+	exf -
8	81	M	360°	corneal pigm; PCL	pigm 2+	exf -
9	74	M	>180°	iris pigm	pigm 2+	exf -
10	75	M	>180°	iris pigm	pigm 3+ SPL	exf -
11	68	M	360°	lens pigm	pigm 2+	exf -
12	60	M	120°	lens pigm; lens striae	pigm 2+	exf -
13	65	M	>90°	-	pigm 1+	exf -
14	68	M	360°	-	pigm 1+	exf -
15	70	M	>180°	iris pigm; lens pigm	pigm 2+	exf -
16	75	F	>180°	corneal pigm	pigm 1+	exf -
17	77	M	>90°	-	pigm 1+	exf -
18	71	F	>180°	-	pigm 2+	exf -

Key: PRL: pupillary ruff loss in degrees; M: male; F: female; pigm: pigmentation; exf: exfoliation; PCL: precapsular layer of the lens; SPL: Sampaolesi's line.

Fig 10.1: This table shows the clinical signs of each patient in group II (possible exfoliation glaucoma).

No difference was observed between groups A and B and age matched exfoliation glaucoma and POAG patients for sex and systemic diseases: arterial hypertension,(x^2 test $p>0.80$), cardiovascular disorders, (test $p>0.20$), and respiratory conditions ($p>0.10$). With regard to other ophthalmic conditions one patient with exfoliation glaucoma had Fuchs' heterochromic cyclitis.

A positive family history of glaucoma was established in one out of the 26 patients with exfoliation glaucoma and in 14 out of the 74 patients with POAG (x^2 test, $0.10>p>0.05$). With regard to ethnic origin, it is difficult to draw conclusions in the West of Scotland since there is a mixture of Scottish and Irish ancestry. Nevertheless, in group A (exfoliation glaucoma group) 6 patients gave a recent history of Irish and one of Greek ancestry (i.e. parents, or grand-parents born in Ireland, or Greece). In the POAG group six patients gave a recent history of Irish and one of English ancestry. Recent Irish ancestry was more common in the exfoliation glaucoma group ($0.05>p>0.02$). No apparent difference was observed in the incidence of bilateral glaucoma in groups A and B. Twenty-one out of the 26 patients with exfoliation glaucoma and 56 out of 74 patients with POAG had bilateral glaucoma ($p>0.80$).

The presenting clinical features (screening, visual symptoms, pain) were analysed prior and subsequent to age-matching. In 3 out of the 26 patients from group A the cause of diagnosis was loss of vision in the other eye caused by exfoliation glaucoma in two cases and central retinal vein occlusion in

the eye of one patient with untreated exfoliation glaucoma. Patients with exfoliation glaucoma were diagnosed more frequently because of visual symptoms (x^2 test, $p<0.001$ prior to age matching; $p<0.01$ after age matching). In contrast, POAG patients were more often detected due to screening performed by opticians ($p<0.001$). However, when exfoliation glaucoma and POAG patients were matched for age and IOP the difference concerning visual symptoms disappeared.

The untreated IOP at diagnosis in exfoliation glaucoma, 36 mm Hg (S.D., 8.5), was significantly higher than that of group B, 29.6 mm Hg (S.D., 6.2), (t-test, $p=0.0012$). The difference between the two groups persisted at various levels of IOP (higher than 26 mm Hg group, $p=0.014$; higher than 30 mm Hg, $p=0.038$). After age matching, the difference between exfoliation glaucoma and POAG patients concerning the IOP at presentation remained significant: 36.7 mm Hg (S.D., 8.6) in exfoliation glaucoma, compared to 29.0 mm Hg (S.D., 5.7) for POAG, ($p<0.001$). Age-matched exfoliation glaucoma patients exhibited a higher untreated IOP at presentation for various levels of IOP (higher than 26 mm Hg, $p=0.021$; higher than 30 mm Hg, $p=0.04$). There was no difference between males and females in each of the glaucoma groups. When age matched male and female patients with exfoliation glaucoma were compared separately with their POAG counterparts, a higher IOP at diagnosis was found for both male ($p=0.028$) and female ($p=0.006$) exfoliation glaucoma patients.

At the time of listing for surgery, the mean treated IOP of age matched exfoliation glaucoma patients, 34.2 mm Hg (S.D.

12.5) was significantly higher than the mean treated IOP for comparable POAG patients, 24.6 mm Hg (S.D., 6.1), (t-test, p= 0.002). No difference for sex was observed in each group. Following age and IOP matching, the IOP at the time of listing for surgery was found to be higher in the exfoliation glaucoma group, mean 34.2 mm Hg (S.D., 10.3) compared with 28.2 mm Hg (S.D., 7.0) in the POAG group.

Age matched exfoliation glaucoma patients were treated medically prior to surgery for a significantly shorter period, mean 8.4 months (S.D., 8.3), compared with POAG patients, mean 39.1 months (S.D., 45.0), (t-test, $p<0.001$). However, when the two glaucoma groups were matched for age and IOP at diagnosis the difference in the duration of medical therapy was not significant ($p=0.08$)

The visual field loss at presentation was analysed for the whole group and in stages; prior to and following age and IOP matching. There was no difference at diagnosis, or at the time of listing for surgery in the degree of visual field loss between groups A and B and between age matched exfoliation glaucoma and POAG patients ($p>0.20$). Similarly, no apparent difference was observed following matching for age and IOP at presentation. When the two age matched glaucoma groups were compared with regard to progression of visual field loss from the time of diagnosis to the time of listing for surgery only eyes with POAG exhibited significant deterioration ($0.02<p<0.05$). No difference was observed at diagnosis and at the time of listing for surgery in the cup to disc ratio for the two glaucomas before and subsequent to

age matching and following matching for age and IOP at diagnosis ($p>0.40$).

A comparison of the indications for surgery for groups A and B is shown in Table 10.2. Two patients from each group refused primary trabeculectomy. A larger proportion of patients with exfoliation glaucoma had undergone primary trabeculectomy, ($0.05>p>0.02$). Once suitably matched for age the difference concerning primary trabeculectomy was not significant ($0.10>p>0.05$).

In the failed medical therapy group, exfoliation glaucoma patients were more often operated upon due to unacceptably high IOP (t-test, $0.05>p>0.02$ prior to age matching; $p<0.01$ after age matching). Subsequent to age and IOP matching the rate of primary trabeculectomy and the frequency of surgery due to unacceptably high IOP were similar in the two glaucomas. Progressive loss of visual field without recognised high IOP was more frequent in POAG than in exfoliation glaucoma patients (Table 10.2), ($p<0.01$ before and after age matching).

No difference was noted between the two age matched groups in the rate of poor compliance ($0.10>p>0.05$) and intolerance to antiglaucoma medications ($p>0.30$). The medications used by the group of patients operated upon following unsuccessful medical therapy were further analysed. Exfoliation glaucoma patients were using acetazolamide ($p<0.001$) and a combination of three medications ($p<0.001$) more often than their age matched POAG counterparts. One patient with exfoliation

TABLE 10.2 INDICATIONS FOR FILTRATION SURGERY

INDICATION FOR SURGERY	GROUP A (EXG)	GROUP B (POAG)
Primary trabeculectomy	9	11
Failed medical therapy	17	63
-due to raised IOP	9	17
-due to loss of VF	2	21
-due to raised IOP and VF loss	3	11
-and/or intolerance to medical Rx	5	14
-and/or poor compliance	5	7

Key: VF: visual field; IOP: intraocular pressure; Rx: therapy; EXG: exfoliation glaucoma; POAG: primary open angle glaucoma.

The indications for filtration surgery are shown for all patients in the exfoliation glaucoma study.

TABLE 10.3 GONIOSCOPIC FINDINGS IN OPEN ANGLE GLAUCOMA POPULATION

GONIOSCOPIC FEATURE	GROUP A N=26	GROUP B N=62
Pigmentation		
no pigment	0	13
grade 1+	0	35
grade 2+	10	14
grade 3+	10	3
grade 4+	6	0
Sampaolesi's line	24	1
Exfoliation material	5	0

glaucoma on medical therapy had lost central fixation because of progressive visual field loss. There was no comparable event in the POAG group.

10.2.3 Slit lamp findings

The biomicroscopic and gonioscopic features observed were as follows:

Exfoliation signs

Whitish flakes of such a size as to suggest exfoliation material were found on the central and inferior retrocorneal surface in 9 out of the 26 cases of group A (35%); (Figs 10.1 & 10.2). Clinical examination revealed exfoliation material on the pupillary border of 20 patients from group A (77%); Four of the 6 cases that did not show evidence of exfoliation material on the pupil were from group II. Slit-lamp microscopic examination failed to reveal the presence of a central disc (Fig 10.3) in 5 exfoliation glaucoma patients (17%). The peripheral granular zone was detected in all 22 patients from group I (Figs 10.4 & 10.5), but was absent from the 4 morphologically determined exfoliation-positive cases from group II (possible exfoliation). Solitary flakes of exfoliation material, in association with the central disc, were observed in 6 out of the 26 patients from group A (23%). In one case a bridge of exfoliation material was seen to connect the granular zone with the central disc (Fig 10.5). The configuration of the central disc and that of the granular zone exhibited significant variations (Fig 10.3). A pronounced rolled over edge of the granular zone was observed

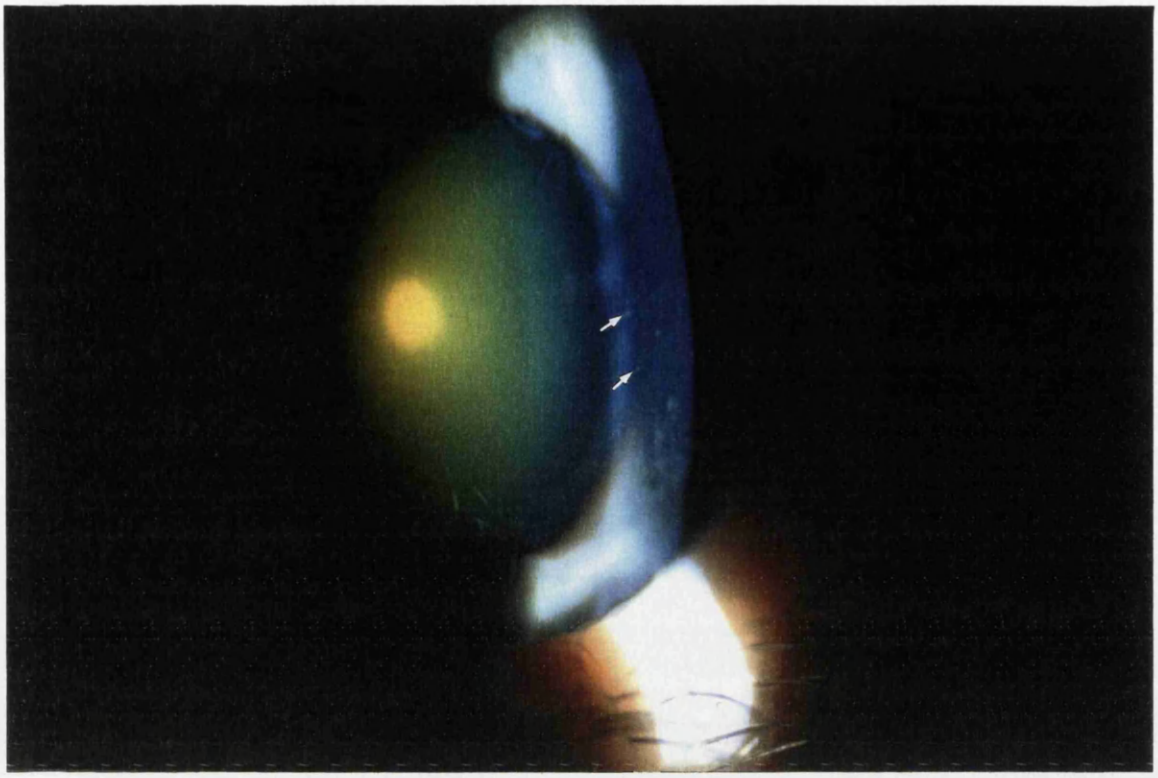


Figure 10.1: Slit-lamp photograph of corneal endothelium in a patient from group A. Note deposition of flecks of exfoliation material (arrows).

Figure 10.2: Slit-lamp photograph of the corneal endothelium in a patient from group A. In this case exfoliation material has formed a large 'dendritic' aggregate (arrow).

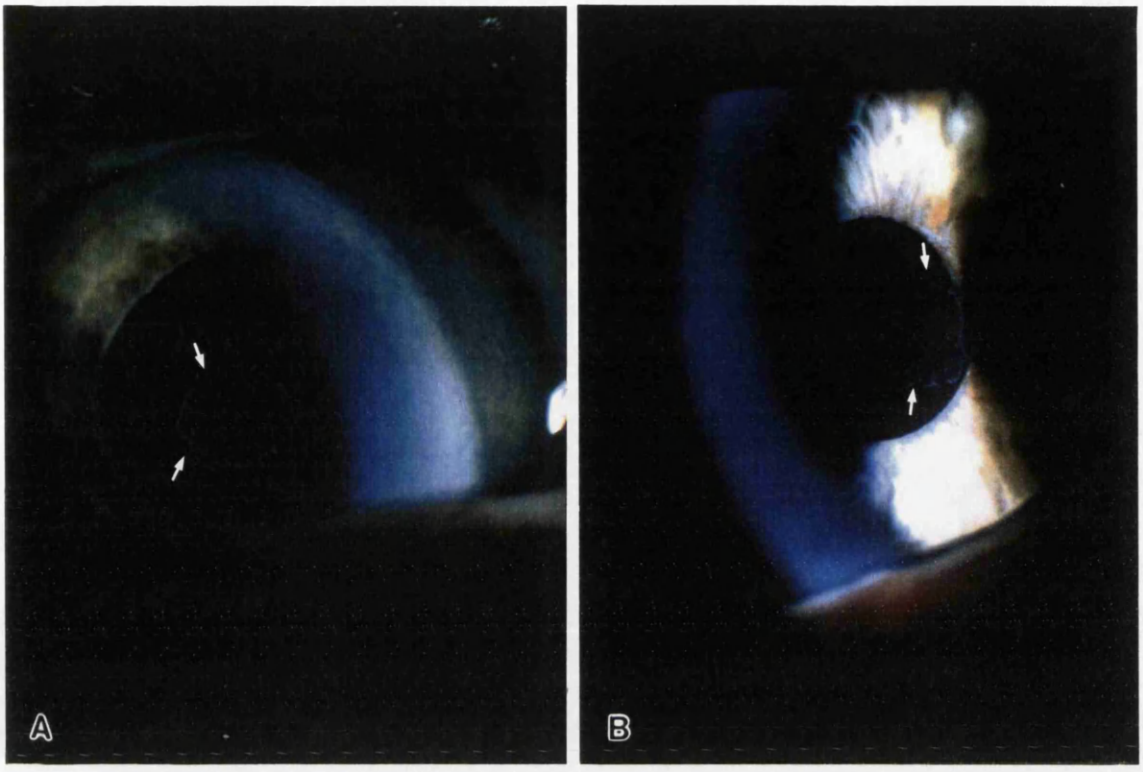


Figure 10.3A,B: Slit-lamp photographs of the central disc (arrows) in group A showing central (10.3A) and eccentric (10.3B) location, illustrating the variability of this clinical sign.

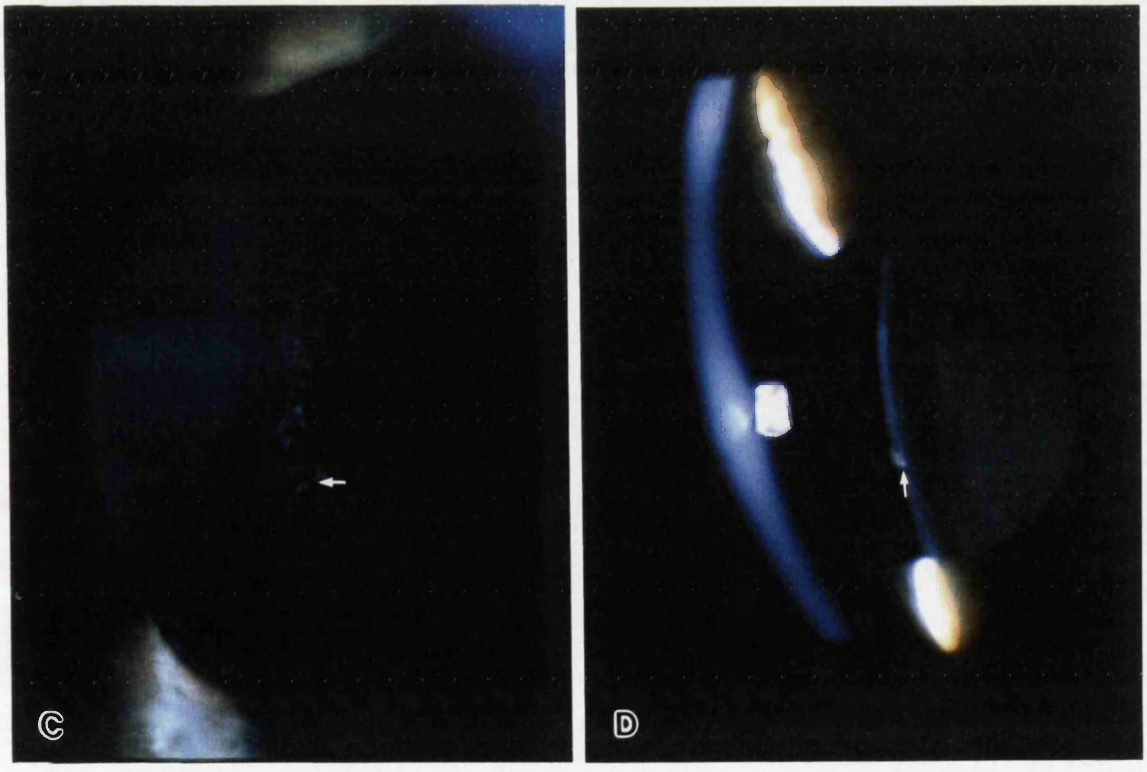


Figure 10.3C,D: Fig 10.3C shows exfoliation material (arrow) in the boundry of the central disc in the same patient as Fig 10.3A. In Fig 10.3D a more conspicuous central disc is demonstrated, with a thickened inferior edge (arrow).

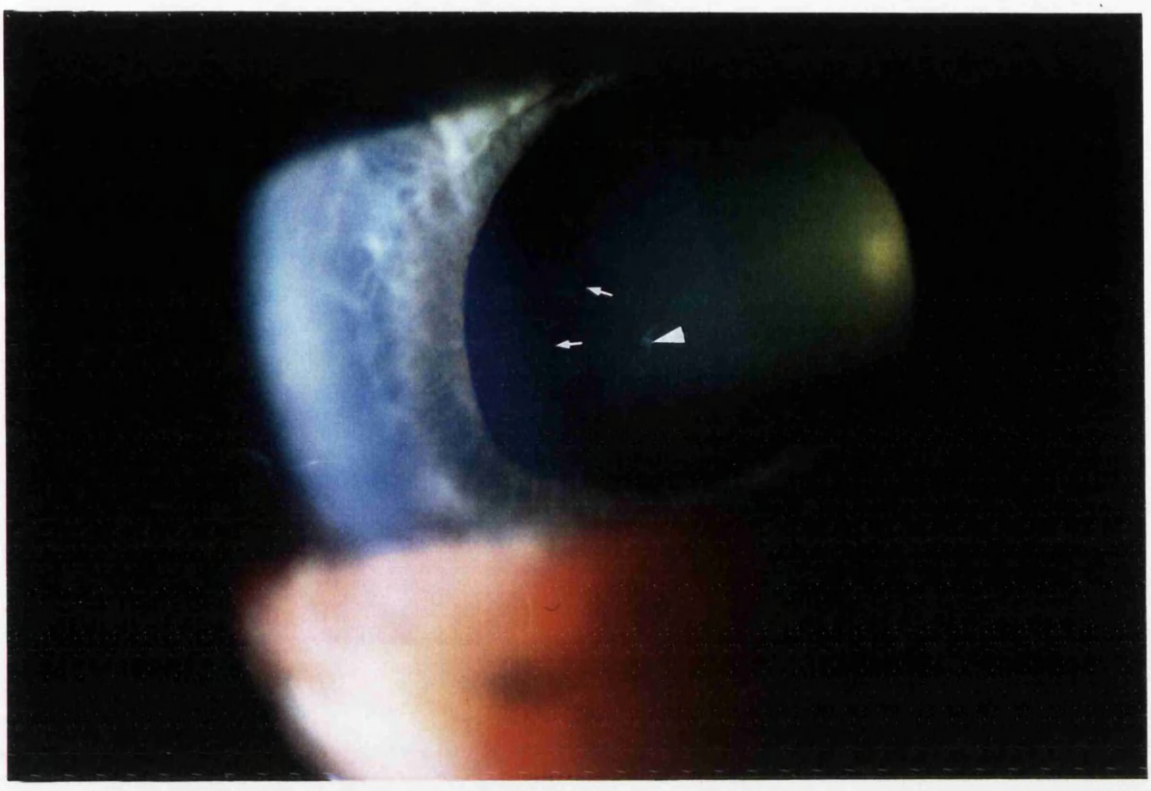


Figure 10.4: Slit-lamp photograph of a patient from group A. No exfoliation material is evident on the pupillary margin but a serrated granular zone is clearly demonstrated (arrows). Note also peeling of exfoliative lens capsule (arrowheads).

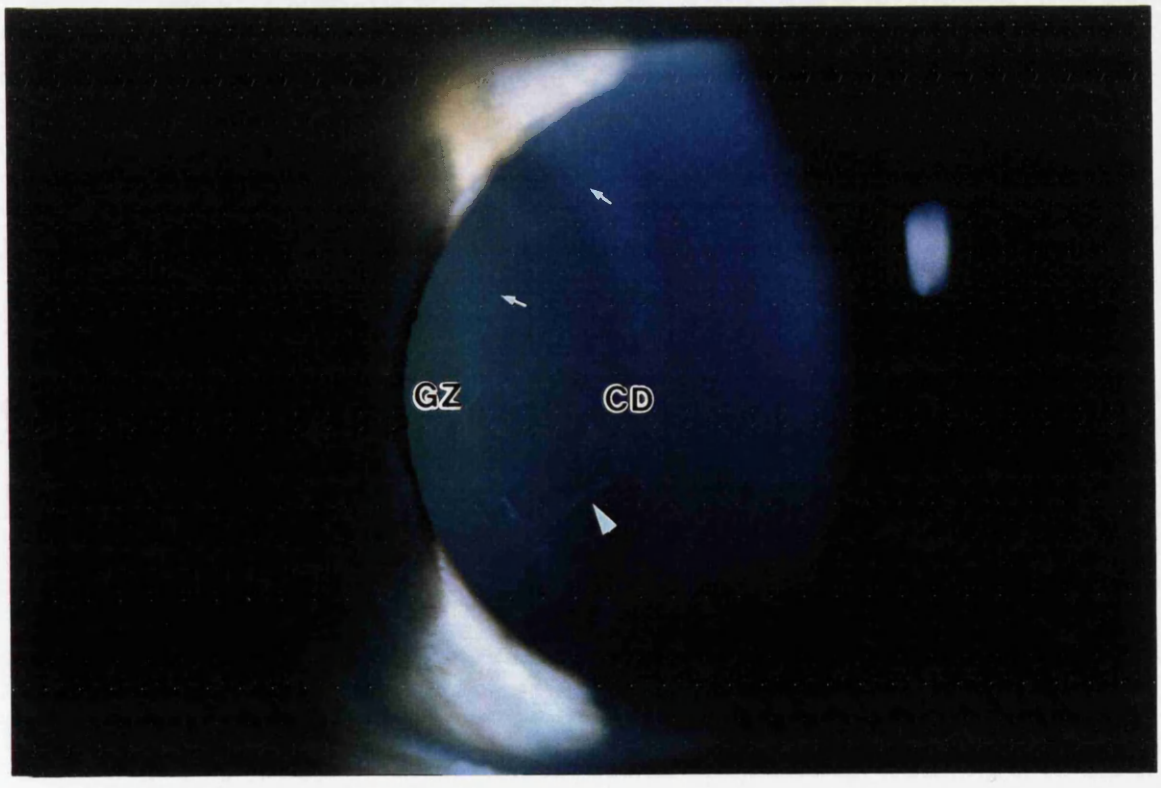


Figure 10.5: Biomicroscopical appearance of the granular zone (GZ) in a patient from group A. In contrast to Fig 10.4 the inner edge of the granular zone is smooth and intact (arrows). A bridge connects the GZ with the central disc (CD) is arrowheaded

in one case. Two cases demonstrated evidence of peeling of the exfoliative lens capsule (Fig 10.4), with a small strip of their lens capsule floating in the anterior chamber.

Pigmentary signs

Discrete pigmentation of the corneal endothelium was detected by slit-lamp examination in 17 out of 26 exfoliation glaucoma patients (65%). A visible pigmented line (extension of Sampaolesi's line) was detected upon the inferior posterior corneal surface in 9 out of 26 cases with exfoliation glaucoma (35%). Twenty-two out of the 26 group A patients (85%) demonstrated subtle pigmentary dust scattered upon the anterior surface of the iris. This feature was more prominent on lightly pigmented irides. A discrete pigmentary cluster localised at the 6 o' clock position in the inferior iris circumference was noted in 14 out of the 26 patients (54%). Biomicroscopically visible fine new vessels were seen on the anterior iris of one patient and exfoliation material upon the anterior surface of the iris was noted in 3 other cases (11.5%).

Well demarcated patches of peripupillary transillumination were noted in 16 out of the 26 patients in group A (62%); (Fig 10.8B). Five of these patients also exhibited transillumination of the iris mid-periphery. All but two patients of group A demonstrated advanced to complete atrophy of the pupillary ruff (greater than 180°). Two patients exhibited moderate atrophy of the pupillary ruff (less than 180°). Discrete pigment granules at the mid-periphery of the lens were seen in 8 cases from group A (31%); (Fig 10.8D).

The 4 cases diagnosed only by morphological examination showed marked atrophy of the pupillary ruff (Fig 10.9). Additionally, there was moderate to dense pigmentation of the trabecular meshwork in all these patients and Sampaolesi's line was present in 3 patients (Fig 10.10). One case displayed fine pigment deposits upon the iris surface and a pigmented trabecular ring gonioscopically, without gonioscopic evidence of Sampaolesi's line.

Other signs

A relative afferent pupillary defect at presentation was noted in 8 out of the 26 untreated exfoliation glaucoma patients. In contrast, only 4 out of the 74 untreated POAG patients demonstrated a relative afferent pupillary defect at diagnosis. The difference was significant (t-test, $p<0.001$). Iridodonesis accompanied by phacodonesis and partial lens subluxation was observed in one exfoliation glaucoma patient (Figs 10.11 & 10.12).

POAG patients

The proportion of POAG patients in this study is shown in Figures 10.8 & 10.9. Pigment deposition upon the posterior corneal surface was noted in 4 out of the 74 POAG patients from group B (5.4%). The difference between groups A and B and age matched exfoliation glaucoma and POAG patients was statistically significant ($p<0.001$). POAG patients demonstrated a significantly lower incidence of subtle pigment deposition on the iris (6.7%), ($p<0.001$); pigment deposition upon the anterior lens surface (5.4%), ($p<0.001$) and peripupillary transillumination (3%) ($p<0.001$). One

patient from group B with a lenticular precapsular film and one with gonioscopic evidence indicating the presence of Sampaolesi's line were subsequently shown on ultrastructural examination to be exfoliation-negative.

A degree of pupillary ruff loss was relatively common in POAG patients. Overall, 38 out of 74 patients from group B (51%) exhibited pupillary ruff loss up to 180°. Further analysis indicated that POAG patients were more likely to exhibit pupillary ruff loss up to 180°, whereas exfoliation glaucoma patients were more likely to manifest pupillary ruff loss greater than 180°, (x^2 test, prior to age matching $p<0.001$; after age matching $p<0.01$).

10.2.4 Gonioscopic findings

The gonioscopic findings of 26 exfoliation glaucoma patients and 62 POAG patients are shown in Table 10.3. The two glaucoma groups were similar with regard to angle narrowness. Exfoliation glaucoma patients were more likely to show moderate to dense pigmentation (grades 3-4+) of the trabecular meshwork (t-test, $p<0.001$) and the presence of Sampaolesi's line ($p<0.001$); (Figs 10.6 & 10.7).

10.2.5 Accuracy of clinical observations

In order to give an indication as to the utility of each clinical sign detected during the course of this study the sensitivity, specificity and predictive value of each sign was calculated. Table 10.4 illustrates the efficiency of

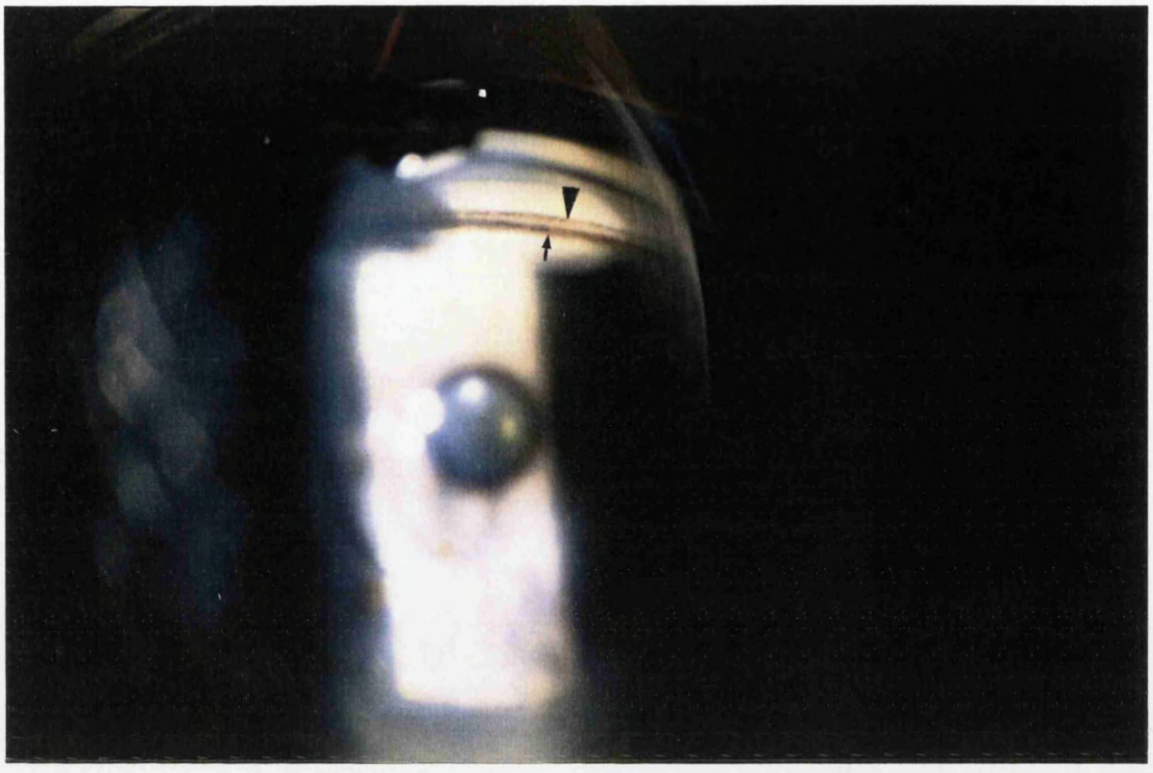


Figure 10.6: Gonioscopic photograph of the inferior angle in a patient from group A. Deposition of pigmentation is greatest at the 6 o'clock position (arrow). Sampaolesi's line is clearly seen (arrowhead).

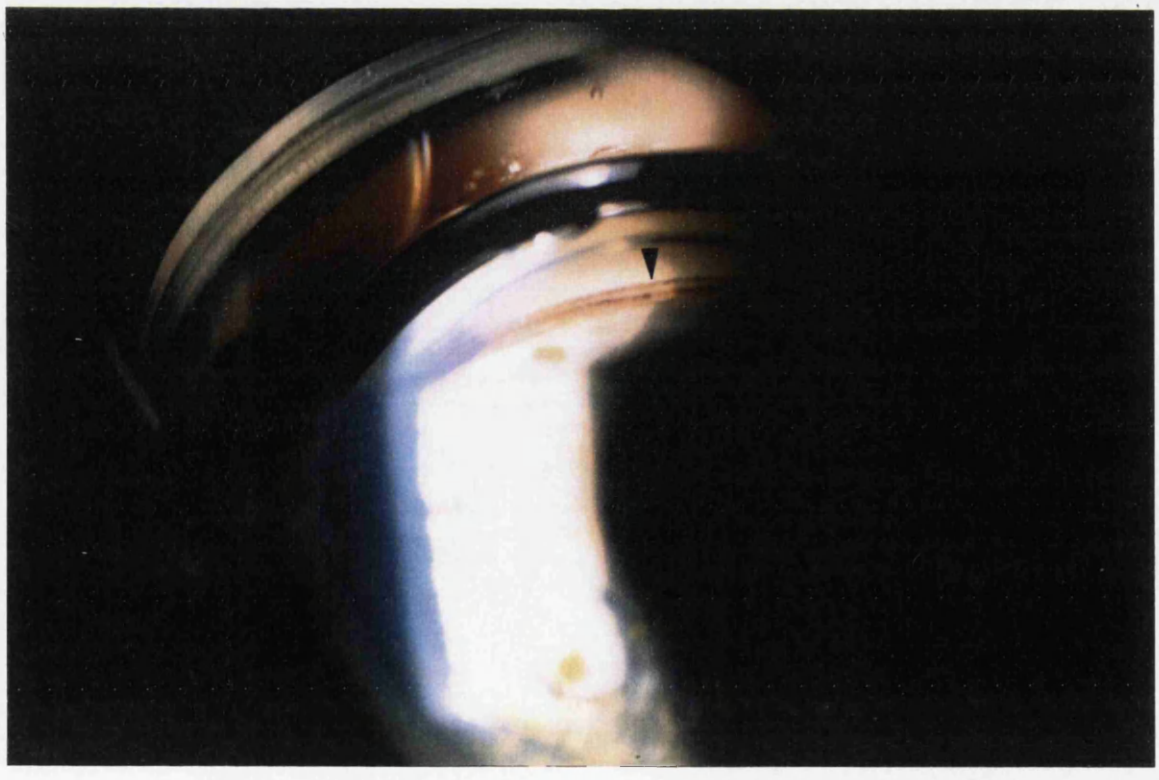


Figure 10.7: Gonioscopic photograph of the inferior angle in a patient from group A, illustrating irregular punctate pigmentation of the trabecular meshwork. Note Sampaolesi's line (arrowhead).

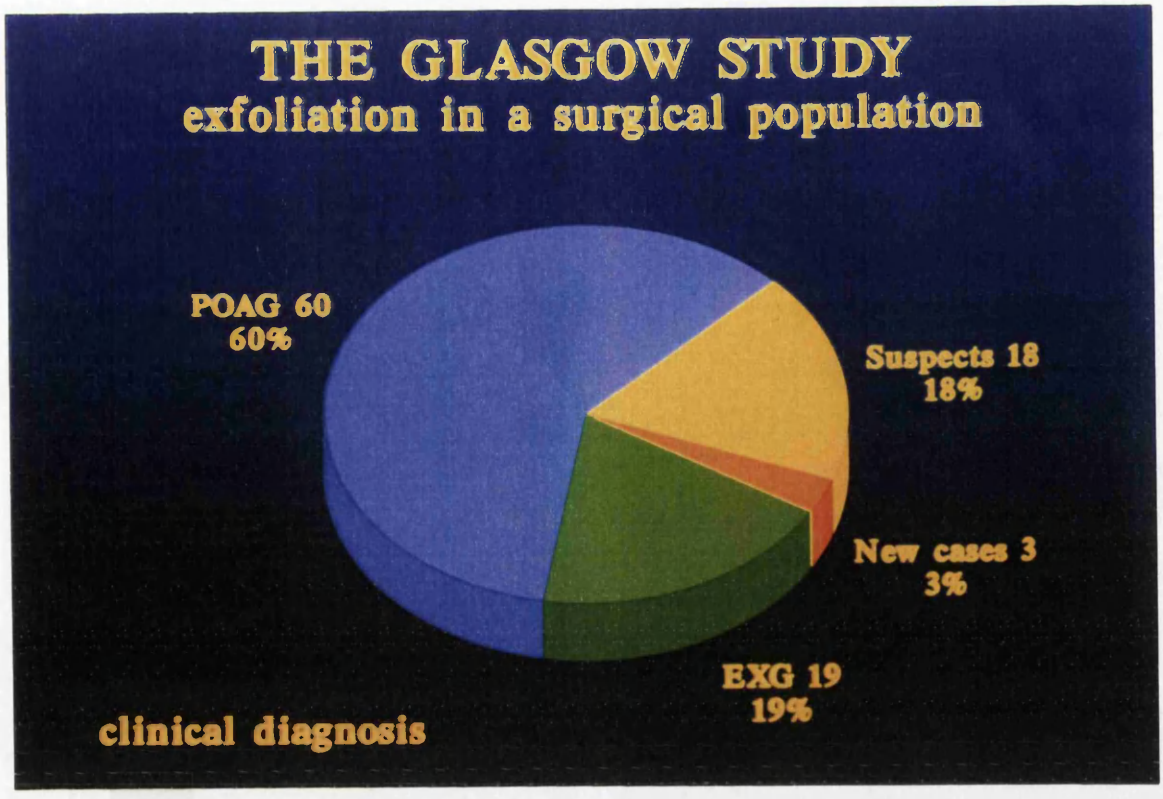


Figure 10.8: Pie chart showing the proportion of patients admitted for trabeculectomy with previously diagnosed exfoliation glaucoma (EXG), newly diagnosed exfoliation glaucoma, possible exfoliation glaucoma (suspects) and POAG.

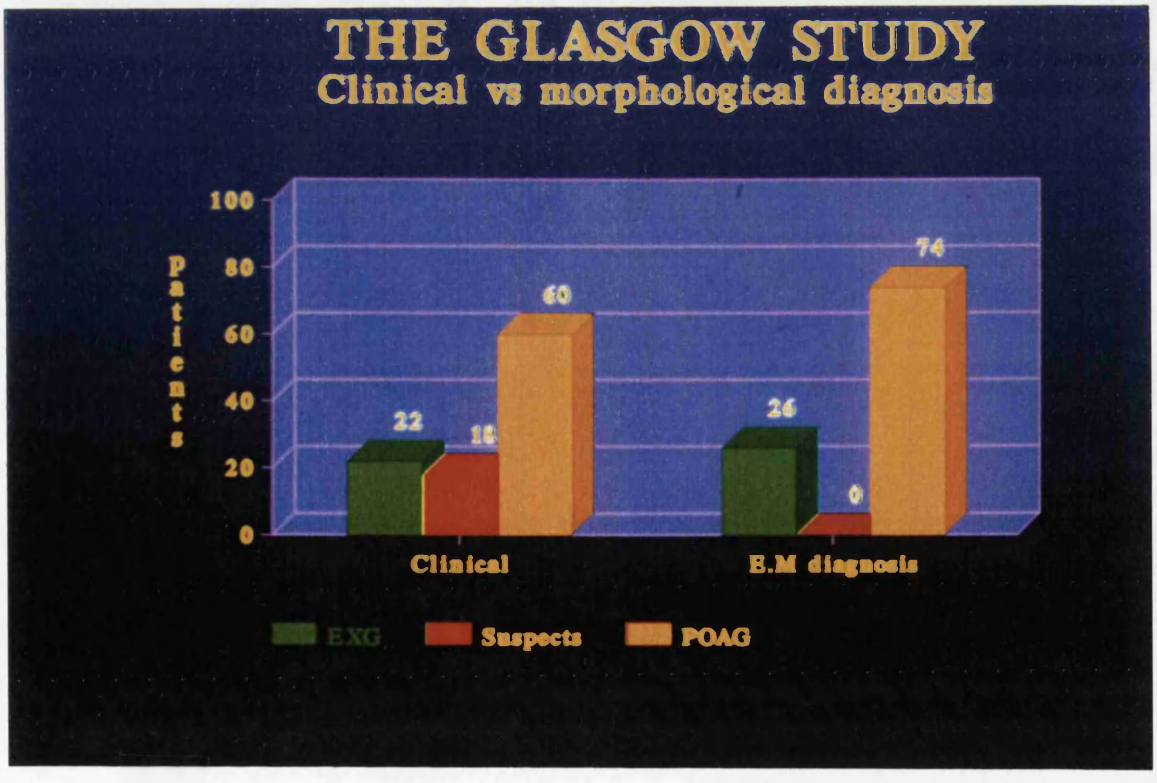


Figure 10.9: Histogram showing the prevelance of exfoliation glaucoma in the surgical cohort according to clinical and morphological criteria. Patients with possible exfoliation (suspects) are subsequently categorised definitively by morphology.

TABLE 10.4 ACCURACY OF CLINICAL OBSERVATIONS

CLINICAL FEATURES	SENSITIVITY	SPECIFICITY	PREDICTIVE VALUE
Exfoliation signs			
Presence of granular zone	100%	95%	85%
Presence of central disc	100%	94%	81%
Exf on the pupil	100%	92%	77%
Exf on the corneal endothelium	100%	81%	35%
Exf in the angle	100%	75%	19%
Pigmentary signs			
SPL in the angle	96%	97%	92%
Loss of pupillary ruff >180°	40%	95%	92%
Iris pigmentation	81%	94%	87%
Corneal pigmentation	81%	89%	65%
Angle pigmentation 3-4+	84%	85%	61%
Tranillumination defects	89%	88%	61%
Iris pigment at 6 o' clock	93%	86%	54%
SPL on inferior cornea	100%	81%	37%
Lens pigmentation	67%	79%	31%

Key: exf: exfoliation material; SPL: Sampaolesi's line.

Table 10.4: The table shows sensitivity, specificity and predictive value for the clinical signs used in the diagnosis of exfoliation glaucoma in the present study.

each individual clinical sign/observation in predicting the 'final' diagnosis (exfoliation glaucoma determined by morphology, group A). Overall, comprehensive clinical examination in this study had a 100% sensitivity, 95% specificity and 85% predictive value.

Group II (exfoliation-negative) patients

Further analysis was undertaken on the subgroup of patients from group II (possible exfoliation glaucoma), who were exfoliation-negative on ultrastructural examination. These 14 patients were compared with the remaining POAG patients from group B and the total exfoliation glaucoma group. They were similar to the remaining POAG patients with regard to sex, degree of visual field loss, level of treated IOP at the time of listing for surgery, frequency of primary trabeculectomy and incidence of surgery due to unacceptably high IOP. They were different from the remaining POAG patients in that they were older, mean 72.8 years (S.D., 6.4), ($p=0.003$); they exhibited a higher untreated IOP at presentation, mean 34.2 mm Hg (S.D., 7.6), ($p=0.012$) and, they were treated medically for a shorter period of time, mean 16.4 months (S.D., 27.5) versus a mean of 43.8 months (S.D., 50.1) for the remaining POAG patients, ($p=0.008$). They were different from exfoliation glaucoma patients (group A) in that they exhibited a lower treated IOP at the time of listing for surgery, mean 25.8 mm Hg (S.D., 6.0) versus 33.0 mm Hg (S.D., 12.3) for group A.

10.2.6 Surgical complications

Patients in group A were compared with those in group B with regard to immediate postoperative complications. No patients required re-operation to correct surgical complications. One patient with clinically significant choroidal detachment was re-admitted to hospital. No infections were observed within the follow up period of this study (6 months). Patients from group A exhibited a significantly higher incidence of fibrinous reaction in the immediate postoperative phase (5 cases versus one for POAG) ($p<0.001$). No significant difference between exfoliation glaucoma and POAG patients was observed for the presence of postoperative hyphaema ($0.10>p>0.05$), postoperative uveitis ($0.10>p>0.05$), temporary reduction of visual acuity ($p>0.80$) and reduction in anterior chamber depth ($p>0.80$). Postoperatively, the ratio of steroid responders in group A (2 out of 26) was similar with that in the POAG group (10 out of 74).

10.2.7 Final follow up

All patients completed the required follow up (6 months) except one patient with exfoliation glaucoma who died one month after surgery and one patient with POAG who was lost to follow up. Of the 98 patients completing the study follow up time, only one patient with POAG (1%) could not be controlled with medical therapy and underwent a second trabeculectomy. One patient with exfoliation glaucoma (4%) and 9 patients with POAG (12%) required supplementary medical therapy (one medication each). Examination of cases in which the IOP

remained controlled after surgery only with the addition of supplementary medical therapy showed that in 6 cases (one with exfoliation glaucoma and 5 with POAG) one medication was added due to an IOP level in excess of 22 mm Hg. In the remaining 4 POAG patients medical therapy was added in an attempt to reduce the final IOP further (prior to therapy postoperative untreated IOP equal or below 22 mm Hg).

The most striking finding at the 6-month follow up, was a significant difference in the mean untreated IOP, including failures, of the two glaucoma groups. Age matched patients with exfoliation glaucoma showed lower untreated mean IOP, 11.8 mm Hg (S.D., 4.4), when compared with similar POAG patients, 15.0 mm Hg (S.D., 4.6), ($p=0.008$). With regard to visual field loss, group A (one case with deterioration, one with improvement in visual field) was similar to group B (5 cases with evidence of deterioration and 9 with improvement in visual field). Progression of lens opacities was observed in two cases in both groups.

10.3 Discussion

To my knowledge this is the first prospective comparative study of exfoliation glaucoma and POAG patients. A definitive diagnosis was obtained in every case by iris biopsy. It was found that exfoliation glaucoma affects almost a quarter of patients requiring surgery. Thus the disorder may be relatively common in the glaucoma population of Western Scotland. Detailed data on the prevalence of exfoliation glaucoma in British surgical cohorts is lacking.

Only Mills (1981) has estimated a prevalence of 3.2% for exfoliation glaucoma in 444 surgical cases in Manchester, but this retrospective study may have underestimated the prevalence of the disorder. The prevalence of exfoliation glaucoma in surgical groups has been reported to vary between 3% and 88% (Aasved 1971, Jerndal & Kriisa 1974, Jerndal & Lundstrom 1977, Mills 1981, Ruprecht et al 1985, Sziklai & Suveges 1988, Konstas & Allan 1989, Psilas et al 1990). It is likely that sampling errors may result in either underestimation, or overestimation of the prevalence of exfoliation glaucoma depending on the diagnostic criteria employed (see section 1.7).

Some of the data presented herein (e.g. IOP at diagnosis, cause of diagnosis, IOP at final follow up) had to rely on retrospective retrieval from case notes. It is recognised that this may have affected the quality of that data. The prevalence of exfoliation glaucoma in surgical patients does not correspond accurately with the prevalence of the condition in a clinical setting or, particularly, in a population sample (Aasved 1971a). The policy of all clinicians in the Tennent Institute of Ophthalmology during the course of this study was to carry out early surgical intervention. The surgical glaucoma cohort investigated is therefore likely to be more representative of a general glaucoma population than cohorts reported in previous comparable studies.

Most authors consider that the presence of exfoliation material is directly associated with the development of

a secondary open angle glaucoma, herein referred to as exfoliation glaucoma. However, this view is not universally shared. Some authors have suggested that the presence of exfoliation material is coincidental and that these patients suffer from POAG (Trantas 1929, Cebon & Smith 1976, Tarkkanen 1984, Wollensak et al 1992). Closed angle glaucoma patients were included in this study as controls to determine whether the presence of exfoliation is linked to the development of open angle glaucoma, or represents a coincidental feature. If the presence of exfoliation material in the aged eye was a coincidental finding similar frequencies would have been expected in the two comparable glaucoma cohorts: the open angle glaucoma (100 cases) and the closed angle glaucoma group (31 cases). The eightfold increase in the presence of exfoliation material in the open angle glaucoma group confirms that exfoliation material is either the cause of glaucoma, or alternatively represents an aggravating factor in an eye vulnerable to POAG.

In this study 3 previously undiagnosed cases were detected clinically by the author and 4 more cases were only diagnosed by iris biopsy. The clinical diagnosis of exfoliation glaucoma is facilitated when a specific comprehensive clinical examination is carried out (section 1.7). The present study suggests that specific clinical examination of surgical patients to identify exfoliation material should detect about 85% of patients with exfoliation glaucoma with no false positives. Surgical patients were used so that biopsy of the iris could be obtained. Exfoliation material was found in all 22 definite exfoliation glaucoma patients,

in 4 out of 18 (22%) possible exfoliation glaucoma patients and in none among the POAG group without clinical suspicion for the presence of exfoliation. Thus, the definite clinical identification as well as the 'absence' of exfoliation material seem reliable. The value and reliability of iris biopsy was demonstrated by the detection of exfoliation material in all 22 cases with the clinical diagnosis of exfoliation glaucoma.

Prince et al (1987) found exfoliation material in conjunctival biopsies in 8 of 23 (34.7%) 'suspect' eyes using similar criteria to group II in this study. The prevalence in group II (22%) was not dissimilar to the prevalence reported by Prince et al (1987), considering the fact that they included both eyes in 7 patients. In agreement with that study, this study suggests that in a group of patients with pigmentary features but no visible exfoliation material the diagnosis of POAG is likely to be inaccurate in some cases.

Accuracy of clinical observations

To my knowledge this is the first study that has attempted to put a quantitative value to diagnostic features. The iris biopsy allowed critical evaluation of the specificity, sensitivity and predictive value of the diagnostic signs. Signs stated in the literature to indicate the positive identification of exfoliation syndrome/glaucoma include the detection of exfoliation material upon the cornea, pupillary margin, angle and lens capsule. Even though these signs exhibit a 100% sensitivity (i.e. all patients exhibiting

these signs actually have the disorder) their clinical usefulness is lessened by their reduced specificity and predictive value. For example, the presence of exfoliation material upon the pupillary margin showed a predictive value of only 77%, that is to say, in 23% of the patients with exfoliation glaucoma this sign was not detectable. Even the presence of the granular zone, considered a pathognomonic feature present in all exfoliation cases (section 1.7.2) showed a predictive value of only 85%.

Similar results were obtained with the pigmentary signs. For example, Sampaolesi's line in the angle and the loss of the pupillary ruff (180° or more) were both found to be of high predictive value (92% and 87%). In the case of the latter however, a low degree of specificity (40%) was observed. Angle pigmentation grades 3-4+ were gonioscopic signs of reasonably high sensitivity and specificity, but of less predictive value (61%). The combination of three of the above signs, namely the presence of the granular zone, Sampaolesi's line and angle pigmentation 3-4+ will assure a 100% accurate index of detection.

Patients with possible exfoliation glaucoma

In the absence of clinically obvious exfoliation material, the presence of exfoliation glaucoma in the other eye (2 out of 2 cases) and the presence of Sampaolesi's line (3 out of 4 cases) were reliable indicators of the ultrastructural presence of exfoliation material. With the latter, one false positive case was found, where the presence of Sampaolesi's line was not associated with the presence of exfoliation

material in the iridectomy specimen. The involvement of the clinically 'normal' fellow eye found in each of the two cases of unilateral exfoliation glaucoma correlates well with previous evidence (Mizuno & Muroi 1979, Konstas 1989, Miyake et al 1989, Hattori 1990, Atsumi et al 1991). For Sampaolesi's line one false positive case was found.

Four cases from the possible exfoliation glaucoma group (group II) were only included in the exfoliation glaucoma group following the morphological study of iris tissue from these patients. In a clinical study such patients would have been accorded the wrong status (POAG). Previous studies reliant solely upon clinical examination for disease classification have no doubt been similarly afflicted.

Age and exfoliation

In the present study patients coming to surgery with exfoliation glaucoma were significantly older than POAG patients. This is in agreement with previous studies (Aasved 1969, Ruprecht et al 1985, Khanzada 1985, Tarkkanen 1986, Forsius 1988). The age difference introduces bias, which has seldom been taken into account. Aasved (1971) established that in both exfoliation glaucoma and POAG that optic nerve damage increased with age. In addition, some studies have suggested increased susceptibility for males to exfoliation glaucoma (Bartholomew 1976, Konstas & Allan 1989, Ayed et al 1990) and a significantly higher IOP in male patients with exfoliation glaucoma (section 1.12), but the present study found no such differences.

Exfoliation glaucoma and POAG

The impressions of previous workers that exfoliation glaucoma is associated with a particularly high level of IOP (section 1.12) have been confirmed in the present study. The untreated IOP at presentation was higher in the exfoliation glaucoma patients than that seen in comparable POAG patients. This difference in presenting symptoms in exfoliation glaucoma is related to this higher IOP as the difference in symptoms disappeared when the groups were matched for level of IOP at diagnosis. This difference was not age, or sex related and may be attributed to the different pathogenesis of exfoliation glaucoma (section 1.5.2). In addition, the mean treated IOP at the time of listing for surgery was significantly higher than that seen in a similar POAG group. Exfoliation glaucoma patients were prescribed more medications, were using acetazolamide more often and were treated medically for shorter periods of time (on average less than a year). Despite this they more often required surgery for unacceptably high IOP. These findings suggest that the pharmacological control of the IOP is more difficult in exfoliation glaucoma and probably justifies the decision to operate earlier.

The similarity between the two glaucomas for visual field loss at presentation is in agreement with a retrospective study conducted by Lindblom & Thorburn (1984b). This study established the same degree of visual field loss at diagnosis for exfoliation glaucoma and POAG, despite a higher mean IOP in the former (42.9 mm Hg versus 34.8 mm Hg for POAG). The similarity of the degree of visual loss at the time of

listing for surgery between exfoliation glaucoma and POAG contradicts previous retrospective studies and may be due to the short duration of medical therapy before surgery (8 months).

The long term results of conventional management of exfoliation glaucoma have been disappointing. Olivius & Thorburn (1978) reported that 5 years after diagnosis, treated exfoliation glaucoma patients exhibited severe visual field loss in 48% of cases compared with only 19% in the POAG group. Furthermore, at the end of their follow up period, (mean 10 years), 40% of the eyes with exfoliation glaucoma as compared with 26% of the eyes with POAG had become legally blind (Olivius & Thorburn 1978). There was a difference in the management of exfoliation glaucoma in that study compared with the present study: surgery in their cohort was employed only as a last resort, whereas in the present study early surgery was often undertaken.

Trabeculectomy creates a guarded outflow passage for aqueous from the anterior chamber to the subconjunctival space (Koryllos 1967, Cairns 1968, Jay 1991). The factors that determine the establishment of sufficient filtration are not completely understood (Herschler 1985, Lamping et al 1986, Stewart 1990). There is evidence that the inflammatory reaction and fibrotic response of the involved tissues influence the final outcome (reviewed by Herschler 1984). Certain preoperative characteristics such as young age, black race, aphakia, re-operation and ocular inflammation may increase the chance of bleb failure (Jerndal & Lundström

1977, Jay & Murray 1980, Konstas 1989, Dereklis et al 1990, Jay 1991). Patients with these risk factors were excluded from this study.

The clinical presentation, time course, indications for surgery and the therapeutic results of trabeculectomy have not previously been documented prospectively in a well defined exfoliation glaucoma group. Indeed, in many studies the surgical glaucoma groups studied represent an ad hoc mixture of different glaucomas. This prospective study confirms the hypothesis that exfoliation glaucoma patients coming to surgery differ from comparable POAG patients in some important respects. The most important difference is the higher level of untreated and medically treated IOP and the lower level of IOP 6 months after surgery.

Surgical complications

As with any form of surgery there may be complications following trabeculectomy. A comprehensive review of these is given by Leblanc & Stewart (1986). However, serious complications after trabeculectomy seem very rare (Jerndal & Lundström 1977, Wilson 1977, Mills 1981, Yamashita et al 1985, Watson et al 1990, Mylopoulos 1991). In the present study the complications of trabeculectomy in exfoliation glaucoma and POAG were assessed. There was no significant difference in the frequency of postoperative hyphaema in this study, but a fibrinous exudate in the aqueous was more common in exfoliation glaucoma patients. This finding has not been previously reported in association with filtration surgery and may be due to the exfoliation vasculopathy (chapter 7).

Postoperative steroid response

It is well documented in the literature (see section 1.12.1) that in exfoliation syndrome/glaucoma there is a lack of IOP elevation following steroid administration and this feature distinguishes exfoliation glaucoma from POAG. In this study no significant difference in steroid response was observed between exfoliation glaucoma and POAG following filtration surgery. Since the trabecular meshwork is the site of the pathology responsible for the IOP elevation (Armaly 1986), it is possible to speculate that filtration surgery eliminates the difference between the two glaucomas.

Final IOP

This study identified a significantly lower untreated postoperative IOP for exfoliation glaucoma than in comparable POAG patients, at approximately 6 months after surgery and this was not related to the duration of medical therapy before surgery. Jerndal & Kriisa (1974) and Jerndal & Lundström (1977) found a favourable IOP lowering effect but did not provide comparison with POAG. Törnqvist and Drolsum (1991) did perform a retrospective comparative study and found that progression of visual field loss occured less often in the exfoliation glaucoma group following surgery compared to age matched POAG patients. It is thus possible that a genuine difference in IOP response following surgery differentiates exfoliation glaucoma from POAG. This concept may be supported by the ultrastructural evidence known about the morphology of the outflow system and the pathogenesis of exfoliation glaucoma (section 1.5.2). A characteristic feature distinguishing exfoliation glaucoma from POAG is the

'mechanical blockade' of the outflow system by pigment and exfoliation material (Rohen 1983, Rohen & Jikihara 1988). It is thus reasonable to hypothesise that filtration surgery can be more effective in exfoliation glaucoma since it relieves the mechanical obstruction caused by particle deposition.

Lavin and coworkers (1990) have proposed that earlier surgical intervention may provide significantly better IOP control. This may be due to a reduction of the duration of medical therapy and a decrease in the adverse influence of pharmacological agents upon the conjunctiva and Tenon's capsule (Lavin et al 1990). Certainly, prolonged medication has been associated with the risk of a pemphigoid reaction in the conjunctiva (Jay 1992) and increased amounts of inflammatory cells have been identified in conjunctival biopsies after prolonged medical therapy (Sherwood et al 1989). In the present study POAG patients had a mean duration of medical therapy of 39 months as compared with a mean of 8.4 months for exfoliation glaucoma patients. In the study by Lavin and coworkers (1990) the medical therapy group (mean 77 months) was treated considerably longer than the primary trabeculectomy group (medical therapy for a few weeks). Therefore, it is considered unlikely that the difference in IOP response in the present study was due to the more prolonged duration of medical therapy in group B.

Many published reports have failed to make a distinction between POAG and exfoliation glaucoma. There are differences between these two open angle glaucomas (see section 1.12). The worse prognosis assumed in exfoliation glaucoma is true

for medical therapy, but may not apply in the case of timely surgical intervention. Early surgery may be an advantage in exfoliation glaucoma.

10.4 Summary

The 'true' prevalence and clinical attributes of exfoliation glaucoma were investigated prospectively in a Scottish surgical cohort. One hundred consecutive patients undergoing trabeculectomy for open angle glaucoma were investigated by clinical examination (biomicroscopy and gonioscopy and classified into 3 categories: exfoliation glaucoma, possible exfoliation glaucoma and POAG. A definitive diagnosis of exfoliation glaucoma was provided by pathological examination of iris tissue. All 22 patients with clinical evidence of exfoliation glaucoma and 4 out of 18 patients with possible exfoliation glaucoma on clinical examination had ultrastructural evidence of exfoliation material. The prevalence of exfoliation glaucoma was therefore 26%.
The sensitivity, specificity and predictive value of each clinical sign was calculated. In comparison with POAG, patients with exfoliation glaucoma had higher untreated IOP, higher IOP on medical therapy and shorter duration of medical therapy. They were more often operated on for unacceptably high IOP. Exfoliation glaucoma patients exhibited significantly lower IOP after surgery.

CHAPTER 11 MODIFICATION OF TRABECULECTOMY TO AVOID POSTOPERATIVE HYPHAEMA THE 'GUARDED ANTERIOR FISTULA' OPERATION

11.1 Introduction

Trabeculectomy is the standard drainage operation for open angle glaucoma (Cairns 1968, Watson 1970, Watson & Barnett 1975, Watson & Grierson 1981, Jay 1991). Its main advantage over full thickness drainage procedures is a reduced incidence of complications (Watkins & Brubaker 1978, Mills 1981, Lamping et al 1986, Konstas 1989, Watson et al 1990). The success and safety of the procedure have been established (Wilson 1977, Zaidi 1980, Watson & Grierson 1981, Jay & Murray 1988), so that recently there has been a tendency towards earlier surgery (Jay & Murray 1988, Jay & Allan 1989, Lavin et al 1990, Jay 1991; 1992).

Nevertheless, filtration surgery may be delayed because of fears that there may be surgical complications. The frequency of postoperative hyphaema varies from 15 to 53 per cent (Ridgway 1974, Watson & Barnett 1975, Wilson 1977, Zaidi 1980, Mills 1981, Shuster et al 1984, Yamashita et al 1985, Le Blanc & Stewart 1986), and although it is seldom a serious complication, it may prolong the patient's stay in hospital. It may also cause temporary reduction in vision which may be a serious handicap if vision is poor in the other eye. This study was designed to confirm and quantify the hypothesis that excising the inner block anterior to the scleral spur reduced the frequency of postoperative hyphaema.

11.2 Results

Patient characteristics

All patients were Caucasians and each group comprised 39 eyes of 39 patients. The patient characteristics for the two groups are shown in Table 11.1. There was no significant difference between the two groups (A and B) for sex, age, reason for diagnosis and presence of systemic diseases (arterial hypertension, diabetes mellitus, haematological disorders). Table 11.2 shows the glaucoma status. At diagnosis there was no difference between the groups for IOP without medication, cup to disc ratio, prevalence of exfoliation glaucoma and duration of medical treatment. Similarly, there was no difference at the time of surgery for intraocular pressure with medication and cup to disc ratio. Neither was there any significant difference for severity of visual field loss at diagnosis and at time of surgery, or for indication for surgery (early trabeculectomy; failed medical treatment).

Twenty-nine patients had their operation performed by three consultants, 12 by two senior registrars and 37 by eight registrars. There was no bias for any level of seniority in the two groups.

Classification

In each group two patients were found to have tissue specimens which did not correspond with the intended style of dissection and these two patients were reclassified according to the histological findings. For the main analysis this

Table 11.1A

	n	Mean age (± SD)	Male/female ratio	arterial hypertension	diabetes mellitus	thrombocytopenia
Group A	39	68.6 (± 10.4)	20/19	12	1	0
Group B	39	67.7 (± 9.1)	21/18	7	1	1

Table 11.1B

		AT DIAGNOSIS		Duration of medical treatment (months)	AT TIME OF OPERATION	
	n	mean IOP (untreated, mm Hg)	C/D ratio		mean IOP (treated, mm Hg)	C/D ratio
roup A	39	31 (± 9.6)	0.6 (± 0.2)	39.7 (± 59)	25.9 (± 6.9)	0.6 (± 0.1)
roup B	39	29 (± 9.4)	0.6 (± 0.2)	27.9 (± 32)	24.9 (± 6.3)	0.7 (± 0.1)

histologically confirmed classification was used but no significant difference in results was found between the histological classification and the classification based on the surgeon's intended dissection.

Frequency and severity of hyphaema

The frequency and severity of hyphaema in the two groups is shown in Figure 11.1. Thirty-five patients (45%) developed a hyphaema: six patients diffuse with no blood level, twenty-one with a level of 0.2mm to 1mm and eight with more than 1 mm blood level. There was no difference in the frequency of grade 1 hyphaemas (three patients in both groups). Group B exhibited a significantly higher incidence of hyphaemas of both grade 2, 0.2mm to 1 mm height, (15 patients versus 6 in group A, $p<0.05$) and grade 3, more than 1 mm height, (7 patients versus one in group A, x^2 test, $p<0.01$).

Considering only hyphaema severe enough to form a detectable blood level, there were 7 out of 39 eyes (18%) in group A, compared with 22 out of 39 eyes (56%) in group B. The difference was statistically significant (t-test, $p<0.001$).

Time of occurence of hyphaema

The time of occurence of hyphaema was analysed for the three grades of hyphaema (Fig 11.2). The few circulating red blood cells which were present in the anterior chamber on the first day in every case were disregarded. Most hyphaemas of grade 1 (5 out of 6) were present on the first postoperative day, whereas only 7 of 21 grade 2 and 3 of 8 grade 3 hyphaemas were present on day one. Grade 1 was therefore significantly

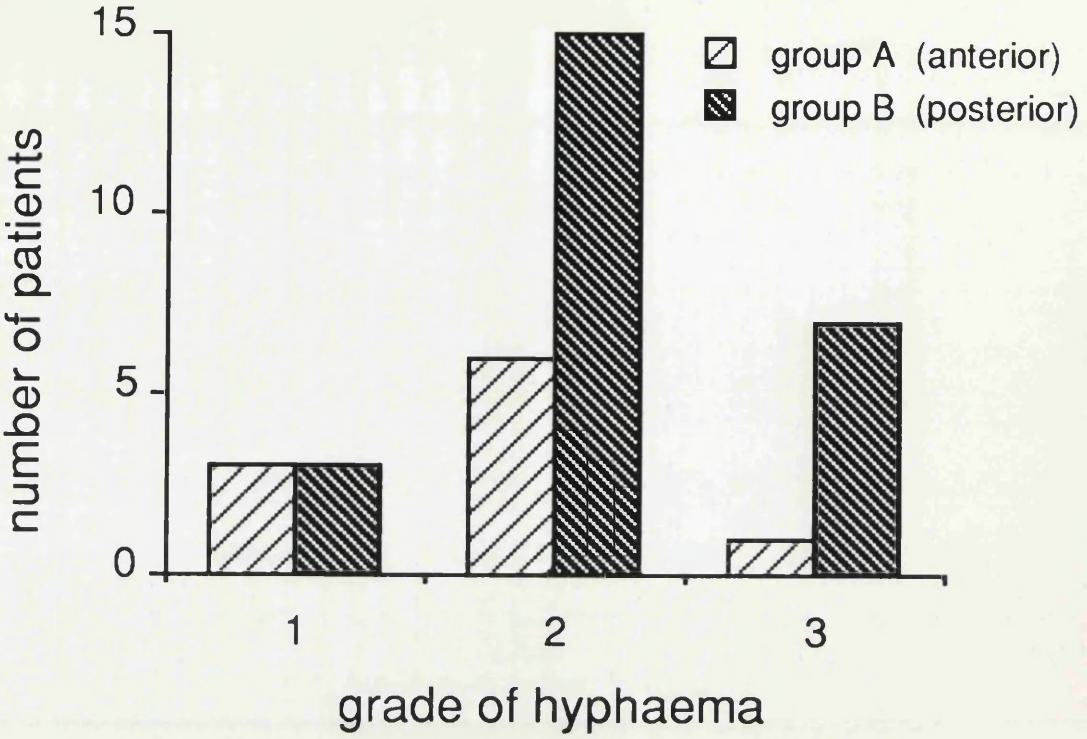


Figure 11.1: Frequency and severity of postoperative hyphaema. Grade 1, diffuse; grade 2, 0.2-1mm; grade 3, >1mm. Anterior dissection (groupA) produced significantly fewer hyphaemas and those that occurred were less severe.

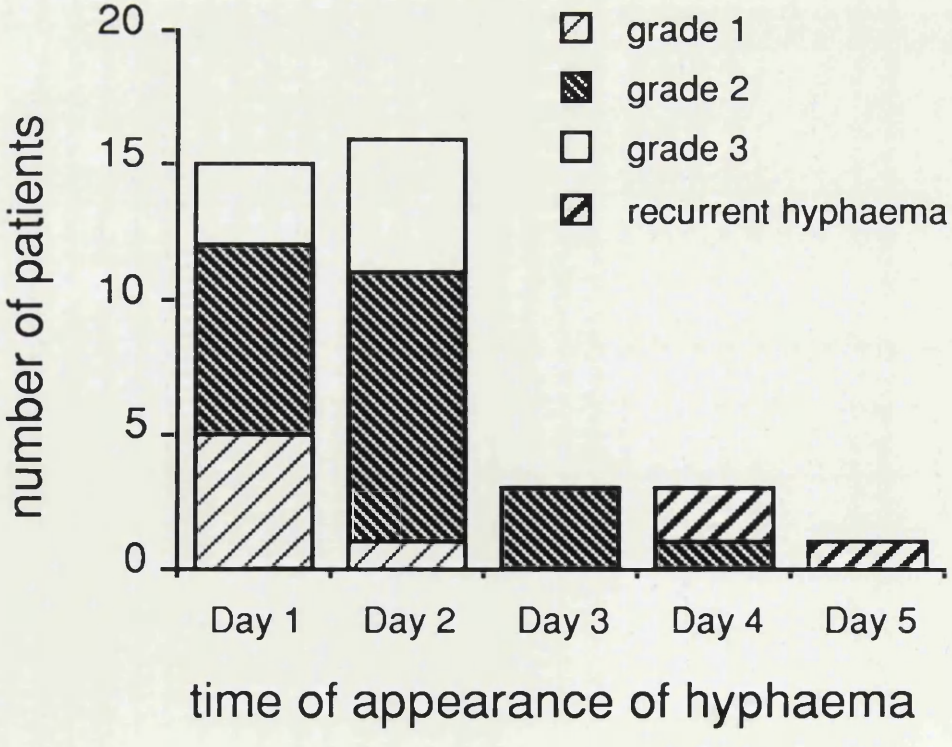


Figure 11.2: Time of appearance of postoperative hyphaemas of different severity

different from grades 2 and 3 in that respect. In the latter grades most hyphaemas appeared on the second (13/29) and third (5/29) postoperative day with only one hyphaema (grade 2) appearing on day four.

Duration of hospitalisation

Patients with hyphaema stayed significantly longer in hospital: mean, 4.4 days (S.D., 2.2), than those without: mean, 2.5 days (S.D., 0.5). Time in hospital was found to be related to the severity of hyphaema. Analysis showed that mean hospital stay for grade 1 hyphaema, did not differ from grade 2 (3.2 days and 3.6 days respectively), but grade 3 patients stayed in hospital significantly longer, mean, 7.7 days (S.D., 2.1). When compared with patients without hyphaema, those with both grades 2 and 3 stayed significantly longer in hospital, but grade 1 did not. Overall, group A patients remained in hospital for an average of 2.9 days (S.D., 1.3), compared with an average of 3.8 days (S.D., 1.9) for group B. The difference was significant (Mann-Whitney test, $p=0.004$).

Exfoliation glaucoma and other features

Patients with each of the three grades of hyphaema were compared with each other and with those without hyphaema to determine possible distinguishing features. Patients with hyphaema were similar to those without hyphaema with regard to age, sex, systemic diseases, intraocular pressure at diagnosis, cup to disc ratio at diagnosis, intraocular pressure at the time of surgery and duration of medical treatment.

Out of the 15 patients with exfoliation glaucoma included in this study 5 (33%) developed a hyphaema postoperatively. There was no case of a severe or recurrent hyphaema. There were 7 patients in group A and 8 in group B who had exfoliation. In group A, one of the 7 patients had a diffuse hyphaema (grade 1) compared with one diffuse (grade 1) and three grade 2 hyphaemas in the eight exfoliation patients in group B. Statistical analysis did not reveal any significant association between the presence of exfoliation and postoperative hyphaema. Considering only the hyphaemas which formed a detectable blood level there was no significant difference between the exfoliation group and the POAG group (x^2 test, $0.05<p<0.1$).

With reference to the individual analysis of the three grades of hyphaema the only significant difference observed was in the sex distribution of grade 1 when compared with grade 2 (5:1 female to male ratio in grade 1 versus 6:15 for grade 2). However, there was no difference in the sex distribution between grades 1 and 3 and 2 and 3.

Recurrent hyphaemas

Three patients, all of them in grade 3, experienced recurrent hyphaemas, two of them on the fifth postoperative day and one on the fourth day. These recurrent hyphaemas were of moderate degree, up to 2.5 mm in height, occured prior to the complete resolution of a pre-existing hyphaema grade 3 and there were not detrimental to the final visual acuity of these three patients.

Postoperative complications

The two groups, A and B, i.e. anterior and posterior dissection and the patients with and without hyphaema were compared with regard to immediate postoperative complications (anterior chamber depth, uveal effusion and pain). No eyes required re-operation to correct surgical complications. There was no significant difference between the two randomised surgical groups and between the patients with and without hyphaema.

Final outcome

At approximately four months after the operation the final outcome was analysed with regard to reduction of visual acuity, final untreated intraocular pressure, rate of postoperative steroid response, and bleb morphology. One patient was lost to follow up and one patient did not have sufficient follow up. Both patients were in group A. No significant difference was noted between groups A and B or between those with hyphaema and those without. The untreated mean intraocular pressure was similar for group A, 15 mm (S.D., 4.1); and group B, 14.9 mm (S.D., 5.5). Similarly, the untreated IOP at four months of patients who had hyphaema, 15.7mm (S.D., 5.3), was not significantly higher than that of the patients who did not experience a hyphaema, 14.2 mm (S.D., 4.1), (t-test, $p=0.18$).

Examination of individual cases in which the IOP remained controlled after the operation only with the aid of medical therapy, showed them to be distributed evenly between groups A and B and between those with and without hyphaema. Three

patients in group A (8%) and four patients in group B (10%) required supplementary medical therapy (one medication each). Similarly, from the hyphaema group three patients (8.5%) required supplementary treatment compared with four patients (10%) in the no-hyphaema group. One patient from group B with a grade 2 hyphaema suffering from thrombocytopenia was the only patient with uncontrolled intraocular pressure, despite medical therapy, after surgery.

11.3 Discussion

This prospective randomised study confirms the hypothesis that excision of the inner block anterior to the scleral spur is associated with a significant reduction in the incidence and severity of postoperative hyphaema. The two groups of patients were comparable, the only difference being the mode of dissection of the inner block. Group B, posterior dissection, was associated with the majority of the more severe hyphaemas (grade 3) and with all the recurrent hyphaemas.

The 'guarded anterior fistula'

The anterior dissection method (group A) should not be confused with the 'Cairns-type' trabeculectomy (Cairns 1968). Cairns (1968) excised trabecular meshwork up to the scleral spur and therefore was likely to have disturbed it in at least some cases. The anterior dissection in this study, termed 'guarded anterior fistula', is further forward and excises only cornea, all anterior to the angle structures.

Incidence of hyphaema

The overall incidence of hyphaema in the present study (45%) is amongst the higher values quoted in the literature (Watson & Barnett 1975, Mills 1981, Shuster et al 1984, Yamashita et al 1985, Watson 1987). This is not surprising as this study includes diffuse hyphaemas (six) and small hyphaemas that might have been overlooked in retrospective studies. Watkins and Brubaker (1978) found a similar incidence of hyphaema (43%) in the partial-thickness group of their study which compared prospectively partial-thickness and full-thickness filtration procedures.

Onset of hyphaema

Most diffuse hyphaemas, grade 1, appeared in the first postoperative day. This suggests that in most cases they represent resolving peroperative bleeding. This is not the case with grades 2 and 3 which appear later and are probably new events.

The anterior dissection, which removes only corneal tissue, (guarded anterior fistula) is associated with a significantly lower incidence of postoperative bleeding. This suggests that the vascular components of sclera and the ciliary body are the most likely sources of hyphaema. This coincides with the occasional postoperative observation during the course of this study of blood streaming from the fistula site into the aqueous. It is also conceivable that in the dissection of the posterior block the peripheral iridectomy may be closer to the iris root. This study can not, however, identify with certainty the source of postoperative hyphaema.

Exfoliation glaucoma and hyphaema

It is interesting to note that although exfoliation glaucoma cases are known to have a varied degree of iris vasculopathy (Vannas 1969, Ringvold 1969, Laatikainen 1971, Ringvold & Davanger 1981, Brooks & Gillies 1987 and chapter 6 of the present work) this did not result in a higher frequency of bleeding. The number of exfoliation glaucoma patients is too small for valid statistical analysis, but the overall incidence of hyphaema and the protective effect of anterior dissection were similar to that for the POAG patients. It remains to be known whether exfoliation vasculopathy may be decreasing the propensity for hyphaema in patients with exfoliation glaucoma.

Postoperative IOP control

The IOP attained with both methods of inner block dissection was similar and within the range quoted in the literature (Ridgway 1974, Jerndal & Lundstrom 1977, Mills 1981, Migdal & Hitchings 1986, Jay & Murray 1988, Lavin et al 1990). The present study confirms that varying the size and site of the fistula does not affect the final IOP (Duzanec & Krieglstein 1981, Starita et al 1984). Reduction of the filtration area (anterior guarded fistula) did not influence the final outcome. It is considered unlikely that the shape of the outer scleral flap would influence the result. The use of triangular flaps has gradually increased in this Department over the last 10 years, without alteration in the rate of either IOP control or complications, including hyphaema and shallow anterior chamber (Jay 1991).

Hyphaema and drainage

It has been suggested that hyphaema might affect drainage (Zaidi 1980). It is also conceivable that suboptimal drainage may influence the severity and speed of resolution of postoperative hyphaema. Nevertheless, in the present study, no significant difference was found in the surgical outcome (untreated mean IOP approximately four months after surgery) between patients with hyphaema and those without. It is conceivable that if with a larger sample size and longer follow up a difference would have been detected.

Advantages of anterior dissection

The main advantage of a modified dissection of the inner block is the lower incidence of postoperative hyphaema. This complication sometimes can cause alarm to the patient and the surgeon and can influence the duration of hospital stay of the patient. In the present study, hyphaemas (grades 2 and 3) were associated with a significantly longer hospital stay and so following the anterior dissection method (guarded anterior fistula) should reduce costs. The extra cost of longer hospital stay after a trabeculectomy has recently been quantified (Ainsworth & Jay 1991).

11.4 Summary

The effect of varying the position of a trabeculectomy fistula on the rate of postoperative hyphaema was studied in a prospective randomised trial. One eye of each of 78 consecutive patients with POAG and exfoliation glaucoma was allocated to one of two groups. In group A the fistula was fashioned anterior to the scleral spur, entirely in corneal tissue. In group B the fistula included cornea and sclera with trabecular meshwork and scleral spur.

Seven out of thirty-nine eyes (18%) in group A developed a postoperative hyphaema with detectable blood level, compared with twenty-two out of thirty-nine eyes (56%) for group B ($p<0.001$). In addition, the severity of the bleeding was greater in group B and the three cases of recurrent bleeding were all in this group. Exfoliation glaucoma patients did not exhibit a higher incidence of postoperative bleeding. Group B patients remained in haspital for an average of 3.9 days, which was significantly longer ($p=0.004$) than the average of 2.9 days for group A. This difference was related to the frequency and severity of the hyphaema. The type of dissection, or the occurence of hyphaema did not influence the IOP at four months after surgery.

CHAPTER 12 FINAL DISCUSSION AND FUTURE STUDIES

The purpose of this chapter is to provide a concise discussion of selected data which have been presented in this thesis and to provide suggestions for further investigations.

12.1 Morphological studies

12.1.1 Final discussion

Basement membranes and exfoliation

The hypothesis that basement membrane constituents comprise part of the biochemical framework of exfoliation material is not novel. Numerous investigators have speculated on the relationship between the biosynthesis and degradation of ageing basement membranes and exfoliation syndrome (section 1.5). The present investigation has found an intimate relationship between iridic vascular basement membranes and exfoliation material (chapter 4). Therefore, it is in favour of the view that ageing cells which form basement membranes are involved in the synthesis of exfoliation material.

The present study was the first to investigate the role of two prominent basement membrane components (laminin and collagen type IV) in normal controls and patients with exfoliation syndrome. Laminin, a principal basement membrane component, was found to be a component of exfoliation material. Collagen type IV was not. Therefore the current study has produced evidence to suggest an association between exfoliation syndrome and basement membranes.

With regard to the elastosis theory advanced by Streeten and coworkers (1986a; 1986b) on the basis of ultrastructural studies on conjunctival tissue, the present study did not find evidence of elastosis in the exfoliative iris. This is not surprising as elastic tissue is absent from the iris (Ringvold 1970a). If Streeten's hypothesis is correct and exfoliation syndrome is a type of elastosis with elastic microfibrills forming the basis for the exfoliative fibres, this process does not apply in the exfoliative iris.

Laminin and exfoliation material

The significance of laminin localisation in the exfoliation material is open to speculation. The present study suggests that in the iris vessels, laminin is incorporated into the exfoliation structural framework by basement membrane producing cells. It is also conceivable that exfoliation material may comprise a different morphological form of laminin, but with the same epitopes for the antibody employed in the present study; for example a substance representing either a precursor, or a breakdown product of laminin. Altered levels of synthesis and turnover of laminin in periods of rapid growth and tissue remodelling have been recently documented (section 2.5). However, the mechanisms which control the synthesis of laminin and the complex modulation of the amount present in ocular tissues remains unknown.

Collagens and exfoliation material

It has been shown in the present investigation that neither collagens I-V, nor any antigenic homologues comprise integral

constituents of exfoliation material. What may be of more relevance to exfoliation syndrome is the associated quantitative collagen changes observed in the iris vascular matrix (chapter 7). An inverse relationship appears to exist between the quantity of collagens type I and IV in the iris vascular basement membranes and the evolution of the disorder. From the evidence presented, it is suggested that exfoliation syndrome affects the biosynthesis of collagen types I and IV in the vascular matrix of the iris. It is not clear why at an early stage of the so called exfoliation vasculopathy, type I and IV collagen labelling increases. It is conceivable that exfoliation syndrome disturbs the synthesis, or degradation of the collagens associated with the vascular matrix in the iris. This lends weight to the hypothesis that local basement membrane-producing cells are involved in the synthesis of exfoliation material.

12.1.2 Future studies

Immunocytochemical studies

Current concepts suggest that qualitative and quantitative variations in numerous ECM components occur in relation to disease processes (chapter 2). It is not known however, whether these changes are primary or secondary to a disease process. Considering exfoliation syndrome, it remains to be elucidated how the changes in the laminin content affect other basement membrane components not investigated herein (e.g. nidogen, fibronectin, thrombospondin and heparan sulphate proteoglycan). The influence of ageing on the ECM of the affected tissues requires further studies.

The study of other integral or trapped basement membrane and ECM components that are not normally related with basement membranes will provide more information concerning the pathogenesis of the disease. ECM changes in the exfoliation syndrome can subsequently be associated with concomitant alterations in the morphology and behaviour of the ocular cells that synthesise the diseased ECM.

In view of the advanced changes observed in the exfoliation glaucoma specimens, early disease (exfoliation syndrome) should in the future be targeted by immunocytochemistry. The use of fresh post-mortem eyes with early disease (exfoliation syndrome, or even preclinical disease) will provide a profitable line of future investigation.

Studies on other body tissues

It has been recently suggested that exfoliation syndrome may be a systemic condition (Streeten et al 1990). However, there is no information on other basement membranes in the body. A future study of kidney tissue (especially glomerular basement membranes) from exfoliation patients undergoing renal surgery would be a promising line of future research.

Other approaches

Advances in tissue culture will permit the use of more sophisticated models to study the synthesis of exfoliation material in vitro. A study of this nature should ideally be carried out on various ocular tissues from the same eye. This strategy may identify the tissues that play a major role in the synthesis of exfoliation material. Other possible

approaches to the problem include 1) separation of exfoliation material from affected ocular tissues and aqueous humour of exfoliation patients (e.g. gel electrophoresis and Western blot analysis). This could then lead to potential determination of molecular weight and ultimately biochemical characterisation. 2) Genetic studies on blood from patients with exfoliation syndrome for location of the gene that determines the disorder.

Preventative studies

Laminin has been found to bind heparin and other structurally similar heparan sulfates (Sakashita et al 1980), but recently an important function was discovered confined to heparin. Heparin was shown to induce a specific alteration of laminin polymerisation in which self-assembly was accelerated (Yurchenco 1990). Future studies in exfoliation patients could conceivably utilise this effect in order to intervene and reverse, or alter laminin polymerisation in affected ocular basement membranes. As yet, the factors that modulate the synthesis of laminin *in vivo* remain unknown. However, a recent *in vitro* study on cultured bovine trabecular meshwork cells has revealed that ascorbic acid promotes laminin production. This key component of the aqueous humour stimulated laminin synthesis in all concentrations tested (Yue et al 1990). Future studies could conceivably test this *in vitro* observation by systemic administration of ascorbic acid in exfoliation patients. It is conceivable that ascorbic acid may increase the production of laminin from iris cohort cells and bring about a reversal of laminin depletion seen in exfoliative vasculopathy (section 7.3).

12.2 Clinical studies

12.2.1 Final discussion

Prevalence of exfoliation glaucoma

The present study has established that exfoliation glaucoma is relatively common (26%) in those Scottish patients coming to surgery in the Tennent Institute of Ophthalmology in Glasgow. The clinico-morphological diagnostic methodology employed herein probably ensures that the figure reflects the 'true' prevalence of the condition in the group studied. It is possible however, that a proportion of the 14 patients with possible exfoliation glaucoma (group II) who were exfoliation negative on electron microscopic examination might develop exfoliation glaucoma in the future.

The present study supports the view that the degeneration of the pigment epithelium of the iris in exfoliation glaucoma and the consequent release of pigment granules into the aqueous circulation probably precede the clinically obvious disease. This view however, can only be confirmed by sequential studies. The present study has dealt only with exfoliation glaucoma patients deemed to warrant surgery. It is reasonable to assume that pigmentary signs are of even greater importance in patients at an earlier stage of the development of exfoliation syndrome.

Exfoliation glaucoma vs POAG

Certain impressions of previous workers concerning exfoliation glaucoma have been confirmed in the present

study. In agreement with previous studies, patients with exfoliation glaucoma were older than their POAG counterparts. Exfoliation glaucoma was confirmed in this study to be a hypertensive glaucoma, characterised by higher untreated and treated IOP in comparison with age matched POAG. Despite the the more frequent use of more medications and acetazolamide the IOP was less amenable to medical therapy in the exfoliation glaucoma group. Consequently, the principal indication for surgery for the exfoliation glaucoma group was unacceptably high IOP. In contrast to other retrospective studies, the higher IOP levels did not result in greater visual field loss at diagnosis, or at the time of surgery for patients with exfoliation glaucoma. From the evidence of the present investigation it is clear that exfoliation glaucoma differs from POAG. It is hoped that the results of the present study will allow a more thorough and critical evaluation of the clinical features of the disorder.

Patients with exfoliation glaucoma were found to have a significantly lower final IOP (at approximately 6 months after surgery) in comparison with suitably matched POAG patients. This may be accounted for by the different pathogenesis of exfoliation glaucoma. Surgery may alleviate the mechanical obstruction of the outflow system caused by exfoliation material and pigment deposition in the cribriform layer. Alternatively, it could be somehow related to the shorter duration of medical therapy in the exfoliation glaucoma group. A significantly lower IOP level has been reported by Lavin and coworkers (1990) for a group of patients with POAG who underwent primary trabeculectomy,

compared with a group of patients who had received at least one year of multiple topical glaucoma therapies before undergoing trabeculectomy. Longer term studies are needed to confirm and quantify the long term duration of this favourable IOP response.

Management of exfoliation glaucoma

For POAG there is considerable evidence from clinical trials on which to base our choice of therapy: medical therapy, argon laser trabeculoplasty, or trabeculectomy. An informed decision can be made for the appropriate form of therapy depending on the stage of the disease, level of IOP, patients' life expectancy and the probability of satisfactory compliance. Such controlled data is currently lacking for exfoliation glaucoma.

The results of the present study together with recent reports in the literature on POAG (reviewed by Jay 1991; 1992) suggest that early surgery (described as a brief trial of medical therapy with one medication, or primary ALT followed by surgery if the IOP is not satisfactory controlled) is the rational choice in the management of exfoliation glaucoma. In some countries and some social circumstances long term medical therapy is impractical and therefore primary surgical treatment (trabeculectomy at diagnosis) may be the best strategy for exfoliation glaucoma. This study suggests that even where there are optimum conditions of glaucoma care, a careful choice should be made depending on the severity of the disorder and the level of untreated IOP.

Primary trabeculectomy may be indicated in the management of exfoliation glaucoma patients with advanced glaucomatous damage. There is now evidence that eyes with advanced optic nerve damage caused by POAG require an IOP well below the arbitrary top level of 20-22 mm Hg, if further field loss is to be avoided (Jay 1991). Increased vulnerability to an IOP in the high 'normal' range may be explained by morphological changes in the region of the lamina cribrosa or diurnal variation in IOP (Stewart 1990, Jay 1991). Odberg (1987) has shown in a retrospective study that the best prognosis in advanced glaucoma (in a mixed group of POAG and exfoliation glaucoma patients) was associated with an IOP less than 15 mm Hg. This level of IOP can in most cases be achieved with drainage surgery.

Conclusion

The results of the first clinical investigation suggest that 1) topical anti-glaucoma therapy is less effective in controlling IOP in exfoliation glaucoma; 2) earlier surgical treatment is more successful in terms of IOP lowering effect in exfoliation glaucoma than in age matched POAG and 3) imply that properly treated exfoliation glaucoma does not carry a worse prognosis than age matched POAG of comparable severity.

The anterior guarded fistula operation

In the second study a modification of 'Cairns type' trabeculectomy with the anterior fistula entirely in corneal tissue significantly reduced the incidence of postoperative hyphaema. The anterior dissection, termed 'guarded anterior fistula' differed from the method described by Cairns (1968)

in that it did not involve excising angle structures and did not disturb the scleral spur. In the posterior group the incidence and severity of postoperative bleeding was significantly higher. The posterior dissection (Watson's modification of Cairns method) proved equally successful in terms of IOP control. Nevertheless, patients who underwent a posterior trabeculectomy stayed in hospital longer. This was due first, to the higher frequency and increased severity of postoperative bleeding and second to the appearance of recurrent bleeding (3 cases, all in the posterior group). The anterior dissection probably diminishes the average cost of surgery in these patients.

Evidence from this study indicates that patients with exfoliation glaucoma do not exhibit a higher frequency of postoperative bleeding. Indeed, it is possible that the reverse may be the case. Although statistical analysis did not demonstrate a significant difference it is possible that the present study did not have the required sample size to have the power to detect a genuine difference.

12.2.2 Future clinical studies

There is much scope for further research on the clinical attributes of exfoliation syndrome/glaucoma. Issues that remain ambiguous include the mode and rate of 'conversion' of the normotensive exfoliation patients to the ocular hypertensive state and subsequently to exfoliation glaucoma. At the present time, our knowledge concerning the clinical attributes of exfoliation syndrome/glaucoma is incomplete.

Long term controlled studies are needed to delineate these parameters. Aspects of the present study which could be extended in the future include the diagnostic methodology (clinical examination followed by ultrastructural assessment) and the clinical examination and analysis of the results in other ethnic groups. This will allow proper assessment of the prevalence and importance of exfoliation glaucoma worldwide. The author has set up a similar protocol investigating the prevalence of exfoliation glaucoma in a surgical cohort in Freiburg, Germany. It is interesting to note that the morphological prevalence established in the German study is similar to that recorded in the present investigation, but the clinical prevalence was significantly lower. A similar study will be set up in Thessaloniki, Greece.

For proper controlled studies first, a random epidemiological survey should be carried out on a homogeneous urban, or rural population. This study ideally would involve screening for exfoliation syndrome/glaucoma, POAG and other ocular disorders to provide information on the 'true' prevalence of the condition, detect ocular pathology at an early stage and lay foundations for a number of subsequent clinical studies. Longer term clinical studies can be performed on suitable cohorts of individuals drawn from this population survey. This strategy would ensure the representative nature of the material under investigation. Such an approach will be pursued in Greece by the author.

ADDENDUM

Since this thesis was submitted another group of research workers has convincingly confirmed that a)laminin is a component of exfoliation material and b)have also identified a number of other basement membrane components (nidogen and proteoglycans) in exfoliation material. In agreement with the current study they suggest that exfoliation syndrome is an aberration of extracellular matrix synthesis (Schlotzer-Schrehardt et al: **Current Eye Research,** 1992; 11: 343-355).

In addition, two groups of research workers have demonstrated that exfoliation material is widely distributed in many tissues in the body (Schlotzer-Schrehardt et al: **Archives of Ophthalmology,** 1992; 110: 1752-1756, Streeten et al: **Archives of Ophthalmology,** 1992; 110: 1757-1762).

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APPENDIX I

TAILS OF CONTROL TISSUE USED IN PART I

	AGE	SEX	DIAGNOSIS	FIXATION PFA 4% plus glutaraldehyde	TISSUE USED	IN STUDY OF COLLAGEN TYPES AND LAMININ
	68	M	CM	0.2% CB	Iris	I-V
	64	F	CM	0.5% CB	Iris	I-V
	62	M	CBM	0.4% PB	Iris	I-V
	64	M	CM	PFA only	Iris	I-V
	64	M	CM	0.2-1% PB	Iris	I-V
	65	M	CM	0.5% CB	Iris	Laminin,I-V
	69	M	CM	0.25% CB	Iris	Laminin,I,IV
	52	M	CM	0.1% PB	Iris	Laminin,I,IV
	81	F	SG	0.2%-1% CB	Iris,cornea	Laminin,I,IV
0	63	F	CM	0%, 0.5% CB	Iris,cornea	Laminin,I,IV
1	78	F	CM	1% CB	Iris	Laminin,I,IV
2	76	M	CM	0.5% 1% CB	Iris,cornea	Laminin,I,IV
3	64	F	Lac Ca	1% CB	Trabeculum	I
4	75	F	CBM	0.2% PB	Cornea	I-V
5	73	M	CM	0.25% PB	Cornea	I-V,laminin
6	78	M	CM	1% PB	Cornea	I-V,laminin

ey: M: male; F: female; CM: choroidal melanoma; CBM: ciliary body melanoma; SG: secondary glaucoma; Lac Ca: lacrimal gland carcinoma; PFA: paraformaldehyde; PB: phosphate buffer; CB: cacodylate buffer.

APPENDIX II

DETAILS OF EXFOLIATIVE TISSUE USED IN PART I

NO	AGE	SEX	DIAGNOSIS	FIXATION PFA 4% plus glutaraldehyde	TISSUE USED	IN STUDY OF COLLAGEN TYPES AND LAMININ
1	79	F	EXS	0.5% CB	Iris	I-V
2	80	F	EXG	0.7% CB	Iris	I-V
3	82	M	EXS	PFA only PB	Iris	I-V,laminin
4	69	M	EXG	0.4% CB	Iris	I-V,laminin
5	68	M	EXG	0.5% PB	Iris	I-V,laminin
6	69	M	EXG	0.25% PB	Iris,trabeculum	I-V,laminin
7	74	M	EXG	0.25% CB	Iris	laminin
8	77	F	EXS	0.1% CB	Iris	laminin
9	65	M	EXG	1% CB	Iris	laminin
10	85	F	EXG	0.2% CB	Iris	I,IV,laminin
11	70	M	EXG	0.3% CB	Iris,trabeculum	I,IV,laminin
12	71	M	EXG	0.5% CB	Iris	I,IV,laminin
13	71	F	EXG	0.2% PB	Iris	I,IV,laminin
14	72	M	EXG	0.5% CB	Iris	I,IV,laminin
15	75	M	EXG	1% CB	Iris	I,IV,laminin
16	79	M	EXG	0.25% CB	Trabeculum	I-V
17	65	M	EXG	0.2% PB	Trabeculum	I
18	68	F	EXG	0.5% CB	Trabeculum	I,laminin
19	79	F	EXG	PFA only PB	Trabeculum	I,laminin
20	74	F	EXG	0.5% CB	Trabeculum	I,laminin

Key: M: male; F: female; EXS: exfoliation syndrome; EXG: exfoliation glaucoma; PFA: paraformaldehyde; PB: phosphate buffer; CB: cacodylate buffer.

APPENDIX III

DETAILS OF PATIENTS FROM WHOM HISTOLOGY WAS DEMONSTRATED IN THE STUDY

FIGURE NUMBER	EM NO	NEGATIVE NUMBER
CHAPTER 4		
4.4	309/90	LM
4.5	309/90	LM
4.6	321/89	LM
4.7	321/89	LM
4.8	141/90	LM
4.9	43/91	LM
4.10	76/90	LM
4.11	76/90	LM
4.12	131/89	42318
4.13	131/89	42310
4.14	174/90	351 (Jeol)
4.15	143/90	322 (Jeol)
4.16	150/90	48237
4.17	270/89	336 (Jeol)
4.18	270/89	331 (Jeol)
4.19	308/89	48260
4.20	308/90	48262
4.21	18/89	47888
4.22	156/90	48221
4.23	189/90	48247
4.24	189/90	48253
4.25	309/90	48244
4.26	145/90	47891
4.27	174/90	47269
4.28	270/89	47934
4.29	355/90	47941
4.30	309/90	48271
4.31	25/90	47939
4.32	191/89	47896
4.33	270/89	47822
CHAPTER 5		
5.1	24/89	42838
5.2	101/88	42452
5.3	24/89	42839
5.4	100/88	42456
5.5	24/89	42836
5.6	100/88	42455
5.7	157/89	42464
5.8	157/89	42851
5.9	101/88	42602
5.10	6/89	42482
5.11	6/89	42478
5.12	150/89	46632
5.13	207/89	43902
5.14	209/89	43889
5.15	150/89	46639
5.16	314/89	46710
5.17	319/89	46703

CHAPTER 6		
6.2	24/89	44322
6.3	101/88	44321
6.4	101/88	44816
6.5	6/89	44389
6.6	101/88	44240
6.7	190/89	44001
6.8	207/89	43870
6.9	190/89	44014
6.10	145/90	44341
6.11	264/89	44339
6.12	264/89	44242
6.13	276/89	46677
CHAPTER 7		
7.1	150/89	46270
7.2	264/89	44334
7.3	207/89	47265
7.4	157/90	46629
7.5	207/89	43901
7.6	150/89	46656
7.7	314/89	46664
7.8	207/89	43896
7.9	150/89	46635
7.10	150/89	46280
7.11	314/89	46669
7.12	6/90	47653
7.14	276/89	46678
7.15	276/89	46724
7.16	157/89	43211
7.17	24/89	43238