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Electron Microscopy of the Limbal Conjunctiva in Eyes with Pseudo-Exfoliation Syndrome (PE Syndrome)

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Summary. The limbal conjunctiva of five human eyes was studied. All eyes showed PE material in the anterior segment by slit-lamp examination. Additionally, electron microscopy of the limbal conjunctiva revealed aggregates of PE material in the connective tissue and within vessel walls. Furthermore, the basement membrane zone of vessels was often disorganized. The demonstration of PE material beyond the intraocular space perhaps indicates that the PE syndrome is of a more general nature than hitherto believed.

Introduction

The name exfoliation syndrome has previously been used to designate an ophthalmic condition characterized by the occurrence of a light grey, flocculent material in the anterior segment of the eye. This term derives from Vogt (1925), who introduced the name "exfoliatio superficialis capsulae anterioris", because he felt that the flocculent material arises as small flecks peeling off from the lens capsule itself. Later, Theobald (1954) showed that the flocculent material is lying on the lens capsule, ciliary body, and zonules, and she denied a true exfoliation of the lens capsule in such eyes. Accordingly, she proposed to call the condition "pseudo-exfoliation of the lens capsule". This term is misleading by placing emphasis on the occurrence of flocculent material on the lens capsule, and a modified form of it will therefore be used in the present study: Pseudo-exfoliation material (abbreviated PE material) means a substance showing an electron microscopic appearance identical to that of the grey, flocculent material in the anterior segment of the eye, and pseudo-exfoliation syndrome (abbreviated PE syndrome) designates the presence of PE material any place in the body.

Numerous electron microscopic investigations have shown that the PE material consists of short fibrils sometimes intermingled with granules. Aggregates of the PE material have been found on and within the lens capsule, on the zonules, on the ciliary body, on the posterior and the anterior iris surface as well as at different locations within the iris itself, and in the trabecular area. Several authors are of the opinion that PE material in the trabecular area interferes with the drainage of aqueous humour, leading to the development of capsular glaucoma (Busacca, 1928; Theobald, 1954; Sunde, 1956; Gifford, 1957; Blackstad *et al.*, 1960; Tarkkanen, 1962; Bertelsen *et al.*, 1964; Ashton *et al.*, 1965; Shakib *et al.*, 1965; Hørven 1966; Dark *et al.*, 1969; Ringvold, 1969, 1970a, 1970b; Ringvold and Vegge, 1971).

Histochemical methods have been applied to elucidate the chemical composition of the PE material. Some reports (Theobald, 1954; Arnesen *et al.*, 1963) state

the presence of an acid mucopolysaccharide in the material, whereas other work (Bertelsen and Ehlers, 1969) stresses the lacking evidence for this statement. The latter authors were of the opinion that the PE material does not contain grater amounts of carbohydrate. Contrary to this, Hørven (1966) classified the material as a glyco- or muco-protein. The Congo red stain was clearly negative both for the PE material (Hørven, 1966) and for the limbal conjunctiva from eyes with PE syndrome (Ringvold, unpublished).

So far PE material has not been demonstrated outside the aqueous humour area, and the PE syndrome has therefore been thought of as a strictly ophthalmic condition. In this connection it should be kept in mind that the aqueous humour is formed by active transport mechanisms in the ciliary body, and it shows a chemical composition different from other fluids in the body (Davson, 1969). One can imagine that an abnormal chemical constitution of the aqueous humour entailed by a failure in the secretion mechanism may cause morphological changes in that tissue, which comes into close relationship with this fluid. However, on the other hand, perhaps reports concerning PE material outside the intraocular space are lacking because nobody has been looking for this material beyond the aqueous humour area. It is known (Bertelsen *et al.*, 1964; Shakib *et al.*, 1965; Ringvold, 1969, 1970b) that the PE material frequently appears closely related to intraocular basement membranes. Since basement membranes are present in all parts of the body, it is here assumed that the PE material may be present not only in intraocular tissue where it is easy to recognize, but also outside sclera. The present study was undertaken in order to test this hypothesis, and it seems reasonable to start searching for PE material just outside the eye, i.e. in the bulbar conjunctiva.

Material and Methods

Five human eyes, all showing PE material on the anterior lens surface by slit-lamp examination, were studied. The patients were 70, 71, 71, 74 and 94 years old at the time of operation.

Three of the eyes were enucleated because of absolute glaucoma with persistent pains. These eyes showed dilated anterior ciliary veins, corneal oedema, moderate aqueous flare, and iridic rubeosis. The patients had received anti-glaucomatous treatment (pilocarpine/adrenaline eye drops, and acetazolamide tablets) for several years. Additionally, the had all been operated on according to Elliot's method because of increased intraocular tension.

The *fourth eye* was removed because of a malignant melanoma of the maxilla. A capsular glaucoma had been diagnosed one year before, and the moderately raised intraocular tension had been kept normal by use of pilocarpine drops three times daily. Vision was unimpaired, and visual field was normal when examined by campimetry.

The *fifth eye* had in the macula region a malignant melanoma, which had caused a gradual loss of vision during the last 6 months. There was no history of increased intraocular tension. As the patient was admitted to the hospital the intraocular tension measured 10,2 mm Hg (Schiøtz), and the visual field appeared normal examined by Donder's method.

Immediately after operation the anterior segments of the eyes were cut off, cut into sectors, and fixed for 1–3 hours in precooled 1% OsO_4, buffered to pH 7.3 with phosphate buffer. Tissue blocks from four eyes were dehydrated in graded acetone solutions, while blocks from one eye were dehydrated in increasing concentrations of alcohol. All blocks were embedded in Araldite. Sections were made with LKB Ultratomes, and stained with an aqueous solution of uranyl acetate followed by lead citrate. Siemens Elmiskop 1b and 1A were used.

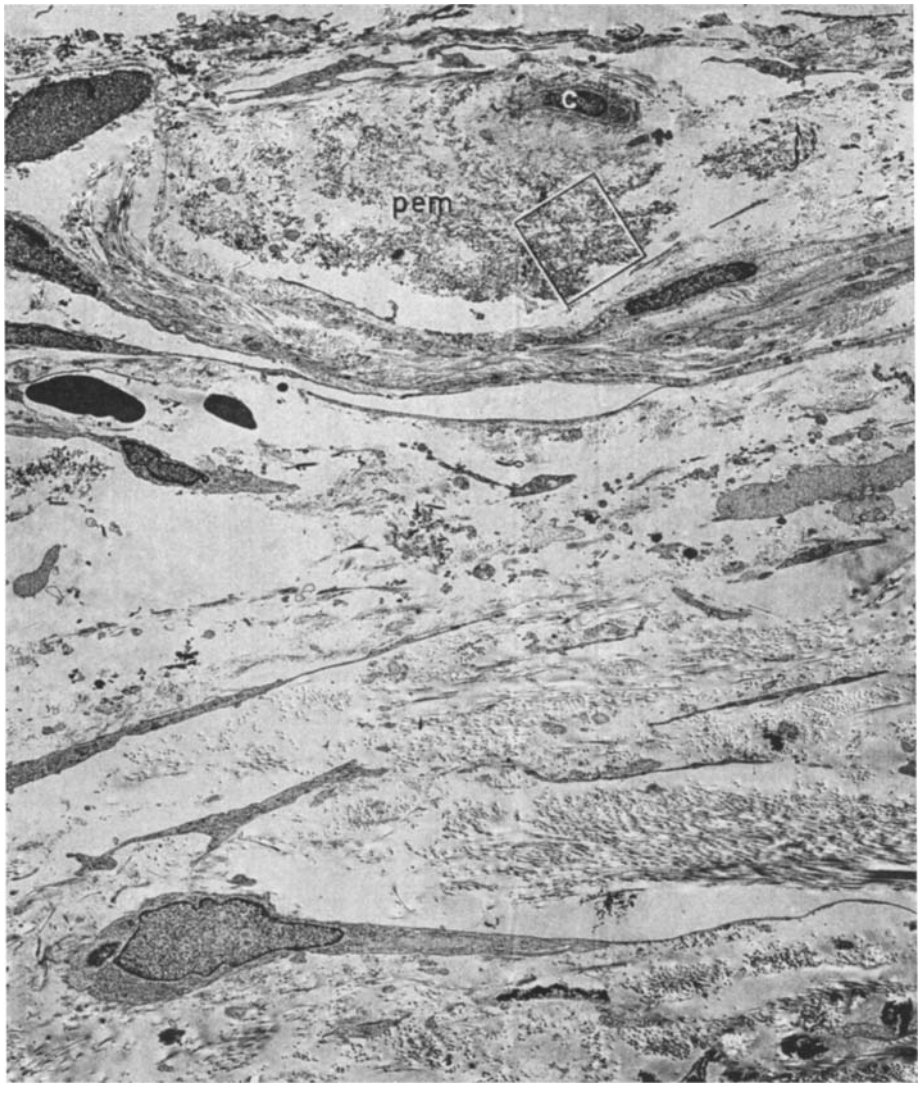

Fig. 1. Large, distinctly limited aggregate of PE material (*pem*) surrounded by a loosely packed connective tissue. Note connective tissue cell (*C*) adjoining the PE aggregate. × 2700

Results

The limbal region of the bulbar conjunctiva was covered by a stratified squamous *epithelium*, which gradually changed into simple squamous towards the fornix. In many instances the epithelium was absent, probably because of mechanical damage during preparation.

The subepithelial *connective tissue* was loosely packed (Fig. 1), and showed the usual extracellular components (Ringvold, in prep.). In addition, an abnormal fibrillar material was observed in this part of conjunctiva in all five eyes (Fig. 1). This material appeared similar to PE material as seen in sections from other

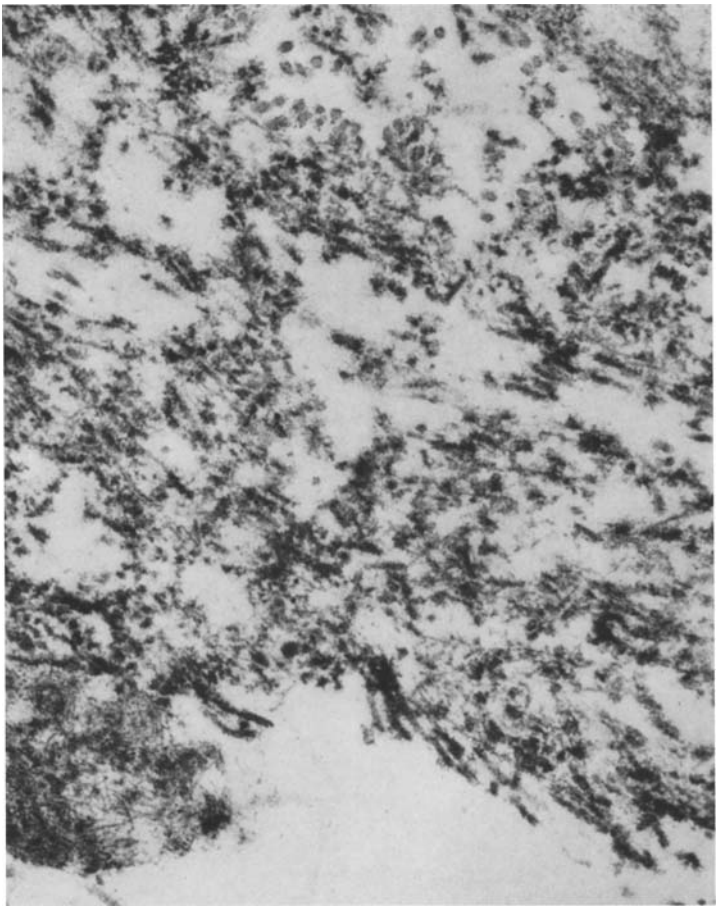

Fig. 2. Higher magnification of boxed area in Fig. 1, showing the irregular course of fibrils within the aggregate. × 30000

locations: Single fibrils were straight, measuring 100–330 Å in thickness, up to 1 μ in length, and some of them showed cross-bands at intervals of about 400–500 Å (Fig. 2). The abnormal material mostly occurred as rather densely packed aggregates (measuring up to 22 μ across), within which the fibrils were irregularly arranged (Figs. 2, 5). Fig. 1 shows that the aggregates were distinctly limited to the surrounding tissue, and some of them were closely associated with connective tissue cells. With the aggregates elastic elements and collagen as well as some granular material were observed. On the other hand, small clusters of abnormal fibrils were also found scattered among the normal extracellular components.

The appearance of many *vessels* corresponded to the description of normal conjunctival vessels (Tamura, 1967; Hara and Hiwatari, 1969). However, in some vessels numerous cytoplasmic processes into the vessel lumen appeared in addition to marginal folds. Some of these processes had a finger-like shape similar to marginal folds, whereas most of them were thinner at the basal than at the

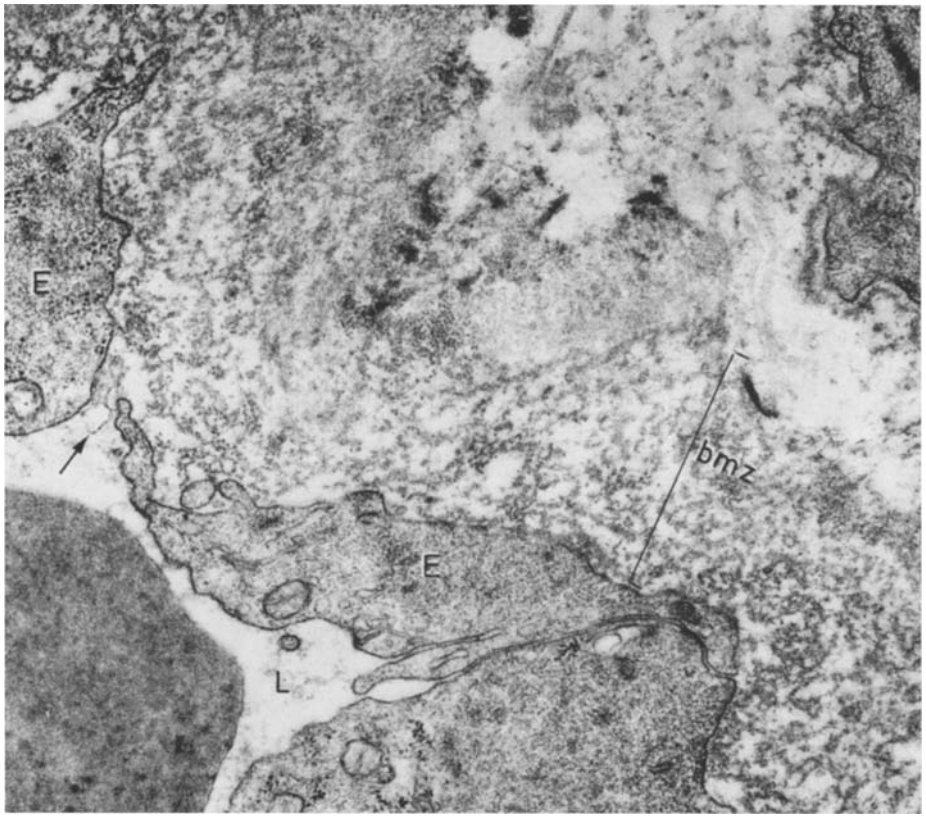

Fig. 3. Mottled basement membrane zone (*bmz.*). The intercellular gap (arrow) should perhaps be interpreted as an artificial opening of the endothelial wall. Vessel lumen (*L*). Endothelial cells (*E*) . × 30000

apical end. Otherwise, cytoplasmic changes of the endothelial cells were not striking. Usually, the vessels showed a continuous endothelial lining, and where section was favourable thigh junctions were observed.

Sometimes the *basement membrane* was changed, i.e. the whole basement membrane zone appeared mottled, containing numerous short, ragged fragments in a random organization (Fig. 3). However, many vessels showed a normal basement membrane, which varied greatly in thickness from one vessel to another (600 Å to 1 μ). This basement membrane often appeared multilayered, and in some instances discontinuities of single layers were observed. Collagen fibrils were found both adjacent to and within the basement membrane. The basement membrane and the connective tissue next to it were sometimes packed with abnormal fibrils (Fig. 4) similar to those of the aggregates described above. The abnormal fibrils partly appeared as dense patches within this zone, which showed a rather abrupt

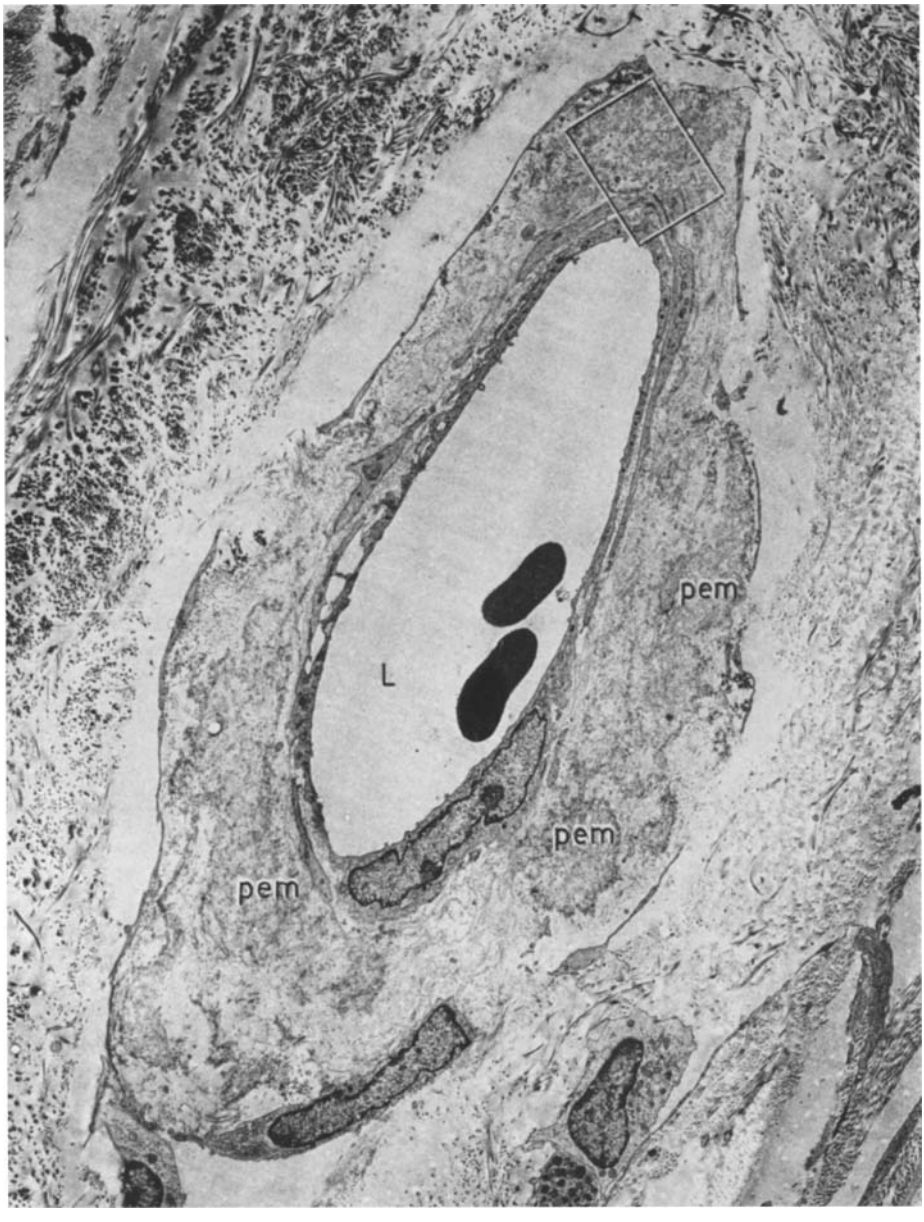

Fig. 4. Abundance of PE material (*pem*) within and adjacent to the vessel wall. Note abrupt transition between abnormal and normal connective tissue components in some parts of the circumference. Vessel lumen (*L*). × 2700

transition towards the normal connective tissue outside (Fig. 4). Furthermore, granules (700–1 700 Å in diameter), sometimes enclosed by a unit membrane, were found mixed with the abnormal fibrillar material (Fig. 5). Pericytes and muscle cells were occasionally surrounded by such abnormal extracellular material.

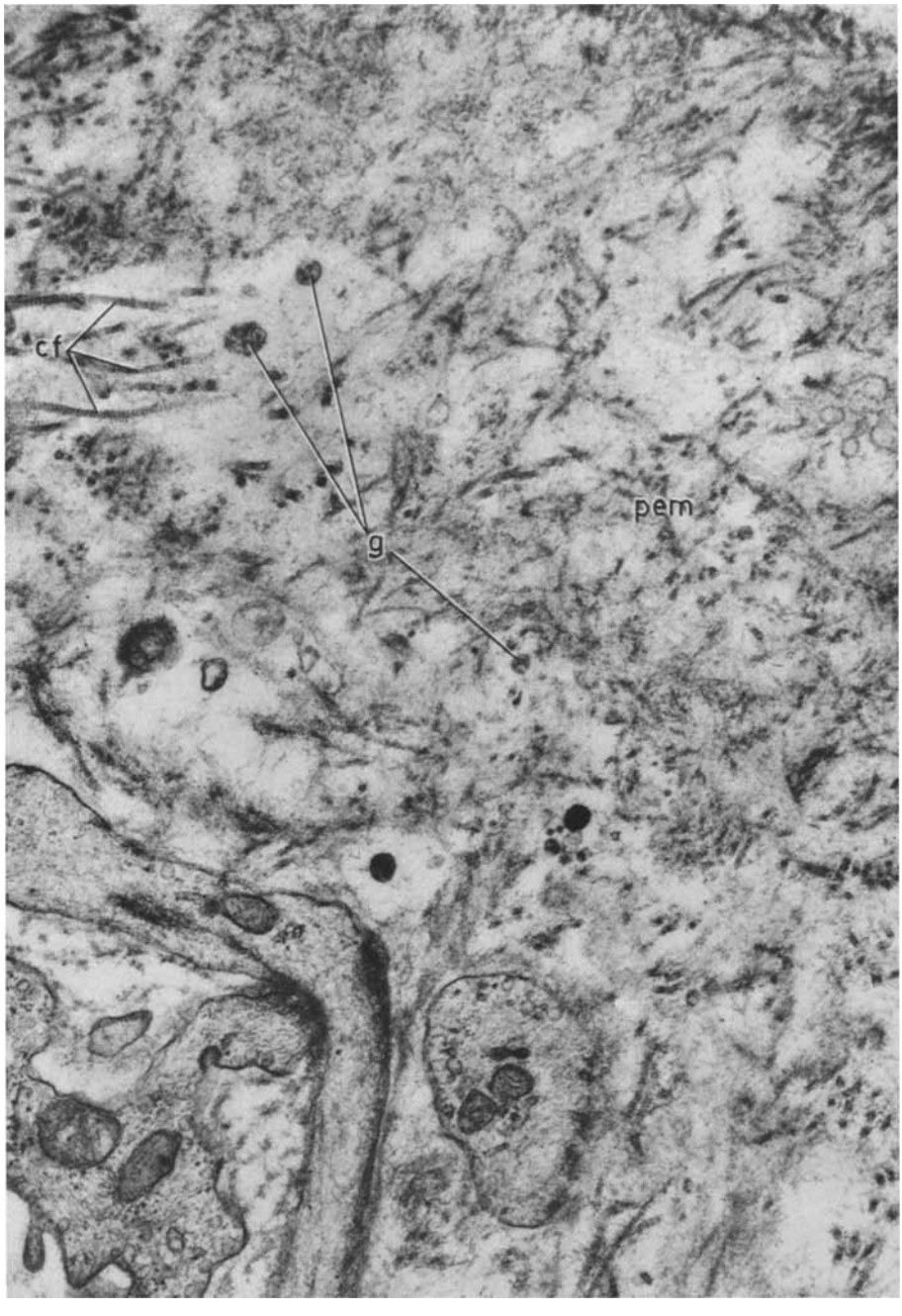

Fig. 5. Higher magnification of boxed area in Fig. 4. PE material (*pem*) containing granules (*g*) of different size. Collagen fibrils (*cf*). × 30000

Discussion

Prior to enucleation, PE material had been observed within the anterior segment of all eyes of the present study. In three of the eyes, this observation has been confirmed in a previous electron microscopic investigation (Ringvold and Vegge, 1971), which showed PE material in the trabecular meshwork. The present study demonstrates the presence of PE material also in the limbal conjunctiva. As far as we know, such material has not previously been found outside the sclera.

Three of the investigated eyes had received surgical treatment for glaucoma (*ad modum* Elliot). This procedure includes trepanation of the juxtalimbal sclera to allow passage of the aqueous humour from the intraocular space to the conjunctiva. Some PE material may therefore have been brought through this artificial scleral opening, thereby contaminating the adjoining conjunctival tissue. It seems unlikely that this mode of transport conveys PE aggregates beyond a very limited area around the scleral opening. However, PE material was found in several blocks from each of the eyes, i.e. in different parts of the limbal conjunctiva. This fact perhaps indicates that a great part of the PE material in conjunctiva of these three eyes is not due to contamination. This interpretation agrees well with the observation that such material occurred in abundance also in conjunctiva of unoperated eyes.

Whether some PE material has been transported from the aqueous humour area by way of the canal of Schlemm and the collector channels to the conjunctival tissue, cannot be settled on account of the present study. The possibility should not be ruled out, since a previous study (Ringvold and Vegge, 1971) showed the presence of PE material in the giant vacuoles of the inner wall of Schlemm's canal. If, on the other hand, the material is synthesized in loco, this would seen to indicate that the PE syndrome is of a more general nature than hitherto believed. The demonstrated changes of conjunctiva should, therefore, stimulate the search for such material in other parts of the body of patients with PE syndrome.

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