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Microfilaments in the cells of the human trabecular meshwork

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SUMMARY In this present study the results are presented of a combined ultrastructural and immunofluorescent investigation of 'smooth muscle' elements within the cytoplasm of human trabecular meshwork cells. The cytoplasm of human meshwork cells both *in vivo* and *in vitro* is replete with 10 nm intermediate filaments and also contains smaller 6 nm filaments which are particularly prominent in the cell processes. By immunofluorescence using sera rich in antibodies to contractile proteins, particularly actin, cultured meshwork cells showed strong cytoplasmic fluorescence. On occasion the cytoplasmic fluorescence was diffuse, but more often recognisable bundles (stress fibres) or a loose fibrillar framework was found. The possible role of structural and contractile cellular proteins in trabecular function was discussed.

It has been shown that trabecular meshwork cells are extremely pliable, and in experimentally induced acute increase of intraocular pressure (Grierson and Lee, 1975; Kayes, 1975; Svedbergh, 1976) or after the application of topical pilocarpine (Grierson *et al.*, 1978) these cells are capable of pronounced attenuation without loss of cell-to-cell contact. Grierson and Lee (1975) suggested that cytoskeletal elements and, in particular, the cytoplasmic microfilaments could be expected to have a key role in cellular pliability, and thus they will have an important function in the maintenance of tissue integrity.

Further, it is known that the structural cytoplasmic proteins of many non-muscle cells, in addition to having cytoskeletal functions, can play a part in cellular mobility and contraction (Pollard and Weihing, 1974). If contractile proteins are a component of the cytoplasm of trabecular meshwork cells, it could be expected that filament-dependent cellular contraction may have an influence on outflow dynamics (Inomata *et al.*, 1972). Indeed it has been shown that the intraocular infusion of cytochalasin B, which disrupts contractile cytoplasmic microfilament systems, produces a marked increase in the facility of aqueous outflow (Kaughman and Barany, 1977).

Clearly structural and contractile cytoplasmic proteins may be of some importance to trabecular function. Therefore the present investigation was conducted to determine the nature and distribution of cytoplasmic microfilaments in the cells of the normal human trabecular meshwork, and an attempt was made to demonstrate the presence of contractile protein antigens within cultured human trabecular meshwork cells by means of an immunofluorescent technique.

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Materials and methods

NORMAL TISSUE

Normal angular tissue for electron microscopy was obtained from 11 human eyes enucleated in treatment of malignant melanoma of the posterior globe. The patients' ages varied between 46 and 76 years. The eyes showed no clinical evidence of abnormalities in the anterior segment. After enucleation they were immersed in 3% phosphate buffered glutaraldehyde and opened by equatorial section; the lenses were removed, and slices of limbal tissue were taken for further processing. After a wash in phosphate buffer the limbal segments were post-fixed in phosphate buffered osmium tetroxide, rewashed in buffer, dehydrated through graded alcohols, cleared with propylene oxide, and embedded in Araldite.

TISSUE CULTURE

The globes for tissue culture were provided by the eye bank and were used from 6 to 48 hours post mortem. The anterior segment of each eye was divided into quadrants, and the iris and ciliary body were removed under the dissecting microscope. Two

parallel cuts were made in the trabecular region, and the strip of drainage tissue was carefully removed with minimal handling. The trabecular meshes were transferred to the cover glass of a tissue culture chamber and embedded in an embryonic plasma clot. The chamber was then sealed and filled with a tissue culture medium. For further details see Tripathi *et al.* (1973).

Outgrowths from the explant spread over the surface of the coverslip, and after 6 weeks to 3 months the primary culture was sufficiently well established for either electron microscopy or immunofluorescent studies. Preparation of the trabecular meshwork cells on the coverslips for electron microscopy was by the technique described by Tripathi *et al.* (1973) and Rahi and Ashton (1978).

IMMUNOFLUORESCENT STUDIES

For this part of the investigation the coverslips containing monolayers of cultured trabecular meshwork cells were washed in isotonic saline and fixed in acetone.

Blood from patients with chronic uveitis or intraocular melanoma containing high titres (greater than 1/160) of antibodies to 'smooth muscle' proteins, particularly actin (Rahi *et al.*, 1976; Rahi and Ashton, 1978) were used as the source of antiserum to localised contractile cytoplasmic proteins. The reactivity and specificity of the sera were judged by examination of frozen sections from composite blocks of rat liver, kidney, skeletal muscle, stomach, and salivary gland which had been treated with the sera (Trenchev and Holborow, 1976).

After treatment with the appropriate sera the method outlined by Rahi *et al.* (1976) and Rahi and Ashton (1978) using fluorescein labelled antihuman immunoglobulins was adopted. A Zeiss epifluorescence microscope equipped with a fluorescein isothiocyanate interference filter was used for routine examination of the tissues.

Results

ULTRASTRUCTURE

The trabecular meshwork cells of the normal human outflow apparatus contained 2 types of cytoplasmic microfilaments of indeterminate length but of different diameters. The larger filaments measured 10 nm in diameter and were most abundant in the main cell body and in the perinuclear zone (Fig. 1). The 10 nm filaments were formed into either sporadically distributed bundles (Fig. 2) or, more commonly, a subcortical network, so that in any one section the filaments were cut both in cross section and in longitudinal section (Fig. 1). These filaments did not make contact with the plasma membrane but did come into close association with the limiting membranes of some cytoplasmic organelles (Fig. 1).

The finer, more delicate filaments measured between 5 and 6 nm in diameter and were found in loose aggregates throughout the cell cytoplasm from the perinuclear zone to the cell periphery. The fine filaments were particularly prevalent in the cell processes, where they were orientated so that they formed an array which ran parallel with the long axis of the cytoplasmic projection (Fig. 3). Unlike the larger filaments the fine filaments came into close proximity with the plasma membrane (Figs. 3 and 4).

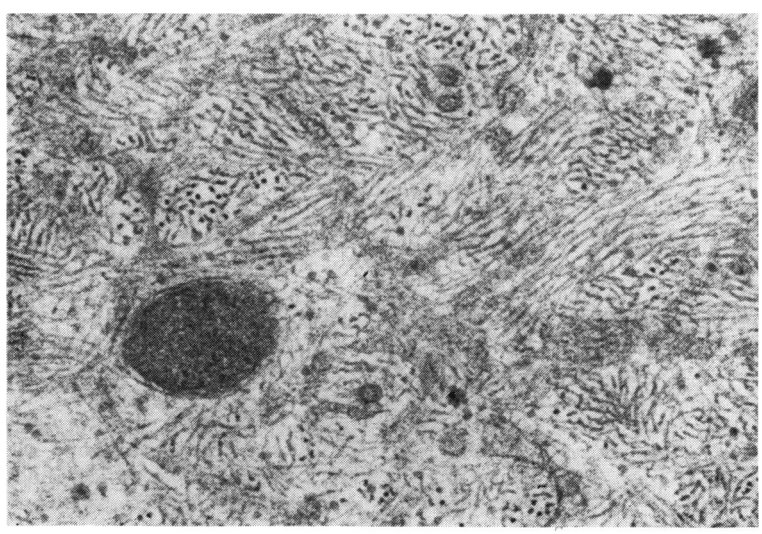

Fig. 1 *Electron micrograph showing 10 nm microfilaments in the cytoplasm of a trabecular meshwork cell. The microfilaments form a network and can be seen both in cross and in longitudinal section* (×60 000)

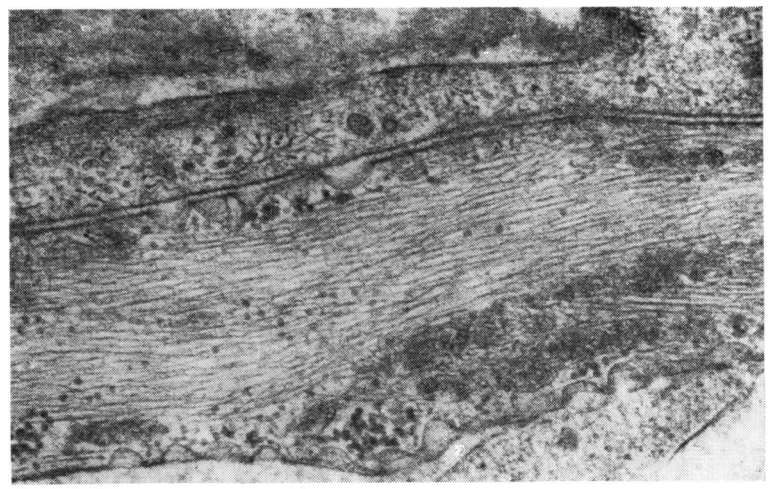

Fig. 2 *Electron micrograph showing a bundle of 10 nm microfilaments in the cytoplasm of a trabecular meshwork cell* (× 50 000)

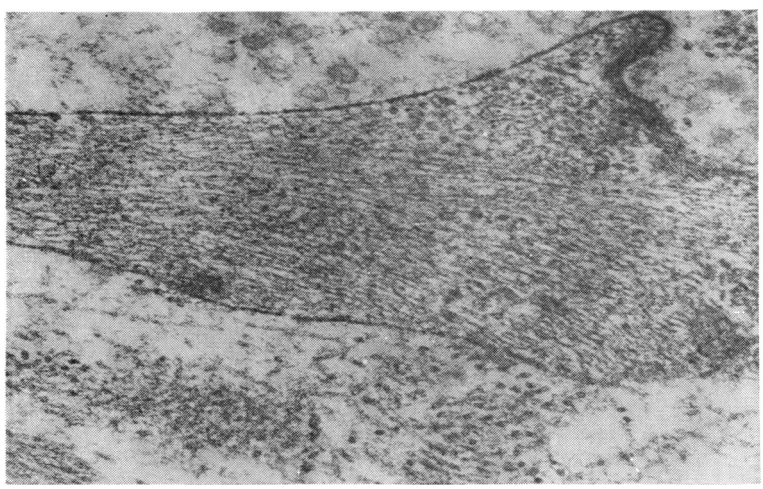

Fig. 3 *Electron micrograph showing abundant 6 nm microfilaments in a cytoplasmic process of a trabecular meshwork cell* (× 68 000)

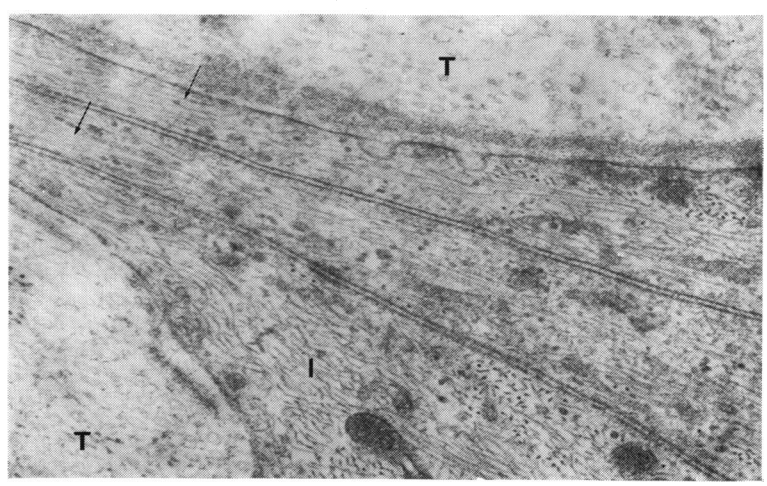

Fig. 4 *Electron micrograph of trabecular meshwork cells, trabecular cores indicated* (T). *In one cell 10 nm intermediate microfilaments predominate* (I), *whereas in the others 6 nm microfilaments can be seen (arrows)* (× 40 000)

Studies of the fine structure of cultured trabecular meshwork cells revealed the presence of both types of cytoplasmic microfilament previously described.

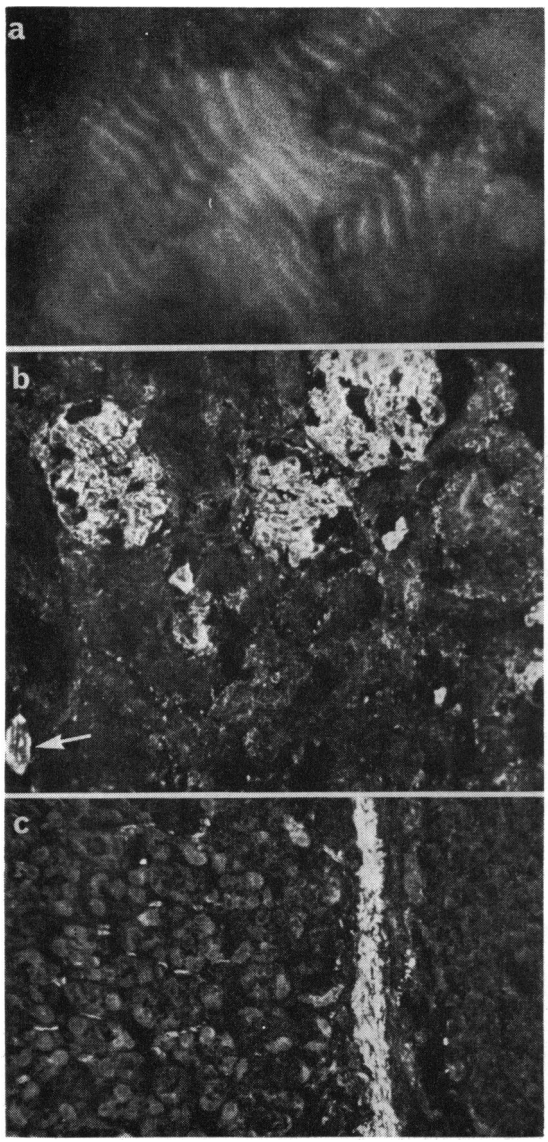

Fig. 5 *Indirect immunofluorescence tests on cryostat sections of rat tissues.* (a) *The anti-actin antibody in the sera reacts at the 'I' band to produce distinctive fluorescence in skeletal muscle* (×1100). (b) *A section through the wall of the stomach shows bright fluorescence at the muscularis mucosa and also in the muscle fibres between the gastric glands* (×190). (c) *In a section of kidney the sera have reacted with contractile proteins of cells in the glomeruli and in the wall of an artery (arrow)* (×190)

IMMUNOFLUORESCENT STUDIES

From the multiple blocks the skeletal muscle showed specific fluorescence for the 'I' bands (Fig. 5*a*). The muscle fibres between gastric glands were strongly fluorescent (Fig. 5*b*), as were the glomeruli and peritubular tissue of the kidney (Fig. 5*c*). In addition hepatocytes showed a polygonal staining pattern. On the basis of these findings it was evident that the sera used reacted strongly with cells which are known to have an abundance of 'smooth

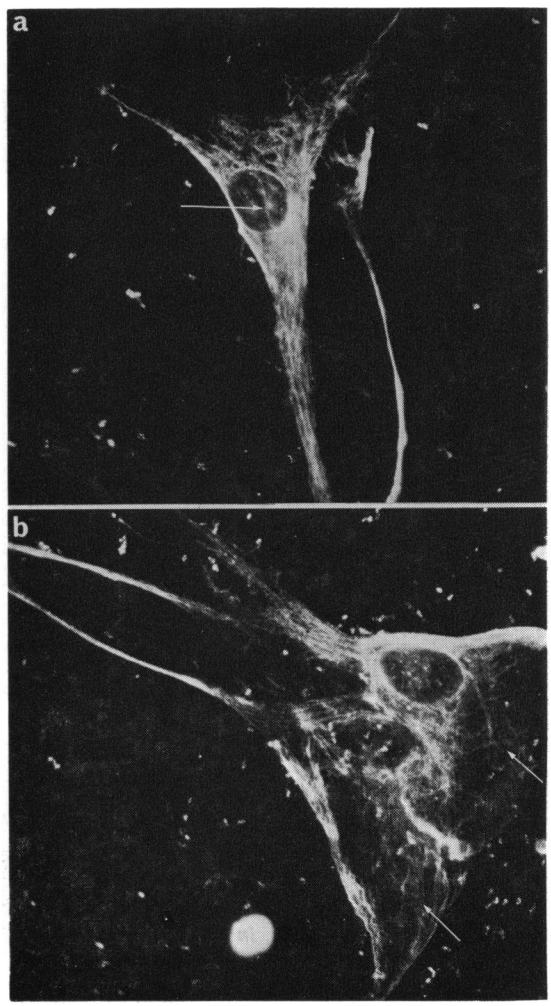

Fig. 6 *Acetone fixed cultured trabecular meshwork cells tested by the indirect immunofluorescence with anti-'smooth muscle' sera.* (a) *Strong cytoplasmic fluorescence in a meshwork cell. Note the unstained cell nucleus (arrow)* (×520). (b) *Two meshwork cells in which there is strong fluorescence in the long extended cell processes. A fluorescence-positive network can be seen in the main cell body (arrow)* (×520)

muscle' proteins and to be particularly rich in actin.

When cultured trabecular meshwork cells were treated with the sera, there was a strongly positive cytoplasmic fluorescence. Cytoplasmic fluorescence was particularly prominent in the cell processes (Fig. 6), at the cell margin, and as a perinuclear halo. At higher magnification recognisable fibres or bundles were found both in the main cell body (Fig. 7*a*) and in the cellular processes (Fig. 7*b*). The bundles were orientated so that they ran parallel with the long axis of the cell, and similar bundles have been described in other cell types, where they have been called 'stress fibres' (Wessells *et al.*, 1971). Stress fibres were not found in all the cells examined; in some there was only diffuse cytoplasmic staining, whereas in others a loose fibrillar framework could be identified (Fig. 6).

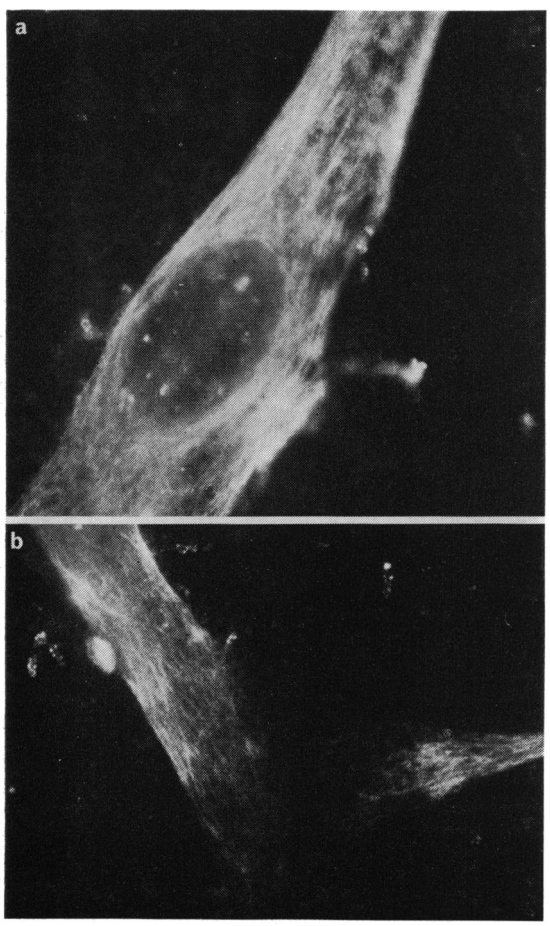

Fig. 7 (a) *Part of a trabecular meshwork cell fixed with acetone and tested by indirect immunofluorescence using serum which contains anti-'smooth muscle' antibody. Subcortical bundles of actin-containing filaments can be seen* (×935). (b) *A trabecular meshwork cell process from a culture which was fixed in acetone and treated with serum containing anti-'smooth muscle' antibodies. Bundles or sheaths of contractile protein are evident* (×765)

Discussion

The cytoplasm of human trabecular meshwork cells is rich in 6 and 10 nm microfilaments. The 6 nm microfilaments were most numerous at the cell periphery, round the cell nucleus, and within cytoplasmic processes. It was therefore of interest to note that the forementioned sites showed, in most meshwork cells, the strongest fluorescence after exposure to sera rich in antibodies to actin and other contractile proteins.

Recently Ringvold (1978) was able to identify 4·5 to 6·5 nm cytoplasmic filaments in the trabecular meshwork cells of the vervet monkey. He exposed glycerinated segments of trabecular meshwork to myosin subfragment (S-1), which forms characteristic 'arrowhead' complexes with actin containing microfilaments. Ringvold found that 'arrowhead' complexes were prevalent on the 4·5 to 6·5 nm diameter filaments and suggested that they were therefore actin filaments. Actin in conjunction with myosin is involved in cellular contraction and motility but also plays a part in other biological functions such as phagocytosis, pinocytosis, and cell adhesion (Allison *et al.*, 1971; Malawista *et al.*, 1971; Wessells *et al.*, 1971; Pollack and Rifkin, 1975; Tilney, 1975).

However, the predominant cytoskeletal component of human trabecular meshwork cells was shown to be 10 nm diameter filaments. These so-called intermediate filaments are of uncertain chemical identity (Lansing-Taylor, 1975). Only 1 component protein within these structures has been described so far and has been called alternatively desmin or skeletin (Kurki *et al.*, 1977). It has been suggested that typically they do not have an actin component (Ishikawa *et al.*, 1969) and that they have a structural rather than a contractile role. On the other hand the distribution of intermediate filaments is altered at the onset of locomotion by leukaemia cells (Felix and Strauli, 1976), and 10 nm filaments in pigment epithelial cells of the retina do contain actin (Burnside, 1976), though this may be an exceptional case. Also 10 nm intermediate filaments are involved in axoplasmic transport in nerve cells (Felix and Strauli, 1976) and have been implicated in the intracellular transport of cytoplasmic organelles in fibroblasts (Goldman and Follett, 1969).

The structural and contractile proteins within trabecular meshwork cells may well be of some

importance to trabecular function, since trabecular cell pliability and contraction may influence the width of intertrabecular spaces and hence affect the rate of aqueous outflow (Kaughman, 1977). However, it is also known that trabecular meshwork cells are latent phagocytes, and in various forms of secondary open-angle glaucoma the native cells of the drainage system engulf considerable quantities of debris (Grierson and Lee, 1973; Van Buskirk and Leure-Dupree, 1978). Recently the suggestion has been put forward that primary open-angle glaucoma may arise as a result of an inability of meshwork cells to phagocytose and get rid of the debris which may accumulate in the outflow system of the older eye (Bill, 1975). If this interesting hypothesis is to be tested, clearly it would be worthwhile to embark on further studies of the filament systems within these cells, which, as has been stated previously, are known to have a key role in the phagocytic process.

Although it has been established in the study reported here that contractile proteins are present in human trabecular meshwork cells, more detailed investigations need to be performed to determine which smooth muscle contractile proteins apart from actin are present in these cells and what role they play in cell contraction, anchorage, and phagocytosis in the normal and diseased outflow system.

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