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Presence of Matrix Vesicles in the Trabecular Meshwork of Glaucomatous Eyes

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Abstract. In the last few years matrix vesicles (M.V.) have been found in the vessel wall in various diseases and are considered to be related with pathological changes of the extracellular fibers of the connective tissue (atypical collagen etc.). Since the trabecular meshwork, at least the cribriform region, may be looked upon as a part of a vessel wall, it was an obvious conclusion to look for M.V. even in this area.

Lysosomal and non-lysosomal M.V. were found in 138 trabeculectomy-specimens of various kinds of glaucoma, located for the most part within the cribriform or juxta-canalicular region of the meshwork. In ten cases small, electron-dense M.V. were found which ultrahistochemically showed a positive reaction for acid phosphatase. This proves that these vesicles are indeed extracellular lysosomes.

Zusammenfassung. Matrix-Vesikel (M.V.) wurden in den letzten Jahren bei verschiedenen Gefäßerkrankungen gefunden und mit pathologischen Veränderungen der extrazellulären Strukturen (atypisches Kollagen etc.) in Zusammenhang gebracht. Da das Trabekelwerk zumindest im Innenwandbereich des Schlemmschen Kanals als Teil einer Gefäßwand angesehen werden kann, war es naheliegend, auch hier nach M.V. zu suchen. In der Tat fanden sich bei Durchsicht von 138 Trabekulektomie-Stückchen verschiedener Glaukom-Formen typische M.V., wobei sowohl lysosomale als auch nichtlysosomale M.V. vorkommen. In 10 Fällen konnte durch den ultrahistochemischen Nachweis von saurer Phosphatase gezeigt werden, daß es sich bei den kleinen elektronen-dichten M.V. in der Tat um extrazelluläre Lysosomen handelt.

Introduction

The presence and development of matrix vesicles (MV) within the connective tissue has been studied with increasing interest by many investigators since it became evident that these vesicles may play an essential role in the pathogenesis of a great number of connective tissue diseases. Riede et al. (1977), Pott and Staubesand (1977), and Staubesand and co-workers (1978, 1980) have shown that MV can develop in the vessel wall, e.g. in cases of renal hypertension, arteriosclerosis or varicosis or in experimental situations in which the vessels are subjected to non-physiological stress.

Matrix vesicles are small *extracellular* bodies, which either derive from intracellular lysosomes or from the cytoplasm itself. The lysosomal MV may still contain lysosomal enzymes such as acid phosphatases, while the non-lysosomal MV do not contain such enzymes and may represent isolated cytoplasmic processes.

In the smooth muscle layer of a diseased blood vessel, the development of MV begins with the transformation of some of the contractile smooth muscle cells into an active non-contractile cell, which is called m-myocyte. This is a metabolically active cell characterized by a well-developed endoplasmic reticulum, Golgi apparatus and many lysosomes (Riede et al. 1977; Staubesand 1978). Such a cell can move freely through the interstitial spaces of the connective tissue, phagocytize foreign particles and under the influence of stimuli develop MV. Recent evidence indicates that not only myocytes but also fibroblasts and macrophages can release MV into the connective tissue layer of the vessel wall.

The trabecular meshwork can, at least in part, be considered a specialized vessel wall. It contains many cells resembling fibroblasts or macrophages, which are capable of phagocytizing particles or macromolecules.

It could therefore be expected that in the trabecular meshwork under pathological conditions such as chronic simple glaucoma, MV may also be found, which in fact, proved to be true.

Materials and Methods

A total of 108 trabeculectomy specimens collected from several eye clinics in the last 10 years was evaluated. In addition, the chamber angle of 30 eyes with normal anterior segment was taken either from eyes enucleated because of choroidal melanoma or during autopsy. The specimens were fixed in 2.5% buffered glutaraldehyde, post-fixed in 1% OsO_4, dehydrated and embedded in Epon in the usual way. Ultrathin sections were made with the Ultratome OM 3 (Fa. Reichert) stained with lead citrate and studied with the Siemens EM (type 101 A).

In 10 cases of chronic simple glaucoma and 4 normal eyes, the unfixed freshly enucleated specimens were stained for acid phosphatase according to the method of Gomori with the Barka modification (1964).

Results

In *normal eyes,* even at advanced age, true MV are rare. The juxtacanalicular or cribriform portion of the trabecular meshwork looked relatively clean. Only occasionally were MV found.

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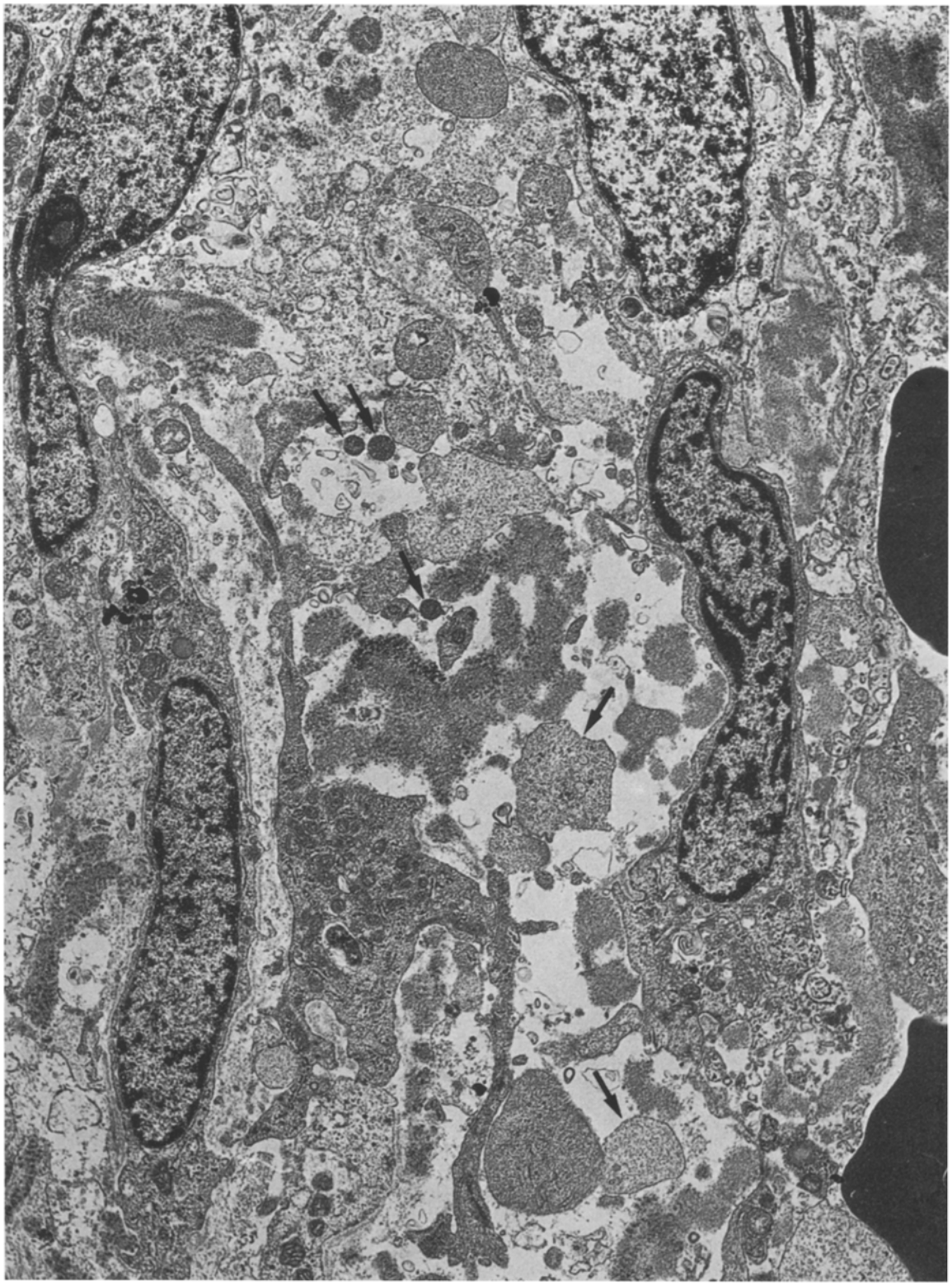

Fig. 1. Electron micrograph of the cribriform region of the trabecular meshwork in a case of glaucoma capsulare (trabeculectomy-specimen, ×10,000). Note several types of matrix vesicles (*arrows*)

In cases of *chronic simple and capsular glaucoma,* however, we often found MV located predominantly within the extracellular spaces of the cribriform portion of the meshwork. Some of these MV appeared as relatively large, round or oval, membrane-bound bodies. Their content was structurally similar to the cytoplasm of the trabecular cells (Figs. 1 and 3). Another form of MV appeared as small, round, electron dense bodies, which are enveloped by a membrane (Fig. 1). Their diameter varies 0.2–1 μm (Figs. 2 and 4).

In addition, vesicles were found that appeared to be partly

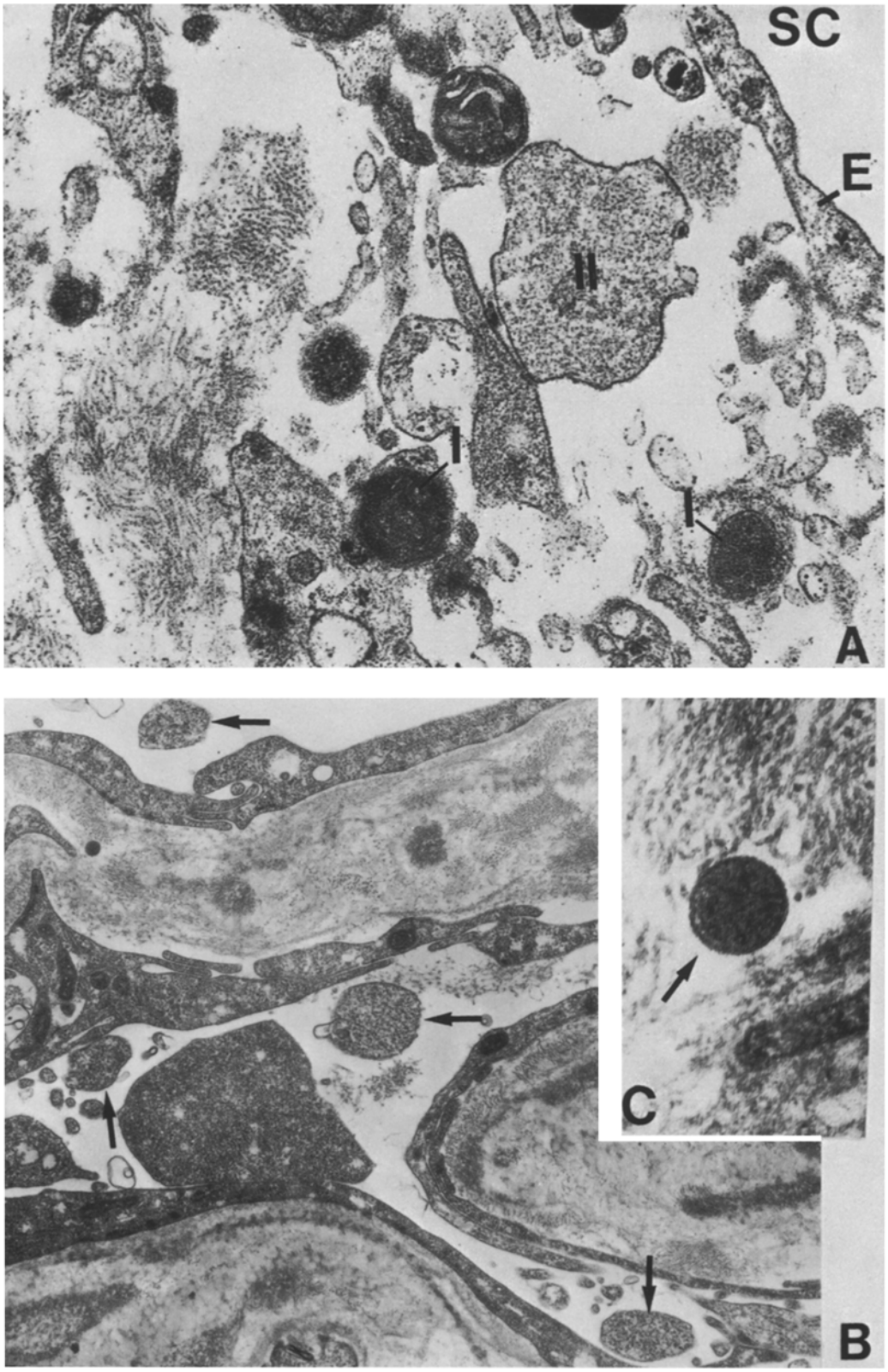

Fig. 2A–C. Electron micrographs of the trabecular meshwork in cases of chronic simple glaucoma (trabeculectomy specimens). **A** Inner wall of Schlemm's canal (*SC*) showing two types of matrix vesicles. *E*, endothelium of Schlemm's canal; *I*, type I MV; *II*=type II MV (×40,000). **B** Corneoscleral part of the trabecular meshwork; several matrix vesicles of type II (*arrows*) are seen in the intertrabecular spaces (×15,000). **C** Extracellular lysosomal matrix vesicle (*arrow*) located in the cribriform meshwork, type I MV (×90,000)

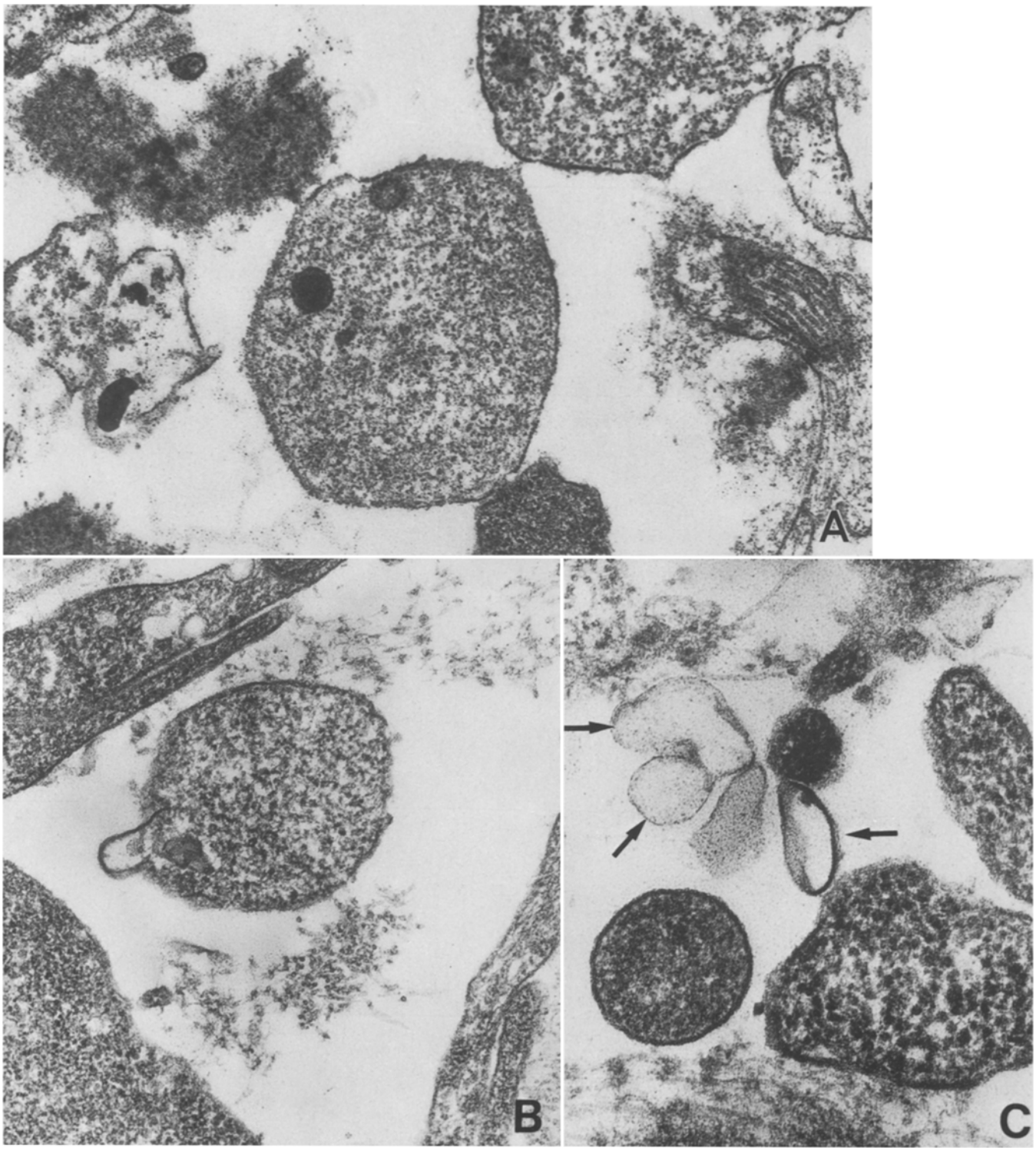

Fig. 3. Matrix vesicles of type II in the cribriform meshwork of glaucomatous eye (trabeculectomy-specimen) **A** ×36,000, **B** ×48,600, **C** ×81,000. Note the empty vesicles in C (arrows)

or completely empty bags with a distinct, sometimes folded membrane (Figs. 3C and 4B, D). In the trabecular meshwork of glaucomatous eyes, two types of MV were found: type I MV are small, membrane-bound vesicles that contain electron dense material resembling extracellular lysosomes. They may also appear as empty vesicles, sometimes still containing electron dense material at the inner aspect of their membranes (Figs. 3 and 4). Type II MV vary largely in size and form. They reveal a weakly osmiophilic structure resembling an undifferentiated cytoplasm (Figs. 1 and 2).

To discover whether the electron dense type I MV are indeed extracellular lysosomes, the presence of acid phosphatases was studied ultrahistochemically. In type I vesicles a blue-black reaction product was found located predominantly in the electron dense matrix material underneath their membranes. In those vesicles that appeared partly empty, only the remnants of the

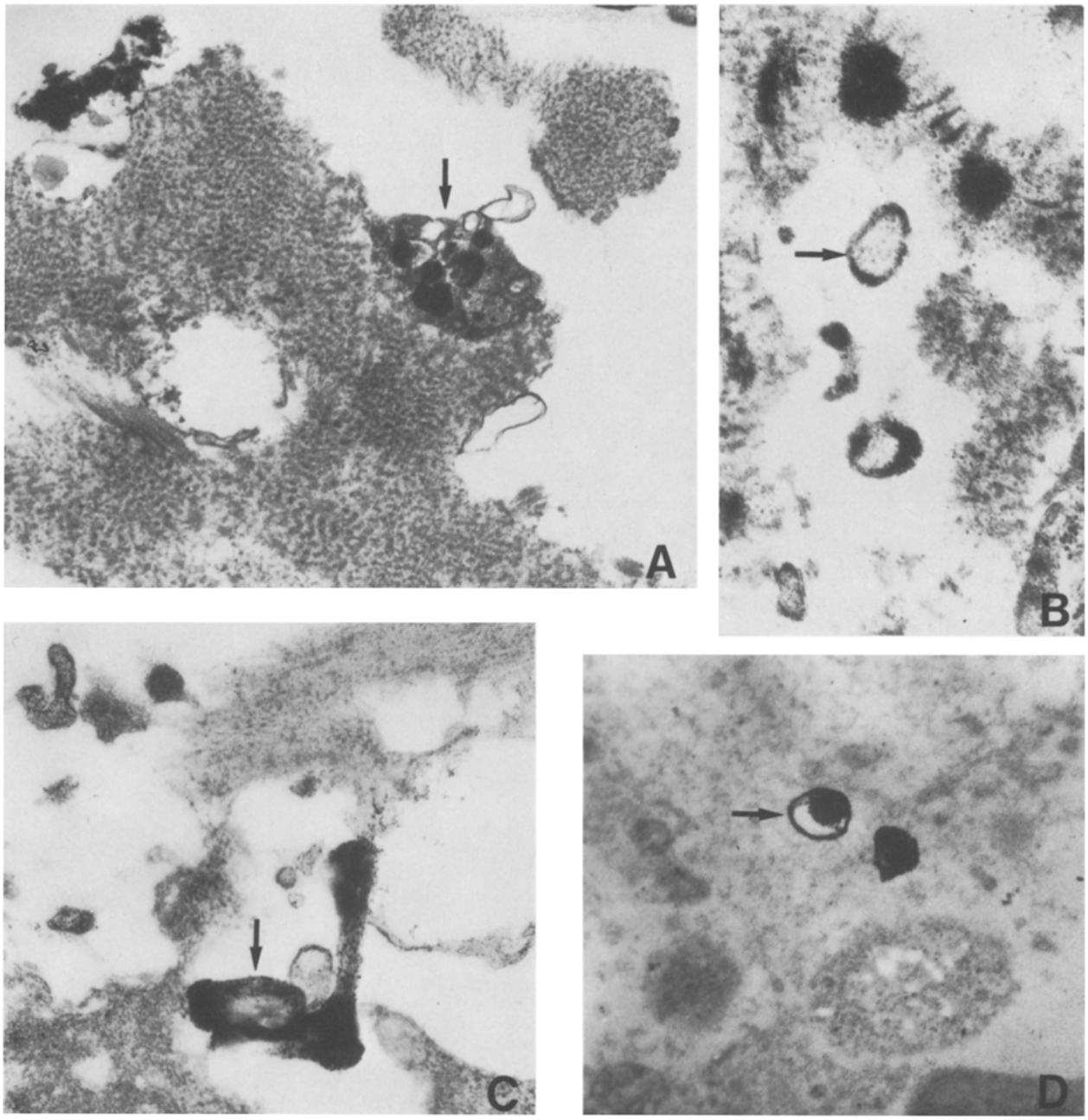

Fig. 4. Electron micrographs of matrix vesicles located in the cribriform meshwork of a glaucomatous eye (trabeculectomy specimen) after staining for acid phosphatase with Gomeri's method according to Barka (1964). Note blue-black reaction product (*arrows*). (**A** ×36,000; **B** ×32,400; **C** ×36,000; **D** ×36,000)

electron dense material attached to their membrane was positively stained for acid phosphatase (Fig. 4B, D).

In trabeculectomy specimens, which incidentally also contained remnants of the anterior tips of the ciliary muscle, we occasionally found muscle cells that had undergone a transformation into the so-called m-myocyts. These cells had lost a great number of myofibrils and had instead developed a cytoplasmic area around the nucleus containing a well-differentiated endoplasmic reticulum, Golgi vesicles and phagolysosomes.

An analogous transformation to metabolically active cells containing a great amount of ER and Golgi material was also seen in the trabecular meshwork cells in cases of chronic simple glaucoma.

Discussion

The staining characteristics, especially the positive reaction of acid phosphatase, prove that the small, electron dense MV of type I are indeed extracellular lysosomes. The empty or partly empty vesicles may derive from extracellular lysosomes that had lost their enzymes and matrix material. The larger type II MV on the other hand, are probably fragments of the cytoplasm from trabecular cells, which are either sequestered from normal cells or left over from deteriorated cells.

In recent literature, three possibilities for the development of lysosomal MV (type I) have been discussed (Staubesand 1978; Staubesand et al. 1980):

1. Production of MV simply by exocytosis of an otherwise intact m-myocyte of fibroblast

2. Sequestration from such cells (microapocrinia)

3. Following a degeneration of a stimulated cell, leaving their lysosomes relatively unchanged because of their more stable membranes

In chronic simple glaucoma the trabecular meshwork shows characteristic changes in the fine structure, especially in the cribriform region (Rohen et al. 1972, 1973, 1981). It was shown recently that the number of trabecular cells is significantly reduced in glaucomatous eyes in comparison to normal eyes of the same age group (Alvarado et al. 1980). It is therefore possible that the relatively great number of MV found in cases of chronic simple glaucoma are the result of cell degeneration. Since lysosomal MV still contain a number of highly active enzymes, these MV are considered to be 'explosive bags', which could seriously injure the extracellular material of the trabecular meshwork. The great amount of atypical collagen (lattice collagen or curly collagen, etc.), the different types of plaque material and the thickening of the elastic-like fiber sheaths found in the ageing and glaucomatous meshwork may be the result of an interaction between MV and the extracellular material (Rohen et al. 1968, 1971; McMenamin and Lee 1980; Lütjen-Drecoll et al. 1981). In cases of renal hypertension or in other pathological conditions, atypical collagenous and elastic fibres of a similar structure were also observed in the areas of the vessel wall where MV had accumulated. The situation in the glaucomatous trabecular meshwork may therefore be in some sense comparable to what is found in the peripheral blood vessels. However, further studies are needed.

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