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AN ULTRASTRUCTURAL STUDY OF CUTANEOUS AMYLOIDOSIS

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Abstract. Ultrastructural studies of cutaneous tumorous amyloidosis in one patient were performed with special reference to mast cells, elastic fibres and calcification. The mast cells were numerous and showed severe alterations including the presence of amyloid fibrils in the granules. Elastic fibres contained amyloid fibrils and were observed in the centre of amyloid masses. Calcification was present around individual collagen fibrils and around and inside amyloid masses.

Clinical, light and electron microscopic studies of 2 patients showing localized tumorous cutaneous amyloidosis of the lower legs have been reported (2). After a plastic operation on one of the patients, a 64-year-old woman, more tissue became available for further ultrastructural studies. In agreement with other reports (11, 14), the previous study (2) presented evidence of local secretion of amyloid substance from reticular cells. In addition, abundant mast cells in close relation to the amyloid substance were found, but their role in the pathogenesis of the amyloid deposition was not explained (2). Furthermore, a close relationship between elastic tissue and the amyloid substance was observed in the light microscope, and X-ray examinations indicated the presence of calcium (2). The purpose of the present study was to further elucidate the relationship of elastic tissue and calcification to the amyloid substance and the possible role of the mast cells in the pathogenesis of cutaneous amyloidosis.

METHODS

The specimens were fixed in a 4% glutaraldehyde solution, buffered at pH 7.4 with phosphate salts for 1 hour at 4°C. For afterfixation, 1% osmic acid solution in 0.1 M phosphate buffer, pH 7.4, was used. After stepwise dehydration in increasing concentrations of ethanol the specimens were embedded in epoxy resin, and ultrathin sections were cut by a Reichert OM 2 ultramicrotome. The sections were stained by a combined technique using uranyl acetate and lead citrate and studied by a Siemens electron microscope (Elmiskop 1A) operated at 80 kV with double condensors.

OBSERVATIONS

Many large masses showing the characteristic fibrillar morphology of amyloid were present in the extremely thickened corium. The masses were separated from each other by spaces containing cells and collagen fibrils, which fibrils were also seen infiltrating the amyloid masses (Fig. 1) and frequently observed in thick closely packed bundles surrounding a few elastic fibres. The collagen fibrils showed distinct bandings but no twistings. The amyloid masses occasionally revealed two zones, i.e. an inner compact and an outer loose (Fig. 1). Often the amyloid masses contained an elastic fibre in the centre (Fig. 1), and amyloid fibrils were observed inside elastic fibres in lines parallel to the axis of the fibre (Fig. 2). The amyloid fibrils could be distinguished from elastic fibrils (microfibrils of the elastic fibre) being thinner, more straight and more tightly packed than the latter. The elastic fibres showed a few round holes evidencing slight "senile" degeneration. Round, electron-dense bodies with granules and needles, indicating calcium apatite crystals in radial and distinct annular arrangements, were occasionally seen inside the amyloid substance without any relation to elastic tissue (Fig. 3).

In the deep corium, electron-dense material was observed in wide areas surrounding amyloid masses and bundles of collagen fibrils (Fig. 4).

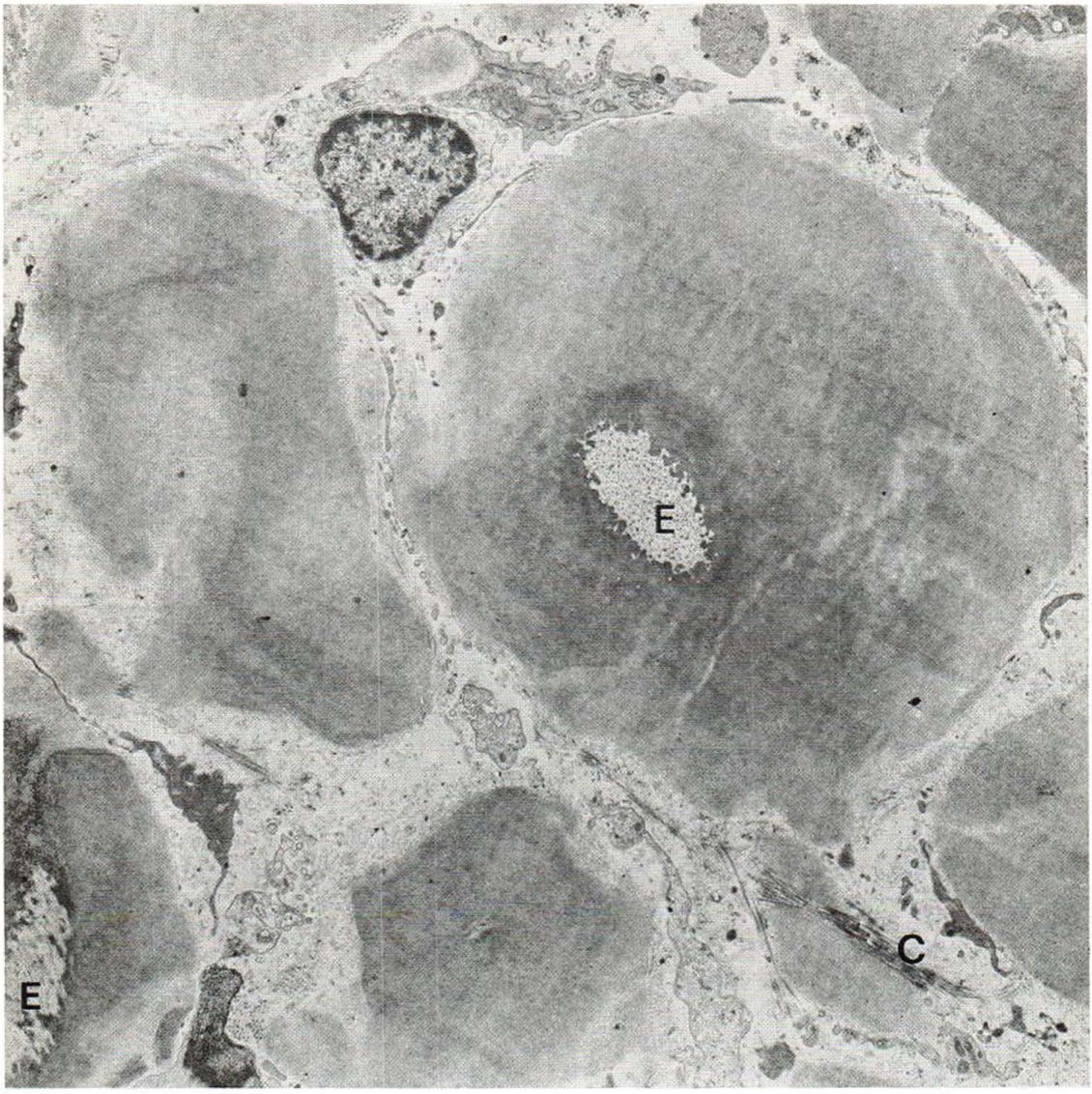

Fig. 1. Large masses of amyloid substance, showing compact inner and loose outer zones. An elastic fibre (*E*) is seen in the centre of two amyloid masses, and collagen fibrils (*C*) are crossing another. × 12 000.

The dense material contained granules and needles, indicating calcium apatite crystals mostly in random arrangement (Figs. 5 and 6). This material was also observed surrounding individual collagen fibrils with needles parallel to the fibrils (Fig. 5) and as round irregular masses inside the amyloid substance (Figs. 4 and 6). Staining with Alizarin red S for light microscopy showed this material to be calcium salts.

Numerous mast cells were located around the amyloid masses and among collagen fibrils. A mast cell was seen almost surrounding an amyloid mass (Fig. 7). In several areas no cell membrane was observed between the amyloid fibrils and the granules or the cytoplasm of this mast cell (Figs. 7 and 8). The mast cells were filled with granules and showed a few, blunt cytoplasmic villi. Most of the granules were either mature or coarse, grainy (Fig. 9). The latter contained lamellae in their peripheral zone and a dense

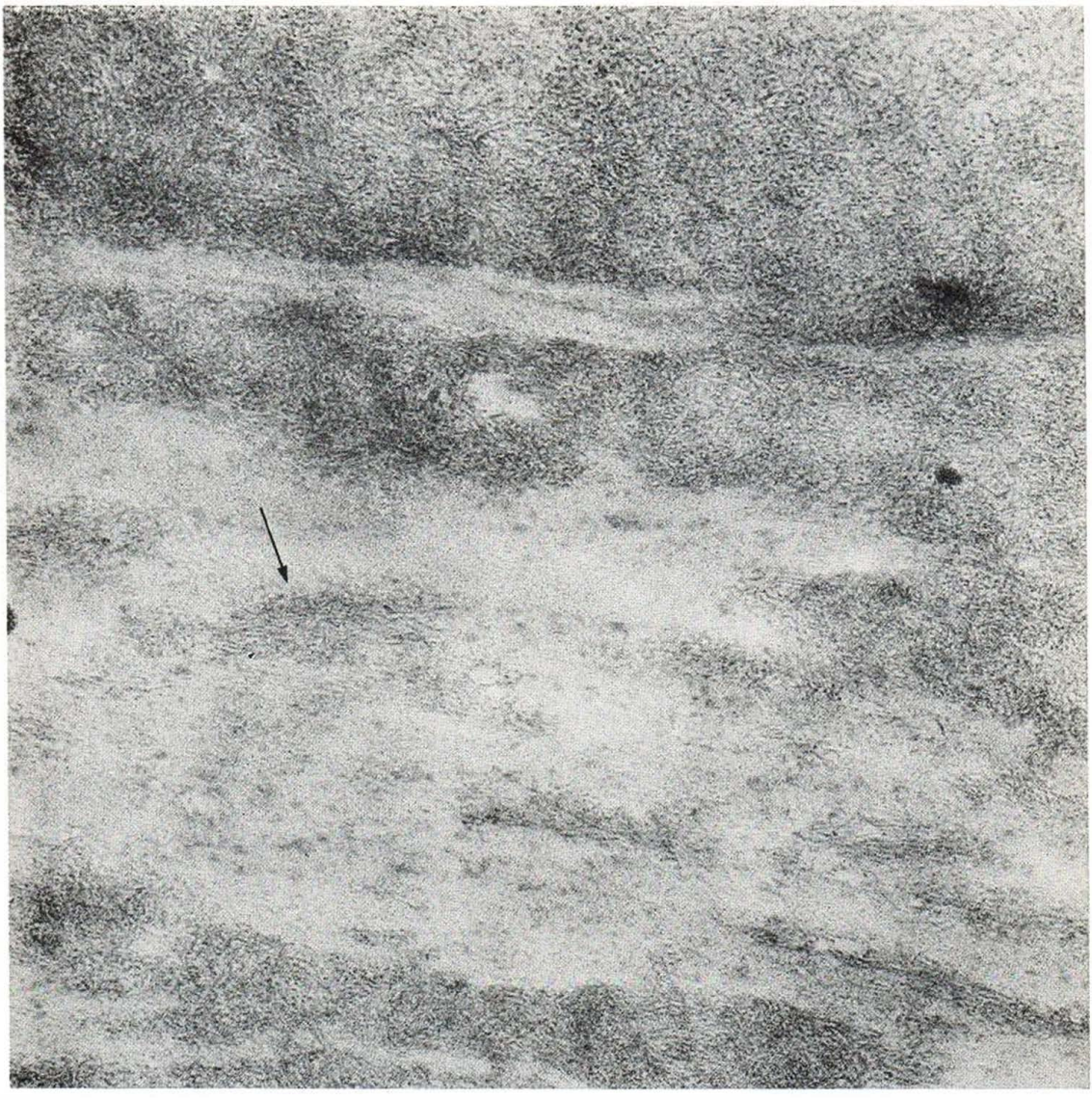

Fig. 2. Amyloid fibrils are seen around and inside (arrow) an elastic fibre. They appear thinner, straighter, and more tightly packed than elastic fibrils. × 60 000.

core with lucent spots in their centre. Other granules showed partially a mature pattern with lamellae and crystalline figures, while the remainder of the same granule was coarse, grainy, with indistinct fragments of lamellae or fibrils, similar in appearance to amyloid fibrils (Fig. 8). A few empty spaces ("honeycomb structures") (Figs. 7 and 8) and extracellular mature or coarse grainy granules were also seen. Besides these cytoplasmic granules, the cells contained mitochondria with indistinct cristae as well as ribosomes (Fig. 9). No phagosome or pinocytotic vesicle was seen in the cytoplasm.

DISCUSSION

The amyloid fibrils seemed to have precipitated around and inside the elastic fibres. It is noteworthy that the chemical composition of elastic and amyloid fibrils has considerable similarity (12,

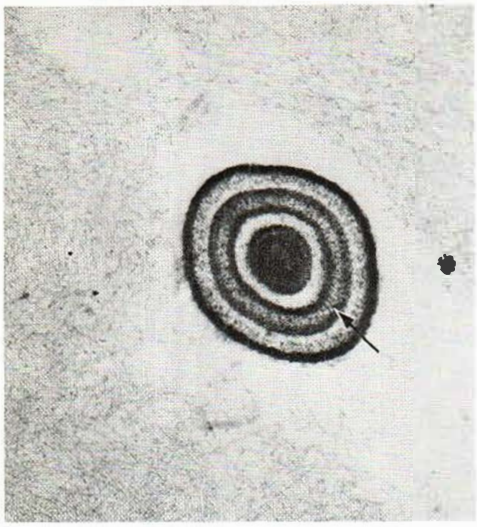

Fig. 3. Round, electron-dense body, showing granules and needles (arrow) in radial and distinct annular arrangement. Amyloid fibrils are seen in the surroundings. × 60 000.

13). The slight "senile" degeneration observed in the elastic fibres was less pronounced than that observed in unexposed skin of patients of corresponding ages (3). The round, electron-dense bodies observed inside the amyloid masses were similar to those observed inside degenerate elastic fibres (3, 4), but they showed more distinct annular structures. The question whether these round bodies are remnants of elastic tissue, or whether they can also develop in amyloid tissue is unresolved. The lack of twisting of collagen fibrils in our case is in contrast to a previously reported case of amyloidosis (9).

The presence of calcium salts around collagen fibrils in the deep corium corresponds to the findings in scleroderma (6). In contrast to the finding in a gastric artery of one patient with pseudoxanthoma elasticum (4), the location of the crystals appeared to be extrafibrillar. Why the pericollagen and the amyloid substance calcify is unknown.

The finding of amyloid fibrils in mast cell granules and the close relationship between the mast cells and the amyloid substance, together with the lack of evidence of phagocytosis, might suggest some functional connection between the mast cells and the amyloid substance. In view of the fact that glycosaminoglycans are present in mast cell granules (1) it is worth mentioning that amyloid contains glycosaminoglycans (10). However, evidence of synthesis of the protein part of the amyloid inside mast cells is lacking. Extraordinarily primitive mast cells have been suggested to possess reticulin-forming properties (5). Coarse, grainy granules with a dense central core have been found in mast cells of squamous cell carcinoma (8). They were interpreted as malformed granules influenced by altered connective tissue metabolism. A few extracellular granules of both mature and grainy type and honeycomb-like structures indicate a slight degranulation activity, although well-developed villi were not seen (7).

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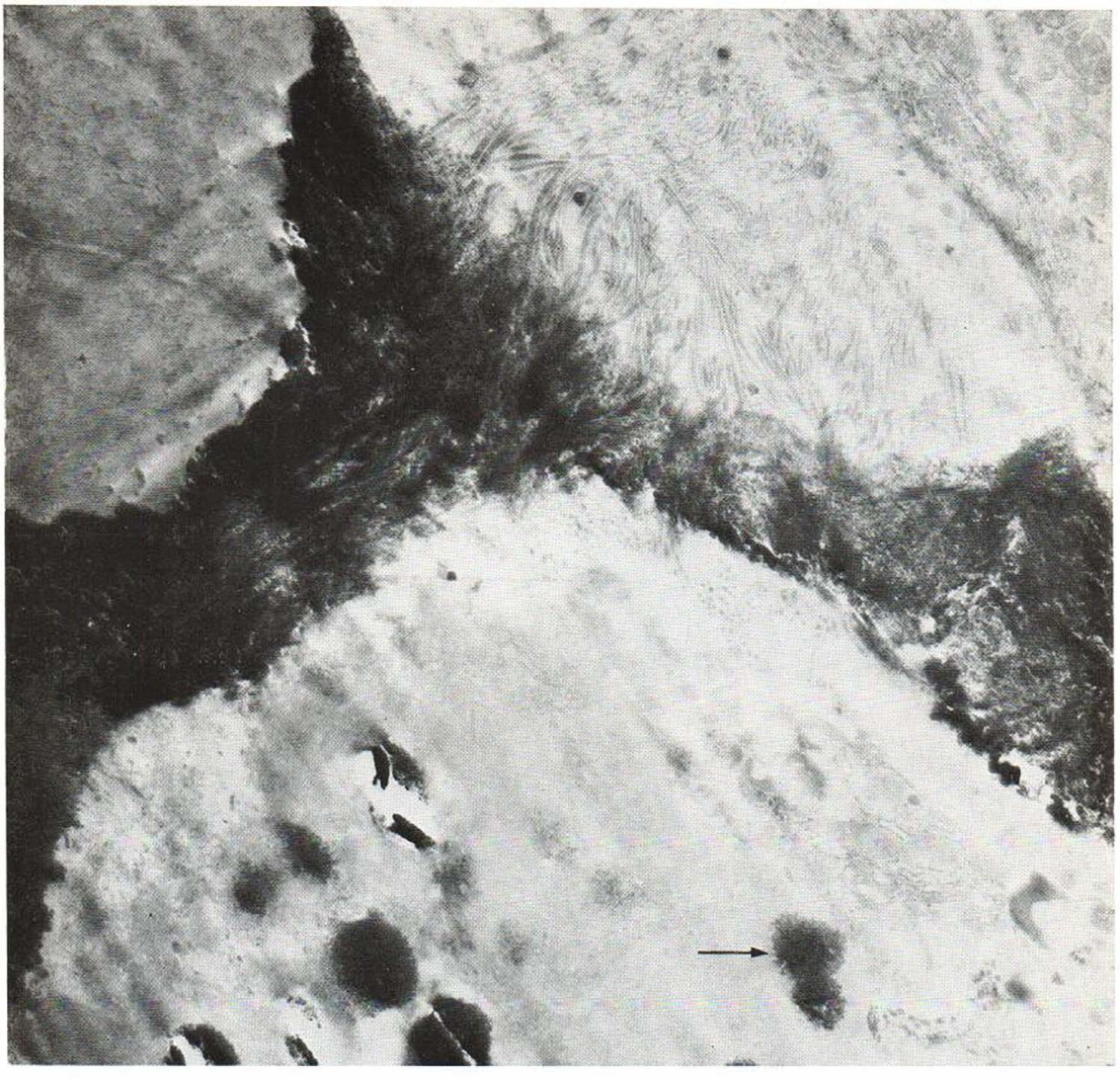

Fig. 4. Large, electron-dense masses around collagen bundles and around and inside (arrow) amyloid masses. × 12 000.

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Fig. 5. Needles surrounding collagen fibrils arranged at random or parallel with the fibrils (arrow). × 60 000.

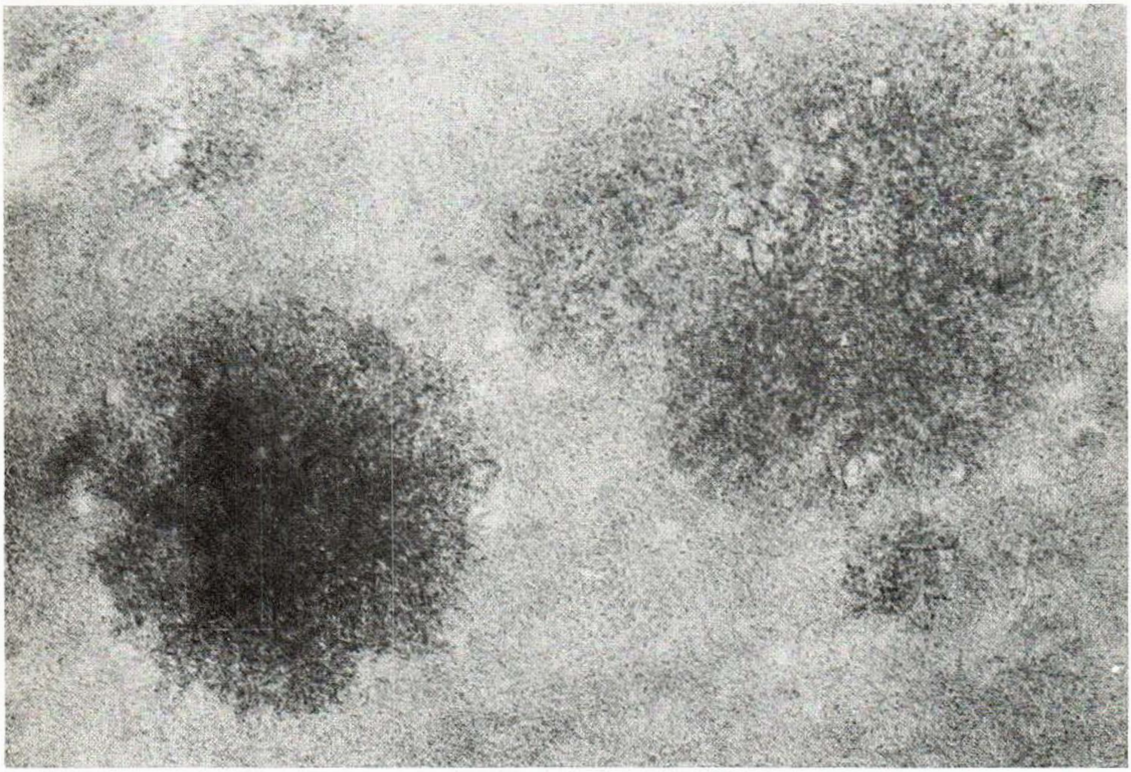

Fig. 6. Granules and needles in mostly random arrangement inside amyloid substance. × 60 000.

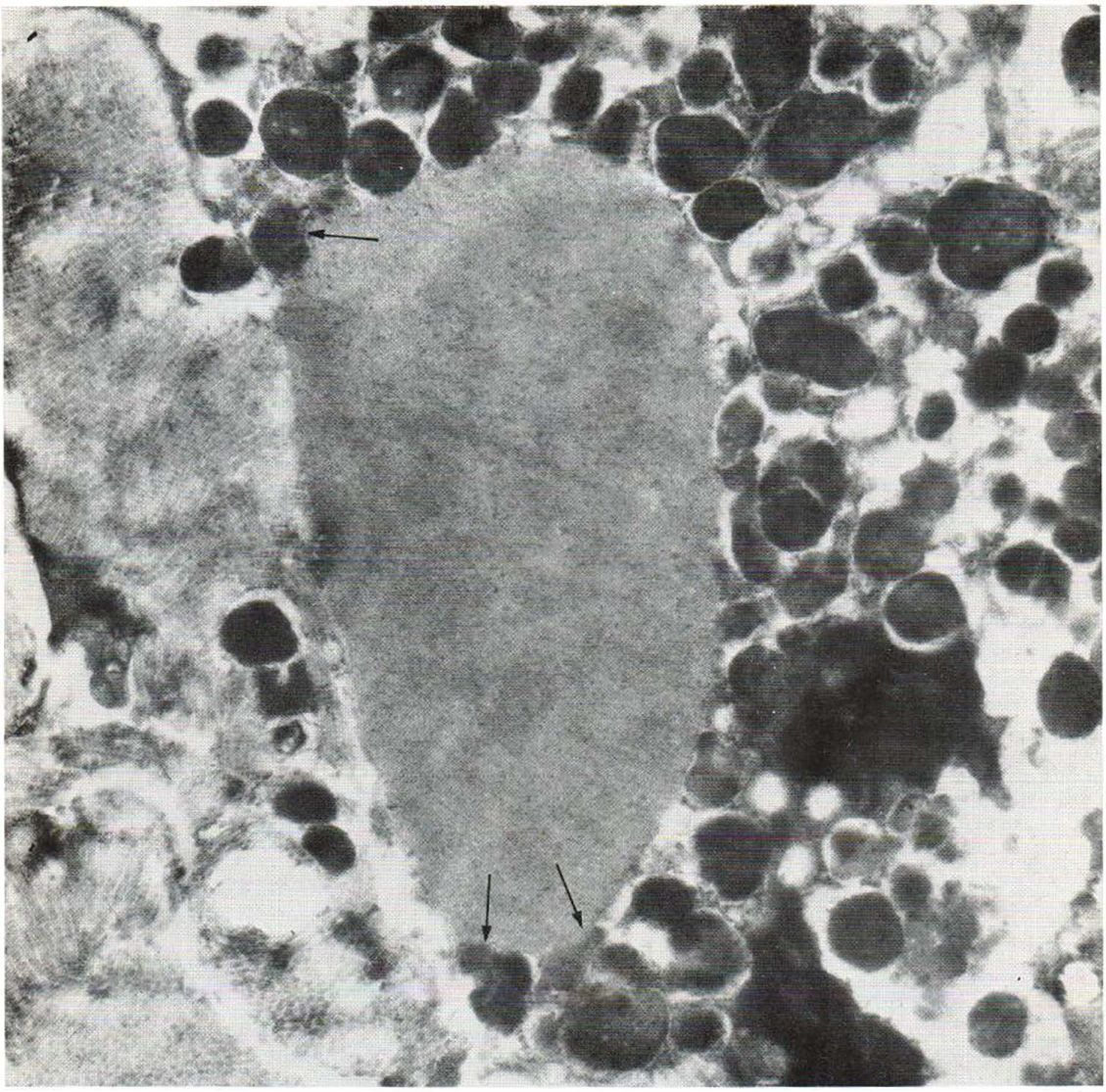

Fig. 7. A mast cell almost surrounding an amyloid mass. In some areas (arrows) no cell membrane is visible between the cell and the amyloid substance. × 24 000.

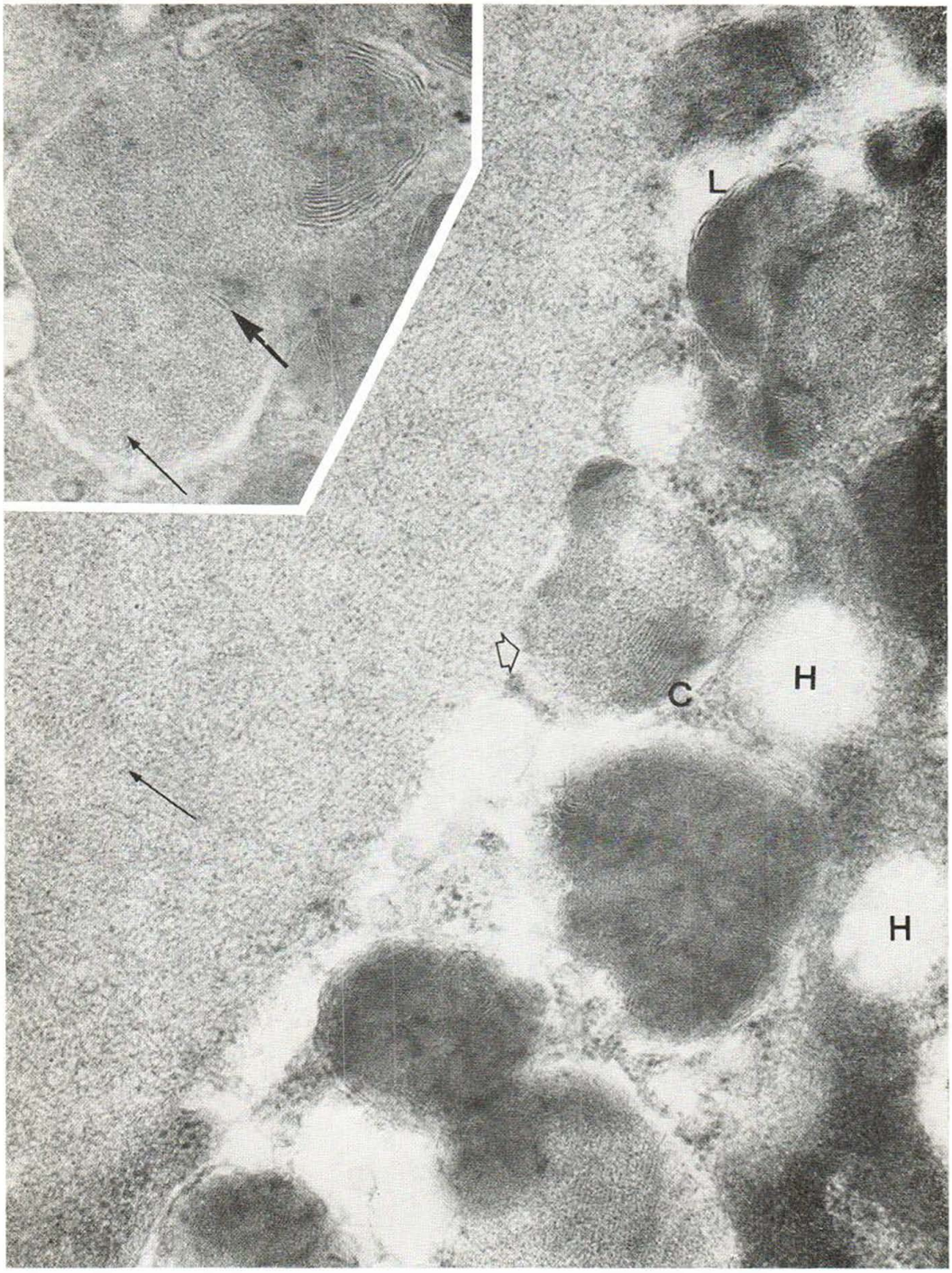

Fig. 8. Area of Fig. 7 at × 60 000 magnification. Some granules have partially a mature pattern with lamellae (*L*) and crystalline (*C*) figures, while the remainder of the same *L* granule is a coarse, grainy material (*outline arrow*). *H* indicates empty spaces ("honeycomb structure"). No cell membrane is seen. A fine arrow indicates an amyloid fibril showing clear banding. *Inset:* An intracellular, enlarged and deformed mast cell granule, composed mostly of coarse, grainy material with indistinct fragments of lamellae (*thick arrow*) and amyloid fibrils with clear banding (*fine arrow*). × 56 000.

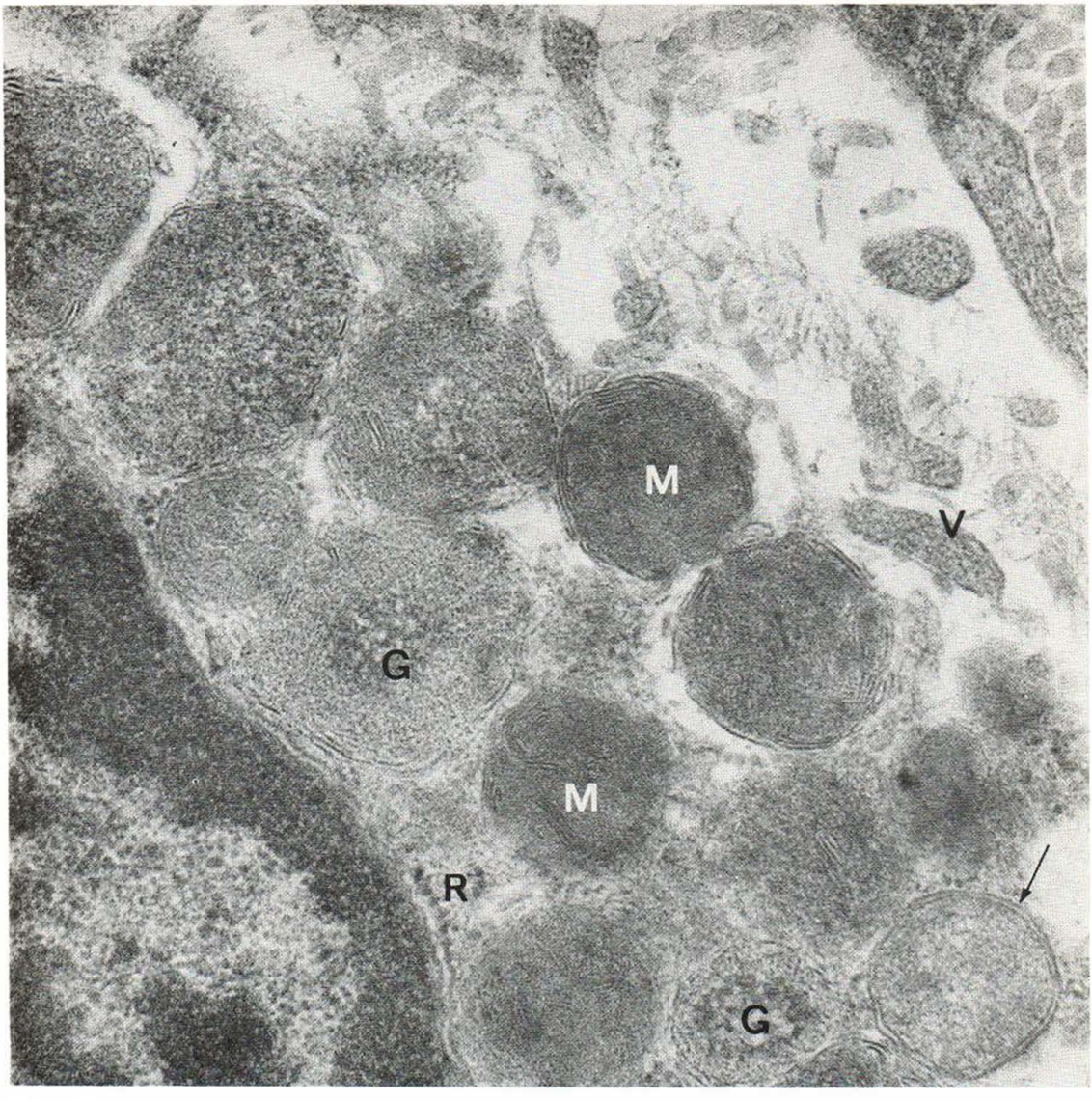

Fig. 9. Mature granules (*M*) and coarse, grainy granules (*G*) of a mast cell. The grainy granules show peripheral lamellae and a central, dense core with lucent spots. Micochondria (*arrow*) contain indistinct lamellae. *R* indicates ribosomes. Villi (*V*) are few. × 60 000.