A STUDY OF THE FINE STRUCTURE OF THE AMYLOID ASSOCIATED WITH FAMILIAL MEDITERRANEAN FEVER

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In the past decade it has become increasingly apparent from the work of Heller and colleagues [1,2] that at least one specific disease entity can be separated from the group of syndromes classified as Periodic Diseases. This disorder, characterized by recurrent attacks of abdominal pain, arthritis and chest pain accompanied by fever has been called familial Mediterranean fever because of its geographic distribution and familial occurrence. Mamou and Cattan [3] recognized that renal disorder was a common part of this syndrome and suggested amyloidosis as a possible cause. Since then, it has been demonstrated that amyloidosis is commonly associated with familial Mediterranean fever and is the usual cause of its accompanying renal disorder.[4,5] Recently Heller, Sohar, Gafni and Heller [4] suggested that the clinical attacks and the occurrence of amyloidosis were genetically interrelated but independent phenotypic characters of familial Mediterranean fever and due to the same presumed inborn error of metabolism.

Amyloidosis is known to occur in a variety of situations and has been classified into specific types according to its tinctorial characteristics, organ distribution and association with other diseases. The designations have included primary, secondary, localized amyloid as well as that associated with multiple myeloma. The validity of this classification has been questioned because of the frequency with which the characteristic features in a given case may overlap several of these types, and because of failure, to date, to demonstrate any distinct chemical differences among the various "types" of amyloid.[6] Recent electron microscopic studies have, in addition, indicated the presence of an apparently iden-

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tical fibrous ultrastructure in amyloid experimentally induced in rabbits and in human primary and secondary amyloid.[7]

The present study was undertaken in order to determine whether the same fibrous component of amyloid was present in the genetically determined familial Mediterranean fever amyloidosis, and to study sites of deposition of the amyloid in this condition.

Methods

Percutaneous renal biopsy specimens were procured with a Vim-Silverman needle and rectal biopsy was carried out according to the technique of Gafni and Sohar[8] in 4 patients with known amyloidosis and familial Mediterranean fever. Within 1 minute of biopsy the tissue was sectioned with razors into 1 to 2 cu. mm. The blocks were immediately fixed in buffered sucrose osmium tetroxide.[9] Tissues were dehydrated in 60, 70, 80, 95 per cent absolute ethyl alcohol, embedded at room temperature in a mixture of n-butyl and methyl methacrylate (90:10), and polymerized with benzoyl peroxide (15 mg. per ml. of methacrylate) at 60° C. overnight.

Absolute ethyl alcohol containing 1 per cent phosphotungstic acid was used as the final dehydrating solution in all instances. Portions of the specimens were also fixed in Zenker's solution at the time of biopsy and embedded in paraffin. Sections of these were cut for conventional microscopy and stained with hematoxylin and eosin and crystal violet reagents. Successive thick (½ to 2μ) and thin (250 to 500 Å) sections were cut with a Porter-Blum microtome. The thick sections were treated with xylol overnight to remove the methacrylate and then stained with periodic acid-Schiff (PAS) and hematoxylin. Sections were examined with an RCA EMU-3F electron microscope.

Results

Light microscopic examination of hematoxylin and eosin stained sections demonstrated glassy, amorphous "hyaline" substance adjacent to the glomerular basement membrane and blood vessels. The "hyalin" was metachromatic when treated with crystal violet. Exact localization of the amyloid, with regard to basement membrane and endothelial cells, was not possible.

The rectal biopsy material appeared to be unremarkable when stained with hematoxylin and eosin. When stained with crystal violet, however, metachromatic material consistent with amyloid was observed (a) in isolated areas of the submucosa; (b) in the submucosa subjacent to the epithelial basement membrane; and (c) in the subendothelial layer of blood vessel walls. There was no evidence of an acute or chronic focal or perivascular inflammatory reaction.

Electron Microscopy

The electron microscopic appearance of the human glomerulus has been well described.[10] In brief, the wall of the glomerular loop contains 3 elements: the endothelial cell, the basement membrane and the visceral epithelial cell. The last (the podocyte) has a remarkable fine structure,

consisting of interdigitating foot processes applied to the basement membrane. The foot processes normally are considered to be distinctly separated from one another although a fine filamentous connection has recently been described.[11] The basement membrane consists of a central dense layer (*lamina densa*) and peripheral non-osmiophilic layers. In the adult human subject, its total thickness is usually about 2,000 Å. The endothelial cell is characterized by a nucleus surrounded by an attenuated cytoplasm which contains a number of pores (diameter about 0.1 μ) where it is applied to the basement membrane.

The patients with familial Mediterranean fever and renal amyloidosis showed marked thickening of the basement membrane. The latter measured over 4,000 Å in width and its larger part was osmiophilic. Striking abnormalities were observed in the epithelial cells. The individuality of the foot process was lost, and a continuous cytoplasmic surface was apposed to the basement membrane. The cytoplasm contained increased numbers of small vacuoles. The endothelial cells appeared to be present in increased numbers, but were somewhat obscured by massive amounts of fine filamentous material (Fig. 1) that occluded large areas of the capillary lumens.

The fibrillar material was identifiable as amyloid by comparison of serially sectioned thick and thin sections, examined by light and electron microscopy respectively. Amyloid fibrils were densely packed and present in greatest concentration on the endothelial side of the basement membrane, although they could also be observed subjacent to the visceral epithelial cells.

Small blood vessels were also found to have large amounts of amyloid deposited about them. It was invariably adjacent to the endothelial cell and appeared to obscure the basement membrane (Fig. 2). Although the vessel wall often appeared to be either completely made up of or replaced by amyloid, the continuity of the endothelial cell separating the amyloid from the vessel lumen was maintained. On the outer surface of the deposit, amyloid fibrils were in close contact with collagen (Fig. 3). There were no fibrils of transitional size between amyloid and collagen, and each type of fibril exhibited a distinctive appearance (Fig. 4). Islands of amyloid fibrils were often seen close to the blood vessels in the connective tissue of the renal parenchyma.

Higher resolution micrographs demonstrated that the amyloid in all instances was made up of fine fibrils. Occasional bundles were observed, but the majority were found in random array. The diameter of the fibrils was less than 250 to 300 Å and they had a beaded appearance at high magnification.

Electron micrographs of the rectal submucosa demonstrated abundant

plasma cells and moderate numbers of mast cells and macrophages (Figs. 5 to 7). The most interesting changes were observed in the small arterioles where the area of the basement membrane appeared to be thickened (Figs. 8 and 9). Higher resolution micrographs demonstrated that fibrillar material was present in this area and possibly in the basal portion of the endothelial cells. Scattered bundles of collagen coursed through this layer. A basement membrane-like structure abutted the smooth muscle cells (Fig. 10). In two instances, fibrillar material was observed in the cytoplasm of the endothelial cell in the portion of the cytoplasm contiguous to the lumen of the vessel (Fig. 11).

Discussion

Familial Mediterranean fever is a genetically determined disease often associated with amyloidosis. The latter is, to date, the only significant necropsy finding in this disease. The amyloid is Congo red-positive and metachromatic with crystal violet. Its anatomic distribution includes organs involved classically by secondary (kidney, spleen) as well as primary (blood vessels) amyloidosis. The liver is rarely affected. The present electron microscopic observations demonstrate that the ultrastructure of the amyloid of familial Mediterranean fever is comparable to that of all other amyloid thus far studied. In the kidney, massive infiltration of glomeruli and blood vessels with amyloid fibrils was observed. The location of the deposit about the basement membrane, primarily on the endothelial side was identical with that seen in rabbits with experimentally induced amyloidosis, and in patients with secondary renal amyloidosis.[12] In the rectal submucosa, small perivascular sites of amyloid accumulation were found. Although it is still not possible to define precisely the exact site of amyloid formation, the presence of fine fibrils in the basement membrane and possibly in the basilar portion of the endothelial cells is suggestive evidence that these cells contribute to its formation. On the other hand, the fibrillar material seen on the lumen side of the cells is not unusual and has been described in other endothelial cells.[13] Other investigators have suggested, on a basis of morphologic and immunologic studies, that plasma cells contribute to the formation of amyloid fibrils.[14,15]

Kennedy has recently suggested (on the basis of sulfur^{35} sulfate labeling and tracer studies of sulfur^{35} methionine and carbon^{14} glycine) that amyloid is an insoluble complex resulting from the interaction of two substances. The first, a glycoprotein formed by plasma cells, circulates and interacts with a sulfated compound formed by the endothelial cells to form amyloid. He, too, felt that the subendothelial distribution of amyloid was of considerable significance.[16] Christensen and Rask-

Nielsen have also emphasized the local cellular production of amyloid associated with casein administration and with that found in murine reticulosarcomas.[17]

It is difficult to delineate the precise structural configuration and dimensions of amyloid fibrils in tissue sections. However, the present studies indicate that within the limitations of fixed and dehydrated tissue, the width of the fibrils (under 300 Å) conforms to those previously described in this and, more recently, in other laboratories.[18-21] In addition, higher resolution studies suggest that the fibrils have a "beaded" appearance. Preliminary electron microscopic observations on isolated amyloid fibrils are in accord with this possibility.[22]

The present study, therefore, provides some added support to the concept that at least part of amyloid may be basically the same or a closely related substance no matter where localized, how it stains or what the clinical expression may be. On the other hand, it is reasonable that the fibrils represent only a portion of what is classified, on a basis of classical pathologic techniques, as amyloid, and that ground substance and other chemical constituents contribute to its total structure.

Summary

Renal and rectal biopsy specimens from 4 patients with familial Mediterranean fever and amyloidosis were examined by electron microscopy.

The amyloid was comparable in its localization (subendothelial) and in its fine structure (thin, delicate fibrils) to the experimental, as well as to human primary and secondary amyloidosis.

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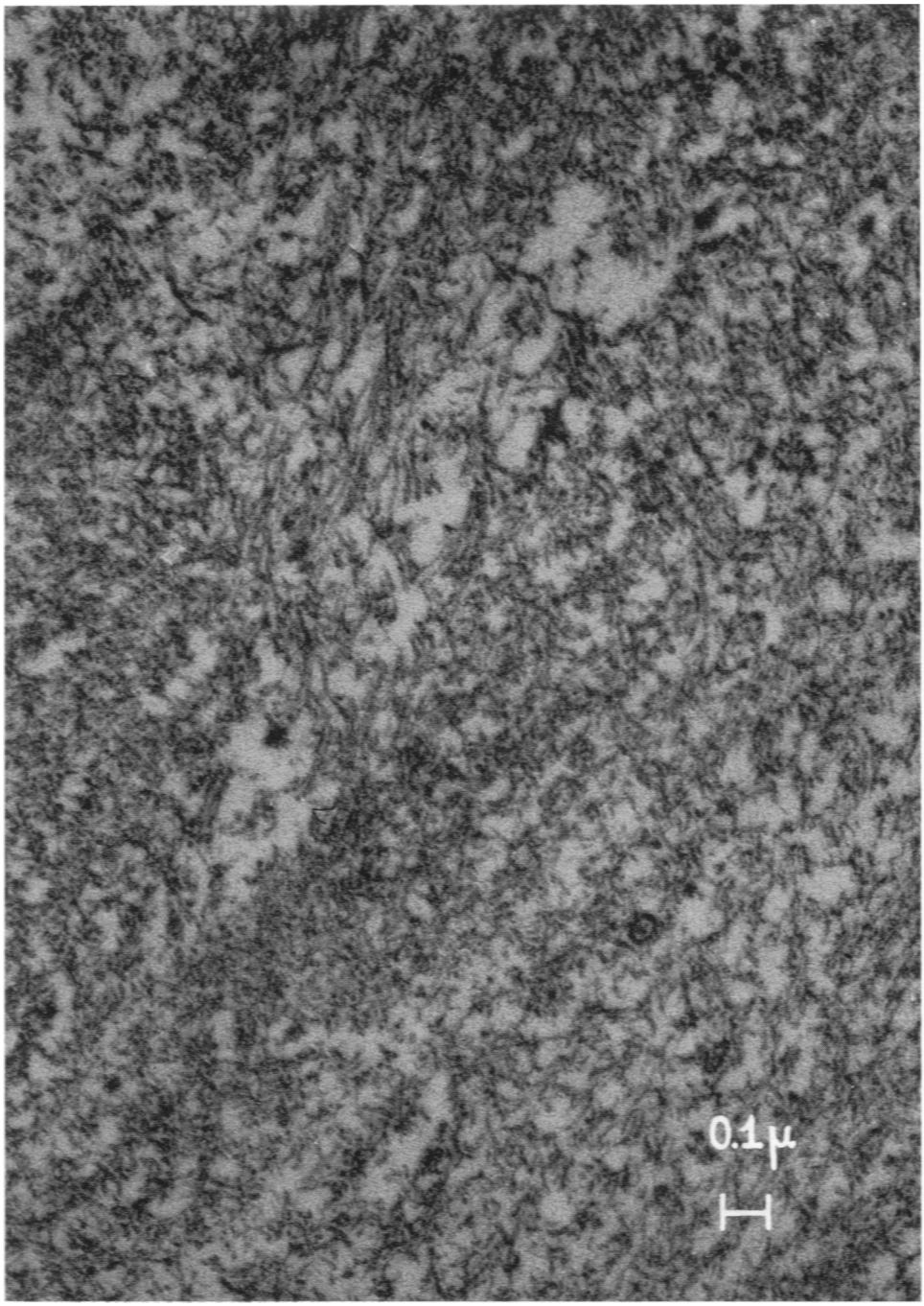
LEGENDS FOR FIGURES

Key:

A = Amyloid
BM = Basement membrane
C = Collagen
E = Endothelial cell
F = Fibrils
L = Lumen
M = Membrane
Ma = Mast cell
MAC = Macrophage
Mu = Smooth muscle cell
PL = Plasma cell

FIG. 1. Amyloid fibrils in a renal glomerulus seen in cross and longitudinal section. × 62,200.



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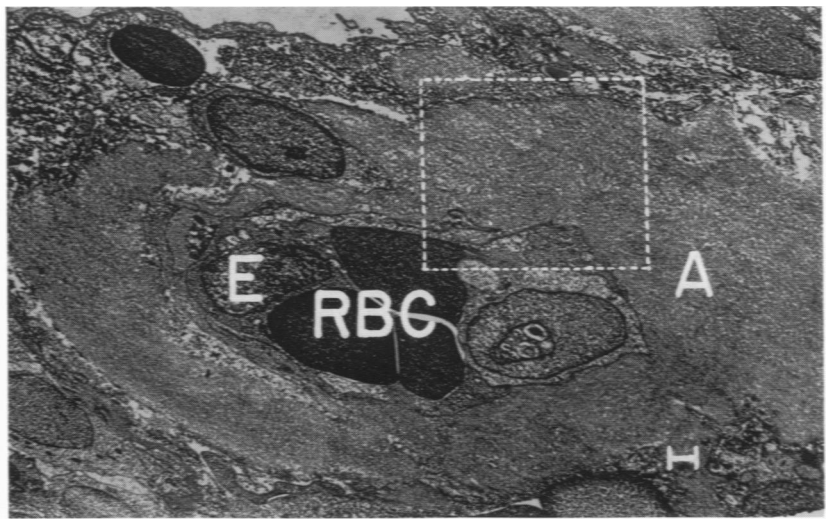

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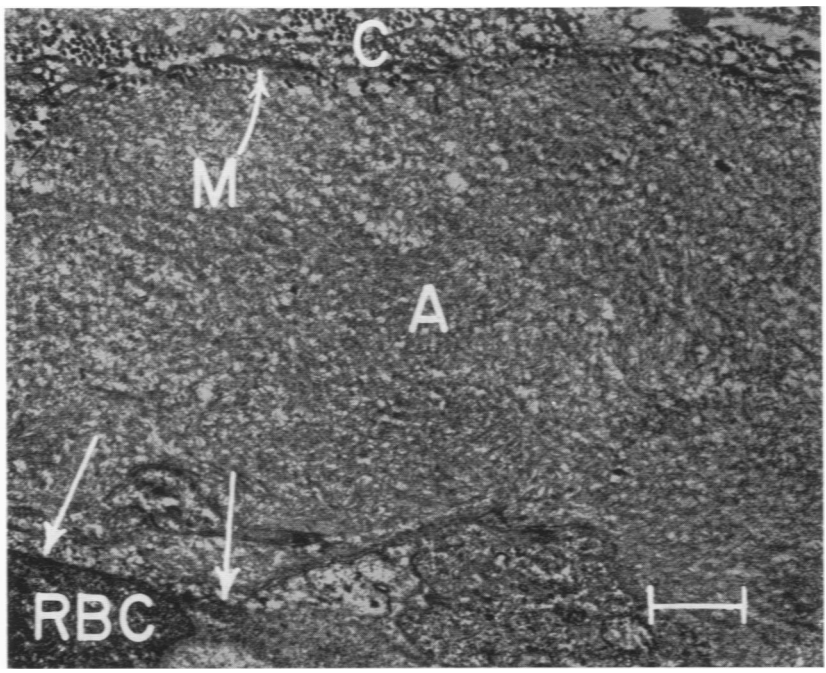

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FIG. 2. The wall of this small renal blood vessel is completely infiltrated with amyloid (A). Red blood cells (RBC) can be observed in the lumen which is everywhere separated from the amyloid by the attentuated cytoplasm of the lining endothelial cell (E). The large amyloid deposit now constitutes the major part of the vessel wall and is in direct contact with the surrounding connective tissue. × 3,400.

FIG. 3. An enlargement of the area in Figure 1 enclosed by dotted lines. Arrows indicate several portions of cell membrane separating the amyloid (A) from the vessel lumen. The amyloid itself has a finely fibrous character. The upper part of the micrograph illustrates the close proximity of the amyloid fibrils to collagen (C) which has been cut in cross section. An incomplete outer limiting membrane (M) can also be seen. × 12,500.

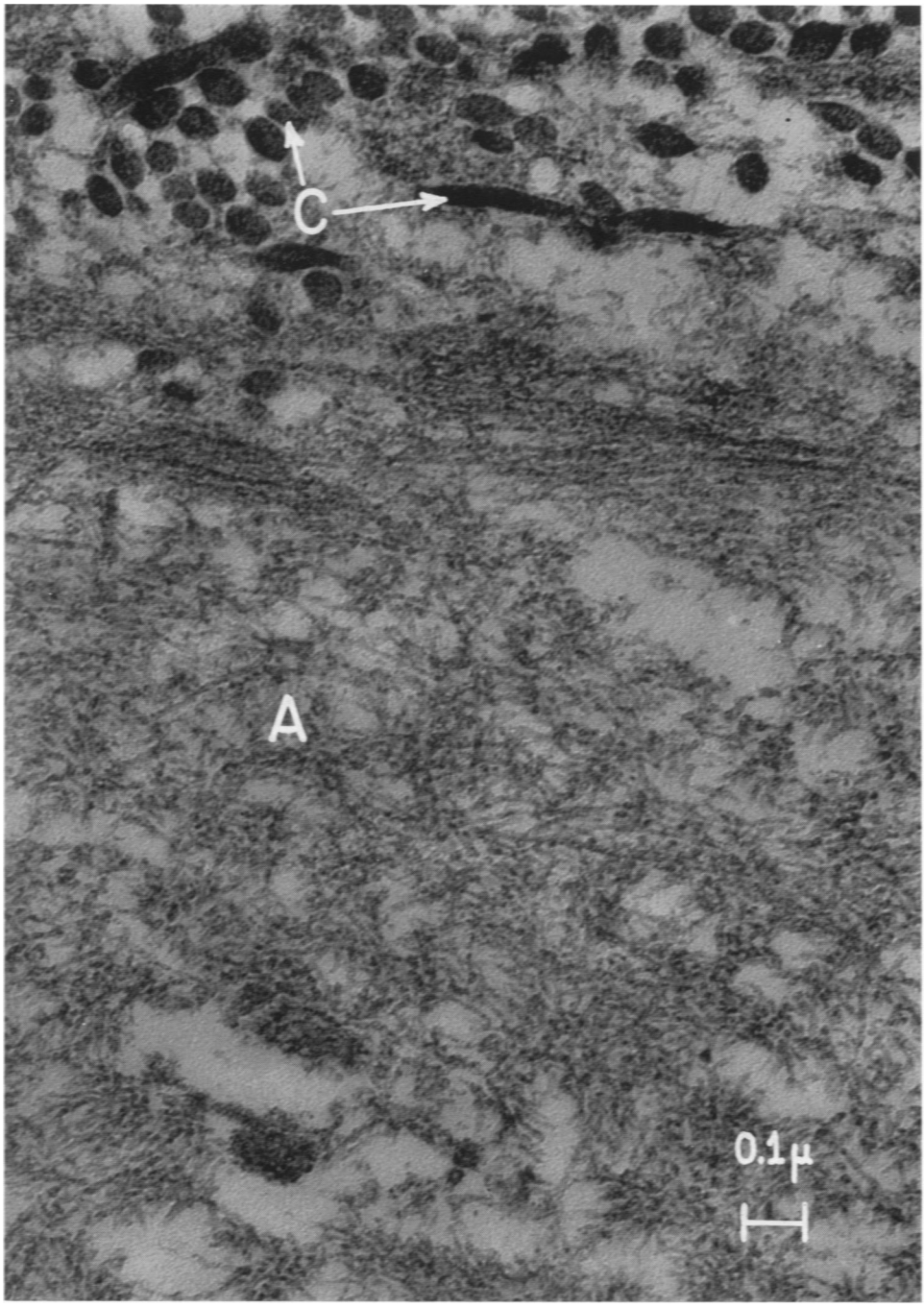
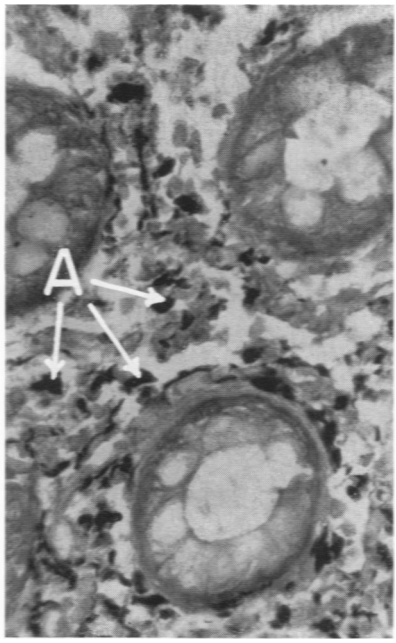


FIG. 4. Enlargement of an area of amyloid (A) fibrils in close approximation with collagen (C). The amyloid fibrils have a diameter of less than 300 Å and at times appear beaded. The collagen cut in cross section demonstrates microfibrils within each collagen fibril. × 85,800.



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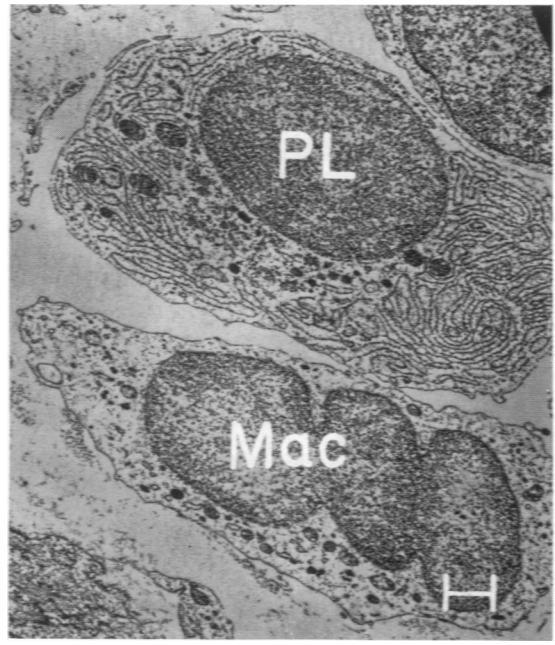

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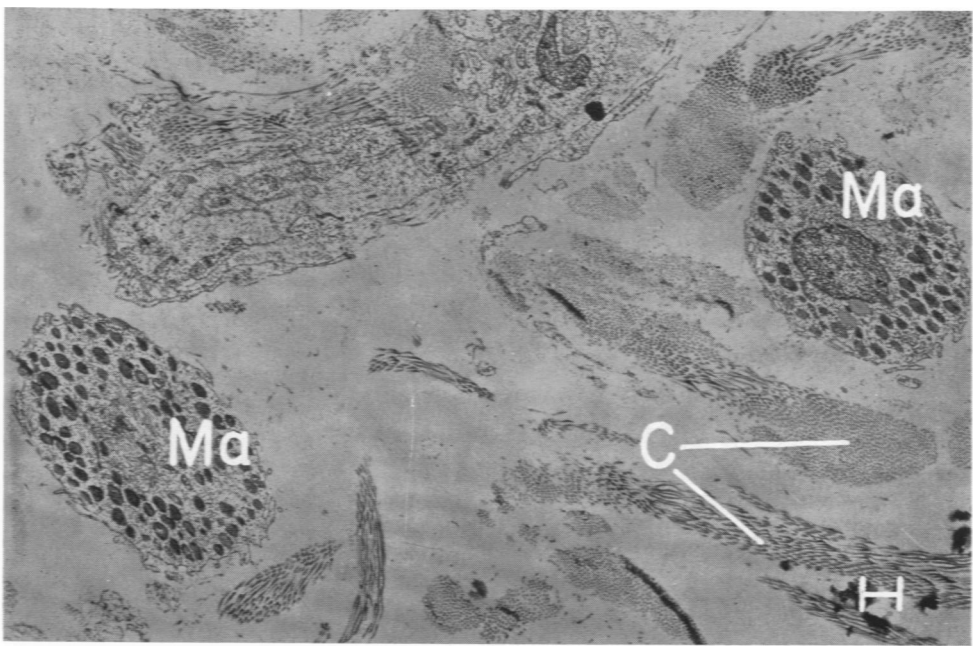

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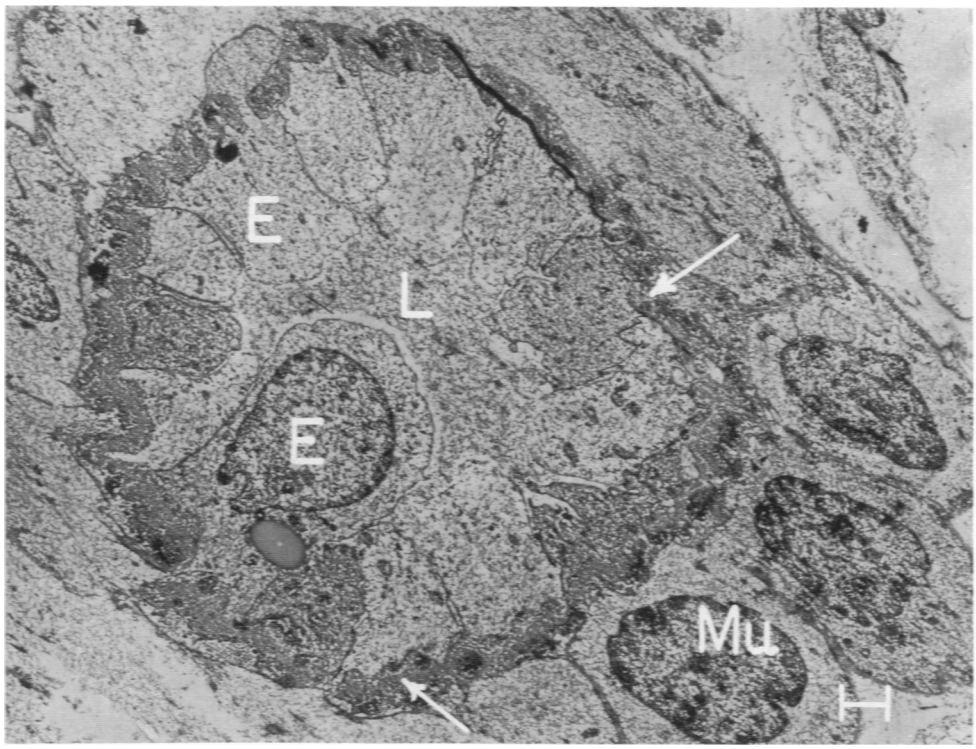
FIG. 5. Light micrograph of a Zenker-fixed section of a rectal biopsy from a patient with familial Mediterranean fever and known amyloidosis. The dark staining material (A) was metachromatic with crystal violet and positive to Congo red stain. Crystal violet stain. × 1,000.

FIG. 6. Typical cells are apparent in the submucosa of a rectum with amyloid infiltration about a blood vessel. The top cell is a plasma cell (PL) with abundant endoplasmic reticulum, while the lower cell is a macrophage (MAC) with an indented nucleus. × 6,200.

FIG. 7. Two mast cells (Ma) in rectal submucosa amidst bundles of collagen (C). × 5,200.



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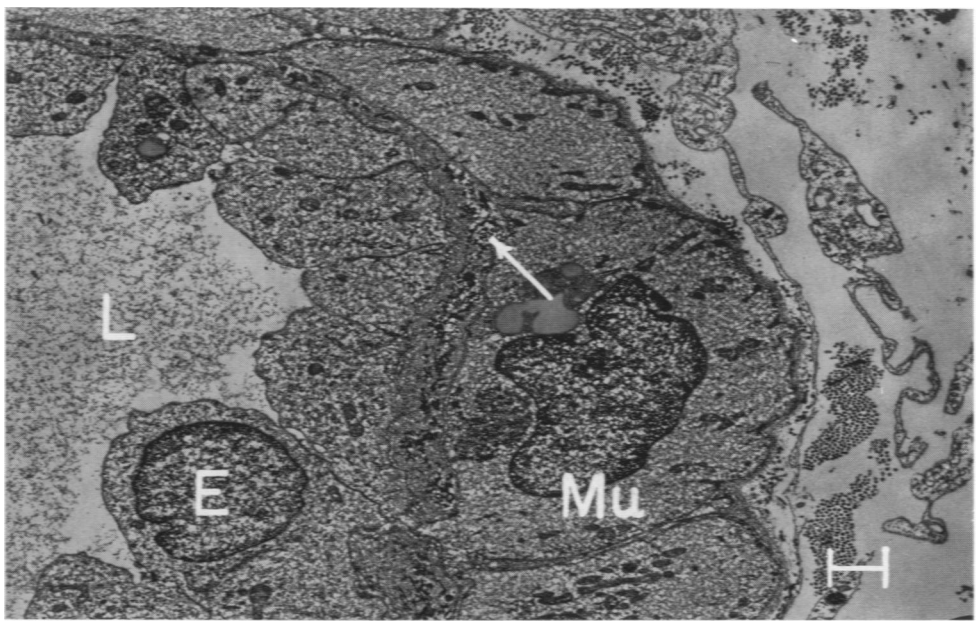

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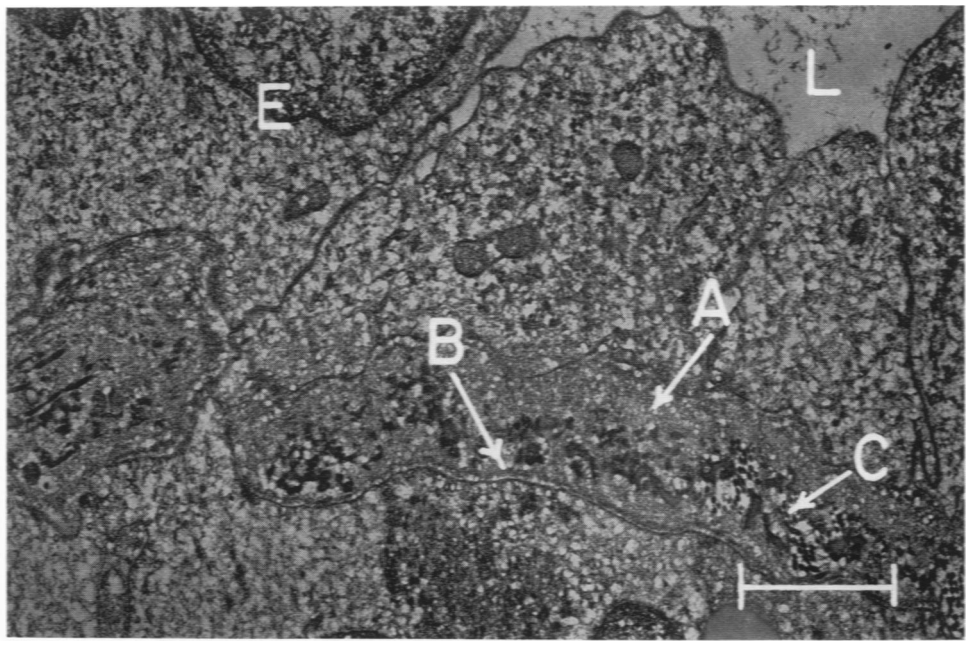
FIG. 8. Arteriole in the rectal submucosa, lined by endothelial cells (E) and invested with smooth muscle cells (Mu). The area of the basement membrane (arrows) is considerably thickened. × 6,300.

FIG. 9. Portion of an arteriole with its lumen (L) lined by an endothelial cell (E). The basement membrane is thickened and interrupted by scattered bundles of collagen (arrow). × 7,700.



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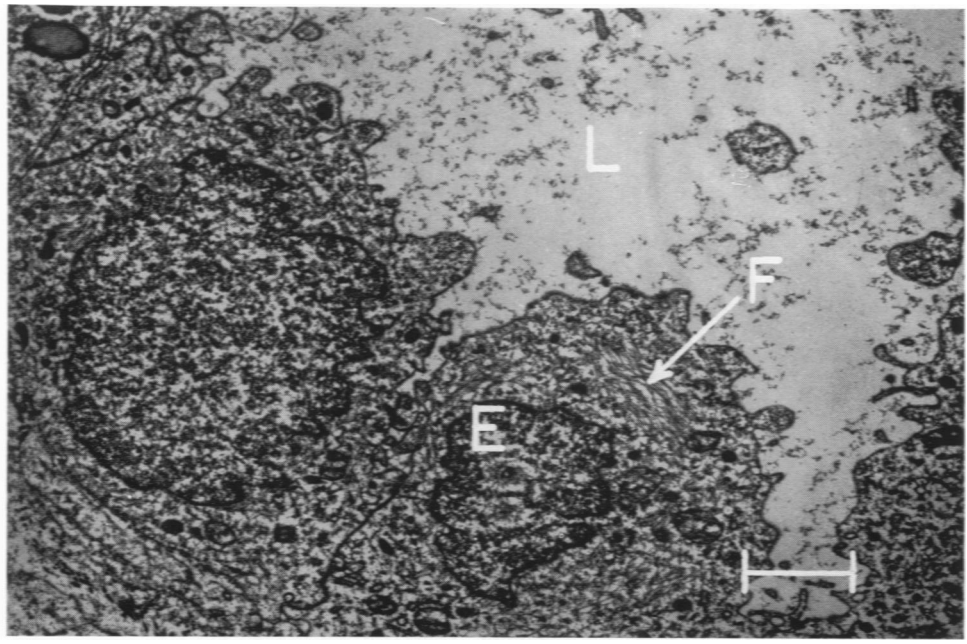

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FIG. 10. Higher magnification of a portion of the arteriolar wall seen in Figure 9. Fine filamentous material (arrow A) is subjacent to the endothelial cells and in some areas a substance of similar density is found in the basal portion of the endothelial cell. Collagen (arrow C) is interspersed through this layer and a basement membrane-like structure (arrow B) abuts the smooth muscle cells. × 20,200.

FIG. 11. Fine fibrils (F) in the upper portion of an endothelial cell. × 14,100.