# The Ultrastructure of Mucoid "Onionskin" Intimal Lesions in Malignant Nephrosclerosis

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**The ultrastructure of mucoid "onionskin" intimal thickening in the intrarenal arteries was studied in 12 cases of malignant hypertension. The thickened areas were found to contain proliferating myointimal cells, basement membrane lamellas, and ruthenium-red-positive proteoglycans. The proteoglycans consisted of granules 15-35 nm in diameter and thin filaments about 3 nm thick. The filaments connected the granules to each other and to the basement membranes of the concentric lamellas, to the basement membranes of the endothelial and myointimal cells, and also to the cell surfaces. This arrangement imparted a loose meshwork pattern to the mucoid layer. The granulofilamentous material is considered to be a structural component of the pathologic lesion distinct from plasma insudation. The relationship between the intercellular substances and the myointimal cells is briefly discussed. (Am J Pathol 1980, 99:67-80)**

RENAL CHANGES associated with malignant hypertension, so-called malignant nephrosclerosis, has been characterized on light microscopy by two types of intrarenal vascular lesions: fibrinoid necrosis of small arteries and arterioles and intimal thickening of the interlobular arteries.[1-3] Histologic and more recently electron-microscopic studies of the vascular lesions have centered mainly on the fibrinoid necrosis.[4] However, only a few electron-microscopic investigations have been conducted of the intimal change in the larger vessels, although this may play a more important role in malignant nephrosclerosis.[1] The intimal thickening shows variable degrees of cell proliferation and basophilic or mucoid change of the intima.[5] It can be either of mixed collagen and elastic type (fibroelastosis or splitting of the internal elastic lamella) or of the "onionskin" mucoid type.[6] The latter has been found to be very prominent in malignant hypertension in the blacks of the United States[6] and may represent the early lesion.[7] Recently several electron-microscopic studies of the arterial lesions in primary malignant hypertension and other diseases accompanied by malignant hypertension have been reported, but the details of

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Supported by Public Health Service Research Grant AM-00918 from the National Institute of Arthritis, Metabolism, and Digestive Diseases.

Accepted for publication November 6, 1979.

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0002-9440/80/0407-0067$01.00

the ultrastructure and the constitution of the large intercellular lucent spaces, the predominant feature of the mucoid intimal change, has not been clarified.[3,6,8,9] Although histochemical studies by light microscopy have demonstrated the presence of acid mucopolysaccharides,[6,8] an ultrastructural histochemical confirmation is still lacking. We therefore undertook to study the intrarenal arteries and arterioles in malignant nephrosclerosis by means of electron microscopy combined with histochemical staining. The histologic description of these vessels is available in the literature [8] and will not be repeated here. The glomerular changes in our cases have been reported elsewhere.[10]

## Materials and Methods

The study population consisted of 12 patients with clinically diagnosed malignant hypertension and diastolic pressure greater than 130 mm Hg. More detailed clinical data have been published elsewhere.[10] Renal tissues were fixed for electron microscopy in 2% phosphate-buffered glutaraldehyde, followed by 1% buffered osmium tetroxide, dehydrated in graded ethanols, and embedded in Epon 812. Vascular lesions were selected from 1-$\mu$ sections. Thin sections were cut with a diamond knife on an LKB microtome, stained with uranyl acetate and lead citrate, and examined with a Philips 300 electron microscope.

For electron-microscopic demonstration of glycosaminoglycans, thin sections were stained with 0.5% ruthenium red in 0.1 N acetic acid for 60 to 90 minutes and washed with 0.1 N acetic acid and distilled water.[11] For demonstration of collagen fibrils and elastic fibers, the grids were stained with 5% phosphotungstic acid in 50% methanol for half an hour.[8]

## Results

Thickening of the arterial intima in malignant hypertension was caused by both cellular proliferation and an increase in the amount of intercellular substances. On the ultrastructural level the characteristic "onionskin" pattern was seen to consist of concentric parallel arrays of cells and electron-dense membranes, usually in an alternating fashion (Figures 1 and 2). The interstitial spaces between these arrays were filled with electron-lucent floccular material. In some cases this material predominated, producing an almost pure "mucoid" thickening (Figures 2, 3, and 7). Since both the onionskin and the mucoid changes usually coexisted, they are described together. These changes were frequently accompanied by endothelial cell proliferation (Figure 4), severe narrowing of the lumen (Figure 1), and occasionally by fibrin thrombi.

### Myointimal Cells

The cells in the thickened intima were spindle-shaped or elongated, frequently showing irregular cytoplasmic extensions. Their abundant cytoplasm contained a large number of cell organelles, particularly rough

endoplasmic reticulum (Figures 1–4). In general, the ultrastructural features were those of smooth muscle cells, manifested by cytoplasmic filaments, dense bodies, plasma membrane caveolae, and a surrounding basement membrane. They are therefore believed to represent modified smooth muscle or myointimal cells.[12–15] Two types of myointimal cells were recognizable: one had quite compact cytoplasm rich in myofibrils, similar to that of mature smooth muscle cells (Figures 2 and 4); the other type had abundant anastomosing rough endoplasmic reticulum with dilated cisternae in the cytoplasm (Figures 2 and 3). Transitional forms were common (Figure 2). Focal cytoplasmic degeneration with accumulation of lipid droplets was frequently seen (Figure 3). The basement membranes surrounding the cells showed many disruptions but maintained a close relation with the cell surfaces and intercellular material (Figure 6).

#### Intercellular Substances

The bulk of the thickened intima was composed of parallel arrays of electron-dense membranes and granulofilamentous material. The membranes were structurally identical with and closely related to the basement membranes of myointimal cells and of the endothelium (Figure 4) and corresponded to the PAS-positive rings seen by light microscopy. They were frequently interconnected, forming a network, particularly in the subendothelial region (Figure 4). The basement membrane surrounding the myointimal cell was often disrupted and detached from the cell surface (Figures 4 and 6). Beneath the detached basement membrane, however, it was also common to see segments of apparently newly formed basement membrane closely apposed to the myointimal cells (Figures 4 and 6). Under higher magnification, the basement membranes were connected with each other and with the myointimal cell surfaces by means of thin filaments (Figure 6).

The electron-lucent spaces between the concentric basement membrane lamellas showed a uniform floccular pattern (Figures 2 and 3). With higher magnification a definite meshwork could be discerned, composed of oval to polygonal granules, 15–35 nm in size, interconnected by thin filaments about 3 nm in diameter (Figures 5–7). The granules were also connected to the myointimal cells by the same thin filaments (Figure 6). The granulofilamentous meshwork obviously differed from plasma insudation. The granules stained well with ruthenium red (Figure 5). The filamentous meshwork bound together all the structures in the thickened intima, including the basement membranes of the endothelium, the newly formed as well as the detached basement membranes of the myointimal cells, and basement membrane lamellas in the intima (Figures 4 and 6).

The lucent mucoid lesions contained few if any microfibrils, elastic fibers, or collagen fibrils. Occasionally these structures were seen in the deep intimal zone. The collagen fibrils in the lucent zones were usually thin, widely separated, and randomly arranged (Figure 7). They were in close relation to the basement membrane lamellas and were frequently associated with the proteoglycans (Figure 7). The cross-striations of the collagen fibrils stained faintly with ruthenium red. Collagen fibrils, microfibrils, and elastic fibers were more prominent in the more fibrotic areas.

## Discussion

It is evident from this study that in malignant nephrosclerosis the "onionskin" mucoid intimal thickening of interlobular arteries has a constant ultrastructural pattern. The main features are proliferation of the myointimal cells and increase in intercellular substances. The latter are composed of roughly concentric lamellas of basement membranes and of granulofilamentous material lying in the lucent spaces. The proliferated myointimal cells and the layers of basement membrane account for the "onionskin" appearance seen by light microscopy.[8] The granulofilamentous material consists of oval or polygonal granules about 15–35 nm in size that have surface projections and are connected with each other by thin filaments 3 nm thick. This gives the lesion a constant and characteristic meshwork pattern. Similar polygonal granules and interconnecting thin filaments have been reported as a structural component of cartilage [16] and of normal embryonal and hyperplastic arteries in animals [17–19] and in other types of connective tissue.[20] Histochemically, they have been defined as proteoglycans (protein–polysaccharide complexes).[17,21] Our results with ruthenium red staining are in keeping with this concept and correspond to the histochemical demonstration of acid mucopolysaccharides on the light-microscopic level.[6,8]

We have found a close spatial relationship between the proteoglycans, the basement membrane lamellas, and the myointimal cells. These substances are the main structural elements of the intimal lesions and are distinct from plasmatic insudates. The close structural relationship of the basement membrane lamellas and the proteoglycans to the myointimal cells and the apparent synthetic activity of their cytoplasm suggest that the intercellular substances are producted by these cells.[22,23] The origin of the myointimal cells is not definitely known, but they are generally believed to derive from the smooth muscle cells of the media.[12,14] The presence of mitoses and penetration of the smooth muscle cells of the media through the gaps in the internal elastic lamella support this possibility.

We have not followed in detail the evolution of the onionskin and mu-

coid lesions, but there is evidence to suggest that microfibrils (10 nm thick), elastic fibers, and collagen fibrils are laid down in increasing numbers, initiating the process of fibrosis. There is a similarity to the development of chick embryo aorta, where proteoglycans predominate in the intercellular ground substance during the early developmental stage,[21] followed by the development of collagen and other fibrillar substances.

The pathogenesis of malignant nephrosclerosis is not definitely known. It has been attributed to angiospasm or to immunologic mechanisms. Although immunofluorescent studies demonstrate fibrinogen, gamma globulins, and complement in the necrotic lesions,[24] these substances are much less frequent in the hyperplastic lesions. Even in the former, it is not clear whether their presence indicates an immunologic injury or simply an entrapment of plasma proteins.[3] Fibrinoid necrosis of small arteries and arterioles could very well be caused by acute ischemia, producing damage to the vascular wall, followed by reflow and by insudation of plasma and fibrinogen.[10,25] It is possible that ischemia is also responsible for the mucoid intimal lesions. The damage is probably less severe than in the more distal arterial segments but sufficient to injure the endothelium and to allow penetration of constituents of blood plasma. Such insudation has been shown to lead to enhanced synthesis of intercellular substances,[22] which probably represents a repair process. Our studies were not designed to demonstrate plasma proteins in the intima, and in any event we could not be sure of the age of the lesions in our patients. The question of initial plasmatic insudation in the development of mucoid lesions can probably be best answered by means of animal experiments. The "onionskin" lesions could very well result from repeated attacks of injury and repair, as suggested by Sanerkin.[26] This idea is supported by the finding of degeneration and of proliferation of the myointimal cells and by detachment and new formation of basement membranes.

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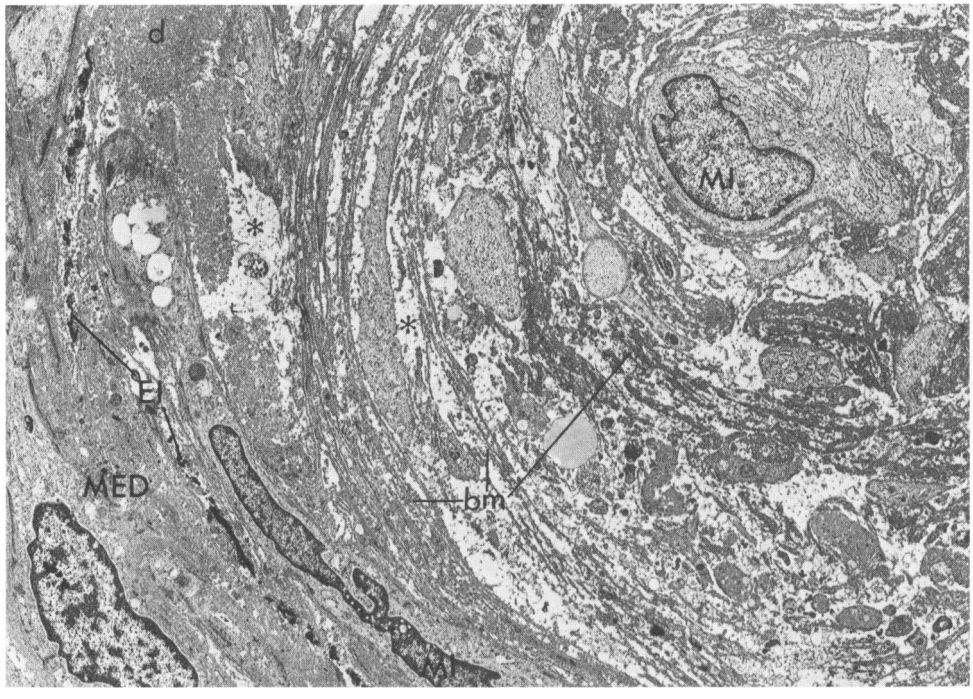
## Acknowledgments

The authors wish to express their appreciation to Norman Katz, A. Prado, and Rosemary Lang for their valuable technical assistance.

[*Illustrations follow*]

**Figure 1**—Typical "onionskin" intimal thickening of the interlobular artery with severe narrowing of the lumen. Concentric lamellaes of basement membranes (*bm*) are interspersed with the cytoplasm of myointimal cells (*MI*) and areas of mucoid material (*). Note also hyaline deposit (*d*) close to the media (*MED*) and internal elastic lamella (*EI*). (PTA and uranyl acetate, ×4500)

**Figure 2**—"Onionskin" lesion similar to that in Figure 1 but showing large amounts of mucoid material. *EI* = elastic lamella. (Uranyl acetate, lead citrate, and ruthenium red, ×3000)

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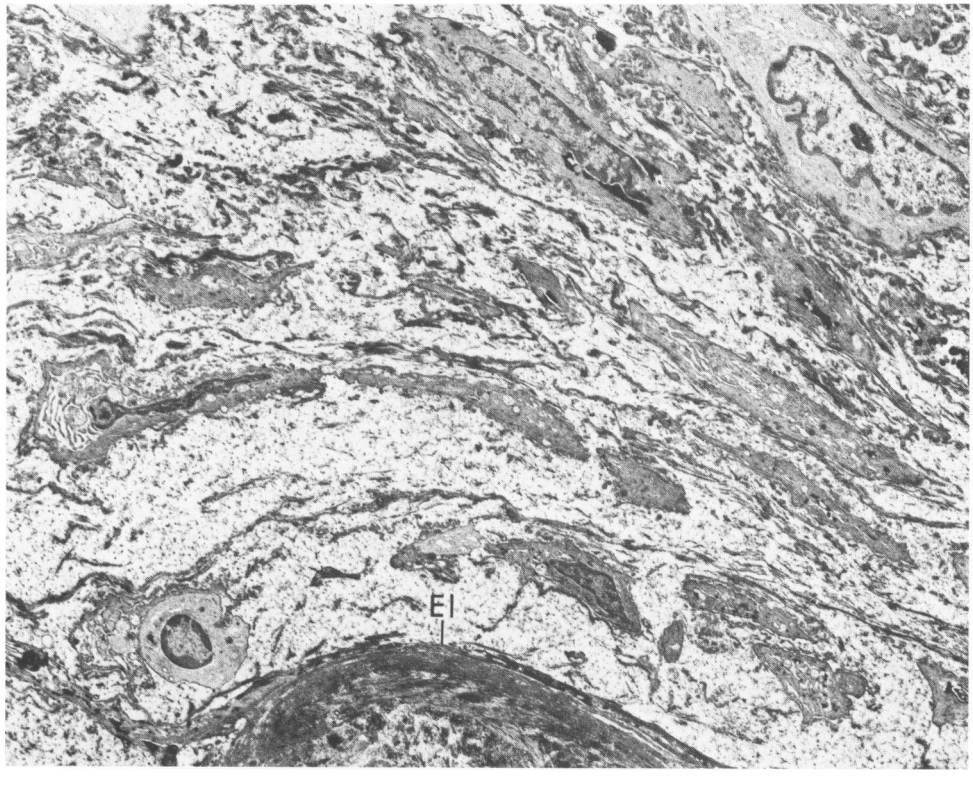


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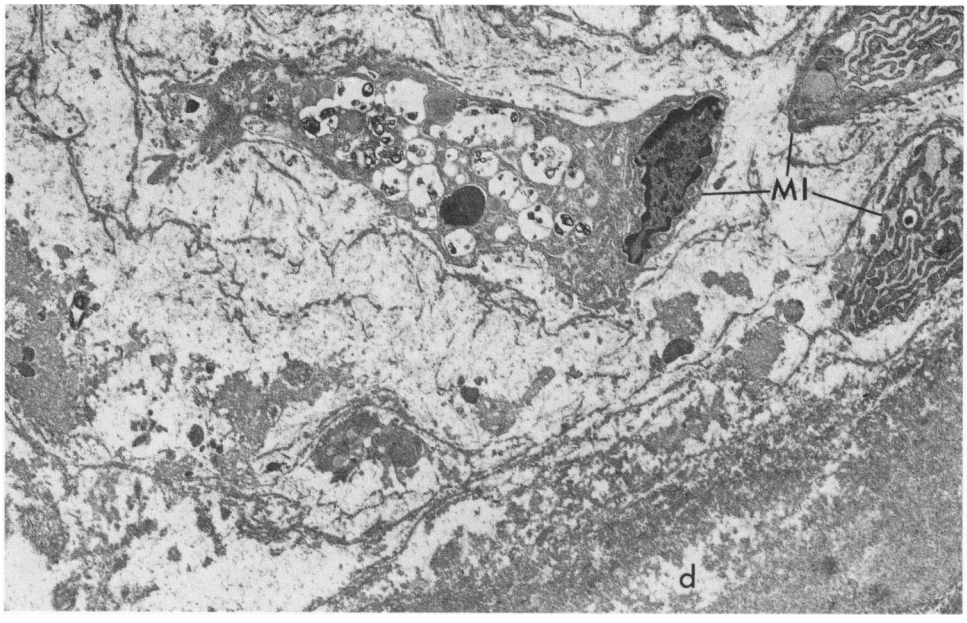
**Figure 3**—Mucoid area in the arterial intima containing proliferating myointimal cells (*MI*) and thin strands of basement membrane. The cell in the center of the photograph shows cytoplasmic degeneration with lipid droplets and myelin figures. Note also a hyaline deposit (*d*) in right lower corner. (Uranyl acetate, lead citrate, and ruthenium red, ×5500)

**Figure 4**—Part of the wall of an interlobular artery showing hyperplastic endothelial lining (*END*), a myointimal cell in the center of the photograph, and reduplicated layers of basement membrane (*bm*). In some areas basement membranes are closely associated with granulofilamentous material representing proteoglycans (*arrowheads*). (Uranyl acetate, lead citrate, and ruthenium red, ×25,000)

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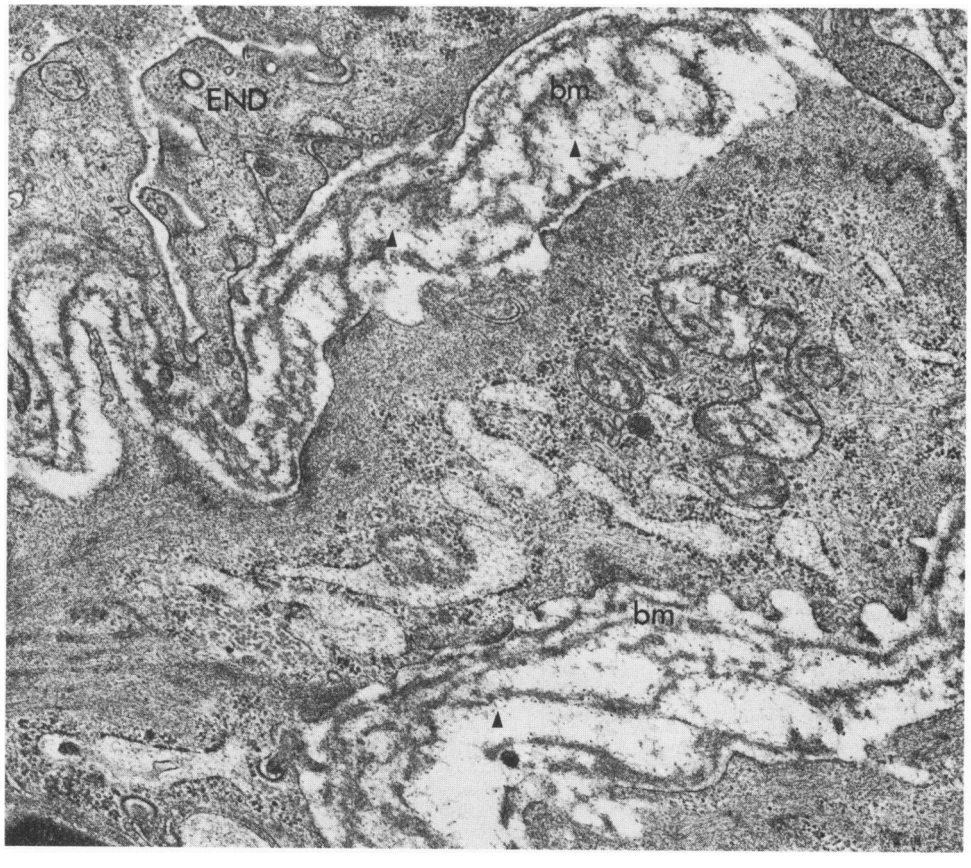


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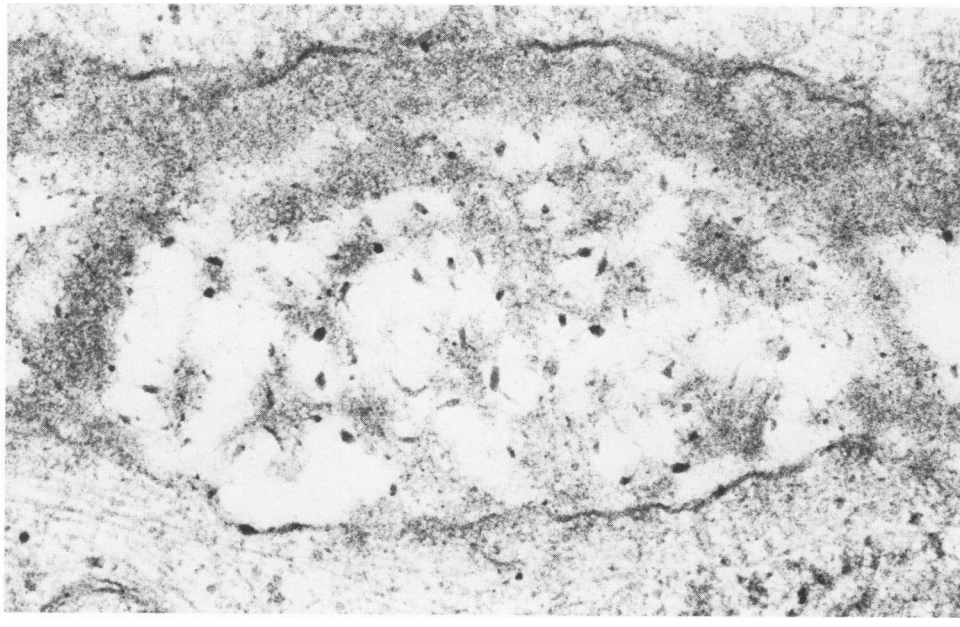

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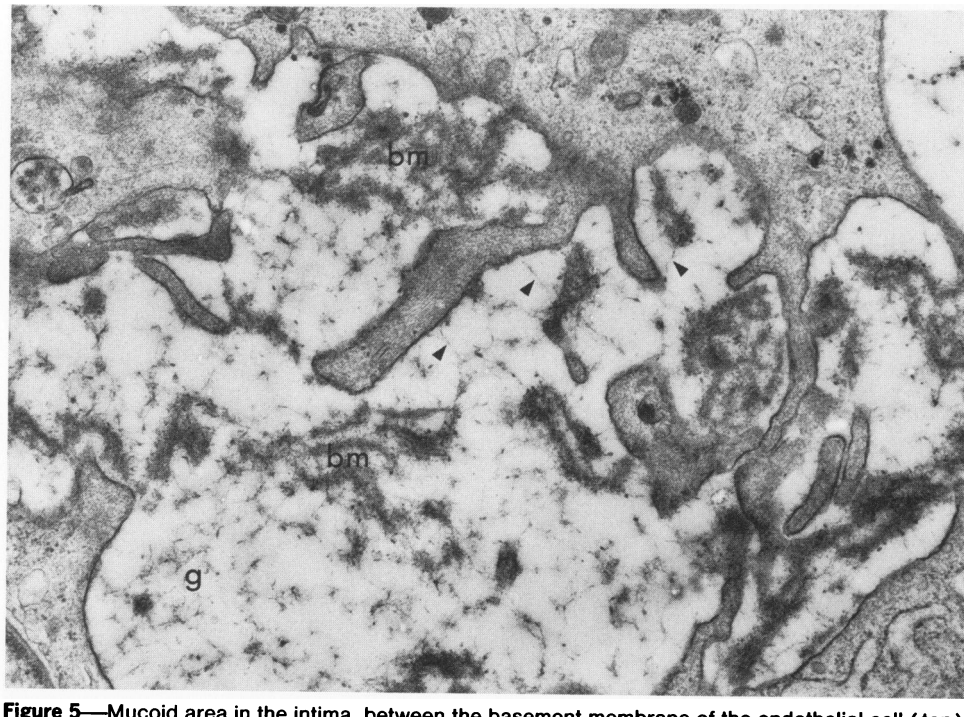


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**Figure 5**—Mucoid area in the intima, between the basement membrane of the endothelial cell (*top*) and a myointimal cell (*bottom*). Granules of proteoglycans and filaments are clearly visible. (Uranyl acetate, lead citrate, and ruthenium red, ×67,500) **Figure 6**—Mucoid area (*g*) showing a pattern of granules and filaments (proteoglycans). Some of the filaments (*arrowheads*) appear to be attached to the cytoplasm of the myointimal cells. Note the disrupted pieces of basement membranes (*bm*). (Uranyl acetate, lead citrate, and ruthenium red, ×20,500)

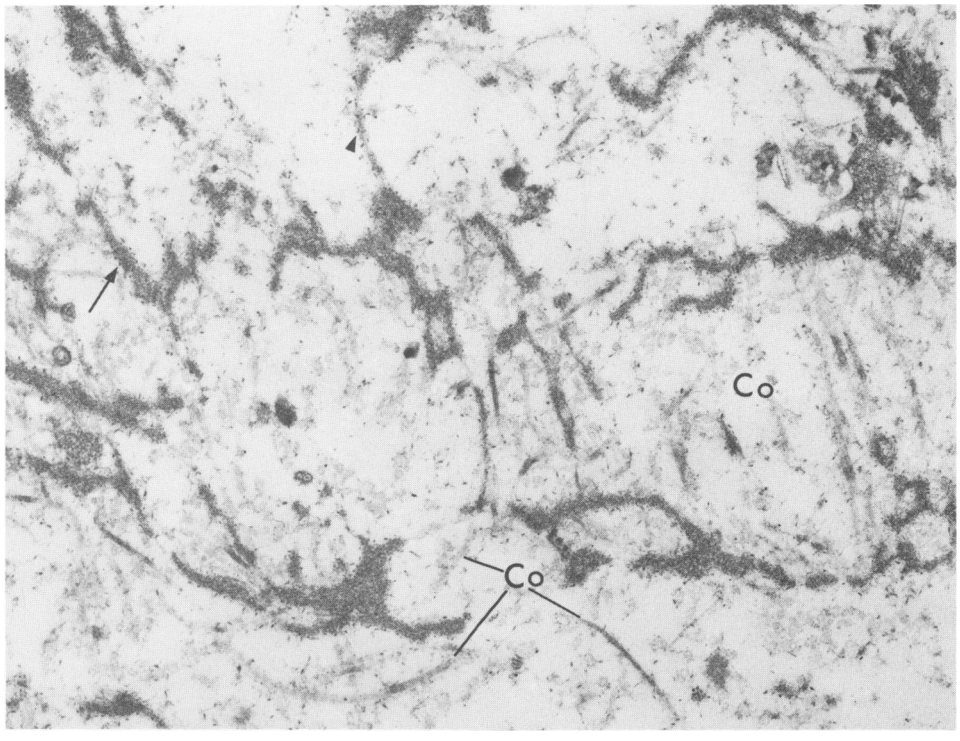


**Figure 7**—Mucoid intimal area containing an irregular network of basement membranes and faintly stained collagen fibrils (*Co*). In some areas close association is noted between the proteoglycans and basement membranes (*arrow*) or proteoglycans and collagen fibrils (*arrowhead*). (Uranyl acetate, lead citrate, and ruthenium red, ×25,000)

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