# LESIONS IN THE RABBIT AORTA PRODUCED BY FEEDING A HIGH CHOLESTEROL DIET FOLLOWED BY A NORMAL DIET. AN ELECTRON MICROSCOPIC STUDY

R. C. BUCK

*From the Department of Microscopic Anatomy, University of Western Ontario, London, Canada*

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THE fine structure of rabbit atherosclerotic lesions produced by feeding a diet containing added cholesterol for a few months has already been reported (Buck, 1958; Parker, 1960). Such lesions differ from those of human atherosclerosis particularly in the greater proportion of lipid-filled cells ("foam cells") found in the rabbit lesions. Constantinides, Booth and Carlsson (1960) have pointed out the remarkable similarity between the lesions of human atherosclerosis and those produced in rabbits by a special regimen which involved the intermittent feeding of a high cholesterol diet over a long period of time. The present report describes the fine structure of rather similar lesions.

## MATERIALS AND METHODS

Rabbits were fed a diet containing 1 per cent cholesterol. The cholesterol was distributed through the food by dissolving it in ether, which was then evaporated. After a period of 4 months the rabbits were placed on the stock diet without added cholesterol. Two years after the experiment was begun only 2 out of 10 rabbits in the experimental group had survived. These 2 animals were then killed.

Only the aortas were examined with the electron microscope. The tissue was fixed in cold 1 per cent osmium tetroxide for 1 hr., stained for 1 hr. in cold 1 per cent aqueous uranyl acetate, dehydrated with alcohol and acetone, and embedded in Vestopal W (Kellenberger, Schwab and Ryter, 1956). Thin sections, cut with a Porter-Blum microtome, were examined with an RCA–EMU 3D electron microscope.

## RESULTS

The 2 rabbits showed remarkably similar lesions. The aortas were dilated, particularly in the thoracic portion, and most of the inner surface was pale and irregular. The livers were of normal colour and consistency, and the only gross evidence, apart from the large arteries, of the previous hypercholesterolaemic state was found in the kidneys. In both animals these showed the pale, streaked band at the junction of cortex and medulla, similar to that seen after a short period of cholesterol feeding. One animal had large, soft calculi in one kidney and a very dilated renal pelvis.

The electron microscopic appearance of the 2 aortas was almost identical in both animals. A typical cross section of the inner part is shown in Fig. 1. The lesions showed a continuous cellular covering which was separated from the tunica media by a thick layer composed of cells and extracellular material.

The extracellular material was of variable composition and included: (1) some amorphous, finely granular material, particularly prominent immediately under

the lining layer of cells, the nature of which is unknown, (2) many unit fibrils of collagen scattered throughout the remainder of the intima, (3) elastic fibres, identified by their rather low density, and (4) granular material of very high density, thought to be mineral salts.

Four types of cells were recognized in the lesions : lining cells, modified smooth muscle cells, fibroblasts and macrophages.

The lining cells, or endothelium (Figs. 1 and 2), formed a single, continuous layer in which adjacent cells were closely applied to each other, frequently showing interlocking by means of a " tongue and groove " arrangement. The luminal and deep surfaces showed few " pinocytic vesicles ". The cytoplasm contained apparently normal, short mitochondria. Smooth surfaced membranes (Fig. 2), interpreted as the Golgi membranes, were often observed to be sectioned several times in a cell. Other small, smooth surfaced vesicles or tubules, some of which contained material of rather high density, were interpreted as part of the endoplasmic reticulum (ER). Rough surfaced cisternae of the ER, with adherent RNP particles, were found occasionally, and these usually contained some finely granular material (Fig. 2).

The modified smooth muscle cells were the predominant cells of the subendothelial tissue. Some of them were of great length, perhaps a hundred microns or more, and they were arranged in a plane parallel to the surface (Fig. 1). Their cytomorphology had certain features in common with that of smooth muscle cells of the media, but showed also certain differences. The myofilaments (Figs. 1, 3 and 4) were confined to a thin layer close to the plasma membrane, and consisted of filaments of 30 to 50 A.U. diameter massed together so that this layer had greater density than the central part of the cytoplasm. The latter contained numerous elongated mitochondria, cisternae of the ER (Fig. 4), some free RNP particles, Golgi membranes and some large, very dense vacuoles (Fig. 1). The vacuoles were thought to contain either haemosiderin or osmiophilic lipid. Also present were other vacuoles, which were really large pockets, the finely granular contents continuous with the extracellular material. " Pinocytic vesicles " were sometimes prominent on the surface of these cells (Fig. 5). Elastic fibres seemed to be closely associated with the cell surface.

Cells interpreted as fibroblasts were only occasionally found, and they were easily distinguished from the modified smooth muscle cells by the absence of the layer of myofilaments (Fig. 6). These cells usually showed tapering cytoplasmic processes and a few dense inclusions, but they had no other remarkable characteristics.

A very few cells with extremely vacuolated cytoplasm were found. These were interpreted as macrophages. Part of the cytoplasm of such a cell is seen in Fig. 1, where it shows only as a lacy membrane enclosing some vacuoles of very low density. A few denser bodies were usually present also (? lysosomes), and the other usual cytoplasmic organelles.

## DISCUSSION

### *Endothelium*

The lining cells of the lesions were essentially similar to those seen in much earlier stages of cholesterol atherosclerosis (Buck, 1958). The prominence of the cytoplasmic organelles, particularly the Golgi apparatus, suggests that these cells continued to be more active metabolically than normal arterial endothelium. In

normal endothelium the Golgi apparatus and the ER are not conspicuous. The suggestion made by Buck (1958) that the material of moderate density contained in the dilated cisternae of the ER might represent a stage in the inhibition of lipoprotein from the serum receives no support from the present study. The cisternae still showed this material, although the serum lipoprotein level must have been virtually normal. The significance of the pronounced change from the normal cytological character of the endothelium thus remains obscure.

*Cells of the subendothelial tissue*

The subendothelial tissue of the 2 aortas was strikingly different from that seen in animals which were simply maintained on a high cholesterol diet for several months (Buck, 1958 ; Parker, 1960). In the present experiments the amount of collagen and elastic fibres was much greater and the typical " foam cells " or macrophages were seldom observed. The preponderant cell of the lesion was a modified smooth muscle cell.

The early literature on a type of smooth muscle cell of the arterial intima has been reviewed by Altschul (1950, 1954) who has also described and illustrated these cells in arteriosclerotic lesions. They were termed " intermediate " cells because they were believed to have features of endothelium, mesenchyme cells and smooth muscle.

Recently a number of reports have reopened the question of the significance of the smooth muscle cell as a component of the normal and pathological arterial

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EXPLANATION OF PLATES

FIG. 1.—Inner part of an aortic lesion showing the endothelium (E) and a typical region of the subendothelial tissue. Immediately under the endothelium is a layer of finely granular material (FG). Under this are layers of modified smooth muscle cells (some of which are labelled SM). Between the cells there is fibrous and granular material. The fine, uniform dots represent cross sections of unit fibrils of collagen (Col.). The lighter, larger and more irregular masses close to the surface of the smooth muscle cells are elastic fibres (El). The very dense granular material in the extracellular space probably represents mineralization (Ca). Another type of cell extends as a band across the whole field just below the longest of the smooth muscle cells. The lacy plasma membrane, enclosing highly vacuolated cytoplasm, is part of a macrophage (Mac). The arrow indicates a large vacuole in a smooth muscle cell. The vacuole communicates with the extracellular material. ×5,500.

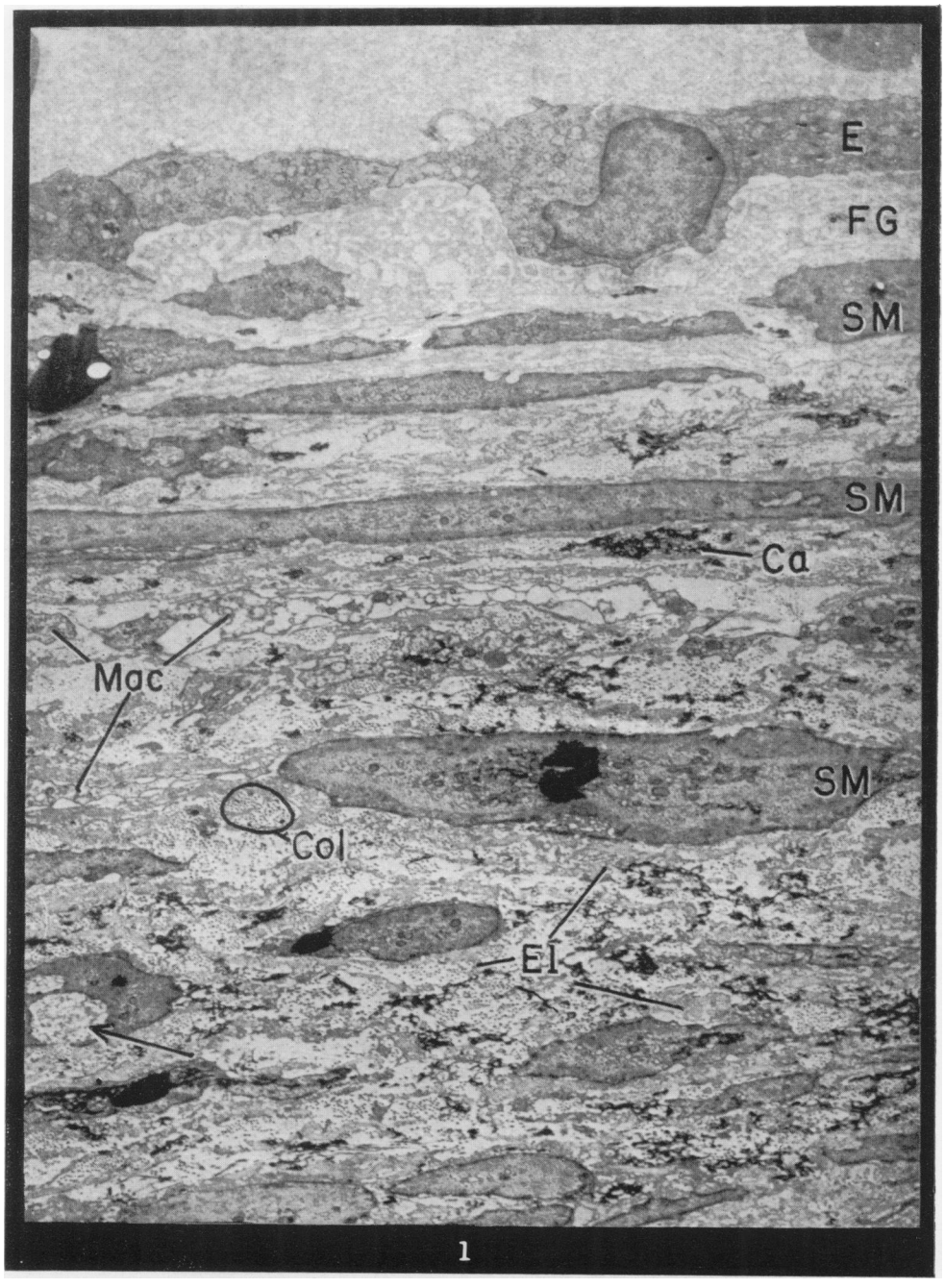
FIG. 2.—Part of an endothelial cell showing the pronounced development of Golgi membranes (GA) and endoplasmic reticulum. The latter is composed of 2 types of cisternae. The rough surfaced cisternae (R) have attached RNP particles and contain some material with definite density. The smooth surfaced membranes (S) enclose material with a different appearance. An edge of the nucleus (N) is seen. The finely granular material (FG) lies immediately under the endothelium. ×29,100.

FIG. 3.—Part of a modified smooth muscle cell showing the narrow band of fine myofilaments (brackets) close to the plasma membrane. The cell has been cut parallel to its long axis. Most of the cell consists of the central part, which contains no myofilaments. Here there are mitochondria (Mit) and membranous structures which are part of the endoplasmic reticulum. ×46,600.

FIG. 4.—Modified smooth muscle cell of the intima cut obliquely or transversely. The sectioned myofilaments at the periphery show as fine dots. The central part of the cell contains well developed membranes of the Golgi apparatus (smooth, parallel arrays) and also cisternae of the endoplasmic reticulum, most of which have attached RNP particles. ×29,100.

FIG. 5.—" Pinocytic " vesicles at the surface of a modified smooth muscle cell of the intima. ×73,800.

FIG. 6.—A fusiform cell of the intima, interpreted as a fibroblast. Parts of the cytoplasm of smooth muscle cells are also seen. ×7,200.

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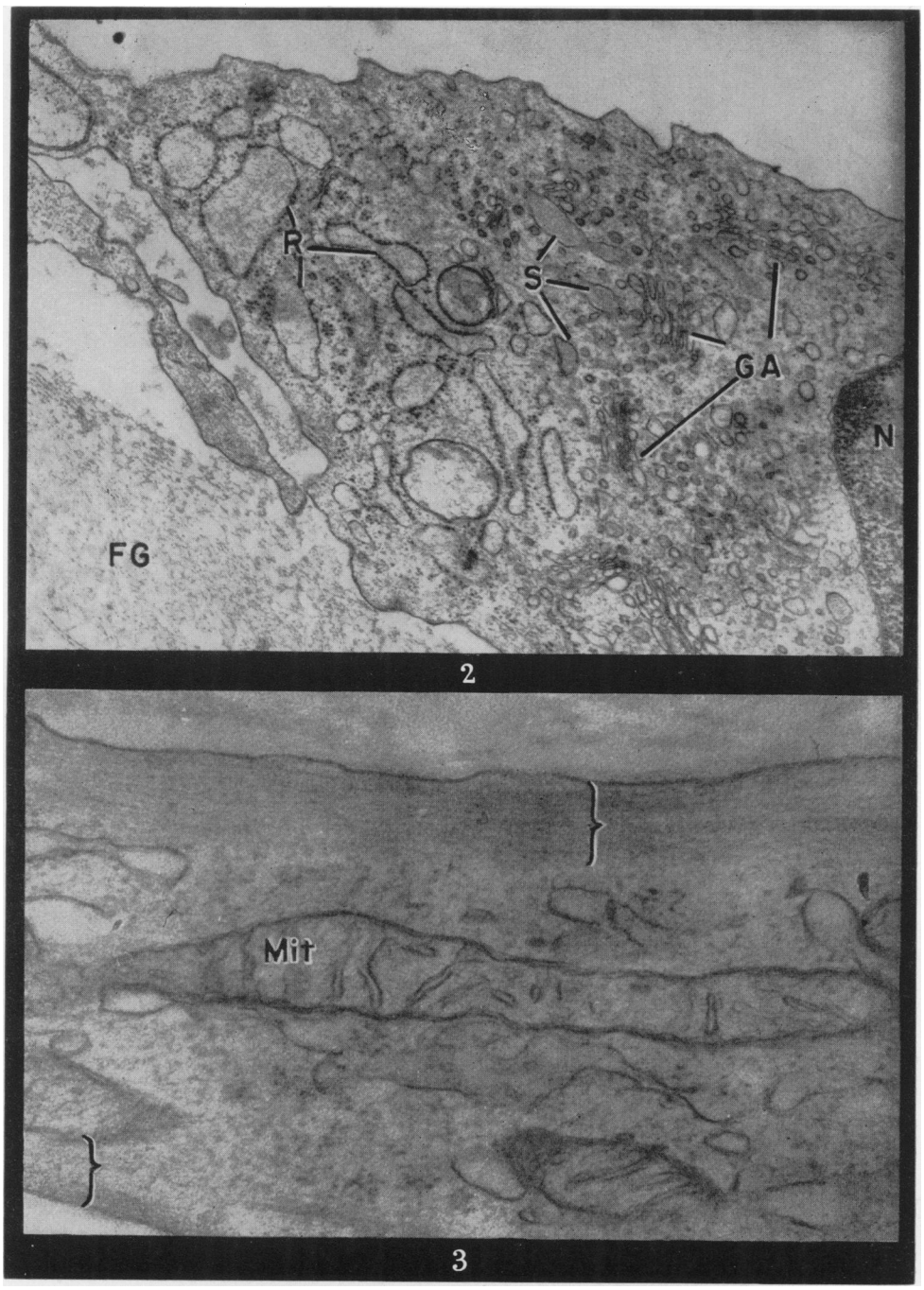


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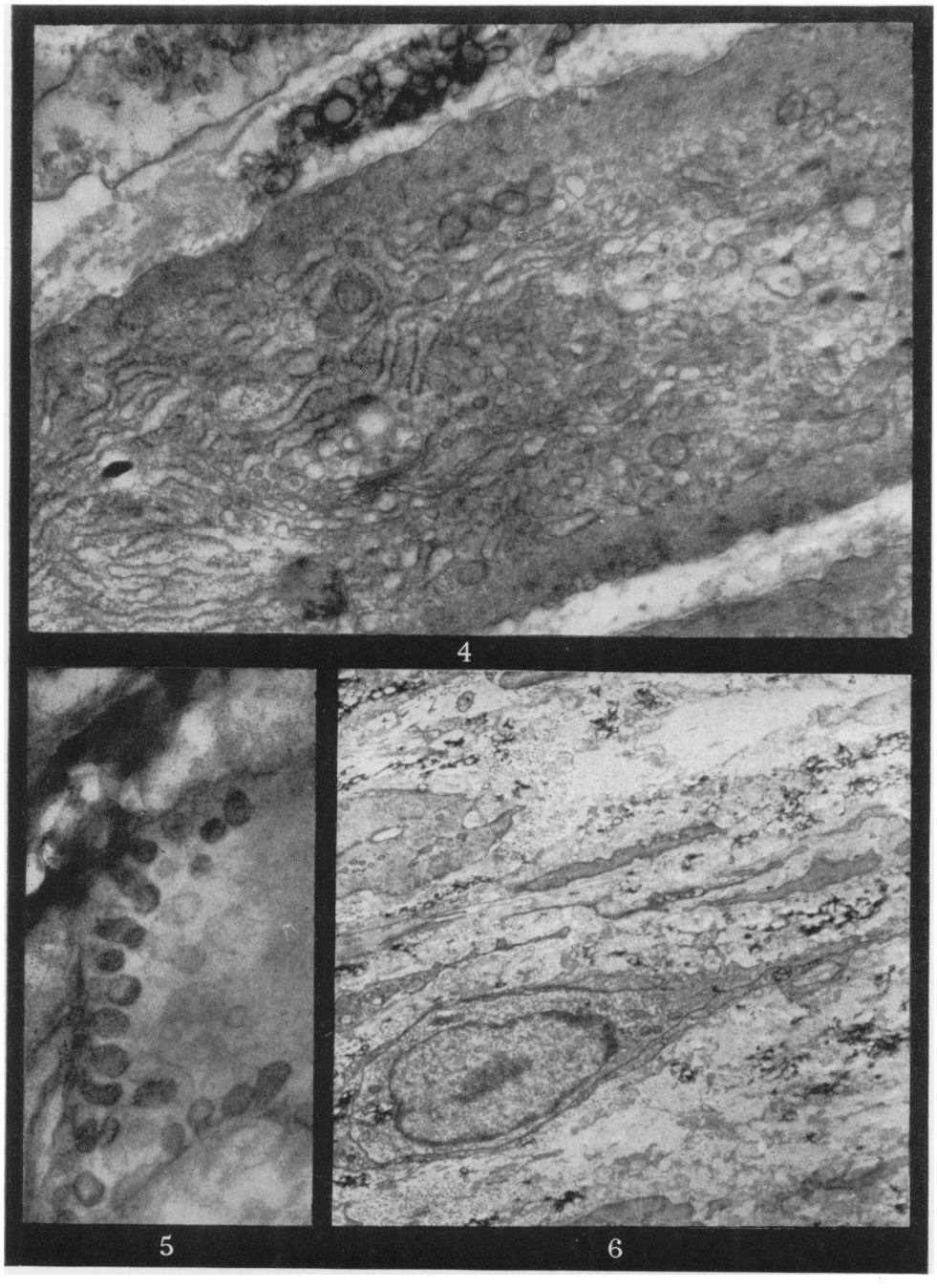

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intima. Parker (1960), using the electron microscope, identified as smooth muscle certain lipid-filled cells of experimental cholesterol atherosclerosis in rabbits. Movat, More and Haust (1958) considered that smooth muscle cells, which they identified with the light microscope, formed an important cellular component of diffuse intimal thickening of human arteries. With the electron microscope Florey, Greer, Poole and Werthessen (1961) have shown that the smooth muscle cell is the predominant type of cell in the pseudointima forming inside a graft of knitted plastic inserted into the aorta of baboons. Buck (1961) found that intimal thickening after ligature of arteries in the rat was due to the growth of modified smooth muscle cells (myo-intimal cells).

The myo-intimal cells of ligated arteries showed certain differences from the smooth muscle cells of the media, although they took origin from the latter. The principal differences were that in the myo-intimal cells the myofilaments were confined to a narrow band against the plasma membrane ; the central cytoplasm, which constituted most of the cell, contained a well-developed ER ; phagocytic vacuoles were commonly seen in the cytoplasm.

The same features of the cytoplasm were found in the cells of the atherosclerotic lesions of the rabbit aortas described in this report. Again, the modified smooth muscle cells, or myo-intimal cells, constituted the principal type of cell in the lesion. Preliminary results of a study of the effects of freezing the arterial wall, being carried out in this laboratory, indicate that the same type of cell appears in the thickened intima produced by this procedure.

The common denominator seems to be the smooth muscle cell in the tissue reaction to freezing, grafting (Florey, Greer, Poole and Werthessen, 1961), ligature (Buck, 1961), ageing (Movat, More and Haust, 1958), cholesterol atherosclerosis in rabbits (Parker, 1960) and recovery from cholesterol atherosclerosis. Perhaps certain other types of arterial lesions also result predominantly from the growth of smooth muscle in the intima, for example, the obliterating sclerosis of ovarian and uterine arteries (Sohma, 1908).

Why does the smooth muscle cell, instead of the fibroblast, appear in response to such " injuries " in arterial intima ? How general is the property of phagocytosis among smooth muscle cells of arterial media and other sites ? Under what conditions does the smooth muscle cell produce elastic fibres and acid mucopolysaccharide ? These questions seem to have no answers at the present time, but the role of the smooth muscle cell in the pathogenesis of arterial disease is obviously a promising field for future study.

*Extracellular material*

The extracellular material of the lesions contained both elastic and collagenous fibres. In the ligated arteries (Buck, 1961) only elastic fibres were present, and in that experiment only smooth muscle cells, and no fibroblasts, were seen. The fact that collagen fibres were found in the aortic lesions is probably related to the presence in them of a small number of fibroblasts. Probably a large proportion of the extracellular material consists of acid mucopolysaccharide, which is known to be in high concentration in such lesions (Buck, 1954), but without special methods this material would not be seen in the electron micrographs. Likewise, cholesterol, which is not osmiophilic, would not be visualized. Some of the granular extracellular material concentrated in a layer under the endothelium may possibly represent the protein component of a lipid-protein complex.

SUMMARY

The aortic lesions of rabbits which were permitted to live for 20 months on a normal diet after a period of 4 months on a cholesterol-rich diet have been studied with the electron microscope. The endothelial cells formed a single, continuous layer, and showed features which distinguished them from normal endothelium, particularly in regard to the Golgi apparatus and the endoplasmic reticulum. The principal cell of the subendothelial tissue was a modified smooth muscle cell, but a few fibroblasts and macrophages were also seen. The smooth muscle cell appears to have a significant but little understood role in the pathogenesis of arterial disease.

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