FINE STRUCTURE OF CANINE EXPERIMENTAL ATHEROSCLEROSIS

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Studies of the fine structure of human [1-7] and experimental [7-14] atherosclerosis repeatedly have called attention to the presence of lipid containing smooth muscle cells in the lesions. It has been proposed that smooth muscle cells may be precursors of the foam cells in the lesions.[1-4,6] Recent studies of human lesions have shown that foam cells derived from smooth muscle cells can be distinguished morphologically from those derived from some other yet to be characterized cell.[4,6] The present investigation was undertaken to describe in the experimental canine vascular lesion the earliest detectable changes and to identify as well as possible the cells participating in the formation of the lesions for the purpose of providing clues to pathogenesis.

MATERIAL AND METHODS

Seven mongrel dogs (5 female, 2 male) approximately 1 year old and weighing between 5.2 and 9.4 kg were fed a basal diet of commercial dog food (Hill's Dog Food, Hill Packing Company, Topeka, Kansas) for 1 month after which cholesterol and 2-thiouracil were added to the basal ration and fed for 4 months. The composition of the diet has been reported previously.[15]

Blood was drawn from the jugular vein at the end of 1 month on basal diet and at monthly periods while the diet was supplemented with cholesterol and thiouracil. At the end of the 4-month feeding period the dogs were killed with an overdose of pentobarbital sodium and necropsied. Tissue blocks from the aorta were fixed in 10 per cent formalin, embedded in gelatin and sectioned in a cryostat. Sections were stained with oil red O-hematoxylin and hematoxylin and eosin. Tissue blocks from other organs were fixed in 10 per cent formalin, embedded in paraffin, and sections stained with hematoxylin and eosin.

Samples of grossly normal aorta and aorta with fatty streaks were taken for study by electron microscopy. Thin blocks were removed from the inner surface of the aorta by undercutting the intima with a razor blade and then slicing the undercut segment into ribbons. The thin ribbons of inner aortic wall were transferred into Hank's balanced saline (Microbiological Associates, Bethesda, Md.) warmed to 37° C. After 30 minutes in balanced saline the tissues were fixed in buffered 1 per cent osmium tetroxide [16] for 1½ hours. Fixed tissues were dehydrated in graded alcohols and embedded in Maraglas.[17] Sections were cut with diamond knives on a Porter-Blum

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microtome (Ivan Sorvall, Inc., Norwalk, Conn.) and stained with either lead citrate[18] or lead hydroxide.[19] Sections were examined and photographed in an RCA EMU 3F electron microscope.

Serum high (alpha 1) and low (alpha 2 and beta) density lipoproteins were separated by dextran sulfate precipitation[20] and the completeness of separation checked by paper electrophoresis. Lipids from the high and low density fractions of the serum were extracted by the method of Folch and associates[21] and dried to constant weight. Total serum cholesterol was determined by the Leffler modification of the Zak method.[22]

Eight dogs fed the same basal ration supplemented with varying amounts of corn oil, butter or cholesterol for 4 months were studied by the same methods as the previous.

Results

None of the 8 dogs fed the basal diet supplemented with corn oil, butter or cholesterol had gross arterial lesions. No stainable lipid was found in their aortas and no abnormalities were detected by electron microscopy.

All 7 of the dogs fed the basal diet supplemented with cholesterol and thiouracil had atheromatous arterial lesions visible to the unaided eye. Thyroid arteries were extensively involved in all dogs. The uterine arteries were severely affected in 4 of the 5 females. Four of the 7 dogs had grossly visible lesions in the coronary arteries, in the distal ramifications more often than in the proximal segments. Other arteries commonly involved were internal mammaries, intercostals, carotids and iliac-femorals.

Elevated, white fatty plaques and streaks were present in all the aortas, but there was marked variation in the extent of involvement. All dogs had lesions in the ascending arch, especially in the sinuses of Valsalva. Three dogs had no visible lesions in the descending thoracic aorta, although one had extensive lesions in this segment which were more severe than those in the abdominal portion. Lesions appeared in the abdominal aorta in 6 of the 7 dogs, and the lesions here were more extensive than in any other aortic segment in all dogs except one.

Primary histologic features were widespread vascular atheroma, thyroid hyperplasia, minimal focal fatty change in the liver and proliferative intimal lesions in the pulmonary arteries secondary to infection with *Dirofilaria immitis*. Microscopic vascular lesions were found in the spleen, pancreas, bronchial artery, aortic vasa vasorum, pulmonary vein and kidney in some dogs. Foci of fatty change in the liver were small and relatively rare. In all instances the liver cells had granular cytoplasm presumably due to glycogen storage known to be excessive in thyroid suppressed dogs fed cholesterol.[23] The only segment of the venous system found to have lesions was the large pulmonary veins; this was noted in 3 of the 7 dogs.

Excessive lipid in tissues other than blood vessels was minimal. Liver lipid content (19.5 ± 2.8 mg per gm dry liver wt) was normal.[15] In 2 dogs small foci of foam cells were seen in the zonae fasiculata and reticularis of the adrenal. Sections of lymph nodes showed no foam cells.

Serum total lipids rose from an average control value of 711 ± 96 mg per 100 ml (range 627 to 870) to 4792 ± 1411 mg per 100 ml (range 3308 to 7781) after cholesterol and thiouracil feeding. The high density lipoprotein fraction concentration decreased from an average control value of 483 ± 78 mg per 100 ml (range 367 to 583) to 149 ± 4.7 mg per 100 ml (range 140 to 153) at the end of the experiment. The increase in serum total lipid concentration was due to an increase in the low density fraction from an average control value of 228 ± 50 mg per 100 ml (range 161 to 328) to 4643 ± 1409 mg per 100 ml (range 3162 to 7628). Serum total cholesterol rose from an average control value of 148 ± 8.0 mg per 100 ml (range 140 to 159) to 1991 ± 501 mg per 100 ml (range 1260 to 2780).

Basically, 3 patterns of atheromatous lesions were observed by light microscopy. In the intima and adjacent media, areas of the aorta that appeared normal to the naked eye often showed microscopic foci of tiny droplets of sudanophilic material in the interstitial tissue and about elastic lamina. The small droplets of lipid appeared for the most part to be extracellular. Some, however, were definitely within endothelium and cells with fusiform and stellate configurations. The other two types of lesions were characterized by accumulations of large foam cells and differed one from the other only in the location of these accumulations. In most the foam cells lay in the inner one-third of the media separating the smooth muscle cells and elastic plates. The remaining lesions were characterized by subendothelial accumulations of foam cells that appeared to have lifted the endothelium away from the internal elastic lamina; these infiltrated the media only superficially. The medial lesion was observed far more frequently in muscular arteries than the subendothelial accumulations.

The fine structure of normal dog aorta endothelium did not differ appreciably from that of other species.[1,7,8,24–27] Adjacent plasma membranes of endothelial cells were separated by a clear space measuring approximately 15 mμ in width; no interendothelial attachment plates were found (Figs. 1 to 3). Many endothelial cells overlying accumulations of lipid exhibited intracytoplasmic lipid inclusions, a large Golgi apparatus and increased numbers of profiles of both granular and agranular endoplasmic reticulum (Figs. 1 and 2). The lipid inclusions were moderately electron dense, homogeneous and limited by a single membrane that was usually irregular in contour (Fig. 2). An electron dense

filamentous or flocculent appearing material was seen occasionally in the endoplasmic reticulum of both normal appearing endothelium and that containing lipid inclusions and increased amounts of endoplasmic reticulum (Fig. 1). No evidence of endothelial proliferation into the intima was observed. The endothelium was separated from the underlying tissue space by a thin basement membrane (Figs. 1 and 3).

Grossly normal areas of aorta examined by electron microscopy either revealed no recognizable abnormality or showed the endothelial changes described above associated with dense amorphous material in the extracellular space, occasional cytoplasmic lipid inclusions in smooth muscle cells, or lipid inclusions in round and irregular cells not identified as smooth muscle (Figs. 1 to 4). The extracellular, amorphous dense material corresponded to the fine droplets of oil red O staining substance observed by light microscopy; thus the extracellular amorphous material seen by electron microscopy was presumed to contain lipid. The smooth muscle cells were readily identified by the presence of cytoplasmic myofilaments, numerous pinocytotic vesicles along the plasma membrane and a limiting basement membrane (Fig. 4). Cytoplasmic lipid inclusions in these cells appeared moderately electron dense, homogeneous, irregular in contour and limited by a single agranular membrane (Fig. 4); they were identical to the lipid inclusions encountered in endothelium. The lipid containing cells in the intima and inner portion of the media not identified as smooth muscle were irregular in shape with multilobed nuclei (Figs. 4 to 6); occasional round or ovoid cells contained few lipid inclusions and fewer cytoplasmic organelles than the irregular cells (Fig. 3). In contrast to smooth muscle these cells had no limiting basement membrane, only occasional pinocytotic vesicles along the plasma membrane, and small parallel arrays of cytoplasmic filaments near the nucleus that were two or more times the thickness of smooth muscle cell myofilaments (600 Å vs. 100–300 Å), (Figs. 5 and 6). Cells that appeared to be intermediate between the round or ovoid forms and the irregular cells were observed.

The grossly visible lesions differed by their large content of foam cells with none of the morphologic features that distinguish smooth muscle (Figs. 2, 7, 8 and 9). Lipid inclusions presented a variety of appearances, but two types predominated. One form was the same as that seen in smooth muscle and endothelium (Figs. 2, 7, 8 and 9). Its irregular contour appeared to result from confluence of cisterns of agranular endoplasmic reticulum containing moderately electron dense homogeneous lipid (Fig. 8). The other type was very electron dense, appeared coarsely granular, and strongly resembled the extracellular lipid (Figs. 2, 7, 8 and 9). There was evidence of phagocytosis of extracellular lipid (Fig.

7). The electron dense, coarsely granular lipid inclusions were limited by a single membrane. The larger of these often demonstrated a central homogeneous portion that was only moderately electron dense (Figs. 2, 7 and 8). The relative area of the central portion varied; in some there was only a narrow peripheral rim of electron dense, coarsely granular material (Figs. 2, 4 and 8). Both the homogeneous and dense granular forms of lipid inclusions appeared in the peripheral portion of the cytoplasm. The Golgi apparatus was not seen to participate in the process of lipid accumulation; in the larger lipid containing cells, however, the Golgi apparatus was quite large (Fig. 9).

In addition to dense, amorphous lipid containing material in the interstitial tissue, there was a variable amount of fine filamentous substance that resembled the connective tissue of basement membranes (Figs. 1, 5 and 6). A similar material was occasionally seen in the cisterns of rough profiled endoplasmic reticulum in smooth muscle, endothelium and foam cells (Figs. 1, 4, 5 and 8). Fibrin was not identified in the lesions. Cells conforming to the description of fibrocytes (fibroblasts)[28] were not seen in the dog aorta.

Discussion

Most studies of experimental canine atherosclerosis have employed much longer periods of cholesterol feeding with thyroid suppression than the study here reported. The long feeding period, however, is not required for producing lesions. Dogs on the regimen for as short a period as 2 months[29] may have grossly visible lesions and those fed for 4 months regularly demonstrate gross lesions.[14,29] Whether or not a dog develops lesions and the extent to which they appear (severity) depends on the magnitude of serum lipid and cholesterol levels and the time over which these levels are maintained; lesions are found when the serum cholesterol is in excess of 1,200 mg per cent for 2 or more months.[29]

The anatomic distribution of canine experimental atherosclerosis is highly variable. There is a tendency for lesions to develop in small arteries earlier than in the large ones and for lesions in the abdominal segment of the aorta to be larger and more extensive than in the thoracic segment.[14,29] The distribution found in the present experiment is the same as that reported by others. The only finding in the present study not previously reported is the existence of lesions in pulmonary veins, primarily their larger ramifications. This indicates that intravascular pressure alone cannot explain the failure of most veins to develop lesions; there must also be a tissue factor. A similar conclusion has been reached in pulmonary artery transplantation experiments in the dog.[30]

Correlation of the light and electron microscopic appearances of aortic

lesions where no grossly visible alteration was manifest indicates that the amorphous dense material in the interstitial spaces contains lipid. A similar relationship has been recognized in human lesions [6] and in experimental lesions in the rabbit and baboon.[31] Few studies of fine structure have alluded to the presence of extracellular lipid in early lesions in either human or experimental subjects.[4,7,9,11] Buck described granular material in the extracellular space in rabbit lesions that he stated might represent the protein component of a lipid-protein complex.[9] Still and Marriott encountered a particulate, granular substance in human lesions, "the most conspicuous element," but stated that extracellular material identifiable as lipid was rare.[7] Balis, Haust and More noted extracellular lipid in human lesions and interpreted it as having been derived from lipid containing smooth muscle cells.[4] Thomas and colleagues found irregular masses of electron dense material and considered them to be suggestive of lipid.[11] The fine droplets of lipid observed by light microscopy and their electron dense amorphous counterparts often are concentrated about elastic fibers and plates. The electron dense substance gathered about the elastica appears to be the fine structural counterpart of lipid incrusted elastica interpreted by others to be a degenerative change in elastic tissue.[32] No degenerative elastic tissue alterations were recognized in the present study.

Though proof that the electron dense extracellular material contains lipid is lacking, the correlation of this substance with stainable lipid observed by light microscopy leaves no doubt that it contains lipid. Knowledge of constituents other than lipid, when available, will help to determine its source. It seems most likely from the appearance of the material and the knowledge that blood lipoproteins enter the vessel wall in both dogs [33] and human subjects [34] that it represents precipitated blood lipoprotein.

Evidence today indicates that the bulk of the lipid that accumulates in atheromatous lesions is derived from the blood and enters the vessel wall through the endothelium.[33-38] The transport of blood lipids as lipoproteins from the blood stream into the vessel wall necessitates passage across the endothelium. Either the lipoprotein passes through the cytoplasm or between endothelial cells. Buck reported the presence of dense material in endothelial endoplasmic reticulum in cholesterol fed rabbits; this could be lipoprotein in transit.[25] In a later study, however, he found the same material in endothelium of rabbits who had received no supplementary cholesterol for 20 months, thus casting considerable doubt on the earlier conclusion.[9] A dense material similar to that described by Buck [25] was found in the present study (Fig. 1). To date no one has provided a clue from morphologic studies as to

how lipoprotein crosses the endothelium of elastic or muscular arteries.

Cytoplasmic lipid inclusions found in experimental lesions differ morphologically from those seen in human endothelium.[1,7,10,11,13,25] The studies of Duff with colloidal thorium dioxide showing incorporation of this colloid into endothelium primarily over lesions indicated increased endothelial permeability or transport.[39] With electron microscopy the only evidence for this has been the presence of a large Golgi zone and an increased amount of endoplasmic reticulum. It would appear thus that endothelium is the seat of increased metabolic activity as suggested by Buck,[9] but the nature of the activity remains to be determined. In the dog there was no evidence that the Golgi apparatus was involved in the formation of endothelial cytoplasmic lipid inclusions; this is in contrast to the observations of Still and Marriott in the rabbit.[7]

The grossly detectable canine aortic lesions obviously contained more lipid than was found in grossly normal portions of the aorta. These lesions differed primarily by their large foam cell content. Bevans, Davidson and Abell described in such lesions large numbers of lipid containing fibrocytes and smooth muscle cells spreading apart the medial elastic fibers.[29] In both the present and in a previous electron microscopic study,[14] fibrocytes were not identified in these lesions. The round and irregular cells did not have the morphologic features of fibrocytes.

A fundamental question begging further clarification in the pathogenesis of both experimental and human atherosclerosis is knowledge of the origin of foam cells. This has been a subject of numerous studies. Leary proposed that the reticulo-endothelial system became filled with lipid after cholesterol feeding and some of the engorged cells gained access to the blood stream eventually penetrating the endothelium of blood vessels forming foam cell lesions.[40] Rannie and Duguid elaborated on a similar idea except that they felt that foam cell aggregates came to lie on the surface, were eventually overgrown by endothelium and thus became incorporated into the vessel wall.[41] Altschul hypothesized proliferation of endothelial cells into vessel walls and their subsequent transformation into foam cells.[42] Duff, McMillan and Ritchie found no evidence to support any of these concepts and proposed that a "monocytoid" cell entered the intima from the blood.[43] All these hypotheses were generated largely from studies of experimental rabbit atherosclerosis. More recently, especially from studies of human lesions, the smooth muscle cell has been proposed as another possible source of foam cells.[1-4]

The present study lends greater support to Duff's concept than to any of the other possibilities. The reticulo-endothelial system of the dog does not become as saturated with lipid as in the rabbit. Thus this is not a

source for lipid laden foam cells. Much of Leary's hypothesis was based on a supposed prolonged lag period between the beginning of cholesterol feeding and the development of lesions. Duff and co-workers found lesions in intimal preparations as early as 4 hours after cholesterol feeding [43] indicating that, in the rabbit, saturation of the reticulo-endothelial system need not occur before arterial lesions begin to develop.

No support for the Rannie-Duguid concept or that of Altschul was provided by the present study. With other evidence at hand it seems most likely that the foam cell develops from a blood leukocyte (either monocyte, lymphocyte or both) that has penetrated into the wall of the vessel. The *in vivo* studies by Ebert and Florey [44] showed the blood monocytes to be capable of transformation into macrophages and histiocytes in tissue. Recent studies indicating similar transformation of lymphocytes ("blastogenesis") [45] induced by certain stimuli make it essential that this cell also be considered a possible precursor of foam cells. Assuming that the microscopic lesions found in the grossly normal aorta represent an early stage, it is apparent that foam cells acquire their lipid content within the lesion and do not enter into it as lipid laden foam cells.

There is no question that some foam cells arise from smooth muscle. Recent studies of human lesions, however, have shown that many of the foam cells are not derived from smooth muscle.[4,6] The term "myogenic foam cell" has been proposed to differentiate a lipid laden smooth muscle cell from a "macrophage" form of foam cell.[4] "Myogenic foam cells" in canine experimental lesions constitute a very minor component of the lesions.

Morphologic observations in the dog provide a clue as to the mechanism of lipid accumulation in smooth muscle and foam cells. The homogeneous cytoplasmic lipid inclusion in the smooth muscle cell appeared to form by coalescence of smaller cisternae of lipid containing agranular endoplasmic reticulum. Certain of the foam cell lipid inclusions had an identical appearance (Fig. 8), which suggested that both types of cells were actively synthesizing lipid in the agranular endoplasmic reticulum. The structure provided no clue as to how, or even if, extracellular lipid was the source. It seems likely that extracellular lipid is either a source for the precursors of lipid synthesis or that it stimulates lipid synthesis. Similar lipid inclusions were not found in normal vascular smooth muscle cells.

In addition to lipid accumulation in the agranular endoplasmic reticulum, foam cells exhibited morphologic evidence of phagocytosis of extracellular lipid. The homogenization in the central portion of the phagocytic inclusion is considered to reflect the action of intracellular enzymes in the course of modifying the composition of phagocytosed

lipid material. The macrophage is known to possess both lipases and esterases capable of acting upon lipoprotein.[46,47]

The lesions in the grossly normal aortas contained much less stainable lipid than was observed in the fatty streaks or plaques. Most of the lipid was extracellular, appearing as amorphous dense masses electron microscopically. This arterial lesion represented the smallest departure from the normal encountered in this experiment and is interpreted to be the earliest detectable lesion in experimental canine atherosclerosis. Since normal dog arteries contain no stainable lipid and spontaneous atherosclerosis is very uncommon in the dog even in old age, the designation of the early lesion seems justified. The reason for its focal distribution as well as that of the fatty streaks or plaques is unknown. It is presumed that continued extracellular lipid deposition associated with assimilation of portions of this lipid by blood monocytes or lymphocytes in the tissue results in the gross lesions.

Summary

The earliest detectable lesion of experimental canine atherosclerosis with the light microscope was an accumulation of fine droplets of stainable lipid in the interstitial tissue and in occasional fusiform or stellate cells. With the electron microscope the extracellular lipid appeared as an electron dense amorphous material. Intracytoplasmic lipid inclusions appeared in endothelium, smooth muscle cells and in round and irregular cells which were not of smooth muscle origin.

Gross fatty streaks or plaques differed from the early lesions by their content of large numbers of foam cells. All stages of cellular transformation from small round and irregular cells in early lesions to large round or ovoid foam cells in the more advanced lesions indicated that the former cells were transformed into foam cells. The foam cells as well as their presumed precursors were readily distinguished from smooth muscle cells by the absence of a limiting basement membrane, a paucity of pinocytotic vesicles along the plasma membrane and an absence of cytoplasmic myofilaments. Cytoplasmic filaments near the nucleus appeared to be two or more times the thickness of myofilaments. Smooth muscle cells containing few cytoplasmic lipid inclusions were found in early and advanced lesions, but in both cases accounted for only a very small part of the lipid present. In the early lesions the bulk of the lipid was extracellular in location and in advanced lesions it was intracellular within foam cells.

The origin of the foam cell in the vascular wall remains to be determined. It appears, at present, to be derived from either the blood monocyte or lymphocyte or both.

Morphologic evidence of phagocytosis of extracellular lipid was found

in foam cells and the cells presumed to be the precursors of foam cells. No evidence of phagocytosis of extracellular lipid was seen in endothelium or smooth muscle cells. Lipid accumulation in endothelium and smooth muscle cells appeared to be the result of lipid accumulation (synthesis?) in agranular endoplasmic reticulum with eventual confluence of the cisterns of endoplasmic reticulum forming large lipid inclusions. The same process was observed in foam cells in addition to phagocytosis.

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LEGENDS FOR FIGURES

FIG. 1. Aortic endothelial cell and intima containing elastic fibers (E), collagen fibers (C), fine filaments of connective tissue (F) and an amorphous dense material (D). The endothelial cell has a larger Golgi zone (G) and more numerous profiles of endoplasmic reticulum (ER) both granular and agranular than normal. The number of free cytoplasmic ribonucleoprotein particles in the cell is greater than normal. A dense material (M) is seen within some of the granular ER. A basement membrane (bm) is present between the endothelium and connective tissue space of the intima. The dense amorphous material in the extracellular space (D) corresponds to the fine droplets of extracellular lipid seen by light microscopy. × 23,000.

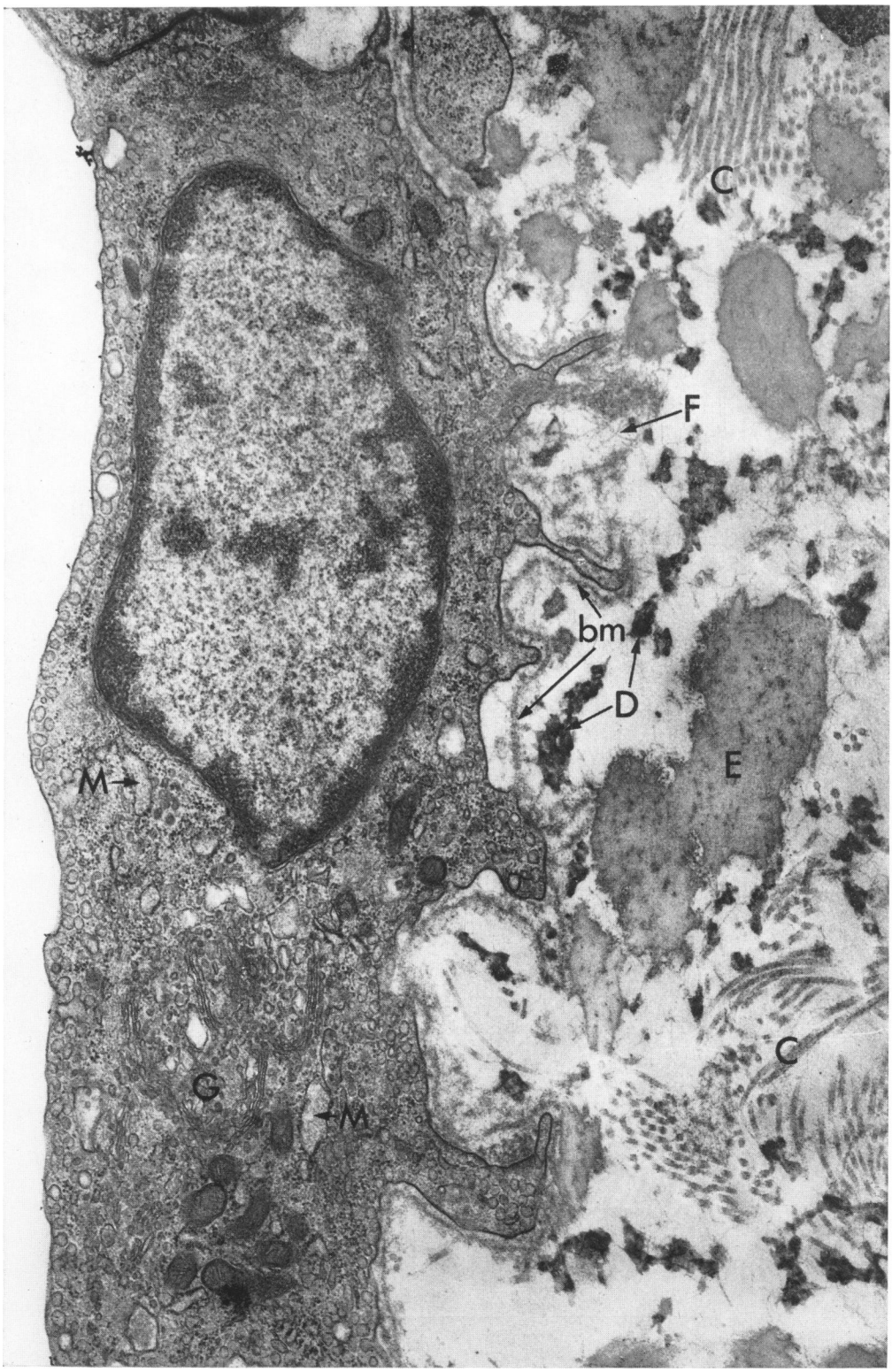
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FIG. 2. Aorta. Endothelial cells (end) contain cytoplasmic lipid inclusions (L) that are moderately electron dense, homogeneous and limited by a single membrane. The central portion of some lipid inclusions is clear possibly due to incomplete penetration of fixative, dissolving away during tissue processing, or dropping out of this area in the process of sectioning and mounting. The endothelial cells contain more cytoplasmic organelles than normal. An aggregate of cells with none of the features of smooth muscle (basement membrane, pinocytotic vesicles, myofilaments) appears in the intima. All these cells except one contain cytoplasmic lipid inclusions (L). Most of the inclusions are identical to those in the endothelium (L); some are very electron dense (EL). The inclusion identified by the letter "R" has a homogeneous moderately electron dense central portion and a narrow peripheral rim of more dense material. × 11,000.

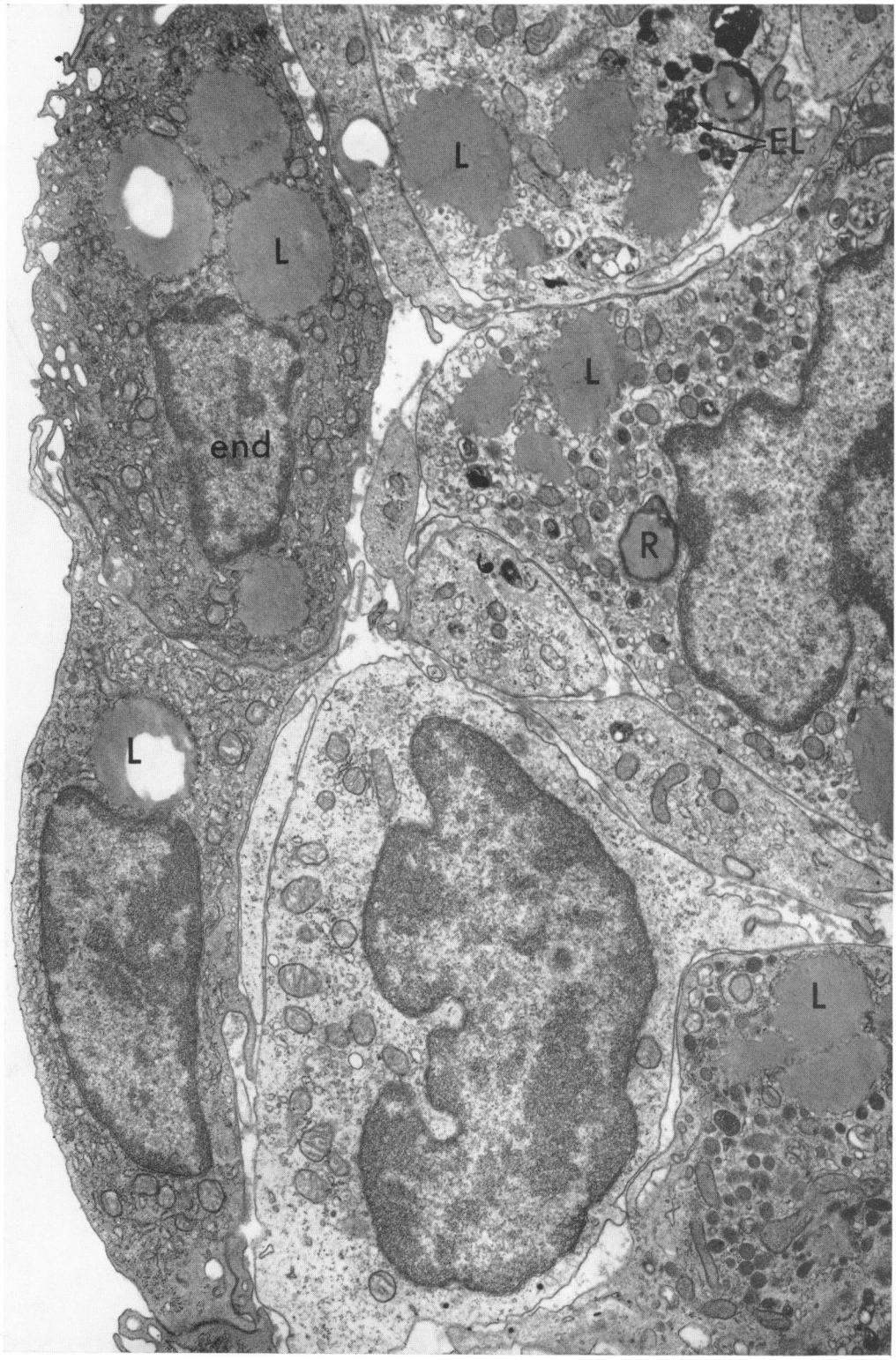

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FIG. 3. Endothelial cell (end) appears normal. In contrast to the endothelium in Figures 1 and 2 this cell shows fewer cytoplasmic organelles and no lipid inclusions. In the intima portions of the internal elastic lamina are seen about which are many masses of amorphous electron dense material representing extracellular lipid. A small ovoid cell (mo) contains few cytoplasmic organelles and small lipid droplets (L). This cell has none of the characteristic features of smooth muscle; it resembles a lymphocyte. Portions of smooth muscle cytoplasm (sm) are seen at the left of the micrograph. × 14,200.

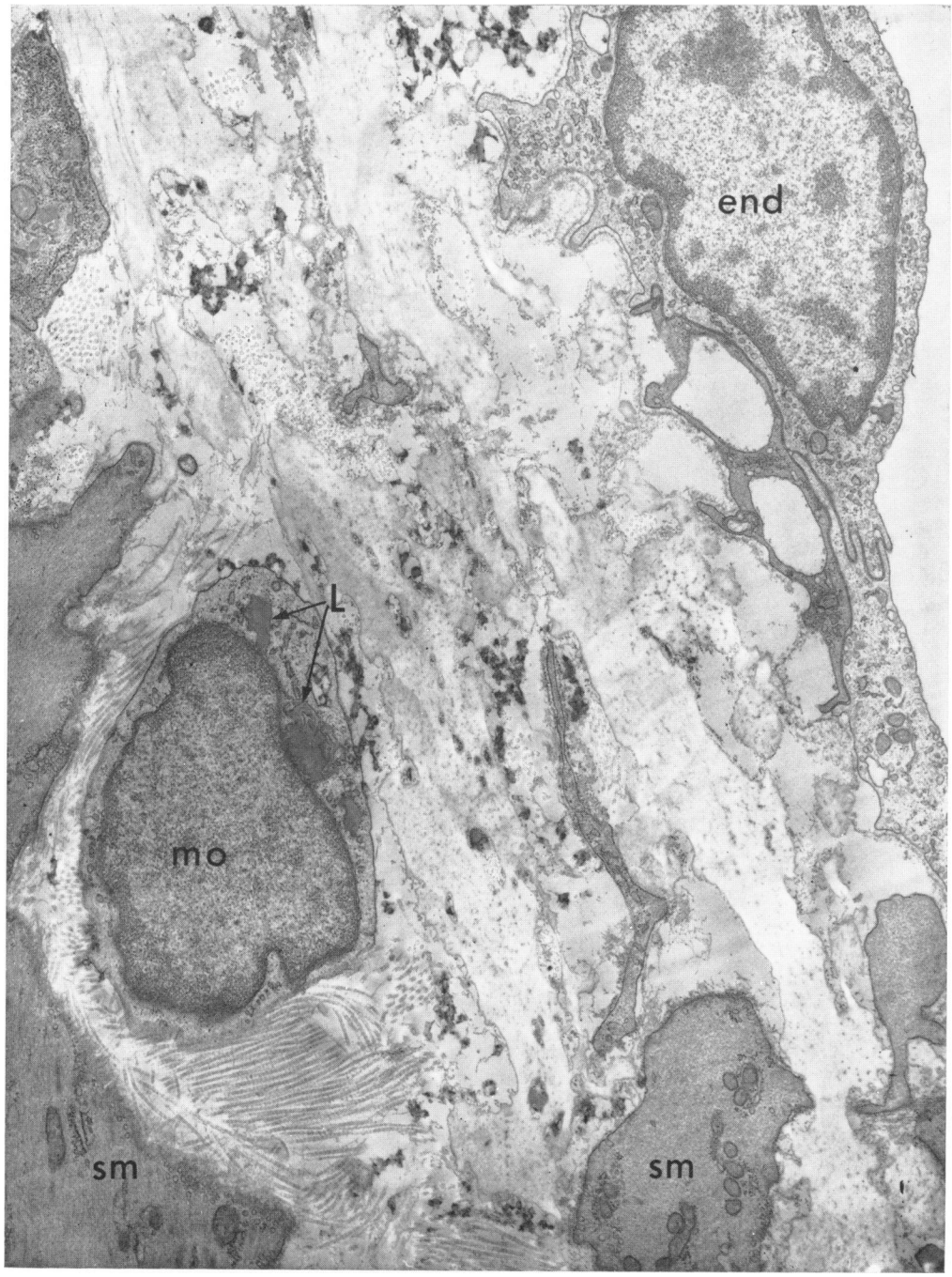

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FIG. 4. Aorta. A smooth muscle cell (sm) contains a cytoplasmic lipid inclusion (L) and an irregular cell (mo) contains numerous cytoplasmic lipid inclusions (EL, L) and amorphous dense extracellular lipid. The lipid inclusion (L) in the smooth muscle cell (sm) is homogeneous, moderately electron dense and limited by a single membrane. There is dense filamentous material (M) in some of its ER profiles. A centriole (c) appears in the Golgi zone. The smooth muscle cell is characterized by intracytoplasmic myofilaments, pinocytotic vesicles along the plasma membrane and a limiting basement membrane. The irregular cell has none of the morphologic features of smooth muscle. The lipid inclusions in the irregular cell (mo) are either homogeneous and moderately electron dense (L) or very electron dense. The larger inclusions containing very electron dense material have homogeneous and moderately electron dense central portions. × 16,500.

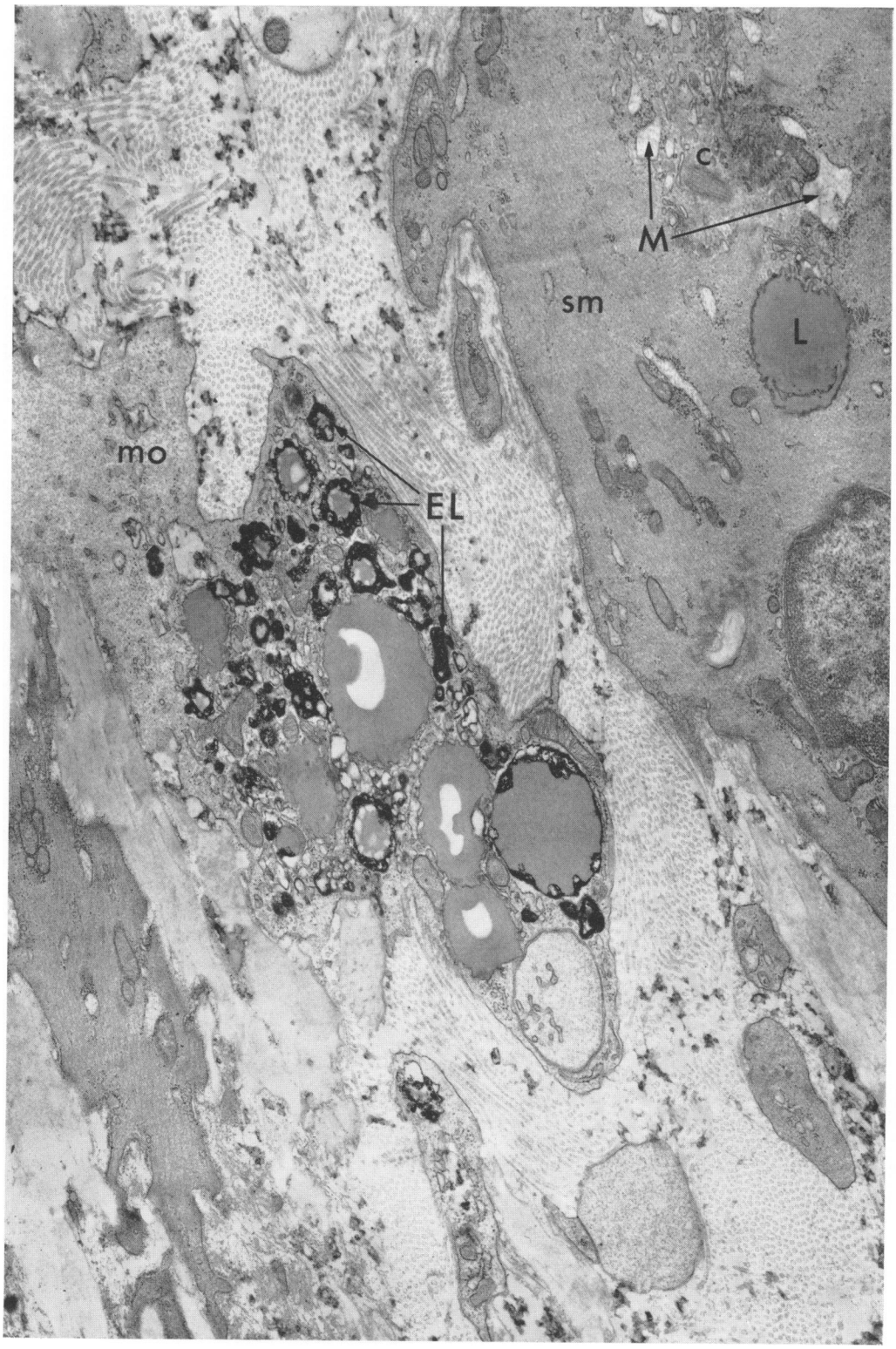

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FIG. 5. Aorta. An irregular cell (mo) contains more cytoplasmic organelles than the ovid cell shown in Figure 3. This cell has no basement membrane, no myofilaments and only rare pinocytotic vesicles along the plasma membrane. Parallel arrays of filaments (f) are seen above and below the nucleus. There is a dense filamentous material (M) in some of the ER profiles. Two lipid inclusions of the moderately dense homogeneous type are evident in the cytoplasm. Fine filaments of connective tissue material are present in the extracellular space; these are similar to those shown in Figure 1 and resemble the filamentous material (M) in the ER profiles. Dense amorphous lipid containing material is present in the extracellular space adjacent to elastic fibers. × 18,750.

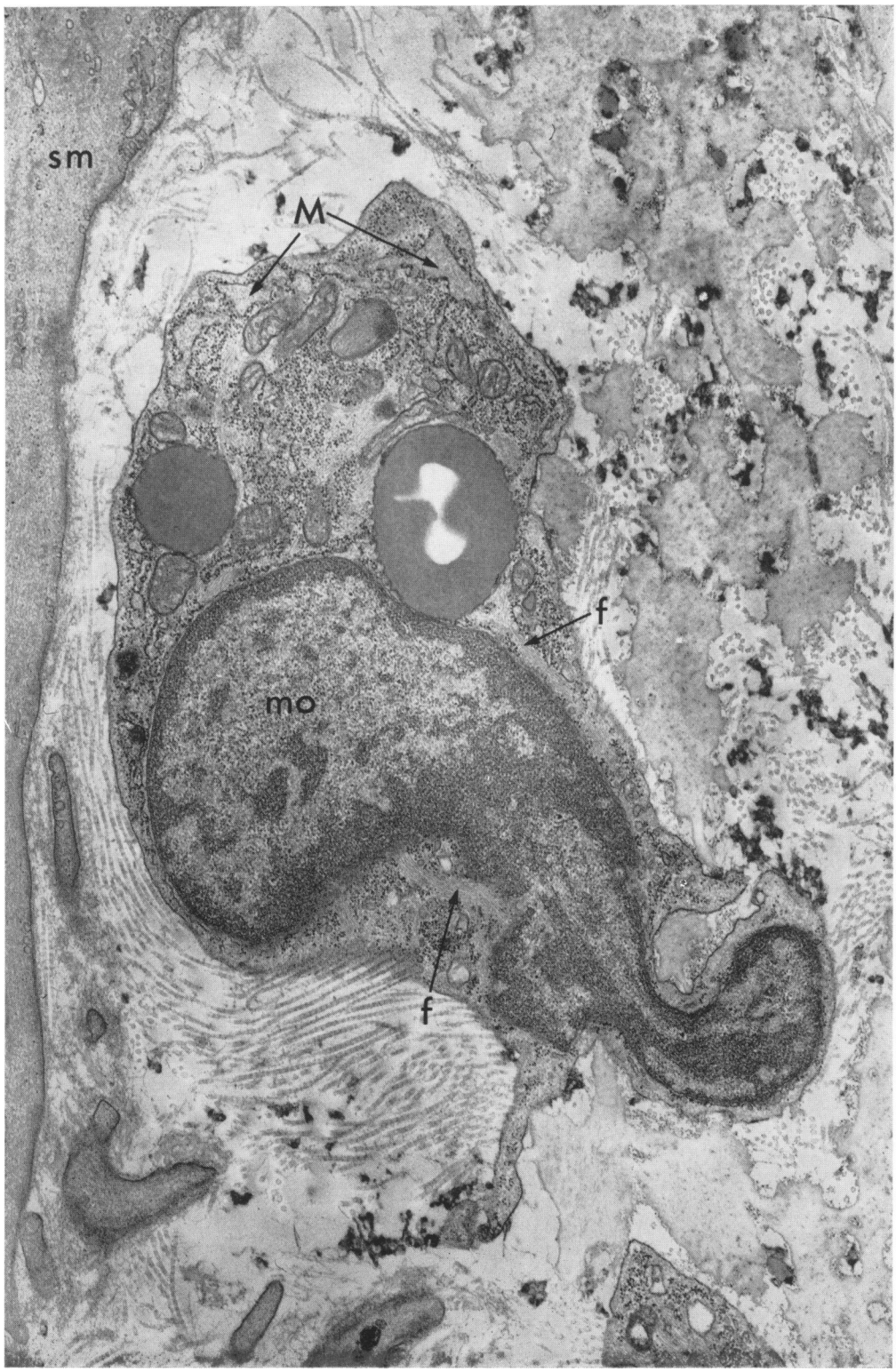

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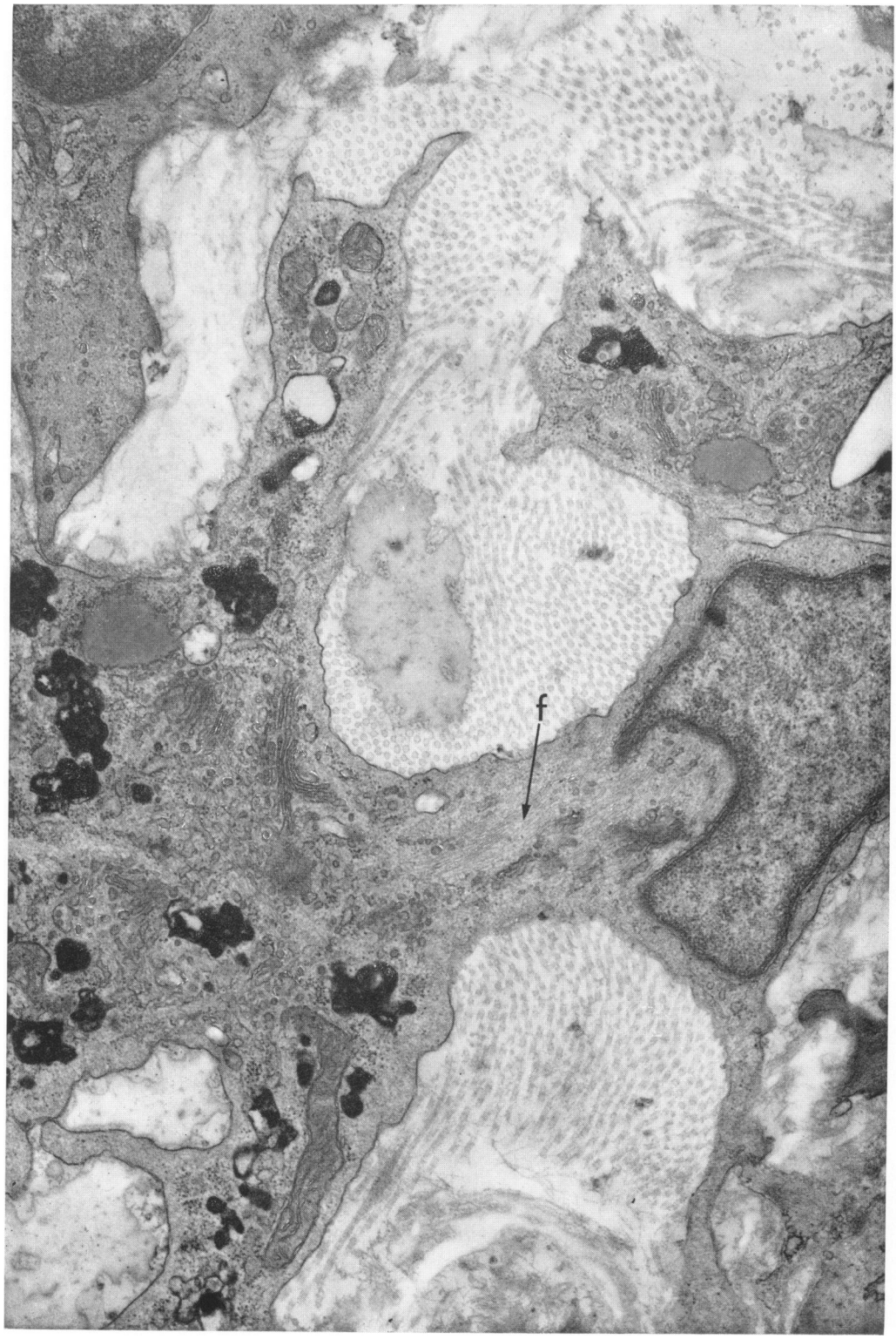
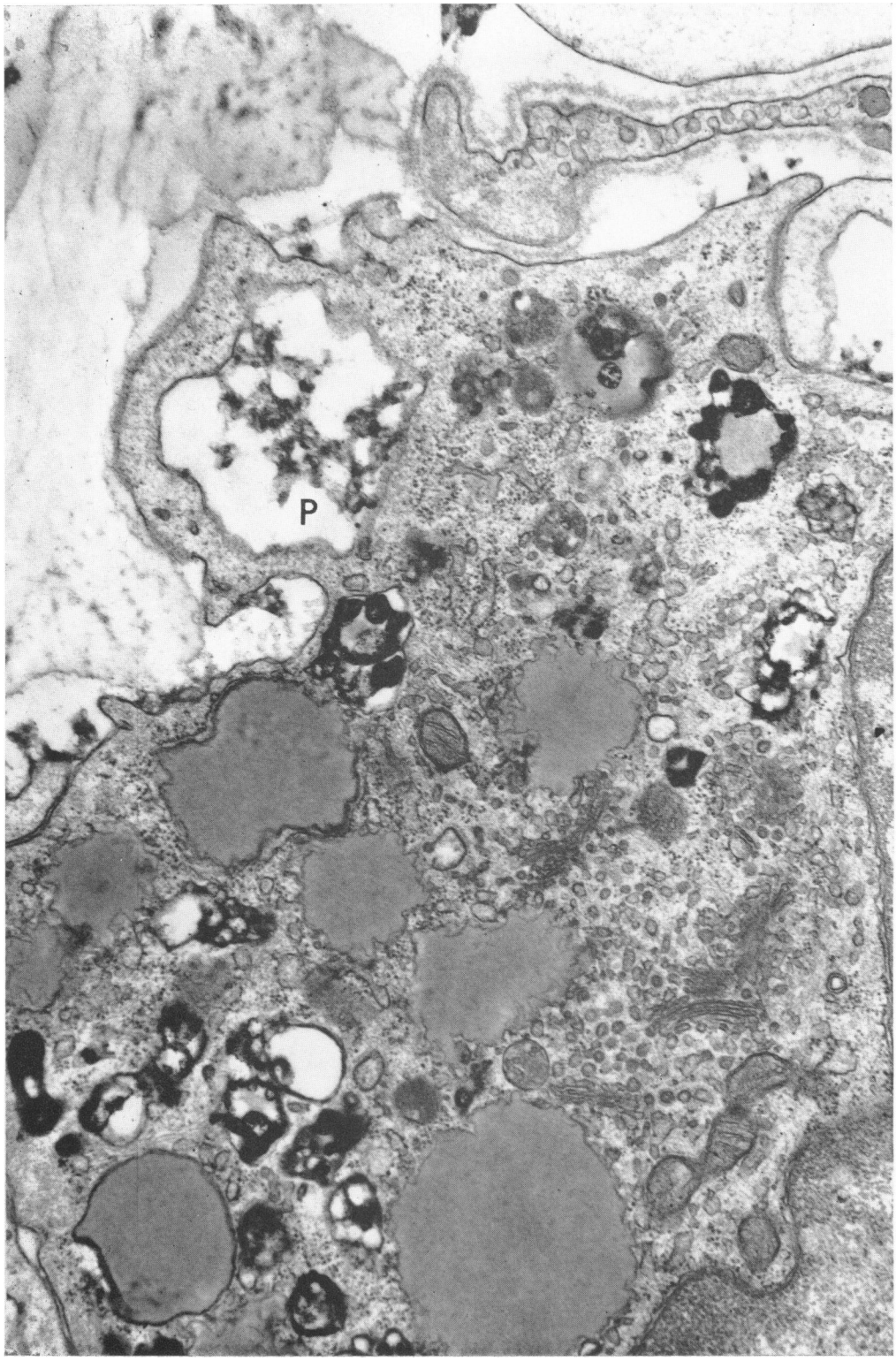
FIG. 6. Aorta. An irregular cell in the interstitial tissue contains no cytoplasmic myofilaments and exhibits rare pinocytotic vesicles along the plasma membrane and no limiting basement membrane. Parallel arrays of filaments (f) are seen in the cytoplasm near the nucleus. Both the very dense and homogeneous cytoplasmic lipid inclusions are present. Fine filaments of connective tissue material are seen in the interstitial space at the lower edge of the micrograph. × 22,500.



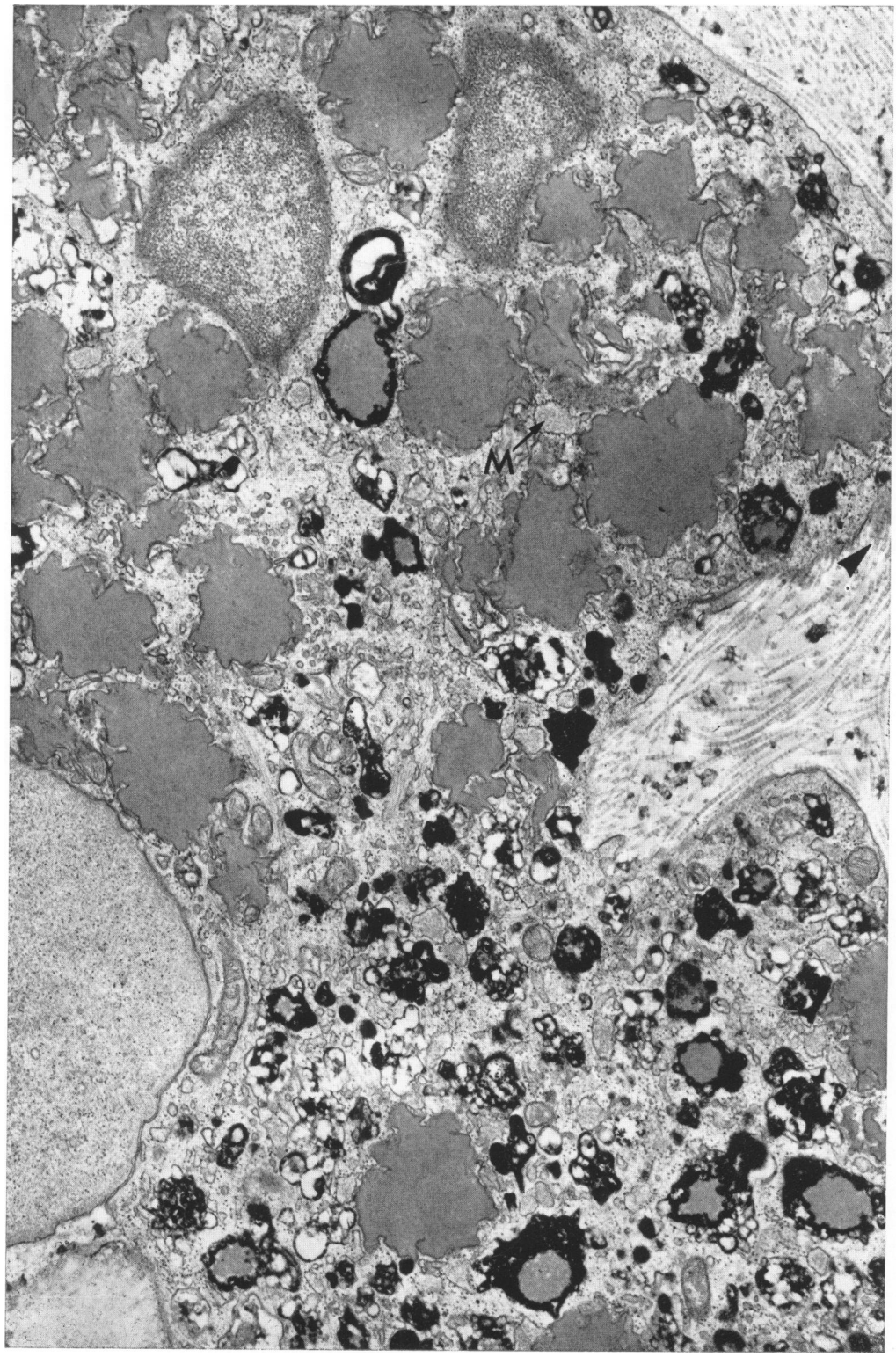
6

FIG. 7. Aorta. A large cell with none of the features of smooth muscle contains numerous cytoplasmic lipid inclusions, both homogeneous and very dense types —a foam cell. Dense amorphous material similar to that seen in the extracellular space in Figures 1, 3, 4 and 5 is contained in a vacuole (P); this is interpreted to represent a phagocytic vacuole. The homogeneous moderately electron dense lipid inclusions are limited by a single membrane and appear to be formed by confluence of smaller cisterns of agranular ER. × 30,000.



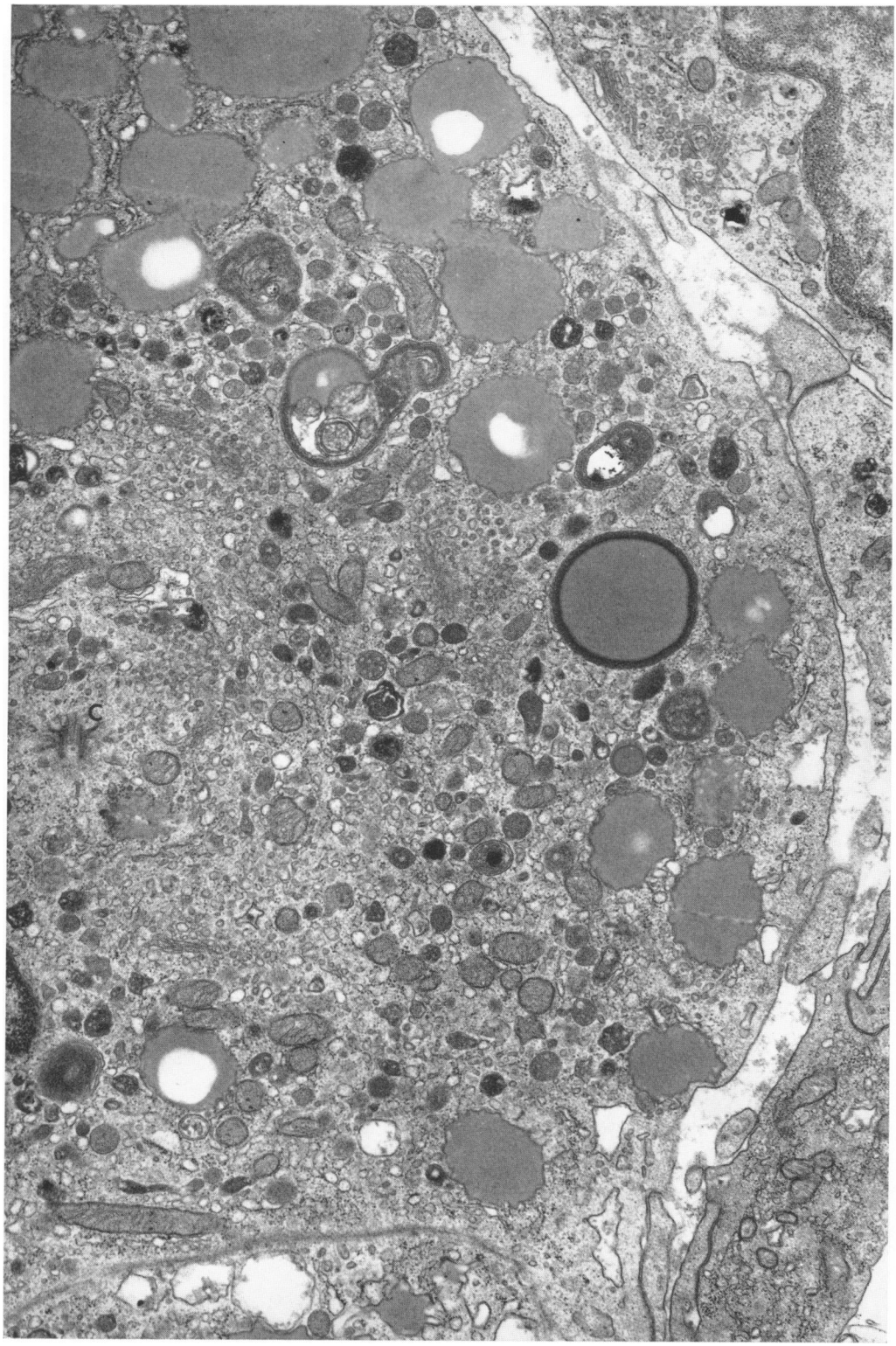
7

FIG. 8. Aorta. A foam cell contains two forms of lipid inclusions. Most of the inclusions in the upper portion of the micrograph are moderately electron dense, homogeneous, limited by a single membrane and appear to be formed by confluence of small cisterns of lipid containing agranular ER. Most of the lipid inclusions in the lower portion of the micrograph appear very electron dense, coarsely granular, and the larger ones exhibit homogeneous moderately electron dense central portions. Filaments in the cytoplasm resemble those shown in Figures 5 and 6. Filamentous material (M) appears in some of the profiles of granular ER. Extracellular lipid is evident among the collagen fibers at the right margin of the micrograph. × 15,000.



8

FIG. 9. Aorta. A foam cell exhibits several types of lipid inclusions in the peripheral portion of the cytoplasm. The Golgi material about the centriole (c) demonstrates no evidence of participation in the processes of lipid inclusion formation. × 17,200.



9