FINE STRUCTURE OF THE GLOMERULUS IN HUMAN AND EXPERIMENTAL RENAL AMYLOIDOSIS

TSURANOBU SHIRAHAMA, M.D., AND ALAN S. COHEN, M.D.

From the Evans Department of Clinical Research, University Hospital, and the Department of Medicine, Boston University School of Medicine, Boston University Medical Center, Boston, Mass.

There is general agreement that in the glomerulus, as in other organs, amyloid has a fibrous ultrastructure.[1] Recent studies on glomerular amyloidosis demonstrate that the fibrils accumulate along the mesangial matrix and the basement membrane. The intimate relationships among the three types of glomerular cells and the amyloid fibrils have also been noted, and the mesangial cell has been designated as most likely responsible for amyloid accumulation. While a basement membrane is present on most occasions, it may be somewhat distorted in heavy accumulations of amyloid fibrils. The similarity of the ultrastructural features between human and experimentally induced amyloidosis, as well as among the different types of human amyloidosis, has been pointed out and no significant ultrastructural difference between them has been reported.[2-16]

In the course of our studies of human and experimental amyloidosis, several new observations have been made on the interrelationships of the various renal glomerular cells, the extracellular elements, and amyloid fibrils. It is the purpose of this communication (1) to re-evaluate the glomerular ultrastructure in amyloidosis, (2) to point out several differences between the fine structure in the human disease and the experimental model, and (3) to describe several new ultrastructural features of glomerular amyloidosis.

MATERIALS AND METHODS

Amyloidosis was induced in a group of New Zealand white rabbits by twice-weekly injections of 12% sodium caseinate as previously reported.[17] Six rabbits had renal amyloidosis and constituted part of this study. Human renal amyloid was obtained by percutaneous renal biopsy * in four instances and at necropsy from six individuals who had died with various forms of the disorder. Of the 10 patients, 3 had no associated disease (primary amyloidosis), 6 had secondary amyloidosis, and 1 had

Supported by grants from the U.S. Public Health Service, National Institute of Arthritis and Metabolic Diseases (Grants AM-04599 and T1 AM 5285), and from the Arthritis Foundation.

Accepted for publication July 25, 1967.

* Courtesy Dr. Ronald Miller, Renal Section, University Hospital and Boston University School of Medicine.

TABLE I

CLINICOPATHOLOGIC CHARACTERISTICS OF PATIENTS STUDIED

Case No.	*Age (Yr.)*	*Sex*	*Clinical classification of amyloid*	*Clinical manifestations of renal disease*	*Glomerular amyloid (Amount)**	*Tinctorial characteristics of amyloid*	
						Congo red	*Green birefringence †*
1	59	M	Secondary (rheumatoid arthritis)	Nephrosis; azotemia	2+	+	+
2	63	M	Secondary (tuberculosis)	Nephrosis; azotemia	3+	+	+
3	63	F	Secondary (tuberculosis)	Azotemia	3+	+	+
4	70	M	Secondary (paraplegia & chronic pyelonephritis)	Proteinuria (<3.5gm./ 24 hr.)	4+	+	+
5	76	M	Secondary (tuberculosis)	Nephrosis; azotemia	4+	+	+
6	68	M	Secondary (bronchiectasis)	Nephrosis	3+	+	+
7	49	F	Primary	Proteinuria (trace)	2+	+	+
8	56	M	Primary	Nephrosis; azotemia	4+	+	+
9	50	F	Primary	Nephrosis; azotemia	3+	+	+
10	80	M	Myeloma associated	Azotemia	4+	+	+

* Estimated replacement of glomeruli with amyloid: 1+, 0–25%; 2+, 26–50%; 3+, 51–75%; and 4+, 76–100%.

† On polarization microscopy after Congo red stain.

myeloma-associated amyloidosis. The clinical and pathologic features of these patients are given in Table I. The amyloid in all instances demonstrated green birefringence on polarization microscopy after Congo red staining.

Small pieces of the renal cortex were fixed in 1% osmium tetroxide in phosphate buffer, or in 3% glutaraldehyde (or 4% paraformaldehyde) in phosphate or in cacodylate buffer followed by 1% buffered osmium tetroxide, and then were dehydrated in graded concentrations of ethanol.[18] After embedding in Epon, thick sections were cut on an LKB Ultratome and were stained with azure II-methylene blue or Congo red.[18,19] Trimming for thin sectioning was oriented by light and polarization microscopy of the thick sections. Thin sections were cut on an LKB Ultratome with glass knives, stained with lead citrate (with or without uranyl acetate),[18] and examined in a Siemens Elmiskop I electron microscope or in an RCA-EMU-3G electron microscope at initial magnifications usually of 2,000–20,000 (and of 40,000–160,000 for high-resolution studies). This report is based on the analysis of more than 1000 micrographs from the present study and also on the review of many micrographs from our previous studies.

Results

General Observations

Except for rare occasions, the amyloid accumulated along the mesangial matrix and the basement membrane. The endothelial-, epithelial-, and mesangial-cell changes will be individually described. Although amyloid fibrils were identifiable in all sections, the distribution was not always diffuse but was occasionally focal and nodular leaving some portions of the capillary wall intact even in the heaviest lesions. The mesangial areas were characteristically more heavily involved. The capillary lumen and the urinary space retained their over-all structural integrity, though they were often narrowed by the dislocation of the endothelial and the epithelial cells secondary to the space-occupying amyloid deposits (Fig. 1 and 14).

Mesangium

Whether the lesions were small (and presumably early) or large, amyloid fibrils were found predominantly in the mesangial area: between mesangial cells, between the mesangial cell and the endothelial cell, and between the mesangial cell and the basement membrane. The mesangial matrix (the loose basement membrane-like component of the mesangium), therefore, often became difficult to distinguish from the accumulated amyloid, since the matrix contains fine fibrous structures, and since the accumulation of amyloid fibrils was usually associated with some amorphous substance (Fig. 1, 2, 5, 7, 8, and 14).

Although the mesangial cells generally had a normal distribution, three types could be defined on a basis of their ultrastructural cytology.

Normal or Resting Mesangial Cell. This type of mesangial cell has been defined in the literature and is characterized by its irregularly shaped nucleus, scanty cytoplasm, numerous long slender cytoplasmic processes, and relatively well-developed cell organelles.[15,20–22]

Active Mesangial Cell. The first modified type of mesangial cell appears to be a functionally active cell, and has been seen in most cases of human and experimentally induced amyloid. The nucleus appears smooth and somewhat lobulated, and contains rich and normally condensed chromatin. The cytoplasm is abundant. Free ribosomes are numerous and are often arranged in polysomal patterns. Granular and agranular endoplasmic reticulum are well developed and often dilated. The Golgi complex is prominent and often multiple. The mitochondria are relatively small and elongated (Fig. 1–6). Lipid droplets have also been found, probably more frequently than is normal (Fig. 1 and 2).

Groups of amyloid fibrils have been found to be highly oriented when they were adjacent to the "active" mesangial cell. When observed in

longitudinal section, these amyloid fibrils have been oriented as a bundle and perpendicularly or radially to the plasma membrane. In most such areas, the plasma membrane was indistinct and often invaginated to make a variably sized "pocket" (Fig. 2–6). Intracellularly, adjacent to such an area, numerous small vesicles often containing moderately electron-dense material (usually homogeneous, but occasionally granular or filamentous) were often observed. Sometimes they appeared to be attached to the cell membrane and to open at the cell surface. These areas also, on occasion, appeared to be related to the presence of a large Golgi complex and well-developed endoplasmic reticulum (Fig. 3, 4, and 6). Dilated cisternae of the endoplasmic reticulum were also seen close by these sites (Fig. 2–4). The portion of the cytoplasm adjacent to the particular site where the plasma membrane and amyloid fibril were in close apposition was often of slightly greater density owing to aggregation of dense, finely granular or filamentous material in the cytoplasm (Fig. 2, 3, and 4). Intracellular membrane-surrounded amyloid fibrils were often seen in this cell type. These fibrils were also highly oriented and the aforementioned close structural relationships were noted between the membrane surrounding the fibrils and the amyloid fibrils (Fig. 3).

These observations on the mesangial cell were made more frequently and were more prominent in the cases of experimentally induced amyloidosis than the human ones.

Degenerating or Phagocyting Mesangial Cell. The other cytologic form of the mesangial cell was found only in the human cases and is distinct from the resting mesangial cell and the active mesangial cell. Its nucleus is irregular in shape and contains a relatively pronounced condensation of chromatin just beneath the nuclear envelope. The cytoplasm, which has slightly increased density, is reduced in volume but shows many long projections into the surrounding matrix and amyloid. The number of free ribosomes is normal or reduced. The profiles of endoplasmic reticulum are few, and the Golgi complex is insignificant to the point of vanishing. The mitochondria appear to be increased in number in a given area of the cytoplasm when compared to the other two types of mesangial cells; the mitochondrial matrix has an increased density. Numerous cytoplasmic vacuoles are present and some of them show the myelin-like figures. Groups of amyloid fibrils have been often seen in this cell type surrounded by a single membrane or engulfed in the finger-like cytoplasmic projections. In this instance, however, the amyloid fibrils have shown neither the high orientation nor the specific relation with the plasmalemma described above in relation to the active mesangial cell (Fig. 7 and 8).

Endothelium

The subendothelial accumulation of amyloid fibrils has also been observed in all cases studied. These deposits have generally been larger and more frequent in areas adjacent to the mesangium (Fig. 1, 9, 10, and 12–14). On occasion, in the subendothelial portion far from the mesangium, the amyloid fibrils have been observed to accumulate in isolation from the juxtamesangial amyloid. This has not only been seen in the usual two-dimensional view, but also has been verified by means of serial sections. In a few instances this type of accumulation has consisted of only a small number of amyloid fibrils (Fig. 11).

The endothelial cells were attached to each other as expected, contained fenestrae, and covered the entire capillary surface, even when considerable amyloid was present. On occasion, the endothelial cell appeared to be detaching from the subendothelial amyloid layer and the fenestra between them was abnormally wide. However, defects of any endothelial cell in the array covering the capillary surface have been extremely rare (Fig. 1, 5, 8–10, 12–14, and 19). In rare instances, the amyloid fibrils appeared to fill the abnormally widened fenestra between the endothelial cells (Fig. 13). No amyloid fibril has been identified with certainty in the capillary lumen during the present study, although occasional fine filamentous, as well as granular, components have been seen in the capillary lumen. Vacuolation and reticulation of the endothelial cytoplasm have been frequently seen in human cases (Fig. 8, 10, 13, and 19), but not in the experimentally induced amyloid renal lesions (Fig. 1, 9, and 12).

The character of the organelles of the endothelial cell has also often suggested that it is functionally more active than normal and is reminiscent of the appearance of the mesangial organelle proliferation (Fig. 1, 9, 10, and 12). Relationships similar to those of the "active" mesangial cell and the tufts of amyloid fibrils have also been found between the "active" endothelial cell and the amyloid fibrils; i.e., definite orientation of the amyloid fibrils, indistinctness and invagination of the plasma membrane, and the intracellular presence of the membrane-surrounded amyloid fibrils. In comparison to the active mesangial cell, however, the invagination of the endothelial plasma membrane in any particular site has generally been shallow, and the intracellular presence of the membrane-surrounded amyloid fibrils has been observed only rarely. Accumulation of the finely granular or filamentous dense material in the periphery of the cytoplasm is comparable in nature but less pronounced than in the epithelial and the mesangial cells (Fig. 9, 10, and 12).

Epithelium

When amyloid fibrils accumulated in the subepithelial area, they showed cellular-extracellular relationships comparable to those of the subendothelial deposits. Amyloid again was seen with greater frequency and in larger amounts in the areas adjacent to the mesangium, while distant areas were less involved. In many locations the subepithelial amyloid fibrils were fewer than those near mesangial and endothelial areas (Fig. 1, 9, 12, 14, and 20). On the other hand, in human cases (though rarely in the rabbits) a larger accumulation of amyloid fibrils was occasionally found below the epithelial cells (Fig. 14, 15, 18, and 19).

Although some epithelial cells were intact in the diseased glomerulus, most showed significant changes. One of the consistent changes has been in the structure of the foot processes which were fused when in contact with the subepithelial amyloid fibrils even when the accumulation was extremely small. In the neighboring area free of fibrils, however, the processes were usually normal (Fig. 1, 2 and 14). In the periphery of the epithelial cytoplasm adjacent to the subepithelial amyloid accumulation, indistinctly defined, irregular aggregations of finely granular or filamentous dense material were usually present. This material appeared similar in its quality to what is found in the normal epithelial cell, as well as in the active mesangial and endothelial cells as described above, but was of much larger quantity. This aggregation has also often surrounded the membrane-bound intracellular amyloid fibrils (Fig. 14–18).

The epithelial cells often demonstrated increased numbers of free ribosomes, as well as polysomes, rough and smooth endoplasmic reticulum, and other changes associated with increased cellular activity. These changes were quite comparable to, though less frequently seen than, those of the active mesangial and endothelial cells. The intimate relation of amyloid fibrils to epithelial plasmalemma characterized by invagination and fuzziness of the cell membrane, as well as condensation of surrounding cytoplasm, was again noted. Here, however, the invagination was often deeper than that previously described (Fig. 14–18). In several remarkable sections, the amyloid fibrils seemed to pass almost from endothelial cell to epithelial cell, bridging the basement membrane (Fig. 20).

In some sections from a case of experimentally induced amyloidosis (after 4 months of casein injections), fibrils structurally quite comparable to amyloid were found in the urinary space. These fibrils, on occasion, have shown sufficient orientation to make a bundle, perpendicular in position to the indistinct and sometimes invaginated plasma

membrane of the epithelial cell (Fig. 14 and 16). Precipitation of fibrin (or fibrin-like material) and other unidentified fibrous structures was also occasionally seen in the urinary space.

Basement Membrane

The portions of the basement membrane free of amyloid were normal. The remainder (at least the lamina densa) was usually well maintained even when it was embedded in a mass of amyloid fibrils. Occasionally, it appeared to be dissociated by the heavy accumulation of the amyloid fibrils and, rarely, it was indistinguishable due to the enormity of the amyloid deposit (Fig. 1, 2, 5, 8–16, and 18–20). At higher magnifications, the finding that the amyloid fibrils passed through the basement membrane was common. In such areas the amyloid fibrils and the basement membrane were clearly differentiated from each other, and no significant change occurred in the basic ultrastructure of each component—the fibrous structure of amyloid and the finely granular and filamentous nature of the basement membrane (Fig. 20).

Ultrastructure of Amyloid Fibrils

The amyloid fibrils in tissue sections were arranged in random array as thin, rigid, and nonbranching rods. In the longitudinal view, the amyloid fibril, which cannot easily or accurately be resolved into subunits by conventional electron microscopic examination of tissue sections, has measured 100 Å in diameter, but occasionally has had dimensions of 200–300 Å (Fig. 21). The length of the fibrils has been found to vary widely from several hundred angstroms to longer than 1 μ (Fig. 20).

On conventional electron micrographs (Fig. 1–20), it has seemed almost impossible to find any regular pattern in subunit structure of amyloid fibrils, even on the micrographs which clearly resolved the unit structure of the plasma membrane. For example, beaded figures were seen in some portions, crossbanding structures were suggested in other areas, and double or multiple longitudinal subunit strands were noted on other portions of the fibrils.

On the other hand, high-resolution electron microscopy of the ultrathin tissue sections which were intensely stained consistently demonstrated a uniform subunit structure in the amyloid fibrils in all cases of experimental and human amyloidosis. In a cross section, this structure was made up of amyloid filaments—circular structures of about 80-Å diameter which appeared singly or aggregated to form fibrils.[5,23–26] Occasionally on this filament, several apparently round subunits 25–35 Å in dimension were delineated. These ring-like structures have been found alone, though occasional pairs or aggregates appeared. When they were

grouped, the center-to-center distance of the ring-like structures measured 80–100 Å (inserts, Fig. 21). In the longitudinal section of the amyloid fibril, 25- to 35-Å-wide linear structures with about 35- to 50-Å periodical beading (which are comparable to amyloid protofibrils[24]) have been often resolved (Fig. 21).

Discussion

Localization of Amyloid Within the Glomerulus

It is generally accepted that amyloid accumulates primarily in the mesangial matrix and along the basement membrane, although minor differences in details of its appearance are reported.[2–16] In the human glomerulus, Suzuki *et al.*[15] described the early deposition of amyloid fibrils to be both in the mesangial matrix and along the basement membrane, though predominantly in the former. Since then, similar observations were made on human amyloidotic glomeruli,[8] as well as experimental ones.[5,9,13,14] In addition, Cohen [5] described the intimate structural relationships of the tufts of amyloid fibrils to the mesangial cell, as well as to the endothelial and epithelial cells, and Shimamura and Sorenson [13] found by serial sectioning that the subendothelial amyloid deposit which appeared to be isolated in a single section had continuity with larger deposits in the mesangium.

The present observations generally support those previous results and emphasize that the mesangial area is morphologically the primary area associated with the deposition of amyloid fibrils. However, the present results also suggest the possibility, especially in the human material, that amyloid fibrils deposit in other areas (subendothelial and even subepithelial), independently from the mesangial deposits. Thus, though serial sectioning has clearly demonstrated the continuity of an amyloid subendothelial deposit with mesangial amyloid,[13] the concept of an isolated amyloid deposit in the subepithelial area (as suggested by Trump and Benditt[16]) may also be a reality. The differences in the results and the interpretations on the localization of amyloid which exist among the different studies are understandable when the following are taken into consideration: (1) the studies prior to Suzuki *et al.*[15] (and some after this[2]) were made without the clear-cut acceptance of the ultrastructure of the mesangium; (2) as will be emphasized below, both the production or accumulation of, and degradation or absorption of amyloid may constantly and simultaneously be in a state of delicate balance in the tissue and involve a variety of cells simultaneously; and (3) the acute appearance of amyloidosis may be easy to observe in experimentally induced amyloidosis, but difficult in human cases in which the chronic accumulation is the situation most often studied.

Unusual areas of localization of amyloid within the glomerulus have also been noted. Sorenson and Shimamura [14] interpreted one micrograph as showing large amounts of amyloid within the vascular spaces. Although the possibility of the existence of amyloid in the capillary lumen cannot be denied, this has not been observed during the present study. Hinglais and coworkers [8,9] reported the amyloid within the urinary space in both experimental and human cases. In the present study fibrils which appeared structurally quite comparable to amyloid were seen within the urinary space in one case of experimentally induced amyloidosis. One also could speculate from the present results that the entry of amyloid fibrils into the urinary space may be a possibility (Fig. 16 and 19). However, some doubt still remains concerning the exact identification of these fibrils as amyloid: this clearly is difficult on the basis of electron microscopic examination of tissue sections alone. To verify whether the existence of amyloid within the capillary lumen or the urinary space is a reality or not, more extensive studies (other than morphologic) will be needed. The evidence for the intracellular amyloid fibrils will be discussed later.

Ultrastructural Aspects of Glomerular Cells

Few details of the ultrastructure of the cells of the amyloidotic glomerulus have been reported. During the present study each of the three types of glomerular cells was found to demonstrate three different cytologic patterns. These are interpreted by their ultrastructural cytology as normal or resting cells, active cells, and degenerating or phagocyting cells. Some of these variations may be found in the endothelial, epithelial, and mesangial cells even under normal circumstances,[15,16,20–22] but the marked nature and distinctive appearance of the changes appeared as a pattern in amyloid disease and are therefore reported in more detail.

The apparently normal or resting cells are those seen in the controls, as well as in those with amyloid disease and are as described in the section on *Results*. Occasionally minor degenerative changes or an increasing development of intracellular organelles is seen.

The active cells—whether they are mesangial, endothelial, or epithelial—contain an abundance of free ribosomes, polysomal aggregates, endoplasmic reticulum, and a Golgi apparatus. This type of organelle system has been associated with the synthesis and intracellular migration of macromolecules of various types.[27–32] One cannot draw any specific conclusions from these data concerning the synthesis of the amyloid fibril except to point out that all these cells, and the mesangial cell in particular, have the necessary synthetic apparatus to manufacture amyloid—if it is indeed produced in situ. It should be noted that, however,

other studies have demonstrated more definitively that in-situ formation, at least in the spleen, takes place.[33] While all the amyloid in the kidney could be there on a basis of its being trapped, its morphologic relation to the cells make this an unlikely possibility. In addition, the distribution of the active-cell population appears to shift almost in parallel with the apparent velocity of progressive amyloid accumulation. That is, the active cells are found frequently and with greater signs of activity in the glomerulus of those rabbits with acute experimentally induced amyloidosis but infrequently in chronic slowly progressive human amyloidosis.

During the present study, the inclusion-containing (phagocytic) mesangial cells have been seen only in the human kidneys. These mesangial cells have the ultrastructural features usually associated with phagocytic activity (Fig. 7). The phagocytic activity of the mesangial cell is not unexpected or specific in this instance, but is predictable from the published literature.[15,20–22] Possible evidence of degenerative changes in the cells, i.e., vacuolation and reticulation of cytoplasm, may be considered as secondary or nonspecific changes associated with amyloidosis for they have been seen in various conditions other than amyloidosis.[16,20,34–38] In addition, they are seen primarily in chronic amyloidosis (in humans) and not in the acute experimentally induced situation.

Thus, it would seem that a reasonable sequence of events is the stimulation—for reasons not completely understood—of resting mesangial (and possibly endothelial and epithelial) cells to become actively synthetic cells, presumably engaged in the production of amyloid fibril precursors and possibly of the fibrils themselves. As the process becomes established, chronic changes take place, including encroachment of the amyloid on the normal cellular function with destruction of some cells and increasing phagocytosis of amyloid by others. In general, simultaneous existence of different phases of cells of the same origin in a tissue, as well as metamorphosis of a cell into the different types, is not unusual and has been investigated in fibrogenesis of collagen and elastin under normal and pathologic conditions.[30,39–42]

Aspects of Amyloid Origin and Absorption Within Glomerulus

The genesis of amyloid has not yet been completely clarified. While many factors have been considered, two that have been of great interest to morphologists have been (1) the concept that a precursor of amyloid exists in plasma and passes through vascular walls to accumulate extravascularly, and (2) the amyloid is formed and deposits in situ. These data have been reviewed[5] and the latter concept has been supported by previous work in our laboratory.[33]

The location of amyloid within the glomerulus itself may be interpreted in various ways. For example, amyloid is not only localized adjacent to the cells, suggesting their intimate relationship to the amyloid formation, but its location along the mesangial matrix and the basement membrane could be considered as a suggestion of its plasma origin, for this is also a well-known site of deposition of such plasma-borne materials.[15,16,20–22,34–38]

The first significant observation concerning this point is the intimate relationship observed between specific areas of the plasmalemma and the amyloid fibrils. This phenomenon may represent a site either of the formation or of the phagocytosis of amyloid fibrils by the cell. The process of amyloid fibril formation may be more likely than phagocytosis for the following reasons: (1) the cell which participates in this phenomenon usually has the active-appearing cytologic characteristics with abundant cell organelles that suggest great synthetic activity; (2) the cell usually lacks the characteristics of the phagocytic cell in its paucity of lysosomal bodies; (3) the plasma membrane (or the membrane surrounding the "intracellular" amyloid fibrils) appears indistinct in these particular sites, in contrast with endocytic processes where the plasma membrane (or the membrane of endocytic vesicle or vacuole) is usually found intact; and (4) the amyloid fibrils are quite regularly arranged in the site.

The intimate structural relationships between the plasma membrane and the tuft of amyloid fibrils such as described in the present study were previously observed in the kidney,[2,5,8,9,14,16] as well as in other organs.[5,43–47] Except for Bergstrand who considered them to be artifacts,[2] most investigators have agreed and interpreted them as evidence of the local site of amyloid production. This concept was substantiated by the study which demonstrated in-situ amyloid fibril production in a spleen explant system by light and electron microscopic autoradiographic techniques.[33] If one may accept this structural evidence for local amyloid production, the idea that all three types of glomerular cells—the mesangial, endothelial, and epithelial cells—participate in amyloid production is also a possibility, though the mesangial cell activity and role in apparent synthesis is most clear-cut.

A feature of interest is the indistinctness of the plasma membrane at the particular site of apparent amyloid formation. Similar ultrastructural features have also been a point of discussion in studies of collagen fibrillogenesis.[30,32,48–50] Whether the membrane is truly altered significantly and momentarily ceases to exist at the site of exit of asymmetric macromolecules such as amyloid or collagen, or whether its fuzziness is an artifact of sectioning at these sites, is not completely clear. In our

studies of both collagen and amyloid fibrillogenesis the latter protein is more consistently seen in this intimate relationship to the plasma membrane.

The second close relationship of cell and fibril is that of the membrane-surrounded amyloid fibrils found within the cytoplasm of the active cells. These are most likely the result of cross sections or oblique sections of the invagination of the amyloid to the body of the cell. This is supported by the great orientation of amyloid fibrils, indistinctness of a part of the surrounding membrane, and the dense accumulation surrounding this structural unit (often seen in the epithelial cells). This type of intracellular amyloid fibril accumulation is distinct from other types either in phagocytic vacuoles or in the cytoplasm without any surrounding membrane (and which may or may not be amyloid).[33,43,44,46,47,51] The latter type of intracellular fibrils were not found in the glomerular cells in the present study or in previously reported studies of the kidney, while they have been found in the cells of spleen and liver by several investigators. In our experience, for example, intracytoplasmic fibrils which have been found in the reticuloendothelial cells of the spleen were structurally strikingly similar to the extracellular amyloid fibrils.[33] This phenomenon has not been seen in the glomerular cells even of the same rabbit. In the present study, other filamentous structures found in the cytoplasm with no surrounding membrane appear by morphologic criteria not to be identical with amyloid fibrils.

The process of amyloid reabsorption has been proposed and phagocytosis may be the major method for its disposal or at least the best studied morphologically.[5,52] Indeed, phagocytosis of amyloid has been suggested in other organs,[33,43,44,46,47] and the phagocytic activity of the mesangial cell is rather well known.[15,20–22] Thus, the presence of the "phagocytic" cells in the present study is not surprising and is presumed to be associated with the phagocytosis of amyloid fibrils, despite the absence of such documentation in previous studies.

Basement Membrane in Glomerular Amyloidosis

The majority of previous investigators have interpreted their data as suggesting that the basement membrane (lamina densa) is not primarily involved in amyloidosis, but is secondarily infiltrated by heavy amyloid deposition.[4–12,14] Bergstrand and co-workers, however, believed that primary involvement of the basement membrane in amyloidosis occurs.[2,3] The present observations support the former concept that the basement membrane is intact. Even in the heavy deposits of amyloid fibrils, the basic structural components of the basement membrane could be demonstrated on most occasions when adequate staining was applied, and the

ultrastructure of the amyloid fibril and the basement membrane can be distinctly identified even in the portions where both structures are enmeshed (Fig. 20).

Ultrastructure of Amyloid Fibrils

Roughly 100 Å has been accepted as the diameter of amyloid fibrils in tissue section by most investigators, although some variation in the measurements, i.e., 50–300 Å, has been found in various reports.[1–16,33,43–47,51,53–63] Based on the observations of the tissue sections, the elucidation of subunit structure of the amyloid fibrils has been attempted by several investigators. A beaded structure was suggested by Cohen and Calkins,[1] and similar observations were also reported by Suzuki *et al.*,[15] Heefner and Sorenson,[46] and Bradbury and Micklem.[54] Gueft and Ghidoni [45] conceived of the amyloid fibril as being composed of two linear components about 25 Å each plus a 25-Å interspace, giving a diameter of 75 Å, and this idea was basically accepted in the investigations of Sorenson, Heefner, and Kirkpatrick; [47] Manitz and Themann; [51] and Terry, Gonatas, and Weiss.[60] Recently, Merker *et al.* reported a banding structure with a periodicity of approximately 55 Å occasionally seen in the amyloid fibrils.[62,63] On the other hand, some investigators noted that no periodicity could be found in amyloid fibrils.[43,44]

After extensive electron microscopic observations of the amyloid fibrils after isolation, as well as in the tissue sections, the present authors have proposed the following configuration.[5,23–26] The amyloid fibril—the fibrous component of amyloid as seen in conventional electron microscopy—consists of a number of filaments assembled side by side. The amyloid filaments are approximately 75–80 Å in diameter and each consists of several subunits—amyloid protofibrils—which are arranged in parallel to each other and almost longitudinally to the long axis of the filament surrounding a central core of 15–20 Å in diameter. The amyloid protofibril is about 25–35 Å in width and appears to consist of 2 or 3 subunit strands helically arranged with a repeat of 35–40 Å (or less likely is composed of globular subunits aggregated end to end). These amyloid subprotofibrillar strands measure approximately 10–15 Å in diameter insofar as they can be discerned by high-resolution electron microscopy.

In the present study, the tissue section analyses of the fibrils (Fig. 21) correspond quite well with the above-mentioned configuration of amyloid fibrils. This ultrastructural organization which we propose [24] is not contradictory to any of the previous observations reported from various laboratories, but rather, in general agreement with all of them. That is, the microtubular structure of the amyloid filament could be demon-

strated on a micrograph as described by Gueft and Ghidoni[45] and by others, and the filament (an assembly of several protofibrils which have a fairly regular structural repeat interval of 35–50 Å) might appear as a beaded or banded structure in some portions of its length. These details are discussed elsewhere,[24] but it is possible to construct models which explain the varying dimensions reported to date, based on the type of staining, the positioning, and so forth.

Thus, due to their complex structure, the amyloid fibrils are not always uniform in appearance though most of them measure roughly 100 Å in width. Their subfibrillar composition is quite difficult to demonstrate by conventional electron microscopy. On the other hand, many other fibrous components exist in tissue which may or may not be related to the amyloid fibril.[64–66] Therefore, a cautious approach should be taken in identifying small numbers of isolated fibrils in tissue or fibrils in an exceptional location.

Summary

1. The ultrastructure of the glomerulus has been studied in 10 cases of human and 6 cases of experimentally induced rabbit amyloidosis.

2. The relationship of the amyloid fibrils to the basement membrane and to the endothelial, epithelial, and mesangial cells is described. The mesangial cell demonstrated the most intimate relation to amyloid fibrils and occurred in 3 forms, i.e., resting, active, and phagocyting (or degenerating).

3. The possible site of formation of amyloid and its structure in tissue section is discussed.

References

1. Cohen, A. S., and Calkins, E. Electron microscopic observations on a fibrous component in amyloid of diverse origins. *Nature (London) 183:*1202–1203, 1959.
2. Bergstrand, A. Discussion to Caesar, R.[44] *Novo Acta Leopoldina 175:*106–111, 1966.
3. Bergstrand, A., and Bucht, H. Electron microscopy and renal function in amyloidosis of the kidneys. *J Path Bact 81:*495–503, 1961.
4. Caesar, R. Elektronenmikroskopische Beobachtungen bei der Nierenamyloidose des Goldhamsters. *Frankfurt Z Path 72:*506–516; 1963.
5. Cohen, A. S. "The Constitution and Genesis of Amyloid." In *International Review of Experimental Pathology* (Vol. 4), Richter, G. W., and Epstein, M. A., Eds. Acad. Press, New York, 1965, pp. 159–243.
6. Cohen, A. S., and Calkins, E. A study of the fine structure of the kidneys in casein-induced amyloidosis in rabbits. *J Exp Med 112:*479–490, 1960.
7. Cohen, A. S., Frensdorff, A., Lamprecht, S., and Calkins, E. A study of the fine structure of the amyloid associated with familial Mediterranean fever. *Amer J Path 41:*567–578, 1962.

8. HINGLAIS, N., and DEMONTERA, H. Étude au microscope électronique de six cas d'amylose rénale humaine. *Path Biol (Paris) 12:*176–191, 1964.

9. HINGLAIS, N., ZWEIBAUM, A., and RICHET, G. Les lésions précoces de l'amylose expérimentale du hamster. Étude au microscope électronique. *Nephron 1:* 16–30, 1964.

10. HJORT, G. H., and CHRISTENSEN, H. E. Histochemical and electron-microscopic investigation of experimental amyloidosis. *Acta Rheum Scand 7:*62–64, 1961.

11. HJORT, G. H., and CHRISTENSEN, H. E. Electron-microscopic investigations on secondary amyloidosis. *Acta Rheum Scand 7:*65–68, 1961.

12. MOVAT, H. Z. The fine structure of the glomerulus in amyloidosis. *Arch Path (Chicago) 69:*323–332, 1960.

13. SHIMAMURA, T., and SORENSON, G. D. Experimental amyloidosis. V. Relationship between experimental glomerular amyloid and the mesangial region. *Amer J Path 46:*645–656, 1965.

14. SORENSON, G. D., and SHIMAMURA, T. Experimental amyloidosis. III. Light and electron microscopic observations of renal glomeruli. *Lab Invest 13:* 1409–1417, 1964.

15. SUZUKI, Y., CHURG, J., GRISHMAN, E., MAUTNER, W., and DACHS, S. The mesangium of the renal glomerulus: Electron microscopic studies of pathologic alterations. *Amer J Path 43:*555–578, 1963.

16. TRUMP, B. F., and BENDITT, E. P. Electron microscopic studies of human renal disease: Observation of normal visceral glomerular epithelium and its modification in disease. *Lab Invest 11:*753–781, 1962.

17. COHEN, A. S., CALKINS, E., and LEVENE, C. I. Studies on experimental amyloidosis. I. Analysis of histology and staining reactions of casein-induced amyloidosis in the rabbit. *Amer J Path 35:*971–989, 1959.

18. PEASE, D. C. *Histological Techniques for Electron Microscopy* (ed. 2). Acad. Press, New York, 1964.

19. SHIRAHAMA, T., and COHEN, A. S. A Congo red staining method for epoxy-embedded amyloid. *J Histochem Cytochem 14:*725–729, 1966.

20. BENCOSME, S. A., and BERGMAN, B. J. "The Ultrastructure of Human and Experimental Glomerular Lesions." In *International Review of Experimental Pathology* (Vol. 1), Richter, G. W., and Epstein, M. A., Eds. Acad. Press, New York, 1962, pp. 139–208.

21. FARQUHAR, M. G., and PALADE, G. E. Functional evidence for the existence of a third cell type in the renal glomerulus. Phagocytosis of filtration residues by a distinctive "third cell." *J Cell Biol 13:*55–87, 1962.

22. KURTZ, S. M. "The Kidney" In *Electron Microscopic Anatomy,* Kurtz, S. M., Ed. Acad. Press, New York, 1964, pp. 239–265.

23. SHIRAHAMA, T., and COHEN, A. S. Structure of amyloid fibrils after negative staining and high-resolution electron microscopy. *Nature (London) 206:* 737–738, 1965.

24. SHIRAHAMA, T., and COHEN, A. S. High-resolution electron microscopic analysis of the amyloid fibril. *J Cell Biol 33:*679–708, 1967.

25. SHIRAHAMA, T., and COHEN, A. S. Subunit structure of the human amyloid fibril. (abst.) *Fed Proc 26:*300, 1967.

26. SHIRAHAMA, T., and COHEN, A. S. High resolution electron microscopic analysis of the amyloid fibril isolated from rabbits. In preparation.

27. CARO, L. G., and PALADE, G. E. Protein synthesis, storage, and discharge in the pancreatic exocrine cell: An autoradiographic study. *J Cell Biol 20:* 473–495, 1964.

28. FEWER, D., THREADGOLD, J., and SHELDON, H. Studies on cartilage. V. Electron microscopic observations on the autoradiographic localization on S^{35} in the cells and matrix. *J Ultrastruct Res 11:*166–172, 1964.

29. HAY, E. D., and REVEL, J. P. Autoradiographic studies of the origin of the basement lamella in Ambystoma. *Develop Biol 7:*152–168, 1963.

30. PORTER, K. R. Cell fine structure and biosynthesis of intercellular macromolecules. *Biophys J 4:*167–196, 1964.

31. REVEL, J.-P., and HAY, E. D. An autoradiographic and electron microscopic study of collagen synthesis in differentiating cartilage. *Z Zellforsch 61:*110–144, 1963.

32. ROSS, R., and BENDITT, E. P. Wound healing and collagen formation. V. Quantitative electron microscope radioautographic observations of proline-H^3 utilization by fibroblasts. *J Cell Biol 27:*83–106, 1965.

33. COHEN, A. S., GROSS, E., and SHIRAHAMA, T. The light and electron microscopic autoradiographic demonstration of local amyloid formation in spleen explants. *Amer J Path 47:*1079–1111, 1965.

34. COMERFORD, F. R., and COHEN, A. S. The nephropathy of systemic lupus erythematosus: An assessment by clinical, light and electron microscopic criteria. *Medicine* In press.

35. FELDMAN, J. D., HAMMER, D., and DIXON, F. J. Experimental glomerulonephritis. III. Pathogenesis of glomerular ultrastructural lesions in nephrotoxic serum nephritis. *Lab Invest 12:*748–763, 1963.

36. MOVAT, H. Z., STEINER, J. W., and HUHN, D. The fine structure of the glomerulus in acute glomerulonephritis. *Lab Invest 11:*117–135, 1962.

37. PARDO, V., and SHAPIRO, A. P. Ultrastructural glomerular lesions produced by synthetic polysaccharides. *Lab Invest 15:*617–628, 1966.

38. STRUNK, S. W., HAMMOND, W. S., and BENDITT, E. P. The resolution of acute glomerulonephritis: An electron microscopic study of four sequential biopsies. *Lab Invest 13:*401–429, 1964.

39. HAUST, M. D., MORE, R. H., BENCOSME, S. A., and BALIS, J. U. Elastogenesis in human aorta: An electron microscopic study. *Exp Molec Path 4:*508–524, 1965.

40. PARKER, F., and ODLAND, G. F. A correlative histochemical, biochemical and electron microscopic study of experimental atherosclerosis in the rabbit aorta with special reference to the myo-intimal cell. *Amer J Path 48:*197–239, 1966.

41. ROBINSON, R. A., and CAMERON, D. A. "Bone." In *Electron Microscopic Anatomy,* Kurtz, S. M., Ed. Acad. Press, New York, 1964, pp. 315–340.

42. SHELDON, H. "Cartilage." In *Electron Microscopic Anatomy,* Kurtz, S. M., Ed. Acad. Press, New York, 1964, pp. 295–313.

43. BATTAGLIA, S. Elektronenoptische Untersuchungen am Leberamyloid der Maus. *Beitr Path Anat 126:*300–320, 1962.

44. CAESAR, R. Elektronenmikroskopische Befunde am Amyloid. *Novo Acta Leopoldina, 175:*87–97, 1966.

45. GUEFT, B., and GHIDONI, J. J. The site of formation and ultrastructure of amyloid. *Amer J Path 43:*837–854, 1963.

46. HEEFNER, W. A., and SORENSON, G. D. Experimental amyloidosis. I. Light and electron microscopic observations of spleen and lymph nodes. *Lab Invest 11:*585–593, 1962.

47. SORENSON, G. D., HEEFNER, W. A., and KIRKPATRICK, J. B. Experimental amyloidosis. II. Light and electron microscopic observations of liver. *Amer J Path 44:*629–644, 1964.

48. CHAPMAN, J. A. Morphological and chemical studies of collagen formation. I. The fine structure of guinea pig granulomata. *J Biophys Biochem Cytol 9:*639–651, 1961.

49. GOLDBERG, B., and GREEN, H. An analysis of collagen secretion by established mouse fibroblast lines. *J Cell Biol 22:*227–258, 1964.

50. YARDLEY, J. H., HEATON, M. W., GAINES, L. M., JR., and SHULMAN, L. E. Collagen formation by fibroblasts. Preliminary electron microscopic observations using thin sections of tissue cultures. *Bull Hopkins Hosp 106:*381–393, 1960.

51. MANITZ, G., and THEMANN, H. Elektronenmikroskopischer Beitrag zur Feinstructur menschlichen Leberamyloids. *Beitr Path Anat 128:*103–121, 1963.

52. RICHTER, G. W. The resorption of amyloid under experimental conditions. *Amer J Path 30:*239–261, 1954.

53. ALBORES-SAAVEDRA, J., ROSE, G. G., IBANEZ, M. L., RUSSELL, W. O., GREY, C. E., and DMOCHOWSKI, L. The amyloid in solid carcinoma of the thyroid gland: Staining characteristics, tissue culture, and electron microscopic observations. *Lab Invest 13:*77–93, 1964.

54. BRADBURY, S., and MICKLEM, H. S. Amyloidosis and lymphoid aplasia in mouse radiation chimeras. *Amer J Path 46:*263–277, 1965.

55. CAESAR, R. Die Feinstruktur von Milz und Leber bei experimenteller Amyloidose. *Z Zellforsch 52:*653–673, 1960.

56. CAESAR, R. Elektronenmikroskoptische Untersuschungen an menschlichem Amyloid bei verschiedenen Grundkrankheiten. *Path Microbiol (Basel) 24:* 387–396, 1961.

57. COHEN, A. S., WEISS, L., and CALKINS, E. Electron microscopic observations of the spleen during the induction of experimental amyloidosis in the rabbit. *Amer J Path 37:*413–431, 1960.

58. FRUHLING, L., KEMPF, J., and PORTE, A. Structure et formation de la substance amyloïde dans l'amylose expérimentale de la souris. Étude au microscope électronique. *CR Acad Sci (Paris) 250:*1385–1386, 1960.

59. LETTERER, E., CAESAR, R., and VOGT, A. Studien zur elektronenoptischen und immun-morphologischen Struktur des Amyloids. *Deutsch Med Wschr 85:*1909–1910, 1960.

60. TERRY, R. D., GONATAS, N. K., and WEISS, M. Ultrastructural studies in Alzheimer's presenile dementia. *Amer J Path 44:*269–297, 1964.

61. THIERY, J. P., and CAROLI, J. Étude comparative en microscopie électronique de l'amylose hépatique primaire humaine et de l'amylose expérimentale de la souris. *Rev Int Hepat 12:*207–251, 1962.

62. MERKER, H. J., SHIBOLET, S., SOHAR, E., GAFNI, J., and HELLER, H. Periodic cross-banding in amyloid filaments. *Nature (London) 211:*1401–1402, 1966.

63. GAFNI, J., MERKER, H. J., SHIBOLET, S., SOHAR, E., and HELLER, H. On the origin of amyloid: Study of an amyloid tumor in multiple myeloma. *Ann Intern Med 65:*1031–1044, 1966.

64. GREENLEE, T. K., JR., ROSS, R., and HARTMAN, J. L. The fine structure of elastic fibers. *J Cell Biol 30:*59–71, 1966.

65. HAUST, M. D. Fine fibrils of extracellular space (microfibrils). Their structure and role in connective tissue organization. *Amer J Path 47:*1113–1137, 1965.

66. Low, F. N. Microfibrils: Fine filamentous components of the tissue space. *Anat Rec 142*:131–137, 1962.

The expert photographic assistance of Mr. David Feigenbaum is gratefully acknowledged. The authors would like to thank Dr. Ronald Miller who performed several of the renal biopsies and Dr. Kenneth Brandt who assisted in obtaining various tissues for study.

Legends for Figures

Key:

A	amyloid fibrils	G	Golgi complex
BC	Bowman's capsule	I	inclusion
BM	basement membrane	L	lipid droplet
C	collagen	Leu	leukocyte
CL	capillary lumen	M	mitochondrion
D	granular or filamentous dense material	Mes	mesangial cell
		MM	mesangial matrix
End	endothelial cell	My	myelin figure
Ep	epithelial cell	N	nucleus
F	filamentous figure	RBC	red blood cell
Fe	fenestrae between endothelial cells	Ur	urinary space

FIG. 1. Experimentally induced rabbit amyloidosis. Survey electron micrograph showing moderately severe glomerular lesion. All components of glomerulus are present. Amyloid fibrils appear along mesangial matrix and basement membrane. They are prominent between mesangial cells, between mesangial cell and endothelial cell, and less frequent between endothlial cell and basement membrane and between epithelial cell and basement membrane (open arrows). Some portions of capillary wall remain intact, e.g., at upper right. Basement membrane is invariably seen even when surrounded by amyloid. Mesangial cells have well-developed cell organelles and frequently contain lipid droplets. Endothelial cells are generally intact except for distortion in some portions. Foot processes of epithelial cell fuse completely when on the subepithelial amyloid accumulation (open arrows), but are quite intact in other areas. Capillary lumen and urinary space are reduced in size. × 4500.

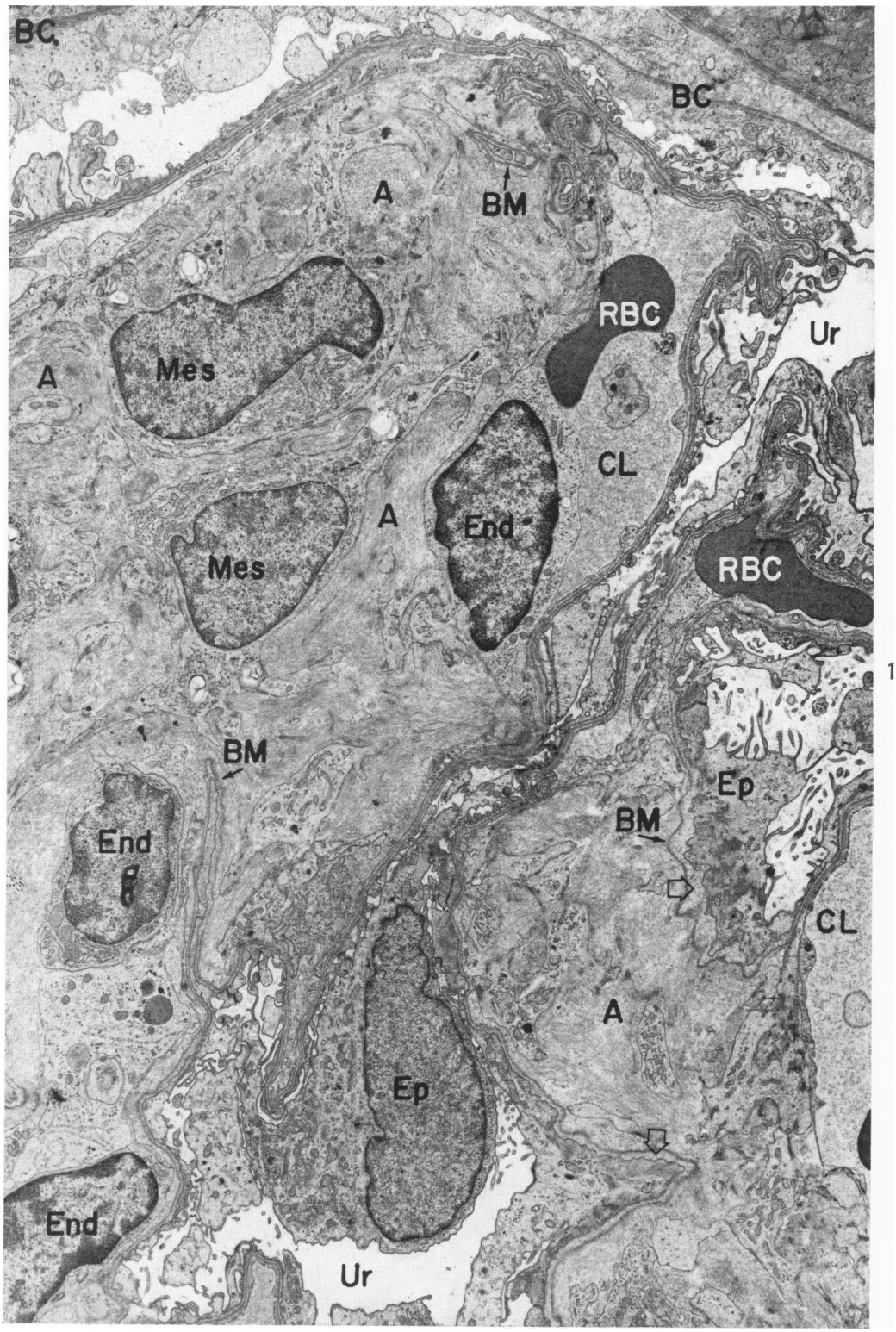
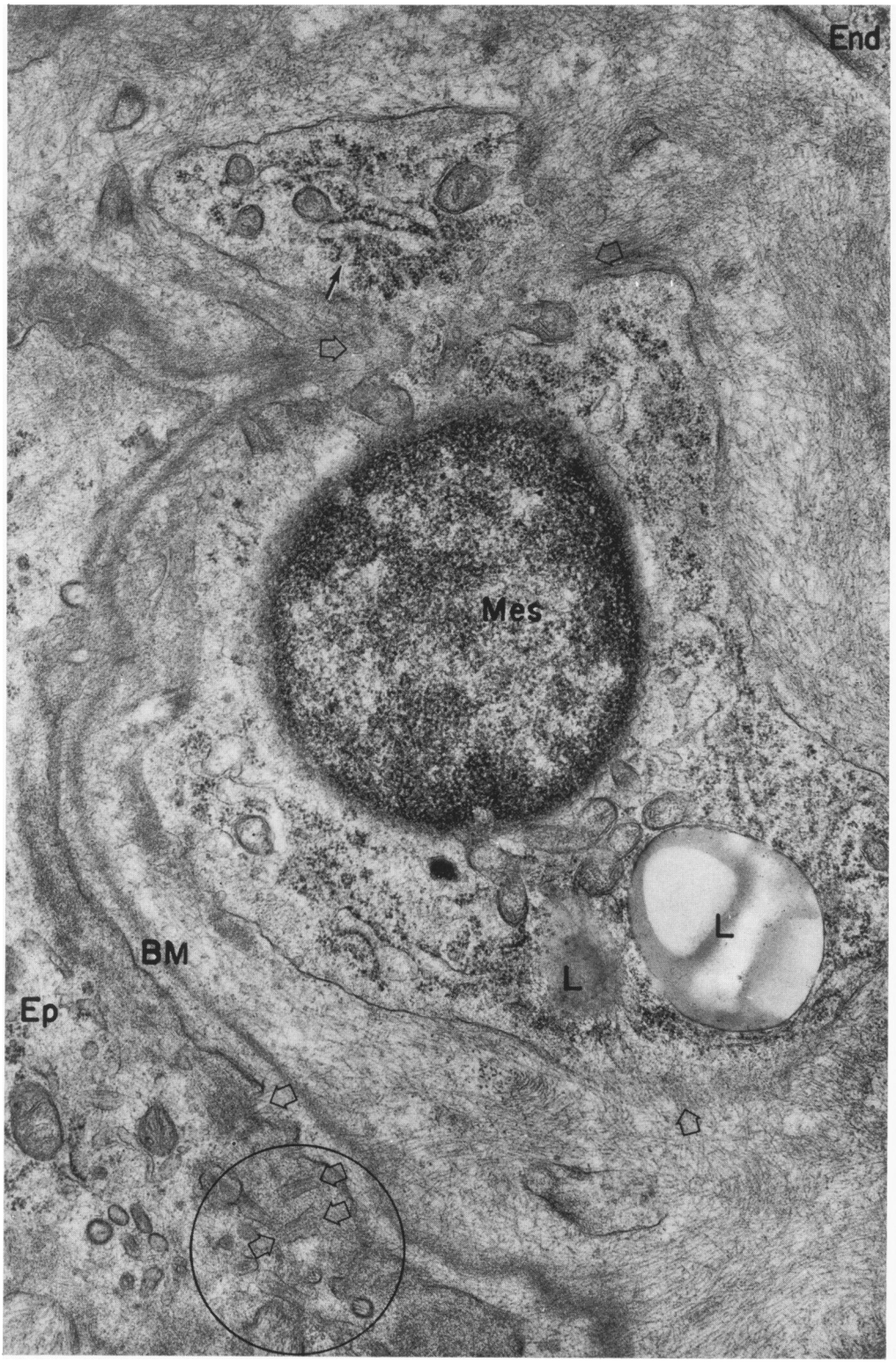

BC
BC
A
BM
RBC
Ur
A
Mes
CL
A
End
Mes
RBC
BM
Ep
BM
End
CL
A
Ep
End
Ur

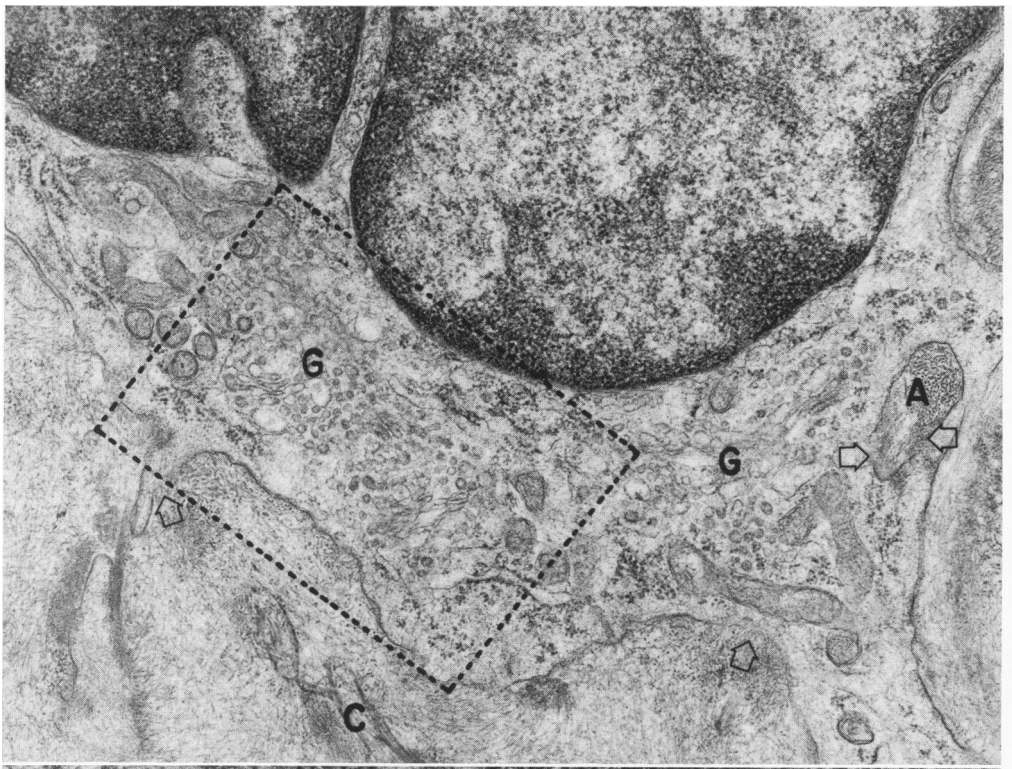
FIG. 2. Experimentally induced rabbit amyloidosis. Most of mesangial cell and portions of endothelial cell, epithelial cell, and basement membrane are present. Cell organelles of mesangial cell are well developed and indicative of considerable functional activity. The cell is characterized by: nucleus with rich and abundant chromatin, lipid droplets, numerous free ribosomes which are frequently organized to form polysomes, moderate number of mitochondria, well-developed and enlarged endoplasmic reticulum (rough and smooth surfaced) whose cisternae contain moderately dense material (usually amorphous or finely granular, but occasionally filamentous) and which occasionally appear to open within cytoplasm (solid arrow). Tufts of highly oriented amyloid fibrils are present in several portions adjacent to plasma membrane of mesangial and epithelial cells. Here, amyloid fibrils are arranged almost parallel to each other and almost perpendicularly to cell surface. Plasma membrane appears to be indistinct and occasionally invaginates as a pocket (open arrows) in these areas. In epithelial cell at pockets of plasma membrane, membrane-surrounded amyloid fibrils are intracellular (circle). At these areas, surrounding membrane is fuzzy on one or both poles (open arrows in circle). Basement membrane is invariably present. Epithelial cell cytoplasm adjacent to basement membrane is dense and has finely granular and occasionally fibrillar appearance. × 25,500.



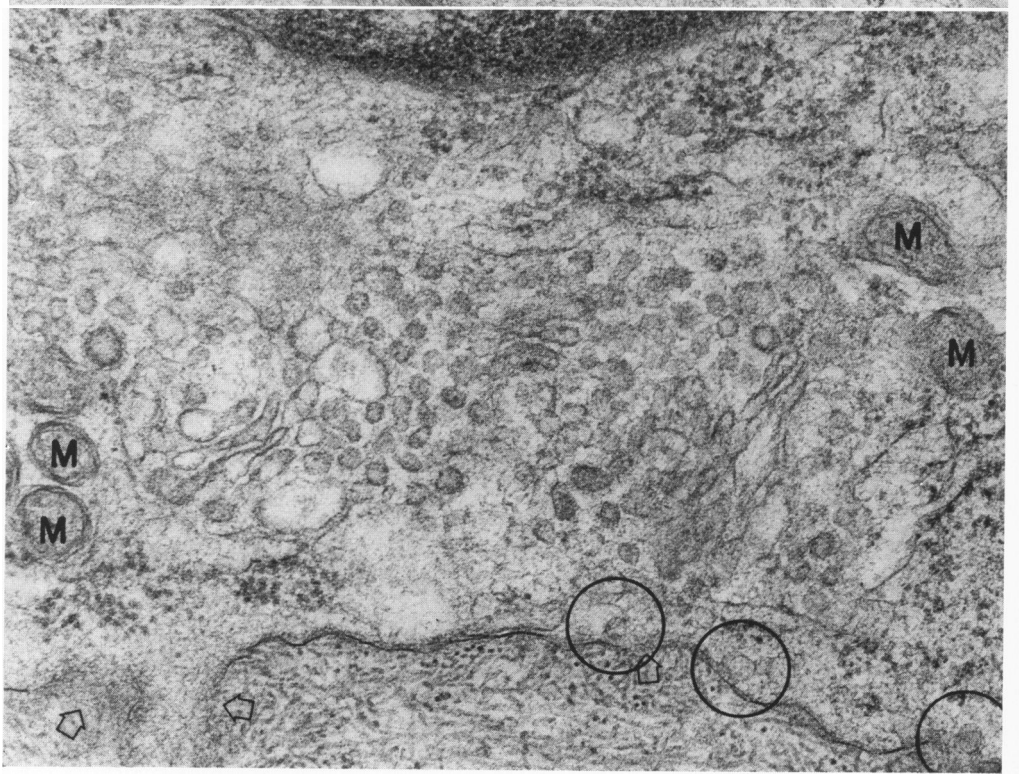
2

FIG. 3. Experimentally induced rabbit amyloidosis. Portion of mesangial cell and associated amyloid fibrils. Mesangial cell contains well-developed cell organelles and amyloid fibrils in cross and longitudinal section surrounded by a membrane. Nucleus is chromatin rich and shows a deep invagination. Golgi complexes are large and multiple; numerous small vesicles frequently contain material of moderate density. Mitochondria are narrow and long, or small and round. Free and membrane-attached ribosomes and endoplasmic reticulum are well developed. Groups of amyloid fibrils are highly oriented in some portions adjacent to plasma membrane, portions of which are fuzzy in such areas (open arrows). Peripheral portion of cytoplasm has a slightly increased density owing to aggregation of dense, finely granular or filamentous material. Collagen fibers are also present in amyloid accumulation. Dotted lines enclose portion enlarged as Fig. 4. × 22,500.

FIG. 4. Higher magnification of portion of Fig. 3. Well-developed rough- and smooth-surfaced endoplasmic reticulum and free ribosomes are present in perinuclear region and in peripheral portion of cytoplasm. From these regions to site where intimate relationship between plasma membrane and amyloid fibrils is observed (open arrows): in sequence, a large Golgi complex, many small vesicles which often contain moderately dense material, indistinct plasma membrane, highly oriented amyloid fibrils. Some small vesicles are attached to plasma membrane; possibly their contents become liberated to the extracellular space (circles). Mitochondria are small and round. × 61,000.



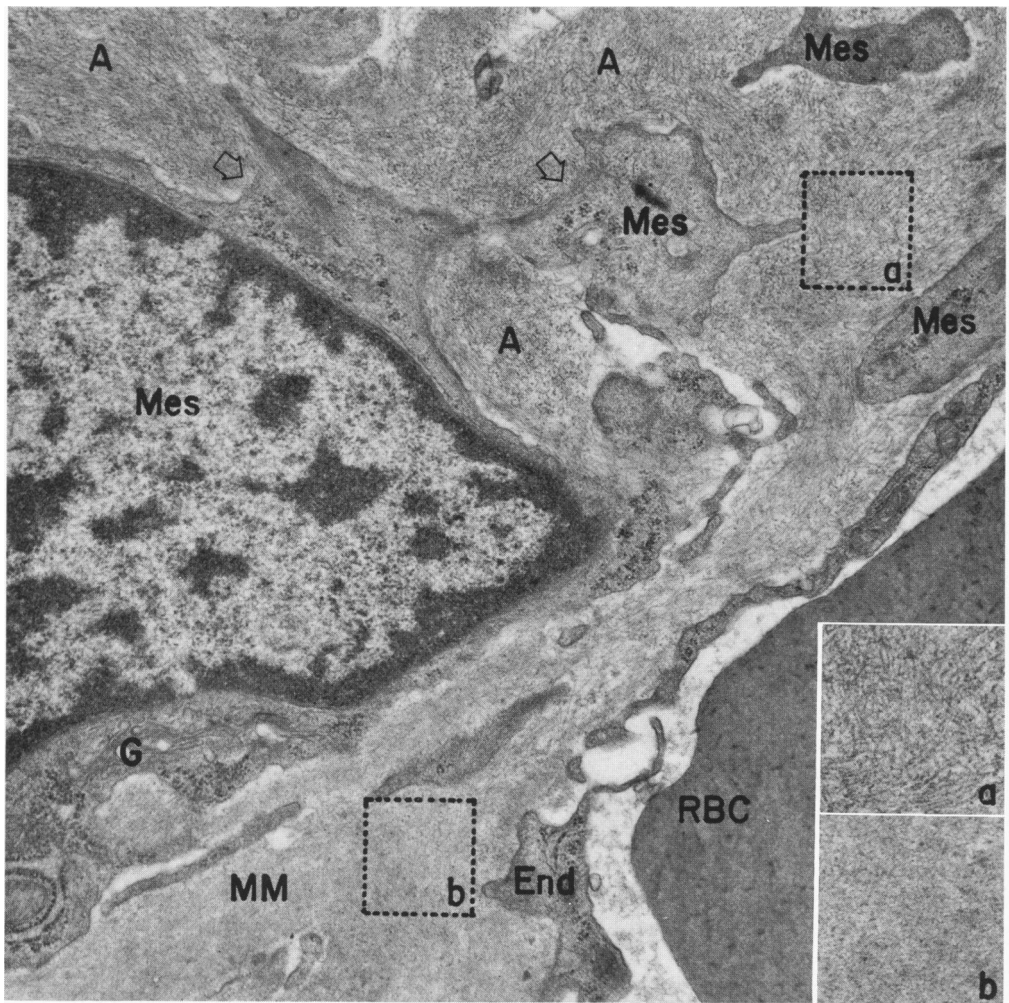
3



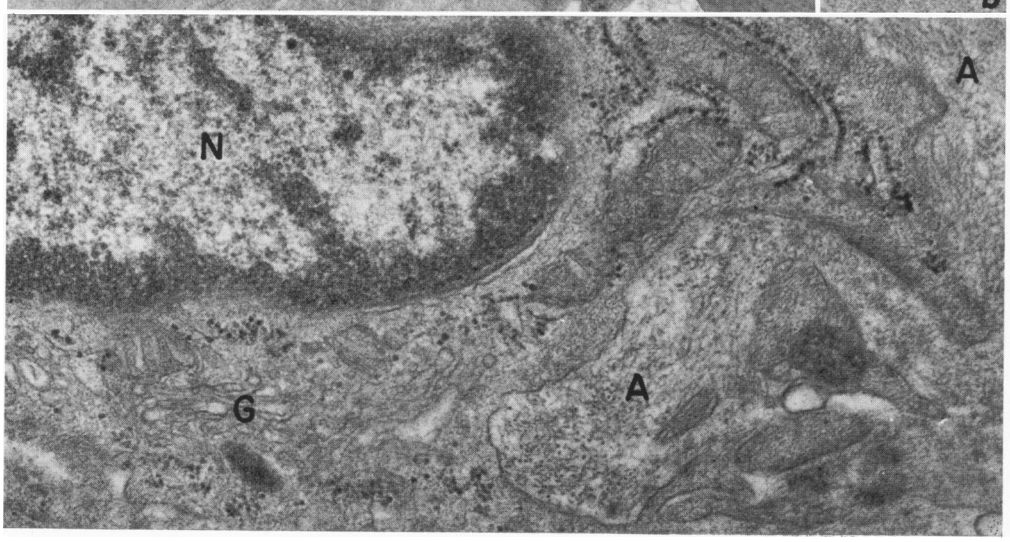
4

FIG. 5. Human renal amyloid. Relatively early amyloid accumulation in mesangial area. Cell organelles of mesangial cell are well developed, but not so obvious as those seen in experimentally induced amyloidosis. In upper two-thirds of micrograph, amyloid fibrils accumulate between mesangial cells (or cytoplasmic processes of mesangial cell), and between mesangial cell and endothelial cell. In portions occupied by amyloid accumulation, ultrastructure of mesangial matrix is barely distinguishable, though it is clearly seen in bottom third of micrograph where it is free from amyloid accumulation. Similar to experimentally induced amyloidosis, in some juxtacellular portions, groups of amyloid fibrils are highly oriented toward particular sites of plasma membrane in which plasma membrane appears to be indistinct and sometimes invaginated (open arrows). However, appearance of as specific ultrastructural relationships between plasma membrane and amyloid fibrils are less obvious in human cases than in experimental ones. × 18,000. Insertions a. and b. are higher magnifications of areas surrounded by dotted line: a, area of amyloid accumulation in mesangial matrix; b, intact mesangial matrix. × 40,000.

FIG. 6. Human amyloidosis. Higher magnification of portion of active mesangial cell. Cell organelles are well developed, show free and membrane-bound ribosomes, mitochondria, Golgi, nucleus. Amyloid found in cross and longitudinal section. × 38,500.



5



6

FIG. 7. Human amyloidosis. Mesangial cell lying in massive amyloid deposit shows a quite different character from those in Fig. 2–6. Irregularly shaped nucleus contains chromatin condensed beneath nuclear envelope. Cytoplasm, with slightly increased density, is reduced in volume and has attenuated tortuous appearance. Free ribosomes are seen. Endoplasmic reticulum is reduced and no Golgi complex is seen. Mitochondria whose matrices have increased density are increased in relative number. Numerous vacuoles are present in cytoplasm, and some show myelin-like figure. Groups of amyloid fibrils are often in cytoplasmic vacuoles and within finger-like cytoplasmic projections but are not oriented with great regularity nor do they fill the whole space of the vacuole. Inclusions other than amyloid fibrils are often found even in same vacuole as the amyloid fibrils. × 23,000.

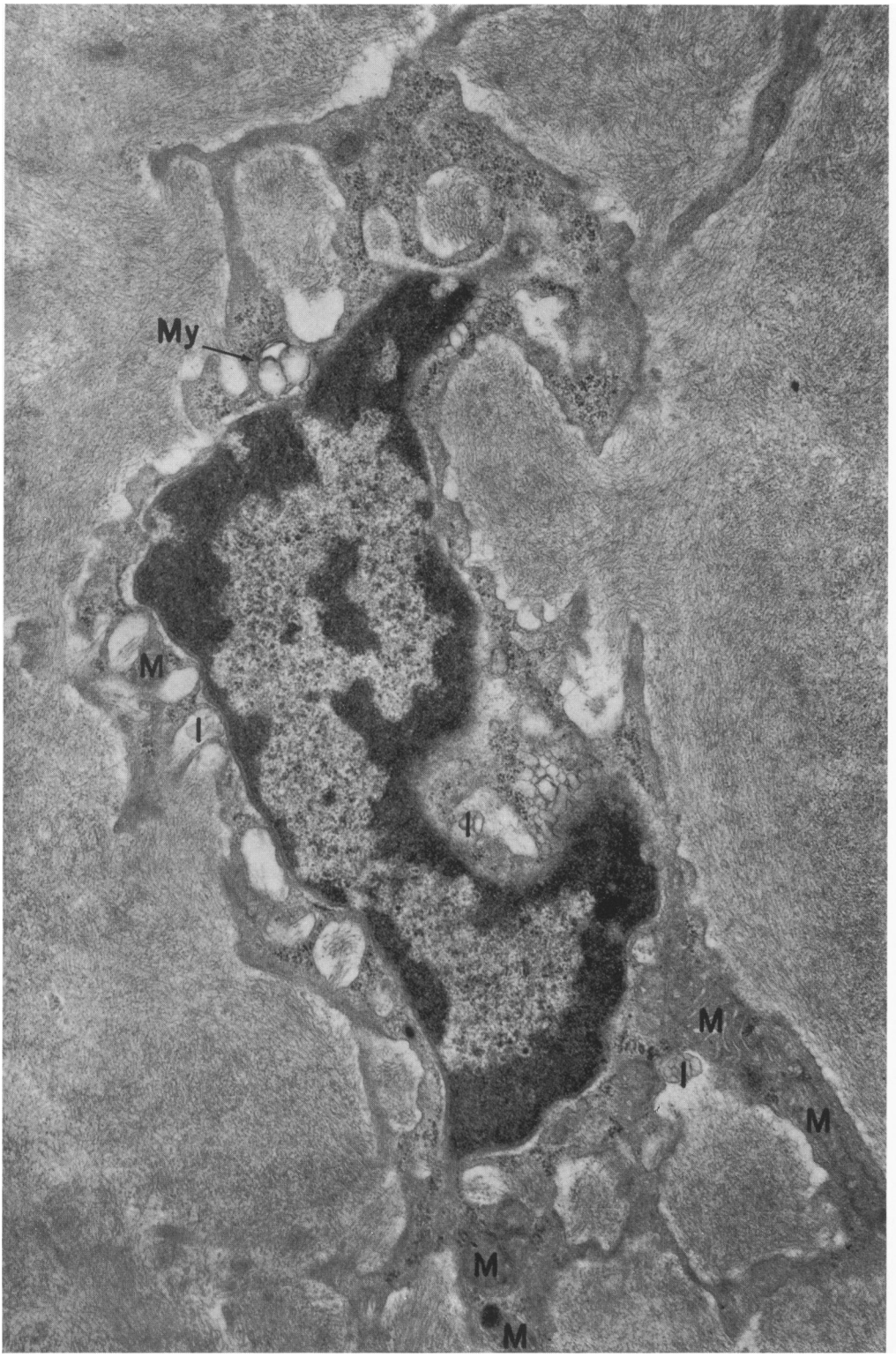

My
M
I
M
I
M
M
M

FIG. 8. Human amyloidosis. Mesangial cell of similar character to one in Fig. 7. Free ribosomes are reduced in number and mitochondrial cristae are not as clearly seen. Endothelial cells are highly elongated but normally cover entire surface of capillary lumen. Structure of fenestra is just visible. Granular and fine filamentous structures are often in capillary lumen. Basement membrane is somewhat distorted, but clearly present. Small portion of epithelial cell is at upper left. Amyloid appears occasionally aggregated in bundles but is usually in random distribution. × 27,000.

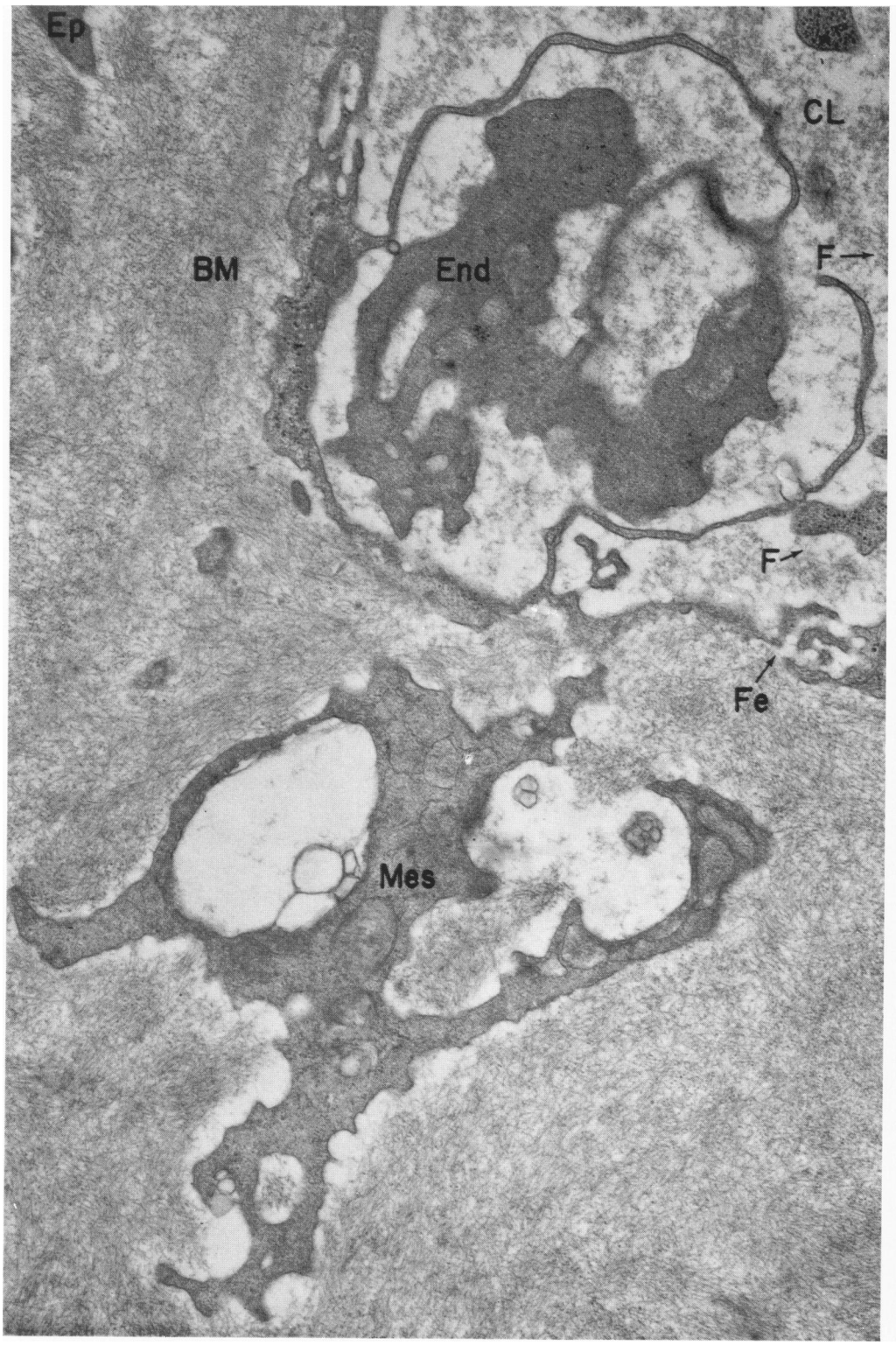
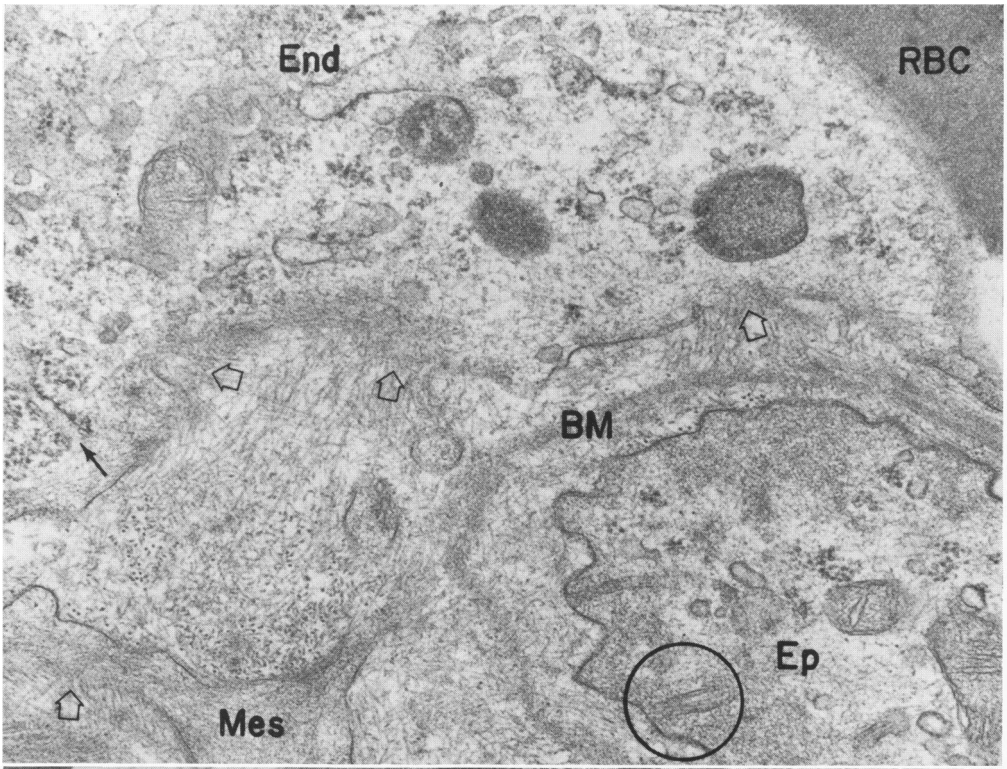

Ep
CL
BM
End
F
F
Fe
Mes

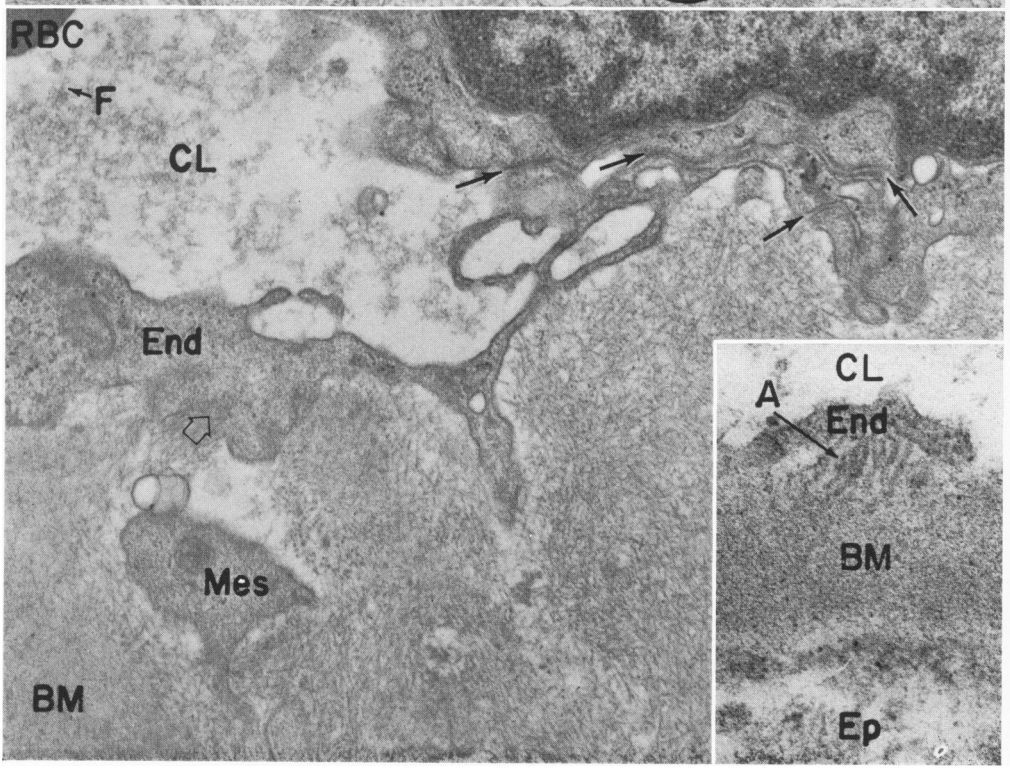
FIG. 9. Experimentally induced rabbit amyloidosis. Early amyloid accumulation is present in area surrounded by mesangial cell, endothelial cell, and epithelial cell. Well-developed cell organelles of endothelial cell suggest it is an active cell. Free and membrane-attached ribosomes are numerous and frequently show polysomal patterns. Well-developed endoplasmic reticulum is often somewhat dilated and contains moderately dense material which consists of amorphous but also granular and filamentous structures. Occasionally, cisterna of endoplasmic reticulum appears to open within cytoplasmic matrix and is especially frequent in periphery of cytoplasm (closed arrow). Mitochondria normally shaped and distributed. Particles in endothelial cytoplasm which are surrounded by single membrane and contain dense material may be of lysosomal nature. In some portions on border of endothelial cell and of mesangial cell, intimate structural relationship between cell membrane and amyloid fibrils can be seen (open arrows). In periphery of cytoplasm of all three types of cells, aggregation of finely granular or filamentous dense material is seen. Membrane-surrounded amyloid fibrils are in epithelial cell (circle). Basement membrane remains intact. × 40,000.

FIG. 10. Human amyloidosis. Endothelial cells and subendothelial amyloid accumulation. Endothelial cells are tortuous and show cytoplasmic processes but appear normal in other respects and have normal contact with each other (solid arrows). In some portions on cell border, intimate structural relationships between plasma membrane and tufts of amyloid fibrils can be seen (open arrow) but are fainter than what has been seen in experimentally induced amyloidosis. Amyloid fibrils are tightly packed and in random array. × 31,000.

FIG. 11. Human amyloidosis. A small number of fibrils (probably amyloid) in subendothelial area. × 70,000.



9

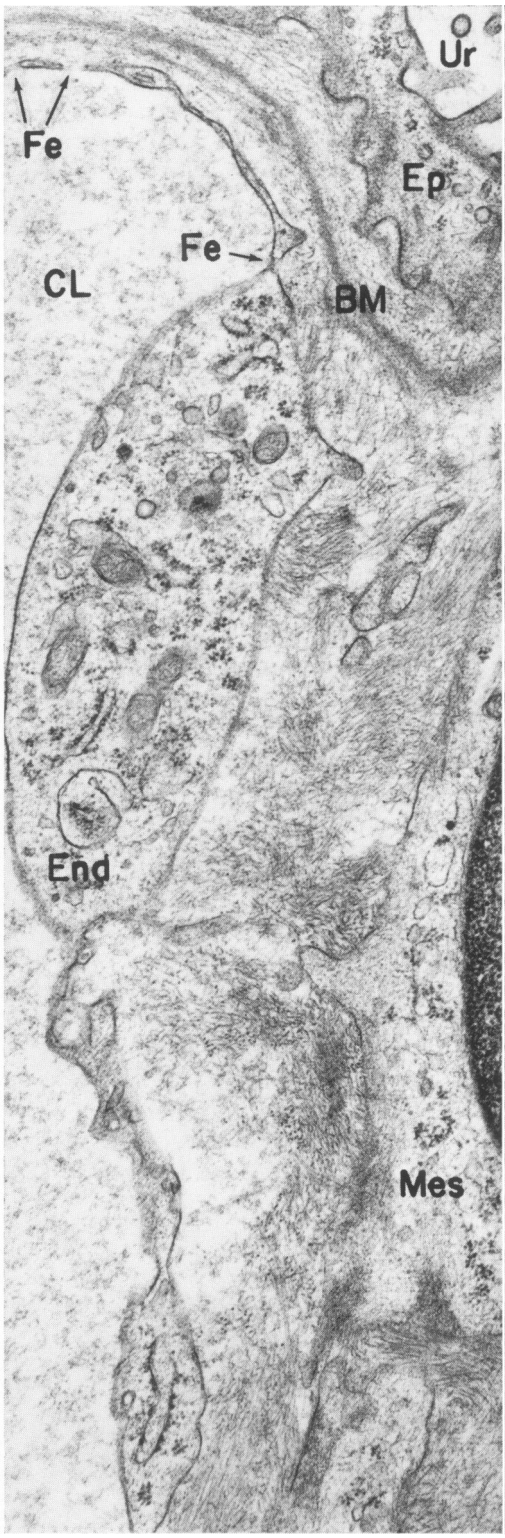


10

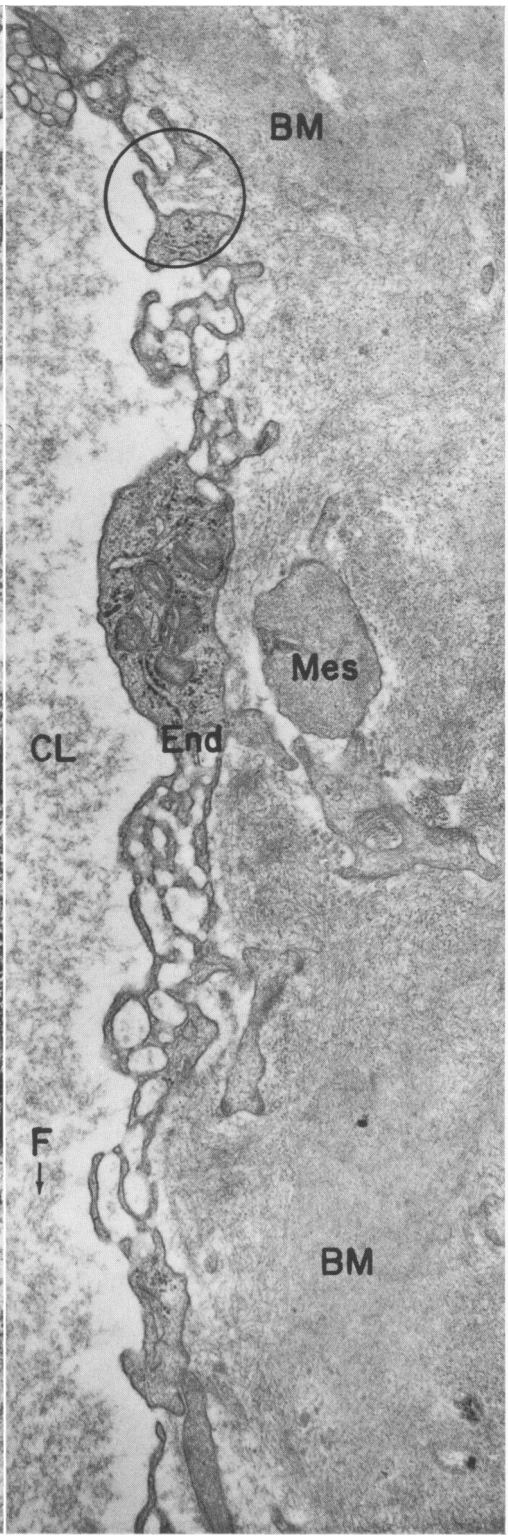
11

FIG. 12. Experimentally induced rabbit amyloidosis. Cytologic character of endothelial cells may be normal or show increased cellular activity (increase of free ribosomes; polysomal aggregates; rough endoplasmic reticulum). In some portions, endothelial cells appear to attach rather loosely to underlying amyloid but they cover the whole capillary surface; fenestrae between endothelial cells are also present in areas where amyloid accumulation is minimal. × 24,000.

FIG. 13. Human amyloidosis. Endothelial cells with reticulated (network) pattern, often in loose contact with underlying layer. Fenestra between endothelial cells appears unusually wide and a number of amyloid fibrils are associated with it (circle). × 23,000.

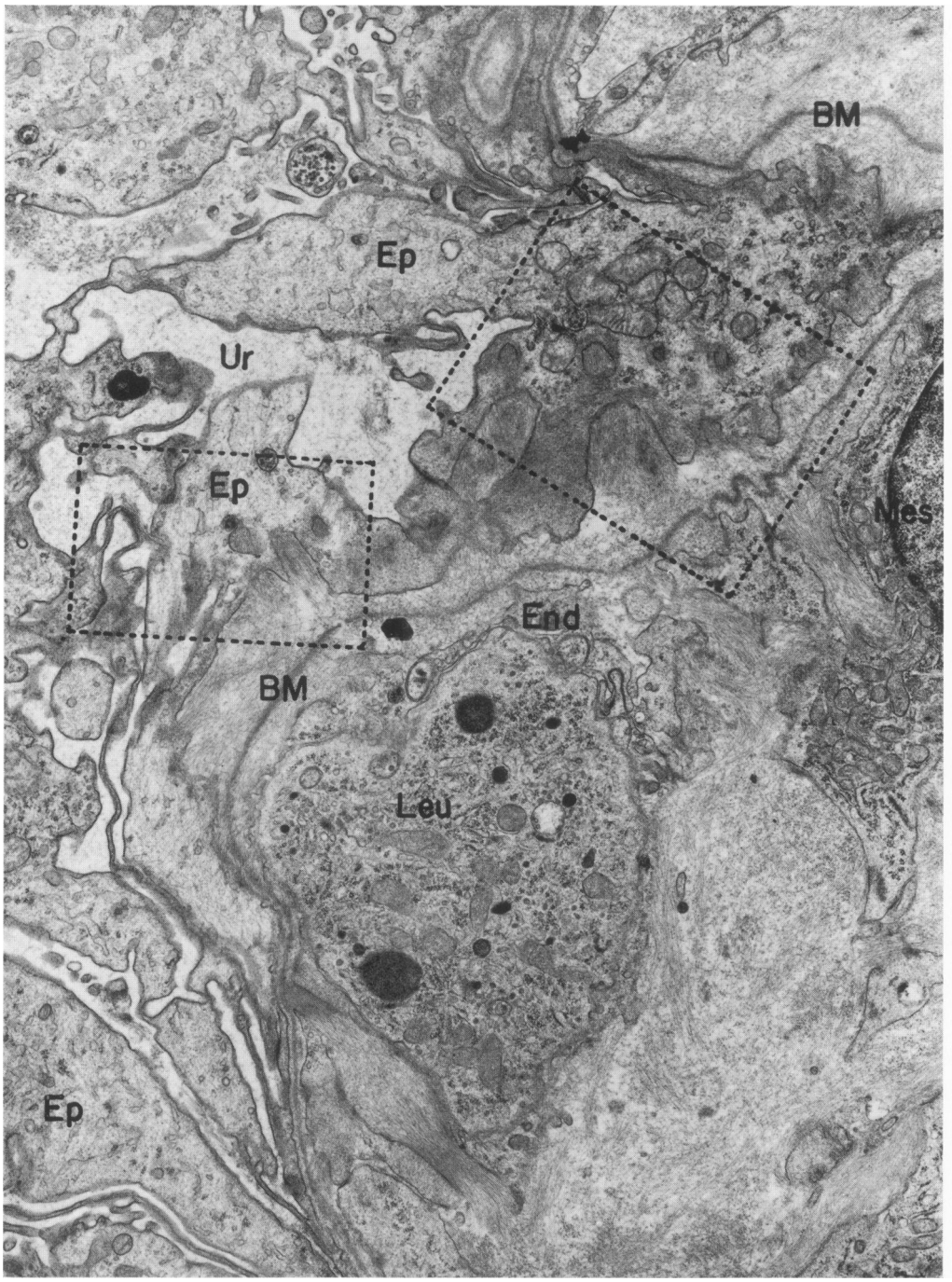


12



13

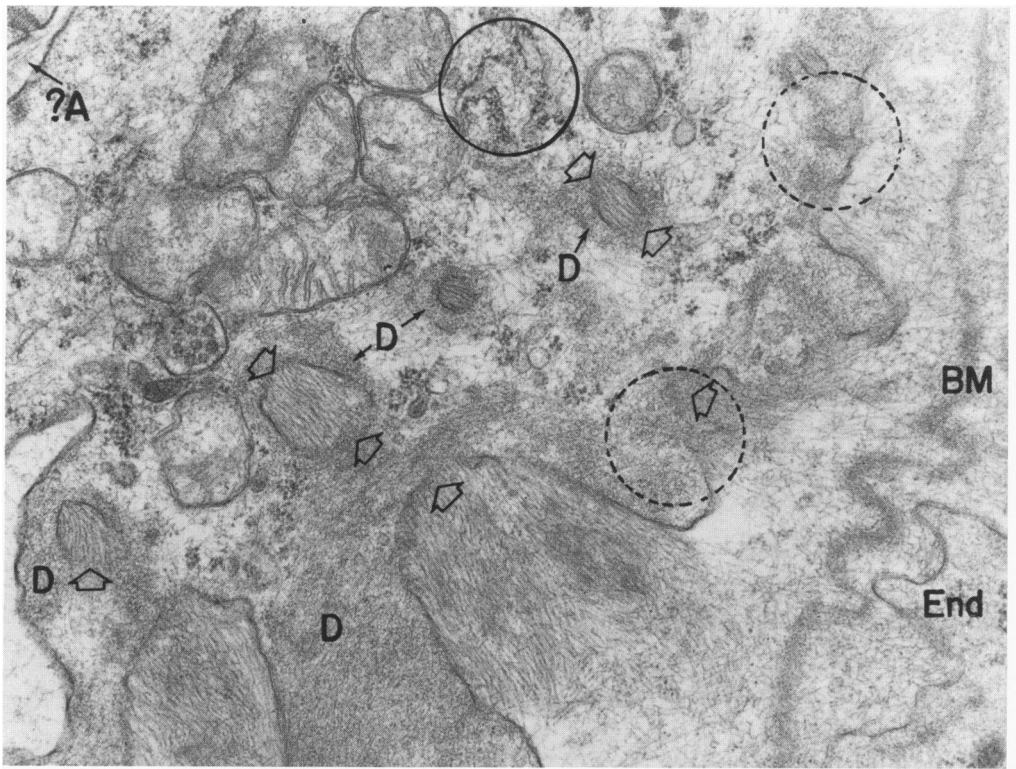
FIG. 14. Experimentally induced rabbit amyloidosis: low-power survey micrograph. Amyloid is present on both sides of basement membrane with larger amount generally seen on mesangial or endothelial surface. At center, however, amyloid, though on both subendothelial and subepithelial areas, is more prominent toward the latter. Cytologic character of mesangial, endothelial, and epithelial cells varies from normal to somewhat increased intracellular organelle activity. Epithelial foot processes are completely fused over subepithelial amyloid accumulation. Basement membrane courses through the amyloid and retains its structural integrity. Fibrils are present in urinary space. Cell filling the capillary lumen presumably is a leukocyte. Areas enclosed by dotted lines are seen at higher magnifications in Fig. 15 and 16. × 12,000.



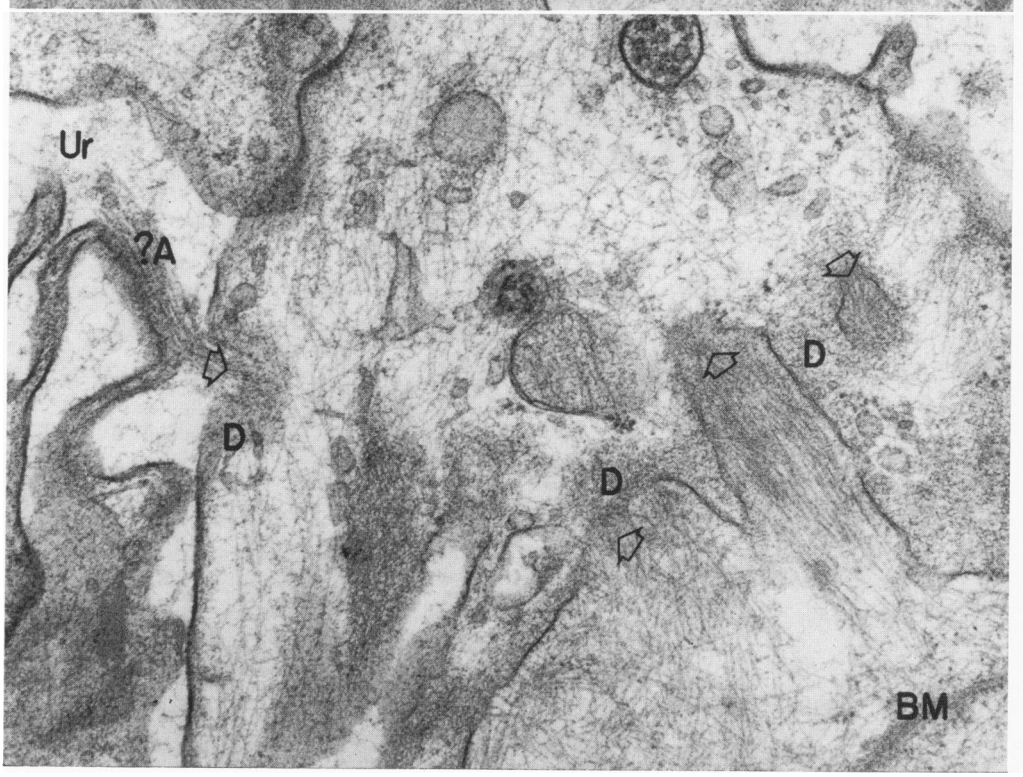
14

FIG. 15. Higher magnification of portion of Fig. 14. Organelles of epithelial cell are well developed. Contents in cisterna of endoplasmic reticulum usually appear to be moderately dense and amorphous or finely granular, but occasionally finely filamentous (closed circle). The numerous filaments presesnt in epithelial cytoplasm are seen in normal epithelial cells as well. Although they appear somewhat similar to amyloid fibrils in dimension and density, they are slightly thinner and "softer" than amyloid fibrils. Amyloid fibrils are seen in subepithelial and subendothelial areas, and in epithelial cytoplasm surrounded by a membrane. A group of fibrils structurally comparable to amyloid (?A) are found in urinary space. Accumulation of finely granular or filamentous dense material exists in periphery of epithelial cytoplasm and around membrane-surrounded intracellular amyloid fibrils. Intimate structural relationships between plasma membrane and tufts of amyloid fibrils are seen (open arrows). Occasionally in such portions, extracellular amyloid fibrils appear to have a close structural relation (? continuity) to the filaments in the dense accumulations and possibly to the usual intracellular filaments normally seen (dotted circles). × 34,500.

FIG. 16. Higher magnification of portion of Fig. 14. Fibrils (?A) in urinary space are structurally comparable to amyloid, and show close relationship with portion of plasma membrane. Relationship seems to be comparable to that seen between plasma membrane and amyloid fibrils in subepithelial area, and between membrane and intracellular amyloid fibrils (open arrows). × 46,500.



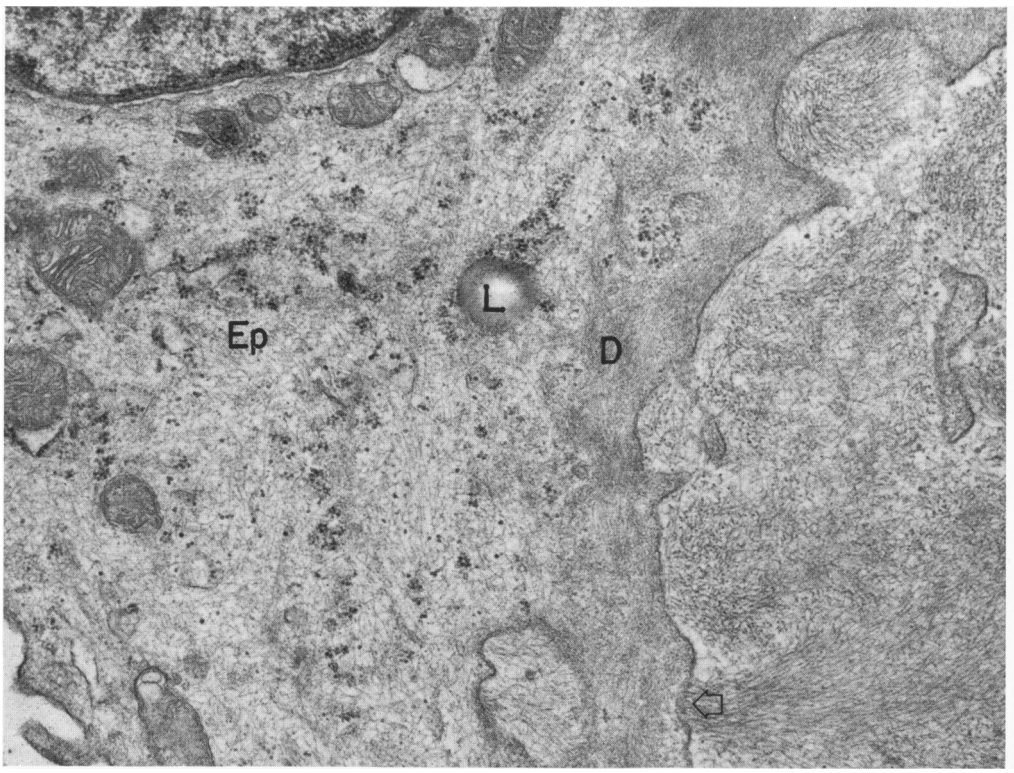
15



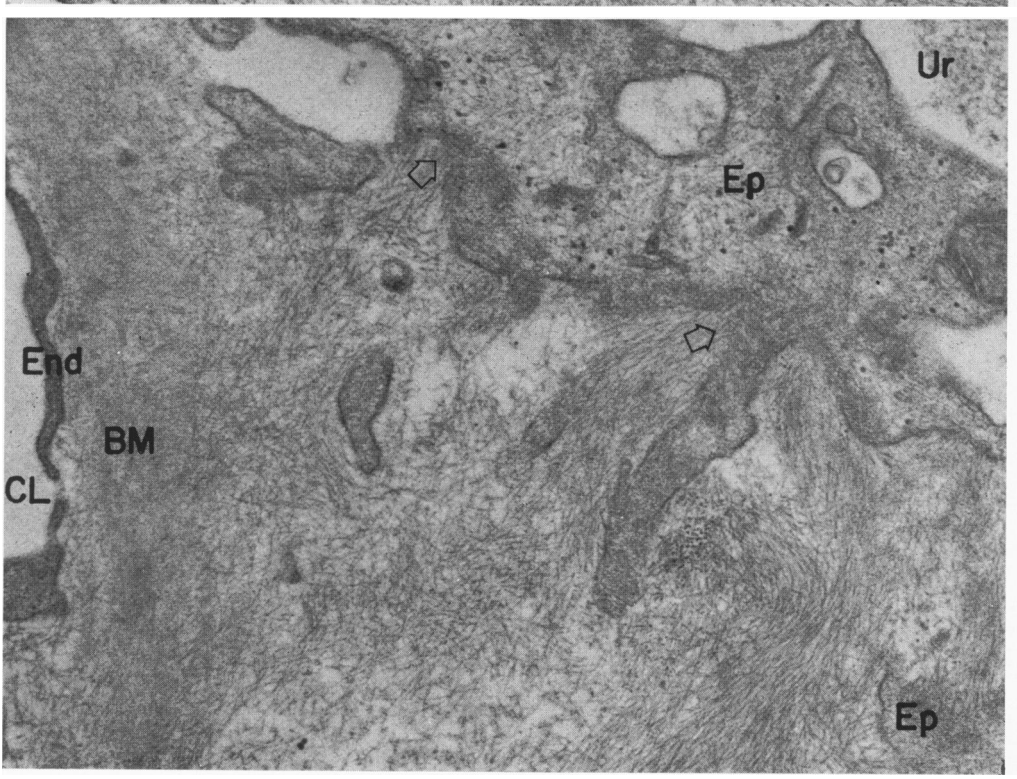
16

FIG. 17. Human amyloidosis. Epithelial cell demonstrates similar cytologic finding to those in experimental lesions. Close structural relationships between plasma membrane and tufts of amyloid fibrils are seen (open arrow). Epithelial cell cytoplasm has many mitochondria; free ribosomes in polysomal aggregates; and intracellular fibrils. × 25,500.

FIG. 18. Human amyloidosis. Complete fusion of epithelial foot processes, but otherwise few intracellular changes in epithelial and endothelial cells are noted. Much greater amounts of amyloid fibrils have accumulated in subepithelial area in contrast to barely recognizable subendothelial accumulation. Several cytoplasmic processes of epithelial cell extend toward basement membrane and through amyloid. Open arrows indicate sites of close structural relationship between plasma membrane and amyloid fibrils. × 30,500.



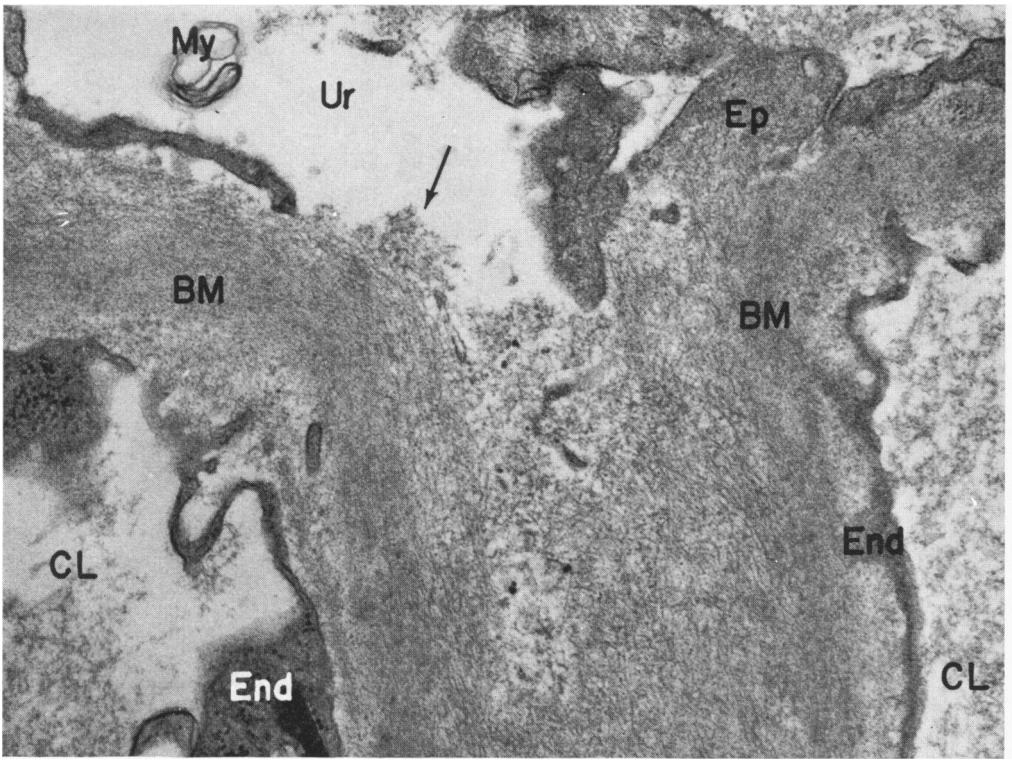
17



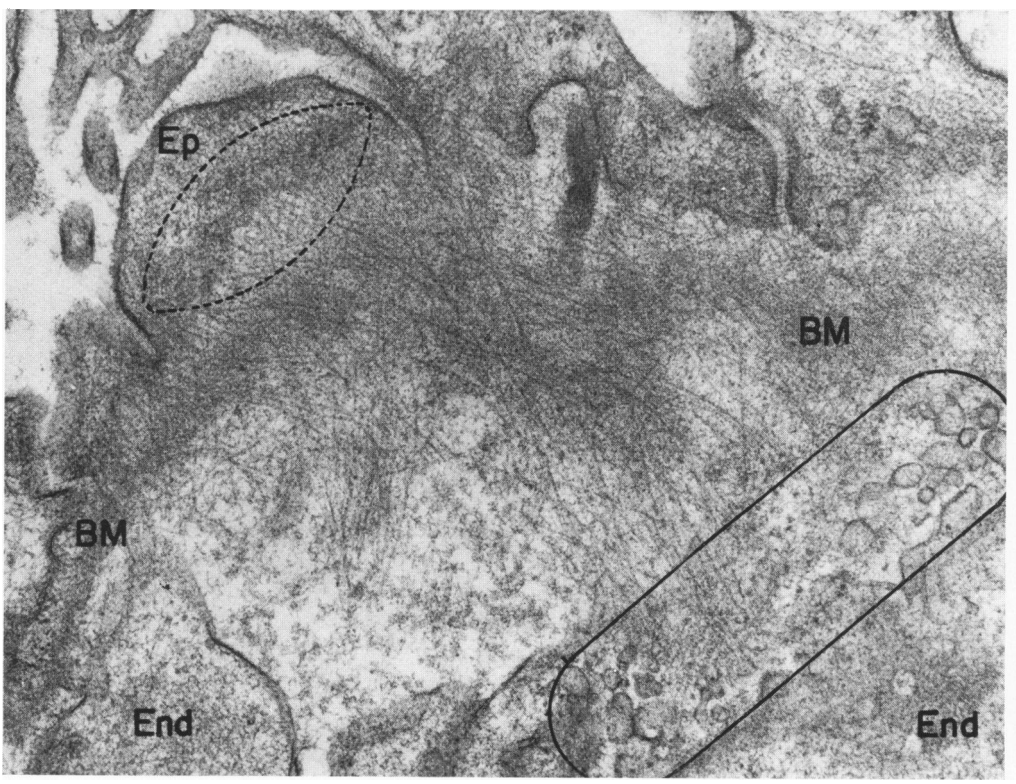
18

FIG. 19. Human amyloidosis. Defect in arrangement of epithelial cells on layer of amyloid fibrils now exposed directly to urinary space (arrow). It is not known whether this represents an area where an epithelial cell underwent degeneration (see free myelin figure), or if it is retraction, or an artifact. Amyloid fibrils accumulate mainly in subepithelial area with almost no subendothelial accumulation. × 30,500.

FIG. 20. Experimentally induced rabbit amyloidosis. Relationship between amyloid fibrils and basement membrane can be seen. Bulk of amyloid fibrils can be clearly followed from endothelial to epithelial side passing directly through basement membrane. Some fibrils measure up to 1 μ in length. Basement membrane (lamina densa) appears to be somewhat dissociated by intrusion of amyloid fibrils, but retains its basic appearance as a moderately homogeneous electron-dense structure. Vesicles (often containing very fine filamentous material) and lack of electron density at junction of amyloid fibril and endothelial cell membrane (closed oblong) contrasts with lack of vesicles and electron-dense character (dotted circle) at epithelial cell-amyloid fibril interphase. × 45,000.

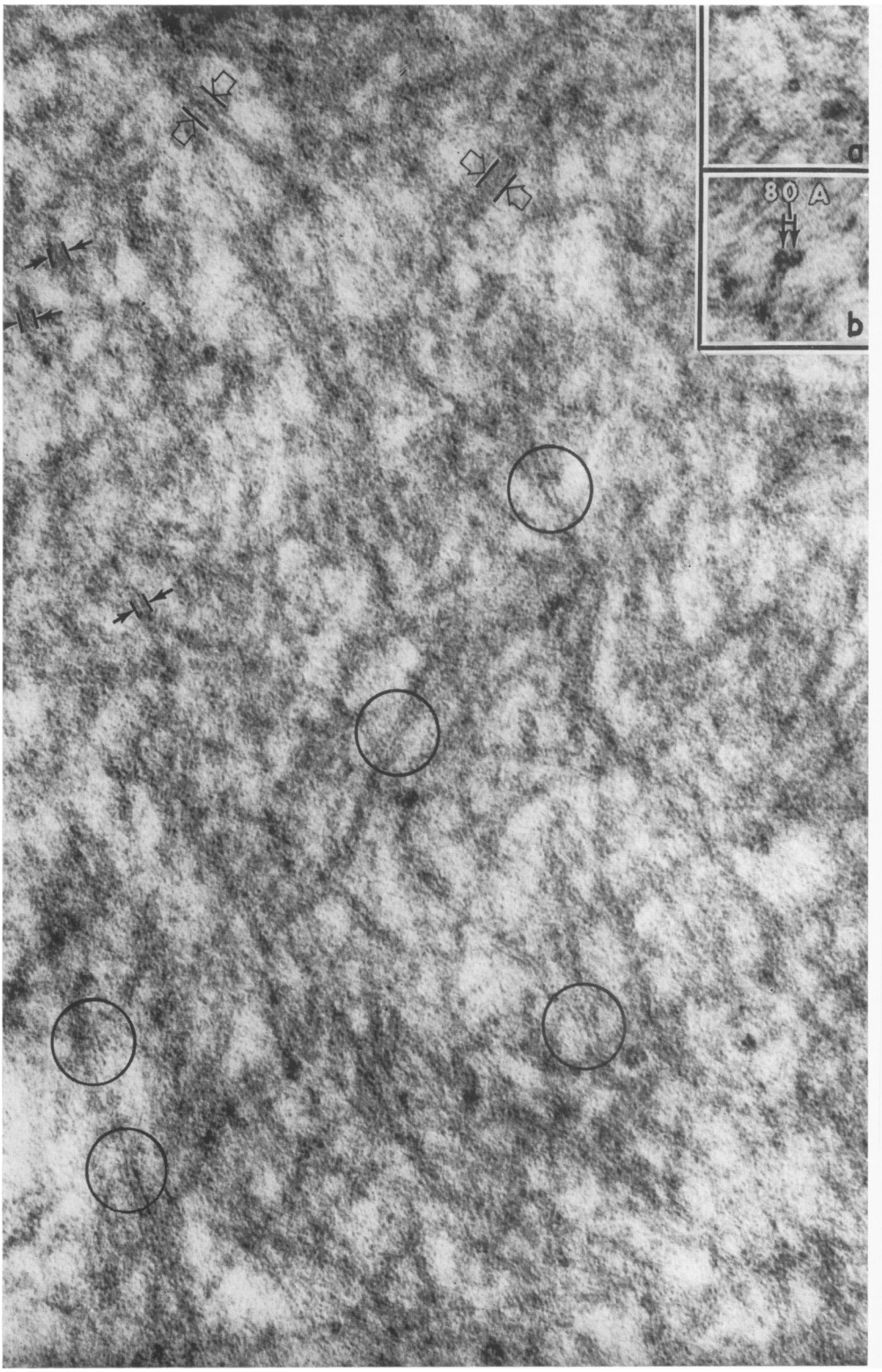


19



20

FIG. 21. Experimentally induced rabbit amyloidosis. Amyloid fibril, though not ideally resolved into subunits by conventional electron microscopy, usually measures about 100 Å in width (solid arrows), but occasionally is broader and measures 200–300 Å (open arrows). On longitudinal view of amyloid fibril, 25–35 Å fibrous subunit structures (amyloid protofibrils) can be demonstrated in portions with a 35–50 Å periodicity along this protofibril (circles). × 200,000. Insert a. Ring-like structure at center presumably represents a cross-sectional amyloid filament measuring about 80 Å in diameter, and shows an electron-lucent central core of about 20 Å diameter. × 200,000. Insert b. Pair of ring-like structures at center. Each is almost identical to the one shown in Insert a. The center-to-center distance also measures approximately 80 Å. × 200,000.


a
80 A
b