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Electron microscopic study on amyloid fibril formation in human lymph nodes

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Summary. The purpose of this investigation was to clarify the mechanisms of amyloid fibril formation in human lymph nodes. In our present study, amyloid deposition was observed diffusely in all compartments of the lymph nodes. The deposition form showed extremely characteristic findings in its morphological features. Namely, amyloid deposits mainly consisted of clusters of round or oval nodules. Each amyloid nodule was frequently enclosed with long-stretched cytoplasmic processes of abutting reticulum cells and/or macrophages. Amyloid fibrils often formed parallel amyloid bundles radiating to outlying sections of the nodule from the center. The amyloid bundles closely adhered to the cytoplasmic membrane of not only the abutting reticulum cells, macrophages and sinus endothelium but also to the lymphocytes and plasma cells. In the central portion of the amyloid nodules, a concentric core was also observed.

The most interesting finding was the intracellular formation of amyloid fibrils in all cells, such as macrophages, reticulum cells, foreign body giant cells and lymphocytes in the process of degeneration. Some fibrils localized in the limited area of the cytoplasm and others appeared in all parts of the cells, including the nucleus. Their cell membranes were missing in several areas and the cell organella had gradually dissolved. Finally the cell residuums were completely replaced by amyloid fibrils and transformed into a nodular structure with radiating bundles of amyloid fibrils.

Key words: Amyloidosis - Human lymph nodes - Ultrastructure

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Introduction

Since Teilum (1956) described the intimate morphological relationship between newly-formed amyloid and reticulo-endothelial cells, many elucidations of the cellular morphogenesis of amyloid fibrils have been reported. However, a satisfactory understanding of the mechanisms of amyloid fibril formation has not been arrived at, compared with the agreement on the biochemical composition of amyloid protein.

It is a common fact that lymph node involvement is concerned in generalized disease but there are no cases of ultrastructural investigation of amyloidosis in lymph nodes backed up by well-documented reports. In the present study, we observed the deposition form of the substance amyloid in human lymph nodes which, we belief, enables a new concept on the mechanism of amyloid fibril formation to be considered.

Materials and methods

The lymph node specimens were obtained from a 75 year-old woman with systemic primary amyloidosis (A lambda). The palpable nodes were extirpated from cervical, axillary, inguinal, pulmonary-hilar, paraaortic and mesenteric regions. Part of each specimen was fixed in 10 per cent formalin and routinely processed for light microscopic examination with hematoxylin eosin and Congo red stain. The remainder of each specimen was placed overnight at 4° C in a fixative composing 4 per cent paraformaldehyde and 2.5 per cent glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4. The specimens were postfixed in 1 per cent osmium-tetroxide in 0.1 M sodium cacodylate for two hours at 4° C. Tissue blocks were then dehydrated through graded alcohol and embedded in Araldyte epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and then examined in a transmission electron microscope.

Results

Lymph nodes exhibited diffuse replacement with nodular deposits displacing a variously-shaped, amorphous, eosinophilic mass under hematoxylin eosin stain. Following Congo red staining, the eosinophilic materials were orange and revealed green birefringence. In a severe case, the deposits of amyloid substance occupied every compartment of the lymph node, with no distinction between the parenchyma, interstitium and sinus. In such a case, we could hardly recognize the residual lymphocytes and non-lymphatic cells comprising the basic framework of the lymph node. However, in some cervical lymph nodes, basic structures remained, thus allowing us to perform ultrastructural investigations.

The chief aims of the present ultrastructural observation focused upon the following points: 1) the deposition form of amyloid fibrils, 2) the relationship between the amyloid bundles and adjacent cells, 3) the intracellular formation of amyloid fibrils.

1. The deposition form of amyloid fibrils

The amyloid deposits in the lymph nodes displayed a round or oval nodular structure. Their size was similar to that of lymphocytes in small deposits. Sometimes they formed a large nodule, about 50 pm in diameter (Fig. 1). Fig. 1 shows the cortical area just below the marginal sinus. There were many amyloid nodules of various sizes and shape. It was presumed that a large nodule was formed by the confluence of small ones.

Some of the amyloid nodules had an electron-dense center, often possessed a concentric circular core and a relatively electron-light peripheral part. At the edge of the nodular deposits, amyloid fibrils formed bundles of fibrils in parallel array which radiated to outlying sections from the center of the nodule. Amyloid nodules were often surrounded by long-stretched cytoplasmic processes of abutting reticulum cells and/or macrophages. When a large nodule was observed, a plurality of abutting cells, linked together by their processes and enclosing the nodule, could be seeh.

The deposition form of amyloid in the secondary follicles also exhibited diffuse accumulation of round or oval nodules. However, the amyloid nodules in the germinal centers were often characterized by their various forms, displaying spindle, polygonal or irregular features (Fig. 2). The amyloid deposition in the germinal centers was more severe compared to that within the lymphocyte corona. In several places, residual dendritic reticulum cells, macrophages and centrocytes could be recognized in the amyloid mass.

Amyloid deposits in the sinus also exhibited a nodular form. A few nodules were set free showing solitary settlement, although in many cases it was wrapped up in the long-stretched cytoplasmic processes of the macrophages. A core was frequently observed in the central portion of the nodules, as were radiated bundles of fibrils in the outlying sections (Fig. 3). A large number of macrophages in the sinus displayed prominent erythrophagocytosis and occasionally contained amyloid fibrils surrounded by a single membrane. This demonstrated that intercellular amyloid fibrils were phagocytized or invaginated into the cytoplasm. Amyloid nodules were also recognized in the sinus endothelium, resulting in nodular thickening of the capsule and irregularity of the luminal surface due to cytoplasmic protrusions of the endothelium into the sinus.

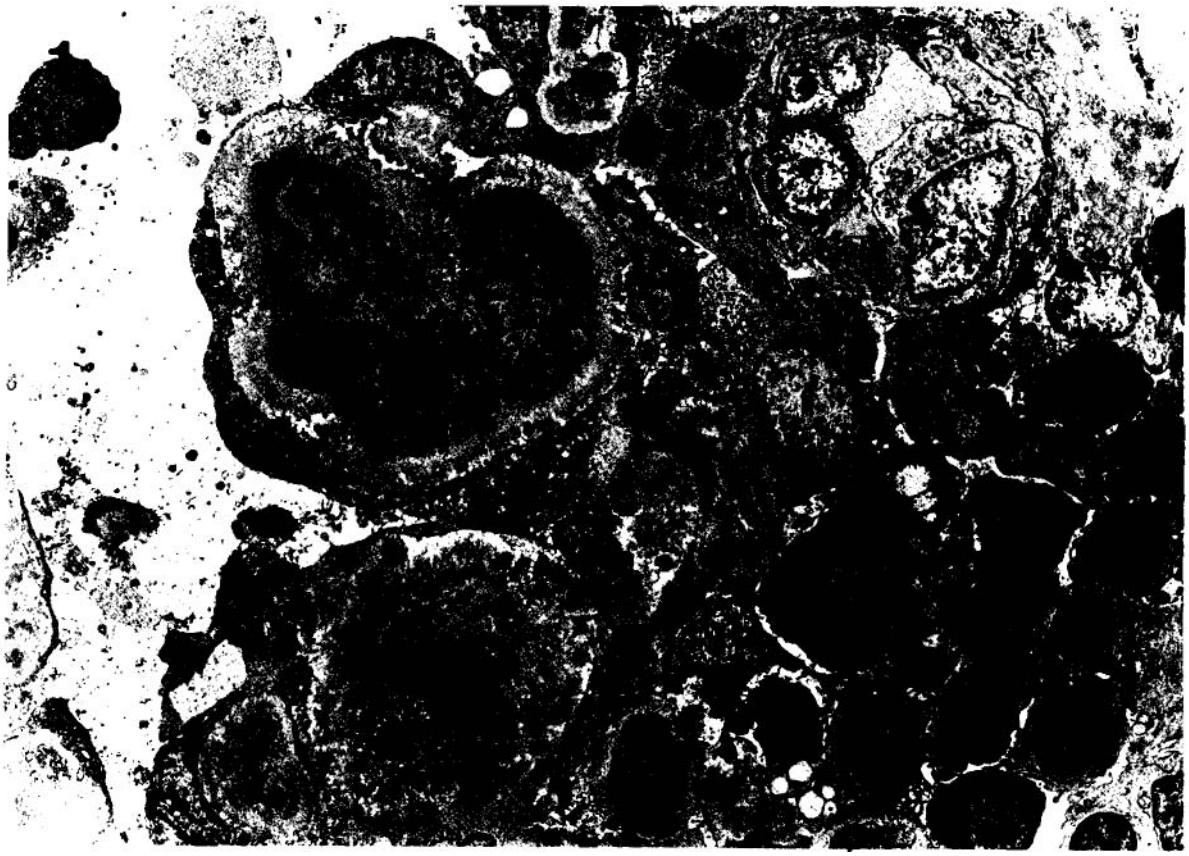
2. The relationship between the amyloid bundles and adjacent cells

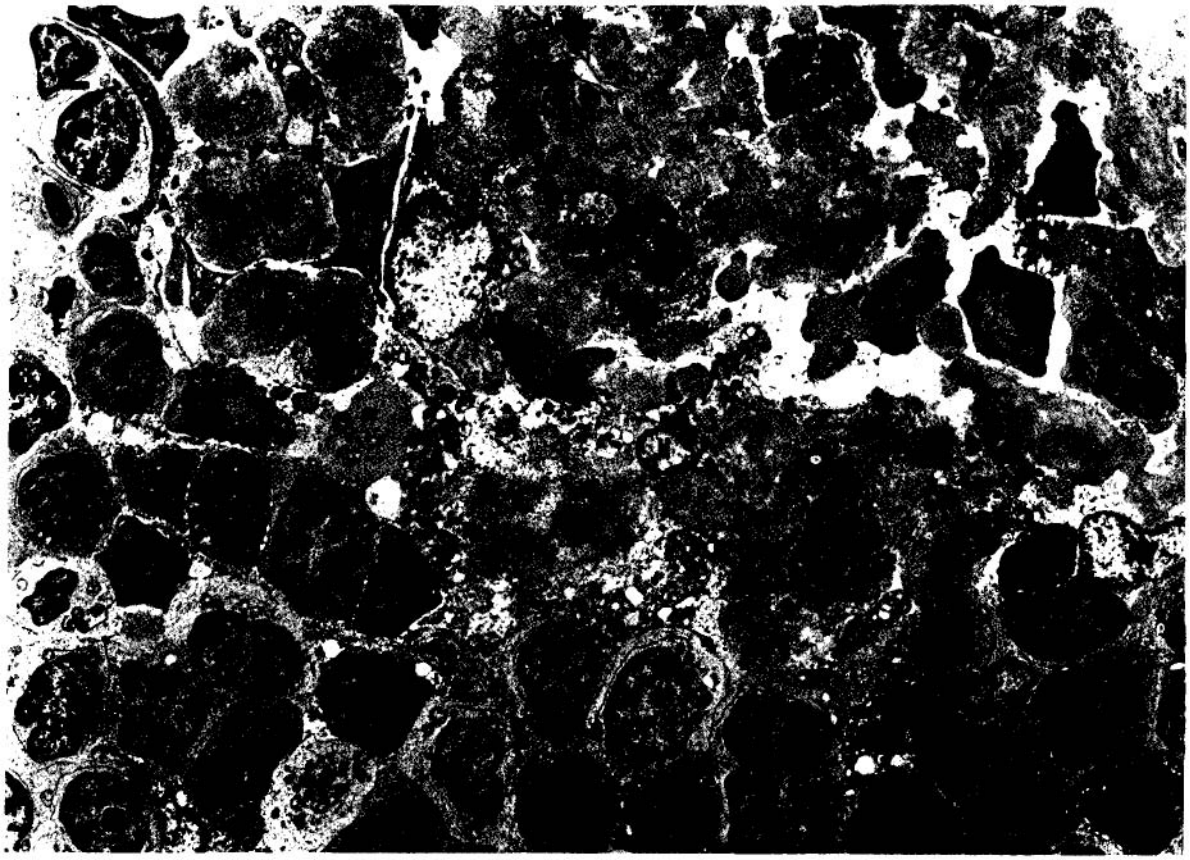
Generally, large round nodules had a tendency to form bundles of amyloid fibrils which displayed a radiating arrangement in the outlying section of the nodule (Fig. **4).** The extreme point of the bundles was closely connected to the cytoplasmic membrane of various cells, and to the reticulum cells and macrophages surrounding the nodules in particular (Fig. 5). Residual lymphocytes and/or plasma cells were also intermingled with necrotic cells and the cell debris in the amyloid deposits. The lymphocytes were adjacent to the amyloid nodule with radiated bundles (Fig. 6). The extreme points of the bundles closely adhered to the cytoplasmic membrane of the lymphocyte, where cytoplasmic indentation was often recognized. There was no hiatus between the bundles and the cytoplasmic membrane (Fig. 7). A similar finding was also observed in plasma cells, where the cytoplasmic membrane closely adhered to the bundles of amyloid fibrils (Fig. 8). In several areas, the cytoplasmic membrane was missing; it was thus difficult to distinguish whether the terminal top of the bundles was embraced into the cytoplasm (Fig. 9).

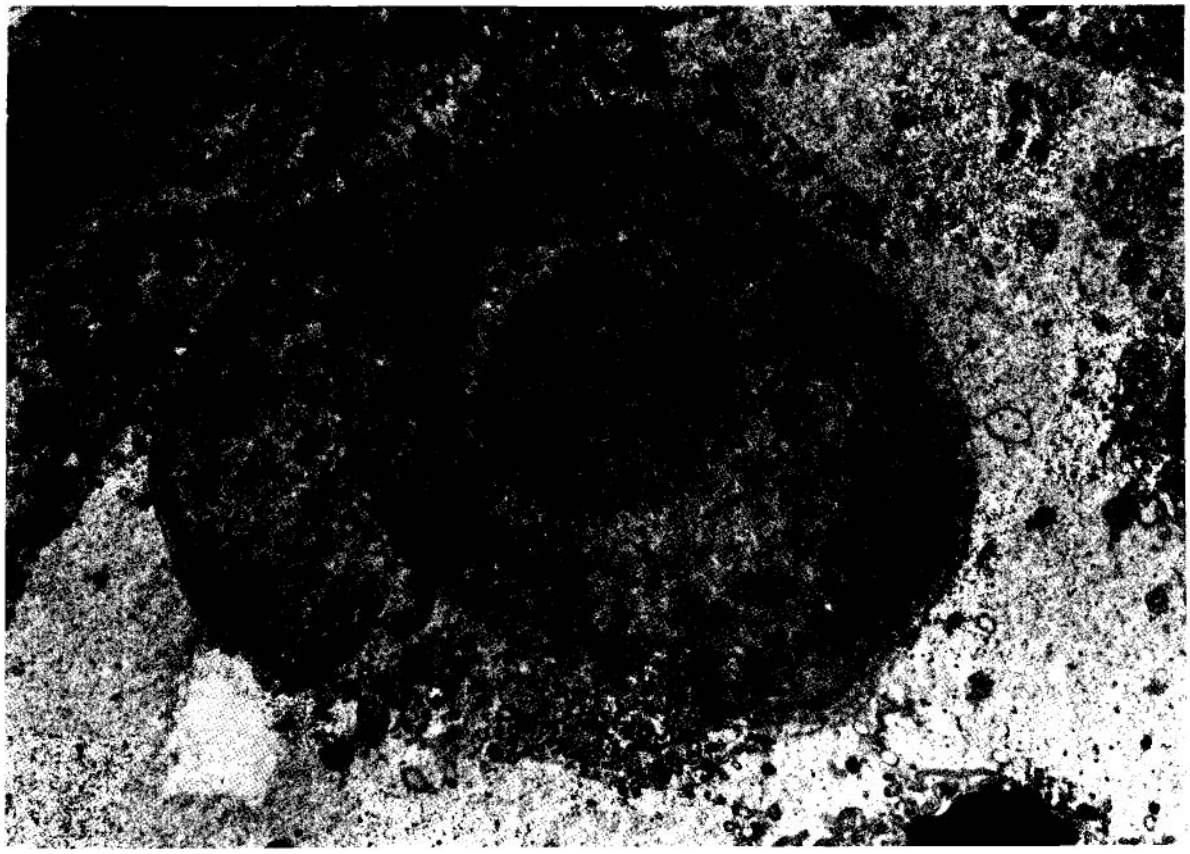
3. Intracellular formation of amyloid fibrils

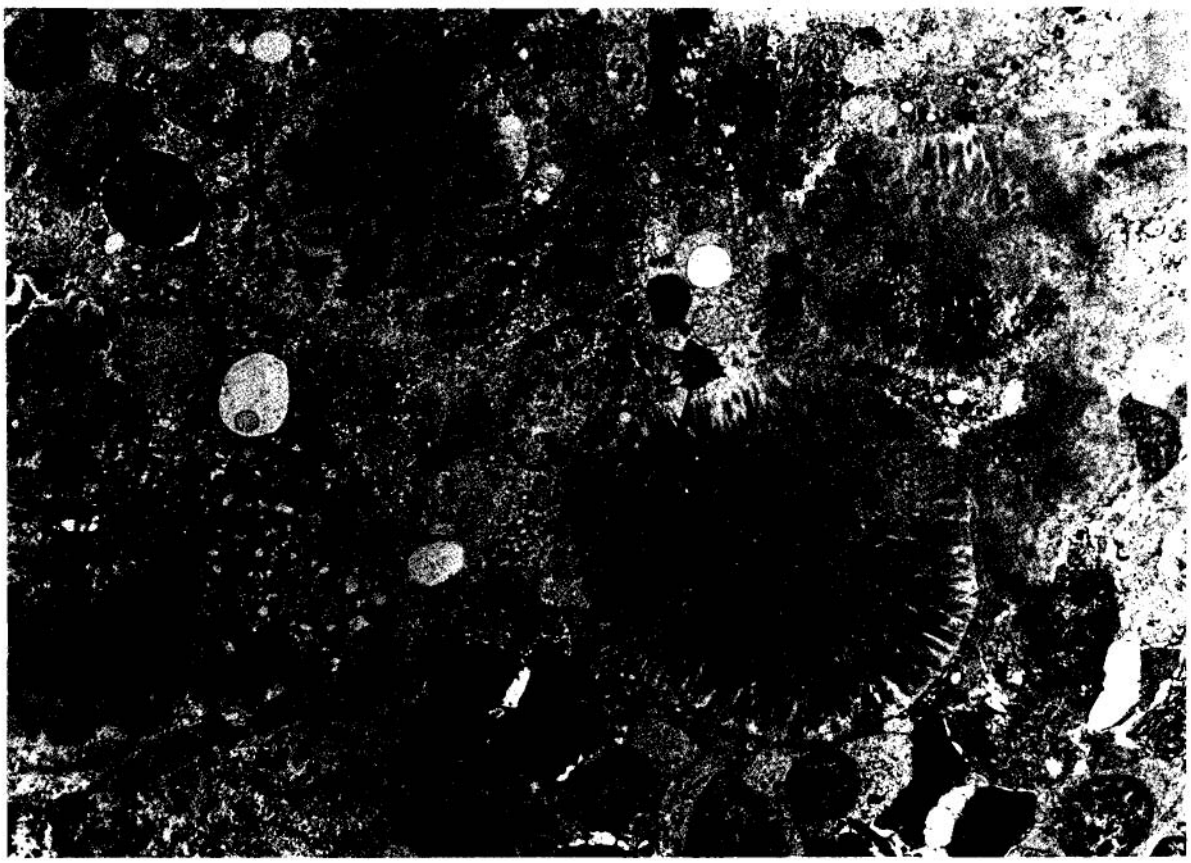
Displaying, in some places, a remarkable formation of amyloid bundles, a number of cells were under degeneration showing cellular necrosis and dissolution in various stages. The first signs of degeneration were the increase of vacuoles, the distention of mitochondria and the swelling of rough endoplasmic reticulum (rER). Subsequent findings showed little change of the nucleus and vanishing of the cytoplasmic membrane. A later stage of degeneration showed pronounced karyorrhexis and complete vanishing of the cell organella. We could, however, find a small number of various living cells intermingled with the amyloid nodules and degenerating cells. A slight degenerative change was observed in a part of the cytoplasm of the living macrophages. The nucleus showed condensed chromatin and the cell organella was localized in a partial area of the cytoplasm (Fig. 10).

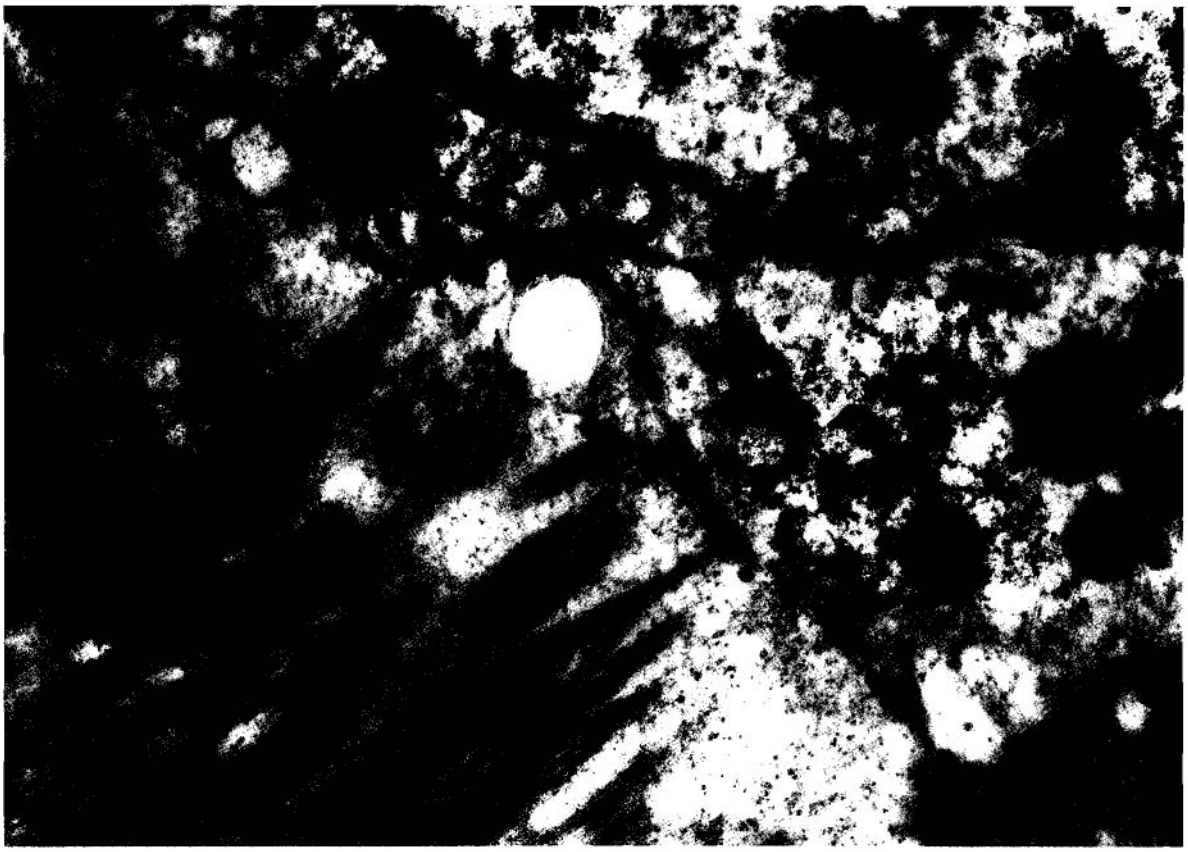
The change was most frequently observed in the cytoplasmic processes where the cell membrane was missing. In these portions, the cellular matrix was replaced by homogeneous amorphous materials (primitive amyloid plaque) of moderate electron density. A few remnants of the organella intermingled with the primitive amyloid plaque (Fig. 11). Similar degenerative findings were recognized in the reticulum cells, lymphocytes and even in the foreign body giant cells. In reticulum cells, vacuolar degeneration was an early sign of morphological change.

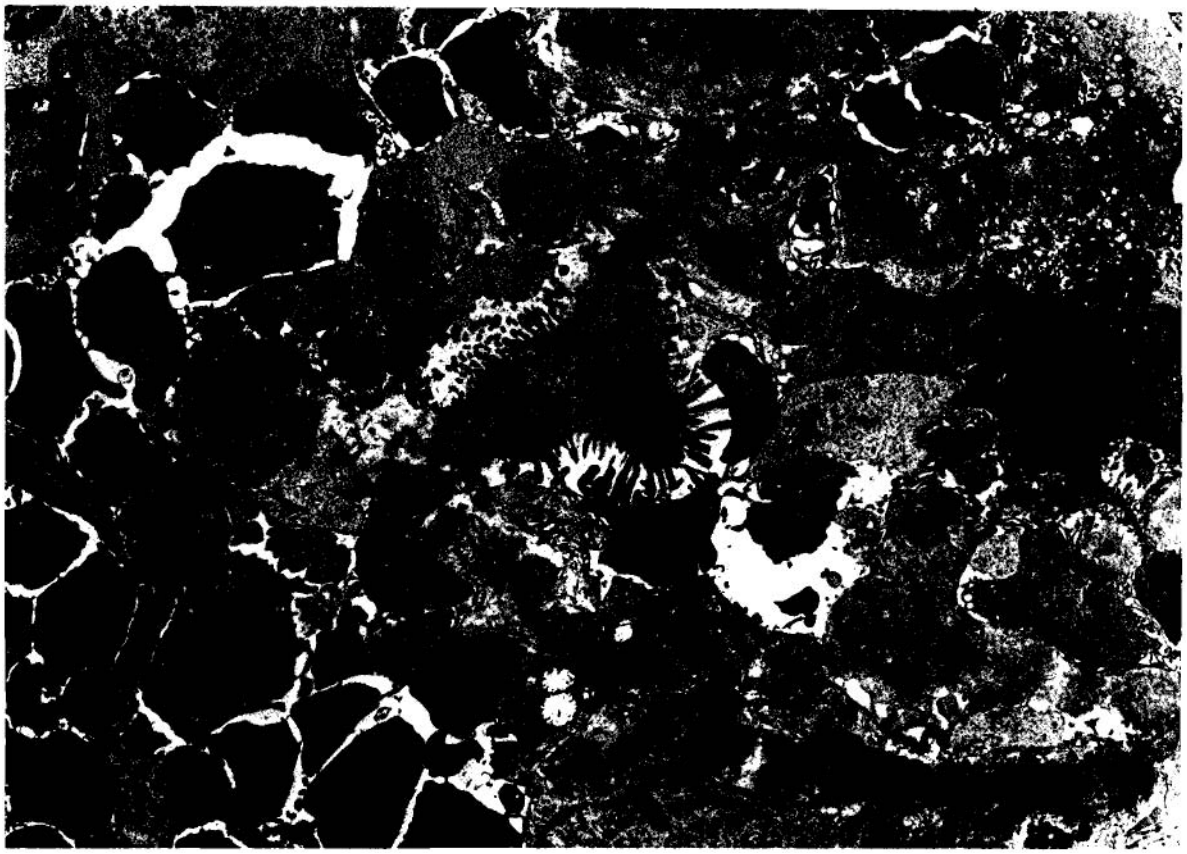


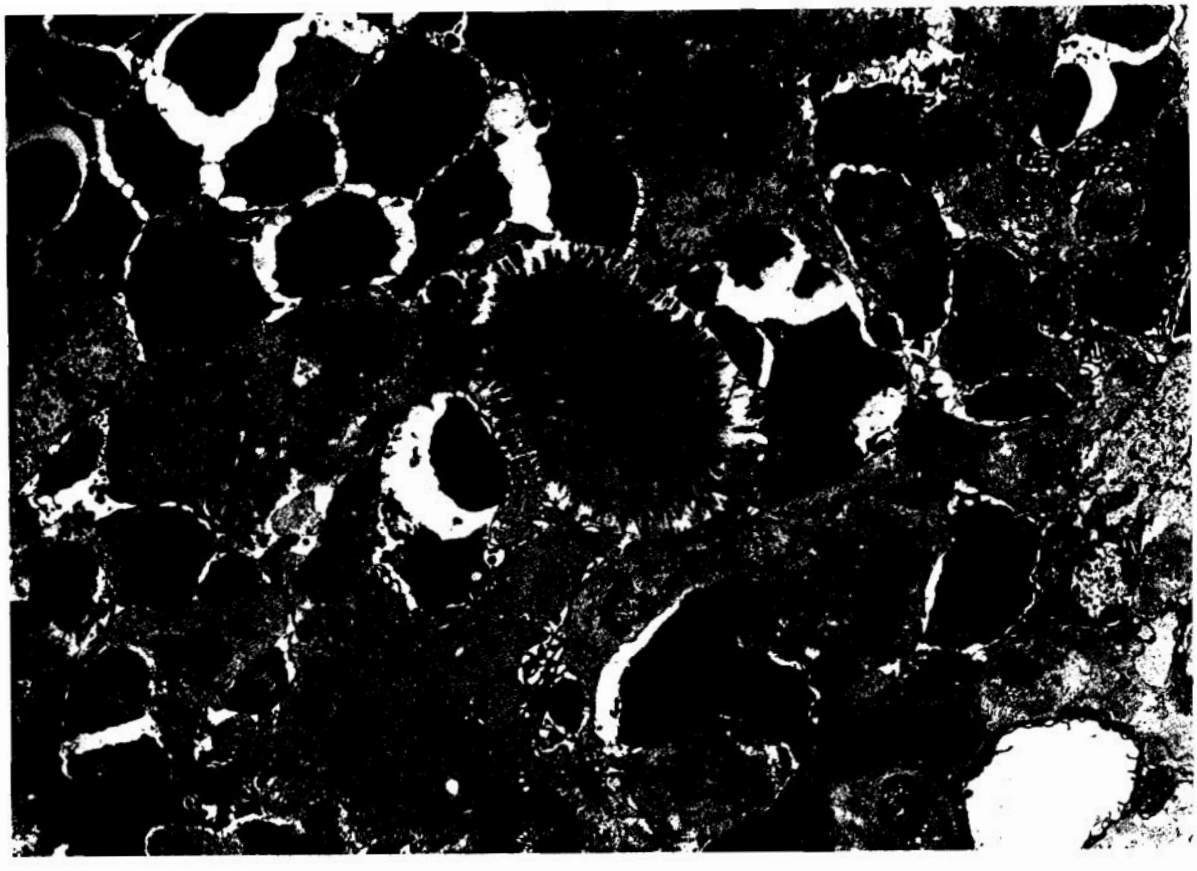


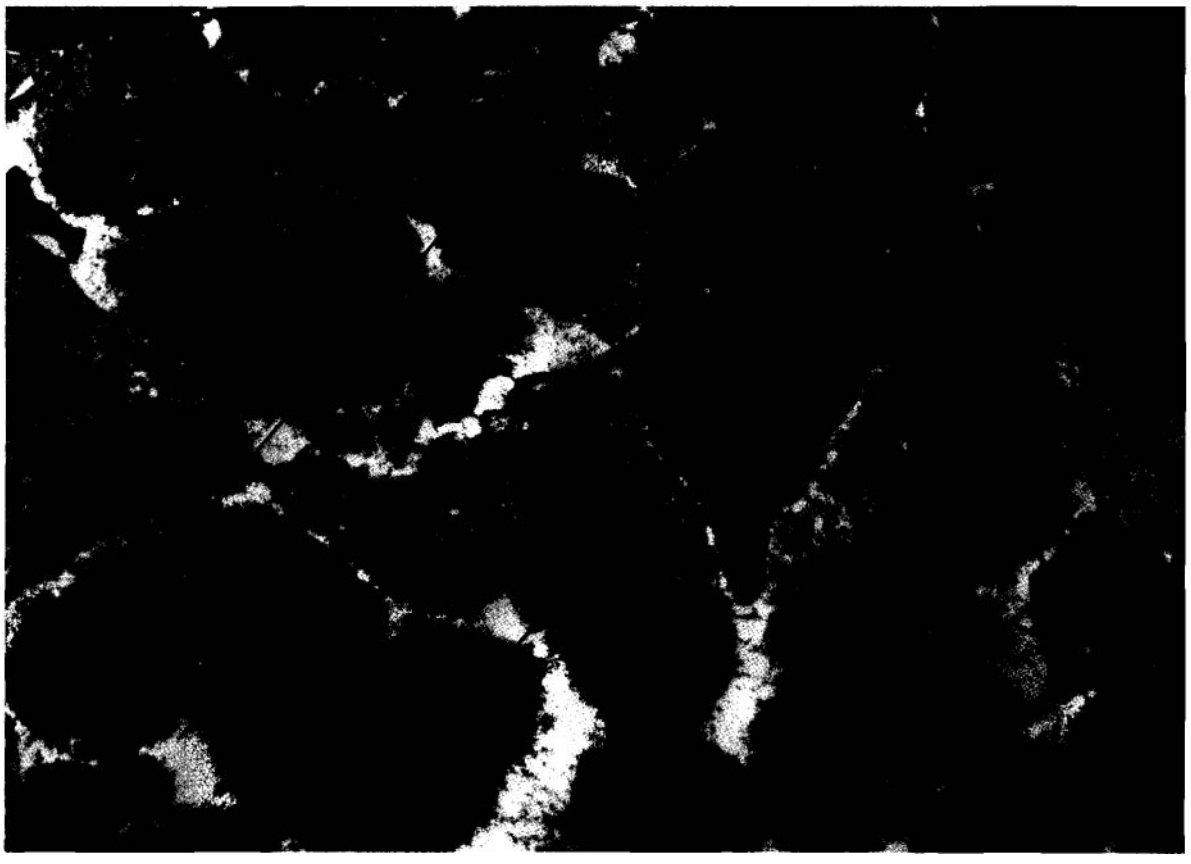


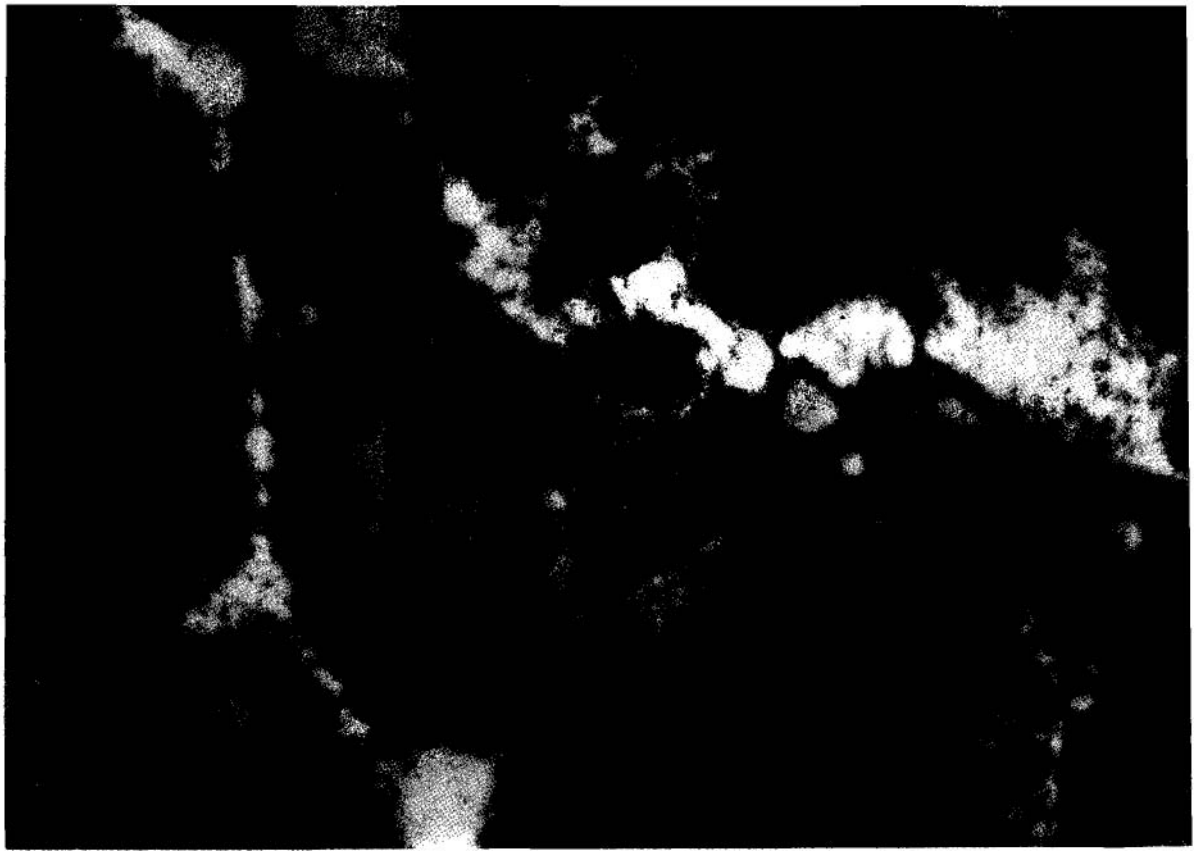


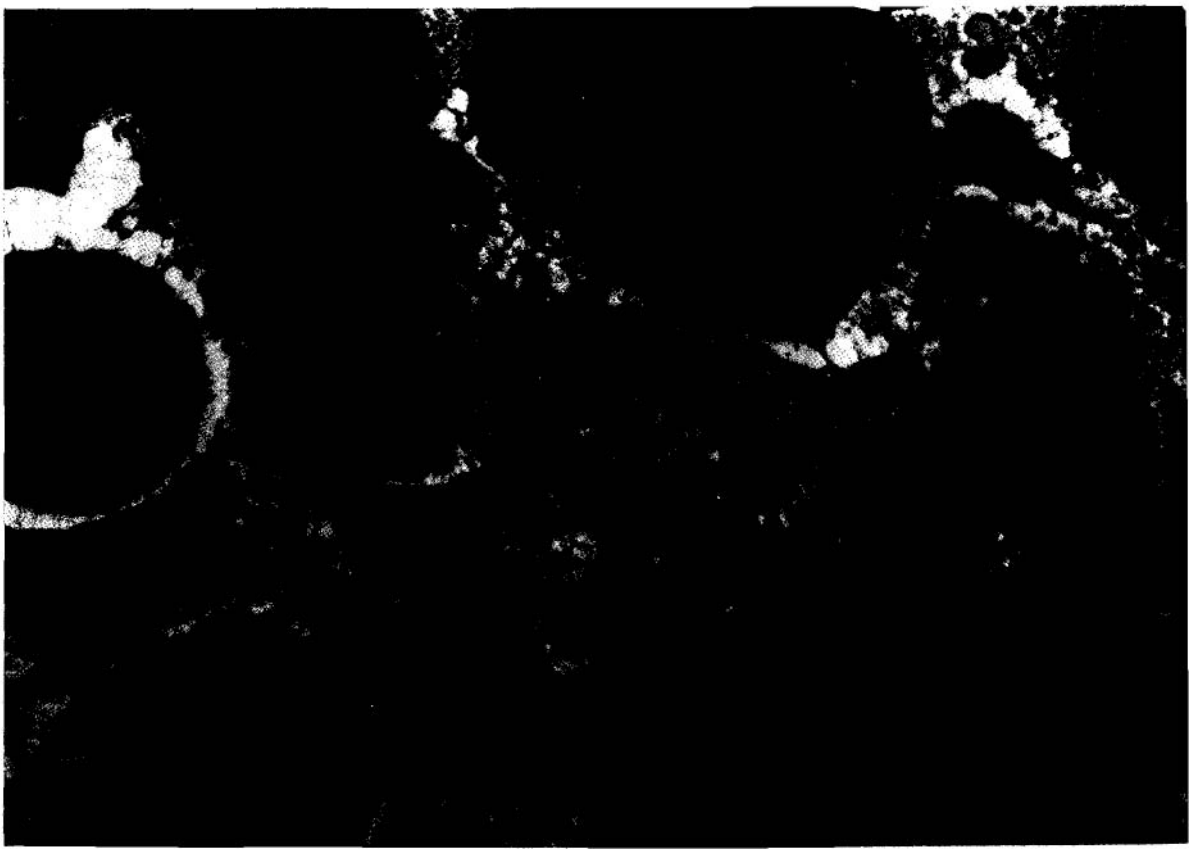












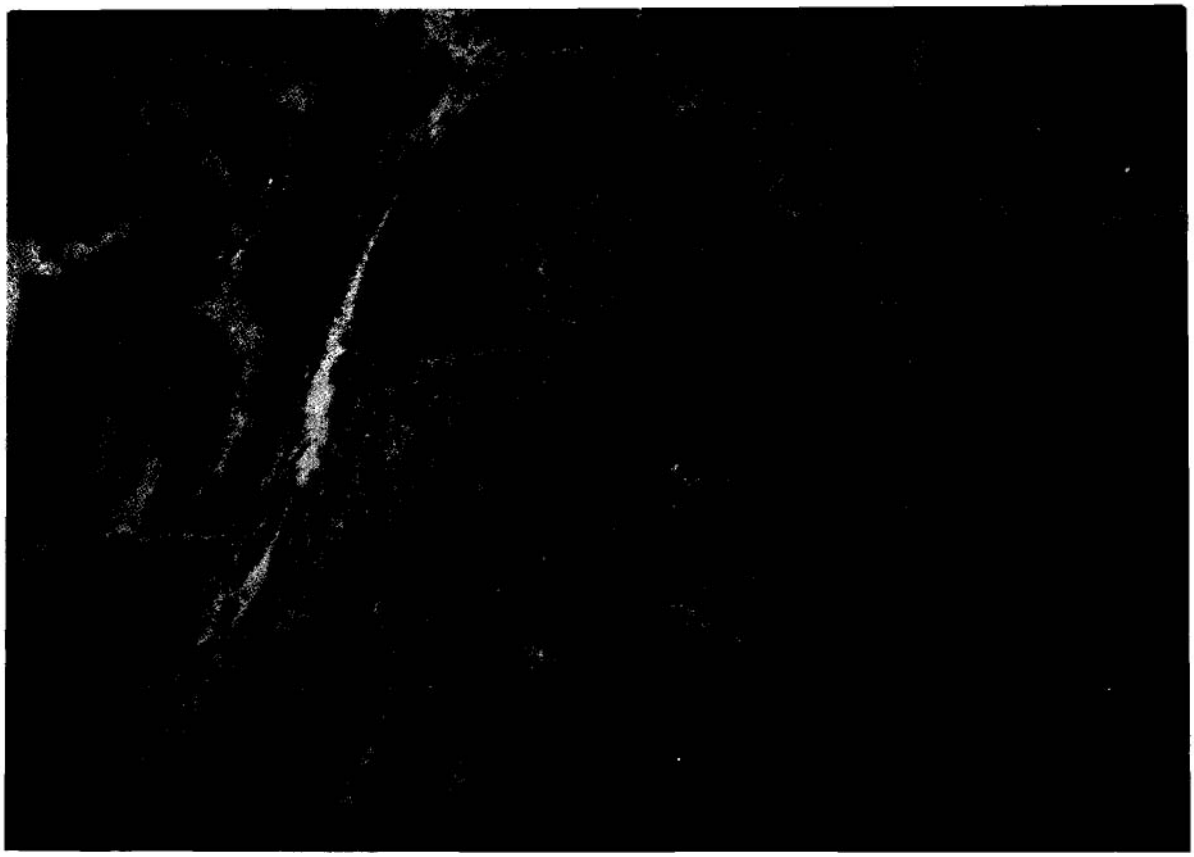


Fig. 1. Cortex of lymph node. Amyloid deposits showing nodular features in various sizes and shapes, often enclosed by reticulum cells (RC) and/or macrophages (M). Concentric circular core (C) and radial arrangement of amyloid fibrils (f) are distinctly seen. x 1,000

Fig. 2. Section from a part of secondary follicles showing marked amyloid nodules (AN) in germinal center but slight involvement of lymphocyte corona. A small number of residual dendritic reticulum cells (DRC), macrophages (M) and centrocytes (Ce) remain. x 1,000

Fig. 3. Section showing amyloid nodule embraced by macrophage in marginal sinus. The nodule consists of prominent core (C) in the central portion and fibrillar composition (f) in the outlying section. x 5,000

Fig. 4. Amyloid nodules (AN) radiating bundles of amyloid fibrils (AB) in the paracortex. Notice various types of cells, reticulum cells (RC), macrophages (M), lymphocytes (L) and plasma cells (PC) abutting the amyloid nodules. x 1,000

Fig. 5. High magnification of Fig. 4. The extreme point of amyloid bundles (AB) is closely connected to the outer membrane of abutting cells. x 20,000

Fig. 6. Section showing the lymphocyte (L) abutting the amyloid nodule (AN). x 1,000

Fig. 7. High magnification of Fig. 6. Amyloid bundles closely adhere to the cytoplasmic membrane of lymphocyte. x 20,000

Fig. 8. Plasma cell (PC) abutting amyloid nodule (AN). x 1,000

Fig. 9. High magnification of Fig. 8. Amyloid bundles (AB) closely adhere to the plasma cell (PC). Cytoplasmic membrane of the plasma cell can hardly be traced because of absence of the membrane. x 20,000

Fig. 10. Residual macrophage (M) in the mass of amyloid nodules. A part of the cytoplasmic process is under degeneration, x 3,000

Fig. 11. High magnification of Fig. 10. Homogeneous amorphous materials (primitive amyloid plaque, AP) are recognizable in the cytoplasm of macrophage in which residual cell organella remain, but cell membrane is partially missing. x 10,000

Fig. 12. Photograph showing degenerative change of reticulum cell (RC). x 3,000

Fig. 13. High magnification of Fig. 12. In the distal portion of the process, cell membrane and organella have completely vanished and intracellular deposition of amorphous materials (AP) are visible, comprising a fibrillar structure (f), suggesting amyloid fibrils. x 10,000

Fig. 14. Section showing a later stage of cellular degeneration of foreign body giant cell. The nucleus is karyolitic and cytoplasm is essentially devoid of cell organella. x 2,000

Fig. 15. High magnification of Fig. 14. The major part of cytoplasm and nucleus is replaced by amyloid fibrils showing nonbranching long-straight parallel arrangement. x 20,000

Fig. 16. Electron micrograph showing the last stage of amyloid fibril formation with appearance of radial bundles (AB) in the outlying section. Original structure of this nodule is not clarified. x 2,000

In the distal portion of the reticulum cell processes, the cell organella and cytoplasmic membrane vanished and primitive amyloid plaque simultaneously appeared (Fig. 12). We found fibrillar structures of parallel array in the newly-formed plaque (Fig. 13). With the progress of cellular degenaration, cell death resulted, the cell organella and cytoplasmic membrane of the dying cells completely vanishing. Therefore, we were only able to recognize the traces of the cells by the residuum of the nucleus. In such cases, not only all parts of the cytoplasmic matrix but also those of the nucleus had been completely replaced by amorphous materials (Fig. 14). High power observation showed a nonbranching, long-straight fibrillar structure, in parallel arrangement, with highly compacted electron density (Fig. 15). In the last stage of fibril formation, the radiated bundles of amyloid fibrils appeared at the peripheral edge of newly-forming nodules (Fig. 16). Their original structure was impossible to visualise due to the extensive damage suffered by the nodule.

Discussion

Since Virchow applied the term "amyloid change", because of the reaction against iodine and sulphuric acid, many investigations on amyloidosis have been carried out. As a histochemical method on the substance amyloid, Bennhold (1922) introduced Congo red staining, considered to be the most specific for detection of this pathologic substance in various organs. Ultrastructural observation of the substance amyloid was reported by Cohen and Calkins (1959) as a filamentous structure. Glenner et al. (1974) proposed that amyloid was used primarily as a genetic adjectival term to indicate the presence of nonbranching, 80-100 A fibrillar proteinaceous deposits demonstrating the possession of either Congo red birefringence or a beta-pleated sheet X-ray diffraction pattern with the chemical nature of the fibril. With the advance of biochemical investigation, it was clarified that the substance of amyloid fibrils and its precursor substance consisted of various types of protein. In primary amyloidosis or myeloma-associated amyloidosis, the amyloid fibrils consist of an immunoglobulin light chain or its portions, this being designated AL. Secondary amyloidosis is a condition characterized by the deposition of amyloid protein A and is designated AA. The precursor is recognized in response to chronic inflammatory or neoplastic disease as serum amyloid protein A, named SAA. Consequently, current diagnosis of amyloidosis is based on the biochemical and

immunohistochemical classification of amyloid protein. However, it is not clear how mechanisms participate in the formation of insoluble amyloid fibrils.

Domagk (1924) described marked changes in the reticulo-endothelial cells and the first appearance of amyloid in the neighborhood of these phagocytic cells in the experimental amyloidosis of the spleen and liver. Smetana (1927) strongly suggested that reticulo-endothelial cells were actively concerned in the formation of amyloid. Concerning the pathogenic mechanisms of amyloidosis, Teilum (1956) described the intimate morphologic relationship between newly-formed amyloid and fixed PAS positive reticulo-endothelial cells and considered highly the suggestion of a cellular morphogenesis of this substance. In "The Two-Phase Cellular Theory of Local Secretion", Teilum (1964) concluded that the common fundamental lesion in various forms of amyloidosis was a failure of the normal differentiation and maturation of plasmacytoid and other pironinophilic reticular cells. Experimental investigation concerning amyloid fibril formation in the reticulo-endothelial cells has been particularly successful in Kupffer cells in the liver. Battaglia (1962) observed amyloid in the vacuoles of Kupffer cells and presumed that intercellular amyloid was produced in the cytoplasm of Kupffer cells. Uchino (1967) presumed that the secretion process of amyloid from Kupffer cells may be considered in three steps: ingestion of precursor substance into the cells, synthesis of the substance and the secretion of synthesized material from the cells. Ishihara and Uchino (1975) reported that synthesized abnormal protein (preamyloid protein) in the stimulated Kupffer cells was transported from rER to the Golgi complex and then the secretory granules might be formed. Due to the dysfunction of lysosomal enzyme, the secretory granules may not be digested and preamyloid protein is polymerized forming amyloid fibrils which are released into the intercellular space by eccrine secretion and form the amyloid bundles.

Ranløv (1967) described thick bundles of amyloid fibrils arranged in parallel array radiating out from indentations of the cell border. Gueft and Ghidoni (1963) found amyloid bundles near the nucleus of histiocytes in the spleen of experimental amyloidosis. They supposed that histiocytes secreted an amyloid precursor and that amyloid fibrils would be formed in the invagination of cytoplasmic membrane. On the other hand, Shirahama and Cohen (1971) noted that amyloid bundles were not produced but were phagocytized by the reticulo-endothelial cells. Uchino (1967) observed that amyloid bundles frequently seen in experimental amyloidosis in animals were rarely encountered in biopsy specimens of human cases. These bundles were recognized at the early stage of amyloid deposit which gradually interlaced to form a felt-like structure.

Involvement of the lymphatic organs is common in generalized disease, thus amyloidosis of the lymph nodes is of particular interest. However, there are few reports of well-investigated cases, especially in ultrastructural observation. Newland et al. (1983) reported that, from a total of 40,000 lymph node biopsies in the files of the Lymph Node Registry in Kiel, there were only 10 cases of amyloidosis in which lymph node enlargement was the presenting feature. In the present study, we were able to keep the lymph nodes in a favourable condition. Therefore, we could observe well-preserved amyloid nodules, the cells connected with the bundles of amyloid fibrils and the mechanisms of fibril formation.

The present case was a 75 year-old woman. In the clinical findings, she had no symptoms of myeloma. Bence-Johnes protein was noticed, and the appearance of M protein (IgG lambda) with agammaglobulinemia became evident. Immunohistochemical examination presented a clasiffication into primary amyloidosis (A lambda). The amyloid deposits in the lymph nodes showed a round or oval nodular structure. Up to this time, it has been considered that a nodular appearance is more frequent in primary amyloidosis than in secondary amyloidosis, although this pheriomenon is a matter of course, as parenchymal deposition generally appears in primary amyloidosis and vascular deposition occurs in secondary amyloidosis. Radiated bundles of amyloid fibrils were frequently recognized in the marginal portion of amyloid nodules. The amyloid bundles adhered to the cytoplasmic membrane of the reticulum cells and/or macrophages surrounding the nodules, but sometimes they closely adhered to the lymphocytes and/or plasma cells. These findings show that the amyloid bundles adhering to the cytoplasmic membrane are not specific to the reticulo-endothelial cells and, moreover, they exhibit neither an active producing picture of amyloid fibrils nor a releasing feature of that from the cytoplasmic membrane. It is beyond doubt that macrophages phagocytize the extracellular amyloid fibrils. We have observed active phagocytosis of amyloid fibrils in addition to active erythrophagocytosis. In such cases, phagocytized fibrils were enclosed by a limited membrane and rarely showed a bundlar structure.

In the present study, the most interesting finding was the intracytoplasmic formation of amyloid fibrils which appeared in various stages of cellular degeneration. The early image of fibril formation was observed in the long-stretched cytoplasmic processes in which few recognizable residual organella remained. The cell membrane was missing in such portions and the continuity interrupted. At that time, small amorphous plaque with moderate electron-density appeared, gradually forming filamentous configuration resembling that of the extracellular amyloid fibrils.

Kisilevsky et al. (1977) reported that agents causing local inflammation and extensive tissue destruction provoked large quantities of splenic amyloid. Christensen and Hjort (1959) noted the acceleration of amyloid deposition in a few days following X-irradiation in amyloid-laden animals. In an electronmicroscopic study of the calcifying epithelial odontogenic tumor of the maxilla, Page et al. (1975) reported that the formation of extracellular amyloid masses was probably proceeded both by active cellular secretion and cell death. It suggested the possibility of amyloid fibril formation due to insoluble cytoplasmic material liberated by the dying tumor cells. Kumakiri and Hashimoto (1979) described that some of the amyloid substance in primary

localized forms of cutaneous amyloidosis derived from the epidermal cells through filamentous degeneration. Bergstrand and Bucht (1961) reported a homogeneous structure and filament in amyloid and presumed that the homogeneous structure developed into filaments.

In conclusion, we propose a speculation that each individual cell constituting the tissue involves amyloid fibril formation and depends on cellular degeneration and the appearance of a precursor substance. The mechanisms are due to the following steps:

1.— The individual cell falls into degenerative change due to various pathologic causes.

2.— Primitive amyloid plaque appears in the cytoplasm of the degenerating cell.

3.— Some precursor substance mingles with the primitive amyloid plaque, due to the absence of cytoplasmic membrane, and fibrils are formed.

4.— Extracellular deposit appears based on complete cell desolation.

5.— Soluble components of primitive amyloid plaques are washed away and insoluble amyloid fibrils remain, occasionally forming a nodular structure with core and bundles of amyloid fibrils.

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