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*Virchows Archiv A*


*Case Reports*

# Amyloidosis of the Stomach

## Report of a Case with Ultrastructure

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**Summary.** The author reports on a rare case of local amyloidosis (amyloid tumour) of the stomach. The first electron-microscopic study of such case was performed by her. She observed a large number of microfilaments in the mucus producing cells of the stomach mucosa, and assumed this to be indicative of a pathologic mucus secretion. The epithelial cells involved also changes indicating the disorder of protein secretion. She treats in detail the electron-microscopic characteristics of cellular elements found in amyloid. The closest relationship to amyloid deposits was shown by myofibroblasts. They probably play an important role in the formation of local amyloid.

**Key words:** Amyloid fibril – Epithelial cell – Myofibroblast – Intracellular filaments

### Introduction

The localized amyloidosis of the stomach was first described by Intriere and Brown (1956). Up to 1978 a total of five cases was presented in the literature. (Ikeda et al. 1978; Intriere et al. 1956; Hirayama et al. 1957; MacManus et al. 1976; Kakizaka et al. 1972). The practical importance of this rare change lies in that the X-ray and endoscopic pictures are suggestive of a malignant tumour. Differentiation is possible only by histological examination. With the spread of endoscopic examinations, it is to be hopes that even such a rare disease can be diagnosed.

The clinical course of localized tumour-like amyloidosis essentially differs from the generalized form since, while generalized amyloidosis may lead to death within some months, eventually 1 or 2 years, local amyloidosis has a good prognosis (Intriere et al. 1956; Kanada et al. 1979; Schoen et al. 1980).

In the case of a 69-year-old male patient, local amyloidosis resembling malignant tumour was detected. Diagnosis was formed on the basis of gastric biopsy made during surgery. On the basis of additional examinations, generalized amyloidosis could be excluded.

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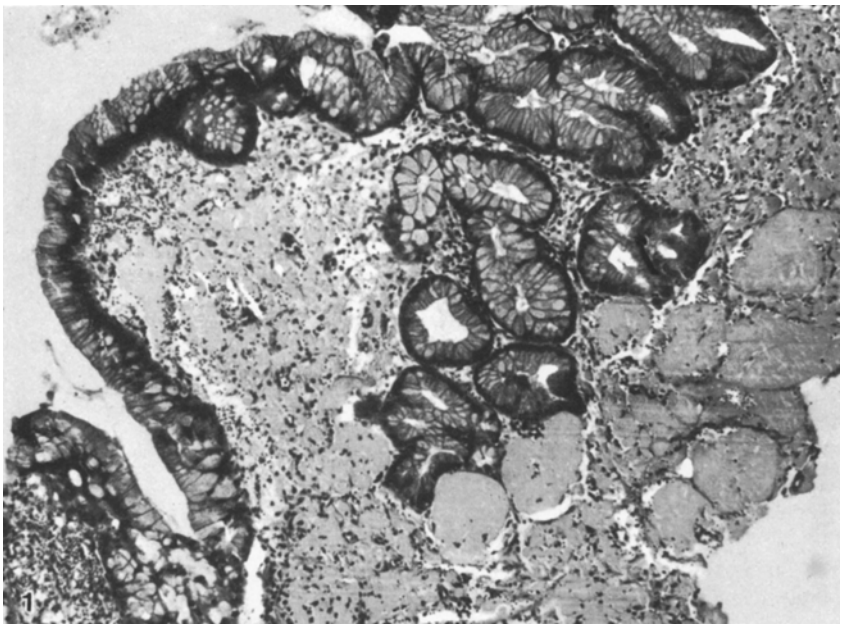

**Fig. 1.** Surface epithelium and glands of the gastric mucosa are recognizable. In the tunica propria there are homogeneous amyloid deposits. Haematoxylin and eosin (HE) × 130

Presentation of our case – beside being a rare one – is indicated by that in the case of local gastric amyloidosis no electron-microscopic examinations have been reported. In our investigations the following questions were to be answered:

1. Is there a characteristic ultrastructural change in the epithelial cells of the gastric mucosa which can be related to amyloidosis?

2. Which are the cells most closely connected with amyloid deposits? Is there a phenomenon in the cells from which the mechanism of amyloid formation can be inferred?

## Case Report

B.J., 69-year-old male patient had no previous history of disease. In January 1979 one night he had a pain in the stomach. Subsequently, he threw up and found some fresh blood in the vomit. After admission to the hospital these complaints did not return.

Physical examination revealed a good general condition. Of his laboratory findings the only pathological value was the positive Weber reaction of the stool X-ray of the stomach and gastroscopy showed a malignant tumour. Based on this finding, on 1st March 1979 he was operated. The serosa of the stomach was found to be intact. The gastric wall was thickened from the cardia to the middle third of the corpus and also soft. Longitudinal gastrotomy was made on the corpus. It was revealed that on the lesser curvature the gastric mucosa had a tendency to bleed and was gelatinous. For histological study material was taken. The change was believed to be a malignant process but, owing to its extension, also to be inoperable. Therefore no resection was made.

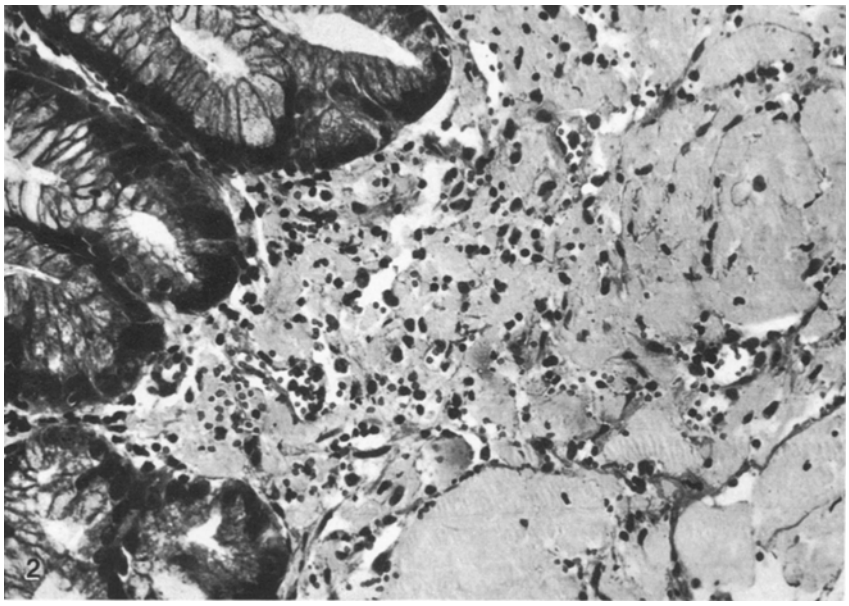

**Fig. 2.** Former change under higher magnification. HE ×180

*Result of Histology.* The surface of the gastric mucosa was covered by columnar epithelium. In the tunica propria and in the site of the tunica muscularis mucosae there was a large homogeneous mass of eosinophil staining (Figs. 1, 2). The gland of the gastric mucosa were well recognizable but were also removed from each other due to the accumulated deposits. The accumulated material was unambiguously proved to be amyloid both by Sirius and Congo red staining and by polarization and fluorescent microscopic examinations.

Postoperative course of the disease was uneventful. In view of the histological diagnosis, generalized amyloidosis was to be looked for. The urine contained no pathologic protein. The serum protein values were normal. The Congo red test gave negative result. Liver function tests and serum enzyme values were neither pathologic. In the biopsy material taken from the rectal mucosa no amyloid could be detected.

The patient was dismissed from hospital on 23rd March 1979 being free of complaints. In 7th July 1979, he appeared for control examination. Then again gastroscopy was performed. The result was as follows. The posterior wall of the upper two-thirds, involving also the lesser and greater curvatures, was covered by a thick, almost "ball" forming, very soft mass of tissue with a tendency to bleed. Biopsy was made for light- and electronmicroscopic studies. The patient was free of complaints, had good appetite and proper working capacity. He did not lose any weight.

*Method of Electron-Microscopic Examination*

1 $mm^3$ pieces of stomach mucosa were fixed in 1% Palade buffered osmium tetroxide, then dehydrated in a graded series of ethanol and embedded in Araldite. The sections were cut on a Reichert ultramicrotome and viewed in a JEM 100 B electron microscope. For orientation 0.5-micron-thick, toluidine blue-stained, semithin sections were prepared.

For control, 3 specimens of normal gastric mucosa were examined by the same method.

## Results

### *Epithelial Cells of the Gastric Mucosa*

The chief and parietal cells of the gastric mucosa did not differ electron microscopically from the normal (Rubin et al. 1968).

In considerable regions of the mucin producing cells the cytoplasm was filled with filamentous substance (Fig. 3). In the regions containing filaments there was a small amount of cytoplasmic organelles (Fig. 4). The filaments were arranged in bundles somewhere in the proximity of the nucleus (Fig. 5a). In other places they showed peripheral arrangement parallel to the cell membrane (Fig. 5b). Near to the cell surface the filaments were irregular (Fig. 6). The mucus secretion seemed to be impaired, the number of secretory granules was small. The epithelial cells contained in several places strongly dilated rough endoplasmic reticulum cysternae (Fig. 7). There were also areas where the cysternae contained an accumulated electron-dense material (presumably protein) (Fig. 8).

### *Cellular Elements of Amyloid Deposits*

In the amyloid mass and adjacent to it two types of cells were dominant: plasma cells (approximately in 30%) and fibroblasts (in about 60%). Beside this, mast cells and macrophages occurred in a smaller number. About 80% of fibroblasts were represented by the so-called myofibroblasts.

The nuclear chromatin of plasma cells had a characteristic pattern. In the cytoplasm there were parallelly arranged rough endoplasmic reticulum lamellae and intact mitochondria (Fig. 9). In several places the rough endoplasmic reticulum was dilated and contained finely granular material (Fig. 10). The cells contained well developed Golgi zones.

The nuclear membrane of the myofibroblasts was serpiginous with deep indentations (Fig. 11). The cell had an irregular shape, the cytoplasm contained mitochondria, rough endoplasmic reticulum and free ribosomes as well as a large amount of fibrillar mass arranged in bundles having characteristic dense regions. Along the cell membrane frequently several pinocytotic vesicles could be seen. The cell membrane of the myofibroblasts was broken in many places and the filamentous content of the cytoplasm mingled with the surrounding amyloid fibrils (Fig. 12). Elsewhere the remnant of myofibroblasts could be observed in the amyloid mass (Fig. 13). Individual filaments of the myofibroblasts measured 40–70 Å, the diameter of amyloid fibrils was 60–85 Å.

In the cytoplasm of the normal fibroblasts there were also several filaments 50–80 Å in diameter (Fig. 14). In the processes of fibroblasts in several places there was evidence of a deterioration of the cell membrane, and also of a close relationship between the cytoplasm and the surrounding amyloid deposits.

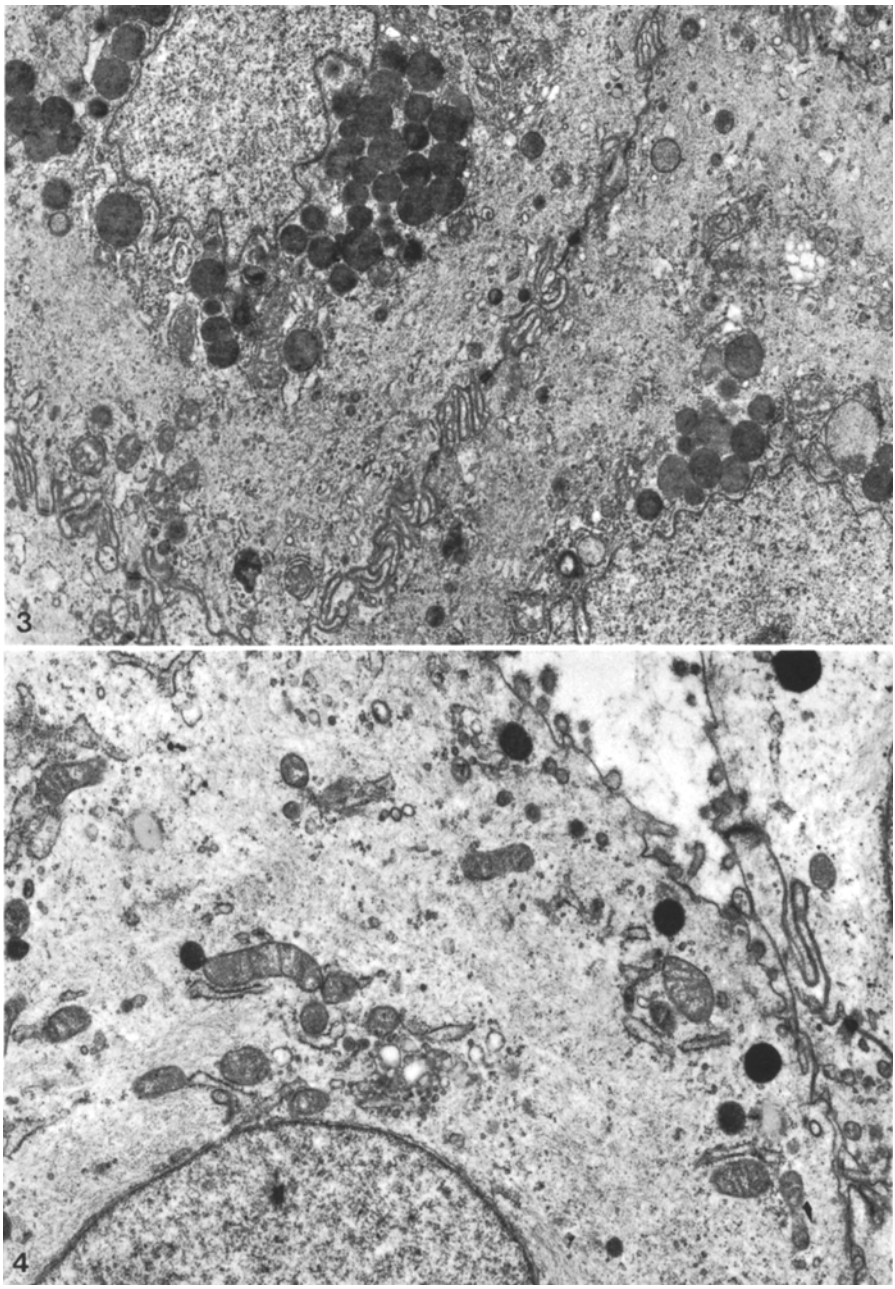


**Fig. 3.** The electron-microscopic picture shows detail of two epithelial cells. The secretory granules are situated near the nucleus. Large regions of the cytoplasm are light and poor in organelles. × 12,500

**Fig. 4.** In the light regions of the cytoplasm there is a filamentous material with scattered mitochondria and some secretory granules. × 16,500

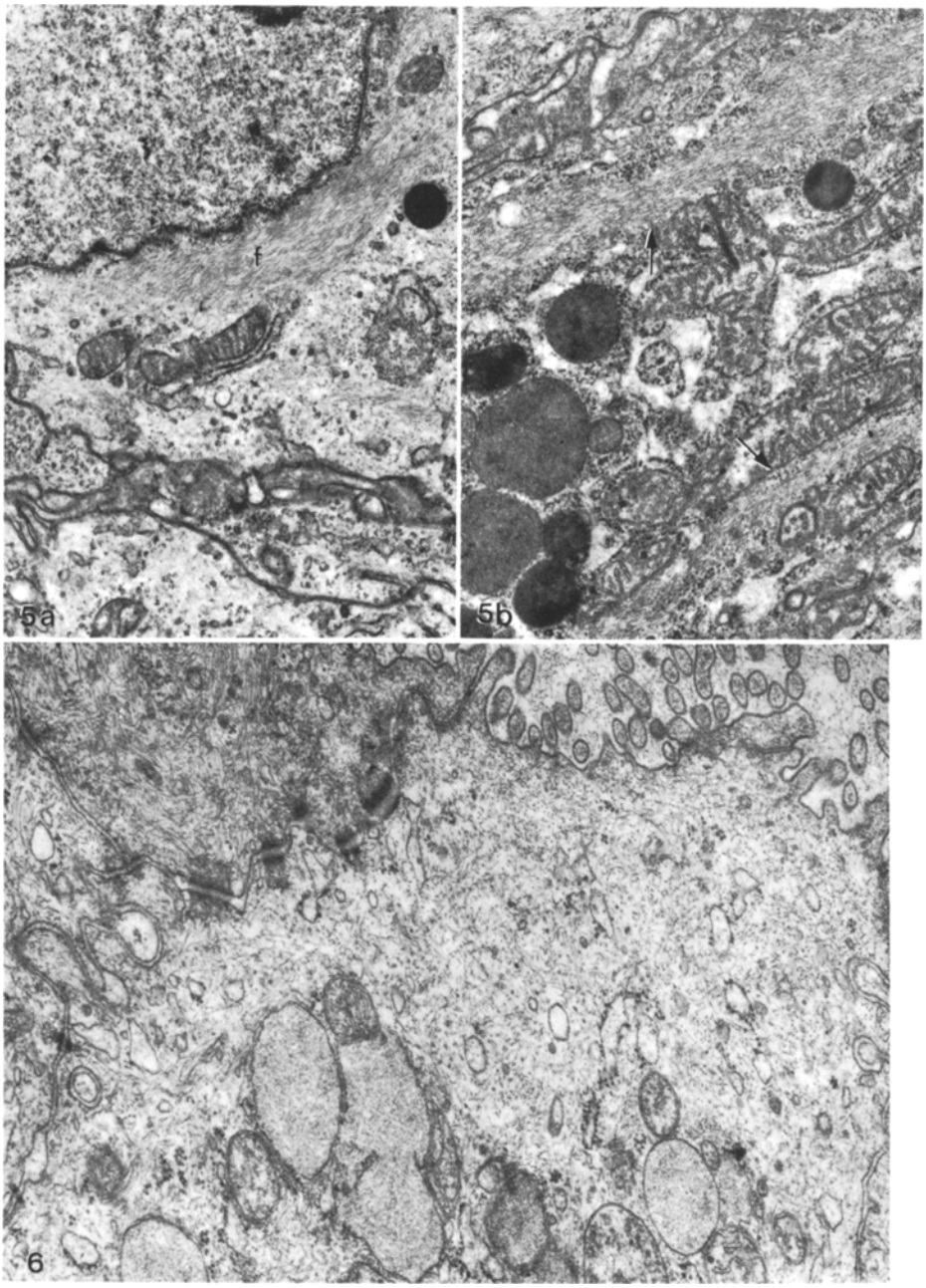


**Fig. 5. a** In the proximity of the nucleus microfilament bundles (*f*) can be seen. ×16,500. **b** Detail of the cytoplasm of an epithelial cell. On the periphery of the cell there are paralelly arranged microfilament bundles (*arrows*). ×25,000

**Fig. 6.** In the epithelial cells, in the regions adjacent to the luminal surface there are irregularly arranged microfilaments. ×31,500

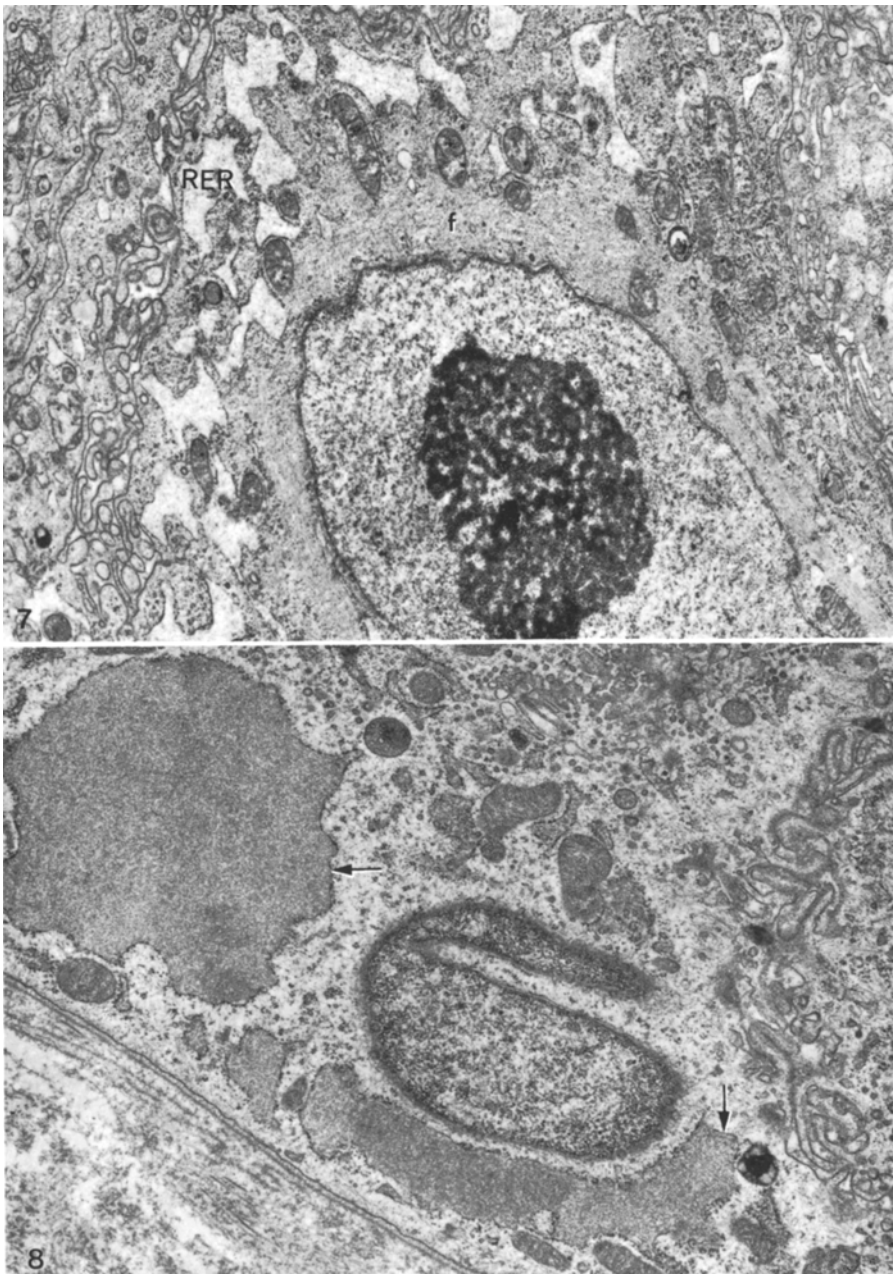


**Fig. 7.** Around the nucleus, filamentous material (*f*), in the peripheral region of the cytoplasm dilatation of the cysternae of the rough endoplasmic reticulum (*RER*) can be seen. × 12,500

**Fig. 8.** In the dilated cysternae of the epithelial cell there is a dense mass (*arrows*). × 16,500

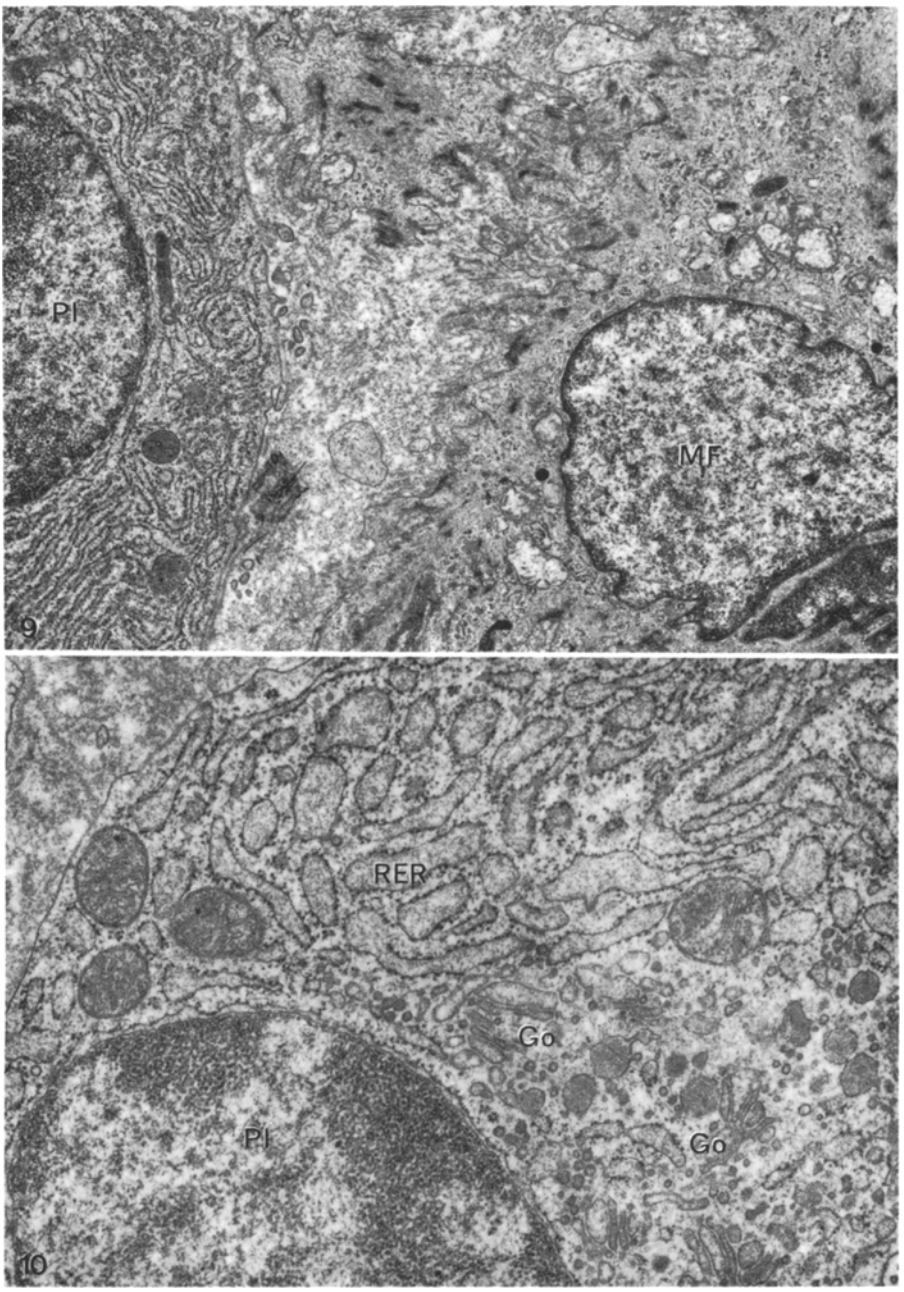


**Fig. 9.** The Figure shows a plasma cell (*Pl*) (*left*) and detail of a myofibroblast (*right*). × 12,500

**Fig. 10.** Detail of a plasma cell. In the cytoplasm the dilated cysternae of rough endoplasmic reticulum can be seen with a finely granular substance and some regular mitochondria and an extended Golgi zone. × 25,000

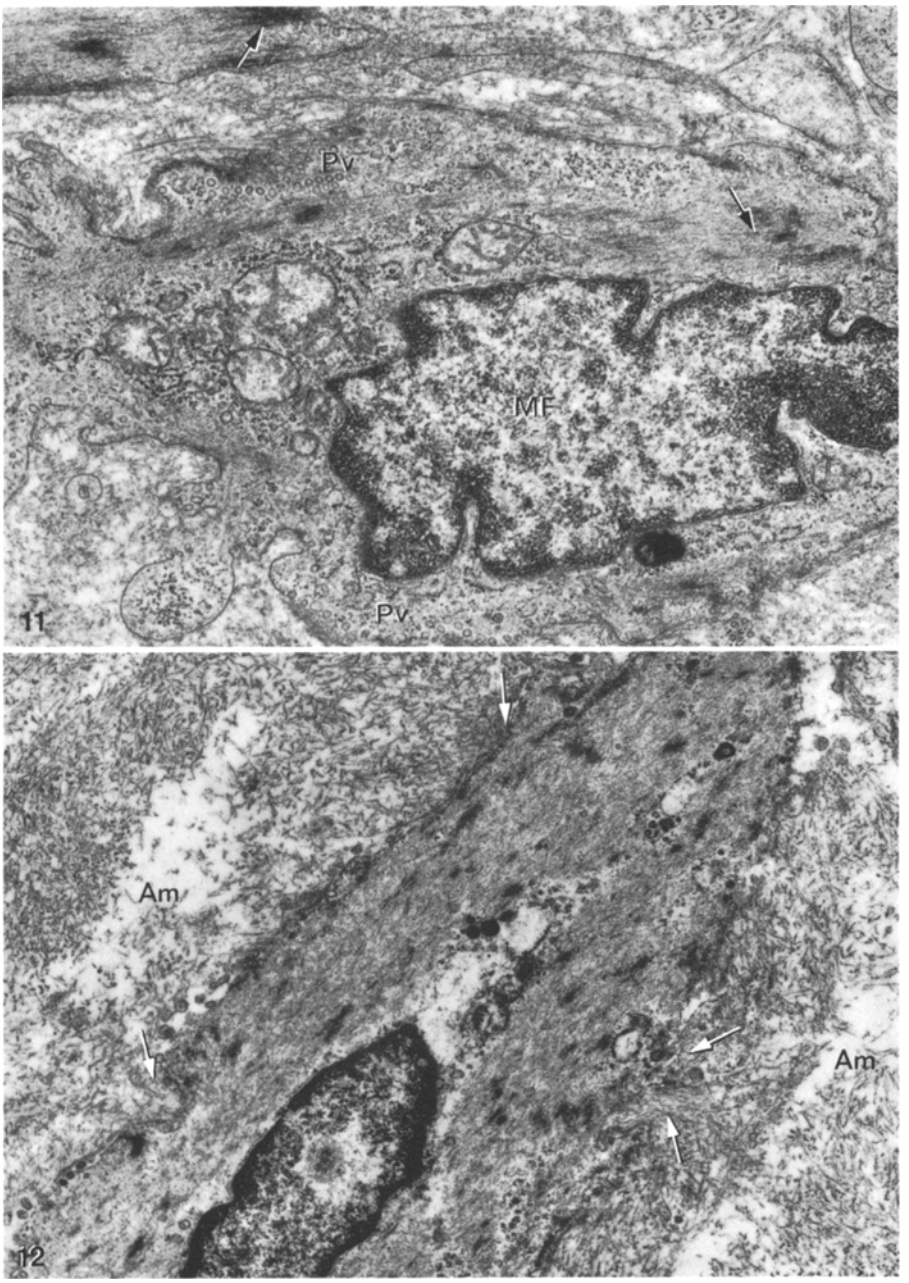


**Fig. 11.** Myofibroblast (*MF*) of a process-like shape, the nucleus containing indentations. In the cytoplasm there are mitochondria, rough endoplasmic reticulum and free ribosome. On the periphery of the cell there are microfilament bundles and pinocytotic vesicles (*Pv*). In between the filaments dense regions can be found (*arrows*). ×27,000

**Fig. 12.** Detail of a myofibroblast surrounded by amyloid fibrils. In the regions marked by arrows, the fibrillar material of the myofibroblast empties and mingles with the amyloid deposit. ×16,500

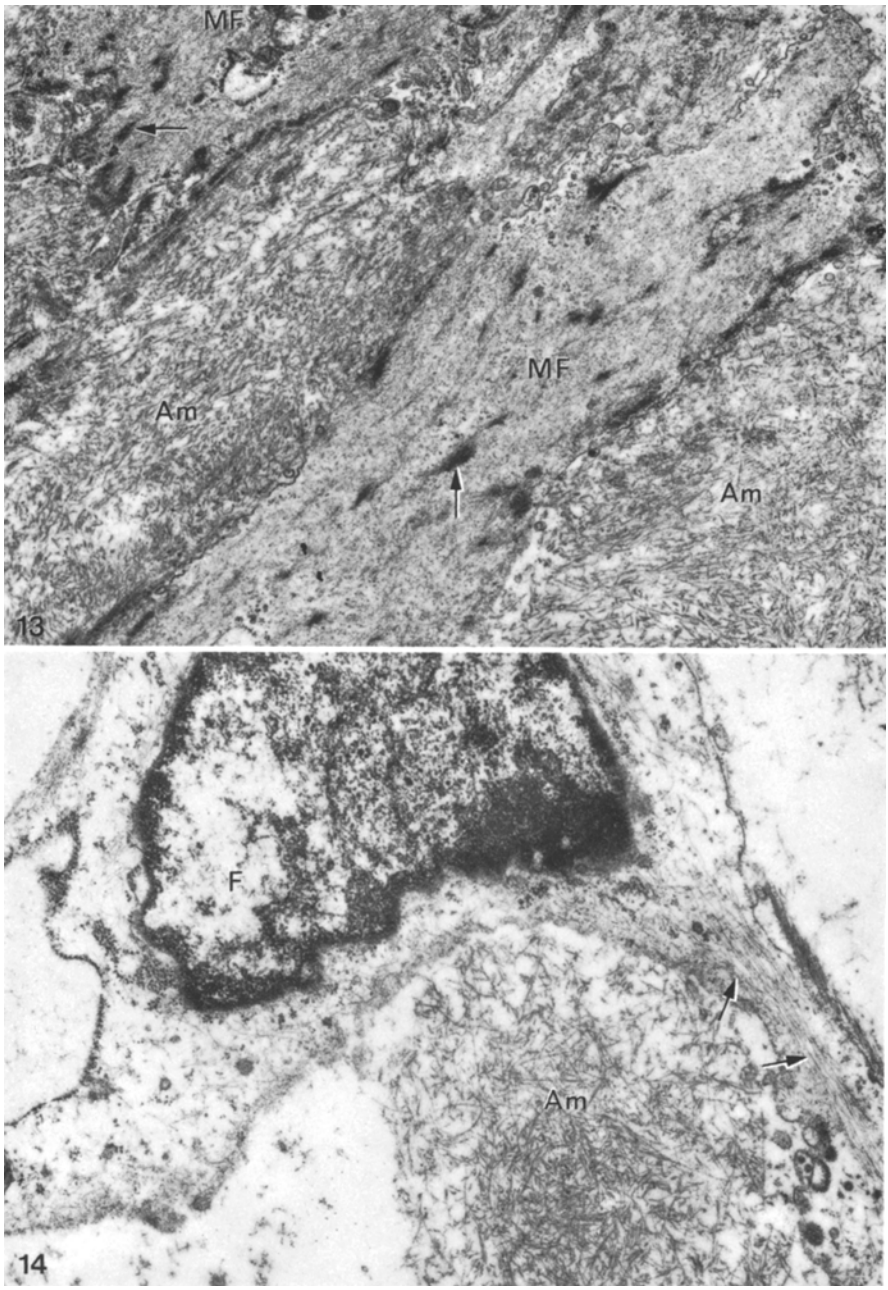


**Fig. 13.** In the amyloid mass (Am), the remnants of a myofibroblast (MF) with dense regions can be seen (*arrows*). × 16,500

**Fig. 14.** Detail of the nucleus and the cytoplasm of a normal fibroblast (F). In the cytoplasmic process, there are fibrils similar to the surrounding amyloid (*arrows*). In the same place, the cell membrane is missing. × 25,000

## Discussion

The chemical composition and morphological appearance of amyloid have been discussed by a great number of reports (Cohen et al. 1959; Holck et al. 1979; Husby et al. 1973; Romhányi 1956; 1972). Recently, summarizing reports have also been published (Cohen et al. 1978; Br Med J 1979; Wuchter 1978). Investigations proved that the amyloid is a complex material the main component of which is a fibrillar protein. The majority of amyloid fibrils can be found extracellularly. However, the exact site of their origin is not known. The generally accepted theory of the pathomechanism is the inadequate immune response to the repeated antigenic stimulus (Machado et al. 1979, Schultz 1977). Nowadays familiarity with the pathomechanism of amyloidosis is not only of a theoretical importance since the therapy attempted on the basis of the above conception seems to be successful both in animal experiments and in humans (Buxbaum et al. 1979; Scheinberg et al. 1976).

Electron microscopically, the amyloid is composed of 75–80 Å thick fibrils, being equally characteristic of each form os amyloidosis (Cohen et al. 1979; Romhányi 1972). Presumably, the material of amyloid precursor originates from the plasma cell but the final formation of amyloid fibrils is largely attributed to the macrophages of the reticulo-endothelial system and the fibroblasts (Ben-Ishai et al. 1968; Machado et al. 1979; Runne et al. 1977; Zucker-Franklin et al. 1970). Some authors reported on the intracytoplasmic appearance of amylois (Kjeldsberg et al. 1977; Michaelis et al. 1979; Zucker-Franklin et al. 1970).

Our investigations revealed that the cytoplasm of the mucus producing cells of the gastric mucosa contained a large amount of microfilaments replacing the cytoplasmic organelles. The microfilaments may play a role in cell motility, migration, phagocytosis, cell division, in changing the size of the cell and in the passage of secretion (Puchtler et al. 1975). They may accumulate under normal and pathological condition in several types of cells (Balázs 1978; Maccartney et al. 1979; Phillips et al. 1975; Welander et al. 1980). Michaelis et al. (1979) found in respiratory tract amyloidosis that, in the epithelial cells of the sero-mucinous glands, there is a large amount of fibrillar material having a continuity to the adjancent amyloid deposits. In our own case we were unable to demonstrateany connection between the filaments of the epithelial cells and the amyloid deposits. It is assumed that the change involving only the mucus producing cells of the gastric mucosa may indicate impaired mucus secretion possibly in response to the antigenic stimulus of unknown origin inducing the local amyloidosis. The secretory disorder was also indicated by accumulation of protein in many epithelial cells.

In the amyloid deposits, and adjacent to them, there were 3 dominant cell types: plasma cells, fibroblasts and myofibroblasts. The plasma cells gave the impression of active protein synthesis. Myofibroblasts had the closest connection to amyloid. The filamentous material of these have entered the extracellular space in many places mingling with amyloid fibrils. Myofibroblasts were described by Gabbiani et al. (1972) in granulation tissues of healing wounds. They play a role in the retraction of scar tissue due to their contractility (Bunt-

rock 1980; Gabbiani et al. 1972; Guber et al. 1978; Rudolph et al. 1977). They occurs also in human and experimental liver cirrhosis (Gabbiani et al. 1974; Toh et al. 1977; Rudolph et al. 1979) in the small intestinal villi of rats (Güldner et al. 1972) and in the stroma of mammary carcinoma (Ohtani et al. 1979). According to Stocco (1979) the increase of myofibroblasts can be regarded as a local immuno-metabolic reaction. Myofibroblasts have the ability to secrete different materials, too, as collagen, elastin and amyloid (Gabbiani et al. 1976; Runne et al. 1977).

In our case, the electron microscopic picture of the myofibroblasts located in the amyloid mass showed active secretion and production of microfilaments. We suppose these cells produce some component of the chemically heterogene amyloid mass. To prove this hypothesis further studies are needed.

Summing up our results, the questions raised in the beginning can be answered as follows:

1. In the case of local gastric amyloidosis, electron-microscopic alteration of mucus production and protein secretion were observed in the mucus producing cells of the gastric mucosa.

2. The myofibroblasts showed the closest contact with amyloid deposits. Their electron microscopic picture revealed considerable secretional activity. It can be suspected that these cells take part in the production of some components of the amyloid mass.

If we accept that the amyloidosis is an inadequate immune response to some sort of antigenic stimulus, than the above described phenomenon can be explained by an identical antigenic stimulus causing the alterations of the mucus producing cells of the gastric mucosa, on one side and the increase of myofibroblasts, on the other.

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