Electron Microscopic Observation on Human Liver Amyloidosis

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Although human amyloidosis is a rare disease in Japan, it is not so uncommon in Europe and America. Therefore, there are a lot of reports on human and experimental amyeoidosis in Europe and America, but in Japan these reports are few.

Authors experienced liver amyloidosis that was diagnosed by light microscopical examination of liver tissue obtained from needle biopsy.

For the second time of biopsy, tissue pieces were examined with electron microscope.

Present report described light and electron microscopic findings of a case of liver amyloidosis. Moreover, this case might be the first report of electron microscopic observation of human liver amyloidosis in Japan, and brief findings of this case was already reported in *Tr. Soc. Path. Jap.*[1)]

CASE REPORT

The pertinent medical history of this case of a 34-year old male began in 1956 with thirst and polyuria. In the Autum of 1958 he admitted to a hospital because of fever, anorexia and epigastric distress. At that time, the liver was palpable 2 fingers breadth below the right costal margin. Urinalyses revealed a 1 plus albuminuria, but urobilinogen and urobilin were negative.

Anorexia, epigastric distress and loss of weight became more severe and the liver was palpable 4 fingers breadth below the right costal margin in November of 1958.

Early in December, he admitted to the 1st medicine of the hospital of Yamaguchi Medical School.

Blood chemistry determinations were shown in Table 1 and urinalyses were as follow; polyuria, 2500 c.c. per day, albuminuria, 250 mg per 100 ml., and specific gravity, 1010.

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Table I. Examination of Blood Chemistry

	9 Dec.	17 Feb.		9 Dec.	17 Feb.
Serum protein (g/dl)	7.7	5.8	Cholesterol (mg/dl)	278	395
Albumin (g/dl)	3.8	1.8	Phenol turb. test (U)		34
Globulin (g/dl)	3.9	4.0	Blood suger (mg/dl)	66	82
Alb./Glob. ratio	0.98	0.45	NPN (mg/dl)	25	25
Icteric index	4	7	Urea N (mg/dl)	11	11
CCFT	—	++	Hemoglobin (g/dl)	12.8	12.4
GPT (U)	14.5	13.0	Hematocrit (%)	38.0	37.1
Cholinesterase (ΔpH)	0.94	0.73	MCHC (%)	33.7	33.4
Alk. Phosphatase (U)	9.6	12.6			

Urea clearance and PSP tests were within normal limits.

A Congo red test showed 100% disappearance after 15 minutes.

The twice liver biopies were performed during the admission in the hospital of Yamaguchi Medical School.

Material and Methods

The 1st needle biopsy was performed on 11 Dec., 1958. Removed liver tissue was fixed in Carnoy's fluid, embedded in paraffin, sectioned and stained with hematoxylin and eosin, gentiana violet, PAS, Congo red, and van Gieson stain for examination in light microscope.

The 2nd of liver biopsy was performed on 4 Feb., 1959. The removed tissue was divided into two blocks of each a few millimeters. One of them was cut immediately small into pieces of about 0.5–1 mm., and those were immersed in cold 1% O_sO_4 buffered to pH 7.4 with a veronal-acetate buffer. Following 1 hour fixation in O_sO_4, the pieces of tissue were washed with physiologic saline solution, dehydrated in graded ethanols, and embedded in a 1 : 4 mixture of methyl and buthyl methacrylate. Thin sections were cut on a SHIMAZU K–1 or HITACHI UM–3 ultramicrotome and a few sections were stained with potassium permanganate.[2] The unstained and stained sections were examined with a JAPAN ELECTRON OPTICAL LAB. JEM 5–HS electron microscope.

The another block was empolyed to examination in the light microscope.

RESULT

Light Microscopy

The hematoxylin and eosin stained sections of the liver block of the 1st biopsy revealed numerous depositions of hyaline-like material between the endothelium of sinusoids and the parenchymal cells (Space of DISSE). The depositions tend to

cause pressure atropy of the liver cell cords. The deposits were colored lightly pink in PAS stain, lightly red in a Congo red stain, redish violet in gentiana violet stain, and lightly yellow in van Gieson stain.

These deposits of hyaline-like material were identified as amyloid substance with above results of staining reactions and the situation in the liver tissue. These amyloid were not observed in the wall of interacinal blood vessels.

Furthermore, the bile thrombi were observed in bile canaliculi there, and granules of bile pigment and lipofuscin were recognized in the cytoplasm of parenchymal cells.

The patient was diagnosed amyloidosis of the liver, according to the above findings.

Electrom Microscopy

Amyloid substance

Amyloid deposits were localized between the parenchymal liver cells and sinusoid lining cells. This finding closely correlated with the areas of amyloid as seen in stained sections by light microscopy. But sometimes the sinusoid lining cells had a imperfect cell membrane immediately adjacent to the amyloid deposits.

In the specimens stained with potassium permaganate, filaments mainly showed haphazard pattern and partly showed in bundles. The individual filaments were approximately 100 Å in width. Length of filaments wes difficuly to measure because of random planes which the filaments pursued. At places the collagenous fibrils were observed within amyloid deposits but these could be identified by size.

Liver parenchymal cell

The cell membrane of each parenchymal cells close to amyloid deposits showed decrease in number or disappearance of microvillis.

The mitochondria were irregular in outline and those appeared slightly enlarged. Those mitochondrial matrix were slightly paler than normal. Occasionally interruptions in the continuity of outer mitochondrial membranes were seen. The rough surfaced endoplasmic reticulums showed a dilatation of the space limited by the paired membranes.

The lipofuscin, bile pigment granules and lipid droplets were observed in cytoplasm.

Frequently, the parenchymal cells showed the deformation caused by the pressure of amyloid deposits.

DISCUSSION

The electron microscopic observations described here on the filamental structure of amyloid deposited in human liver are similar to those by COHEN and CALKINS.[3] A similar filamental appearance has been reported also in experimentally produced

amyloid.[4)~8)] These filaments were somewhat variable but were approximately 100 Å in width. This is comparable to measurements made by COHEN[3)] and others.

COHEN et al[3)] reported that in the material stained with phosphotungstic acid the filaments were more clearly defined than the unstained sections. In the present study the filamental appearance of amyloid deposits was more clearly observed in the section stained with potassium permanganate.[2)]

We have observed an increase in plasma cells in smears of bone marrow, and electron microscopic studies of CAESER[6)] and LETTERER et al[5)] have reported a close relationship of amyloid deposits with plasma cells or thrombocytes. However, such a relationship was not observed with light and electron microscopically in the liver tissuess of the present case.

On the other hand, we have seen sometimes an intimate association of amyloid deposits with endothelial cells of sinusoid. GEER et al[9)] reported that there is an increase in the number of endothlial cells in the glomerular capillaries of secondary amyloidosis and endothelial cytoplasm is continuous with amyloid. But they believe that amyloid is a pathological alteration of the connective tissue ground substance whether condensed into basement membrane or present in usual from because the amyloid is found in the kidney in intimate association with basement membrane or interstitial substance.

HEFFNER and SORENSON distinctly demonstrated that deposits of amyloid appeared to lie entirely within the cytoplasm of splenic or hepatic reticuloendothelial cells in experimental amyloidosis. They suggested a possible relationship between these reticuloendothelial cells and amyloid production.

From present observations we supported the HEEFNER and SORENSON's report.

SUMMARY

Amyloidosis of the liver was diagnosed in a 34-year old male by needle biopsy.

Sections of the liver were observed by light microscopy and electron microscopy.

By electron microscopy amyloid deposits were recognized in the space of DISSE. Amyloid deposits were shown as filamental structure of about 100 Å in width. Amyloid deposits were found contiguous with cytoplasm of endothelial cells of sinusoid. The relationship of amyloid deposits and endothelial cells were discussed.

(The patient died on 4 April 1959. As the result of autopsy, present case was diagnosed primary generalized amyloidosis. Details of clinical and pathological findings were reported in the Clinic of Digestive Disease 2:412–418,;1960. (KANAHARA, Japan.))

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EXPLANATION OF FIGURES

Fig. 1. and 2. Light micrograph of liver tissue. Amyloid deposits can be seen between liver cell cords and sinusoids.

Fig. 3. Electron micrograph of amyloid deposited in DISSE's space. Sinusoid containing a red blood cell (red) becomes narrowed by the pressure of amyloid deposits (amy).

Fig. 4. Electron micrograph of amyloid deposits which are composed of fine filaments. Many filaments are arranged haphazardly. (Potassium permanganate stain)

Fig. 5. Electron micrograph shows collagen fibrils with amyloid deposits. Several collagen fibrils (Col) can be seen at the lower left part of figure.

Fig. 6. Electron micrograph of amyloid (amy) and parenchymal lever cell. Microvilli (mv) of parenchymal cell show deformation and decrease in number. Deposits are observed as delicate filamental pattern.

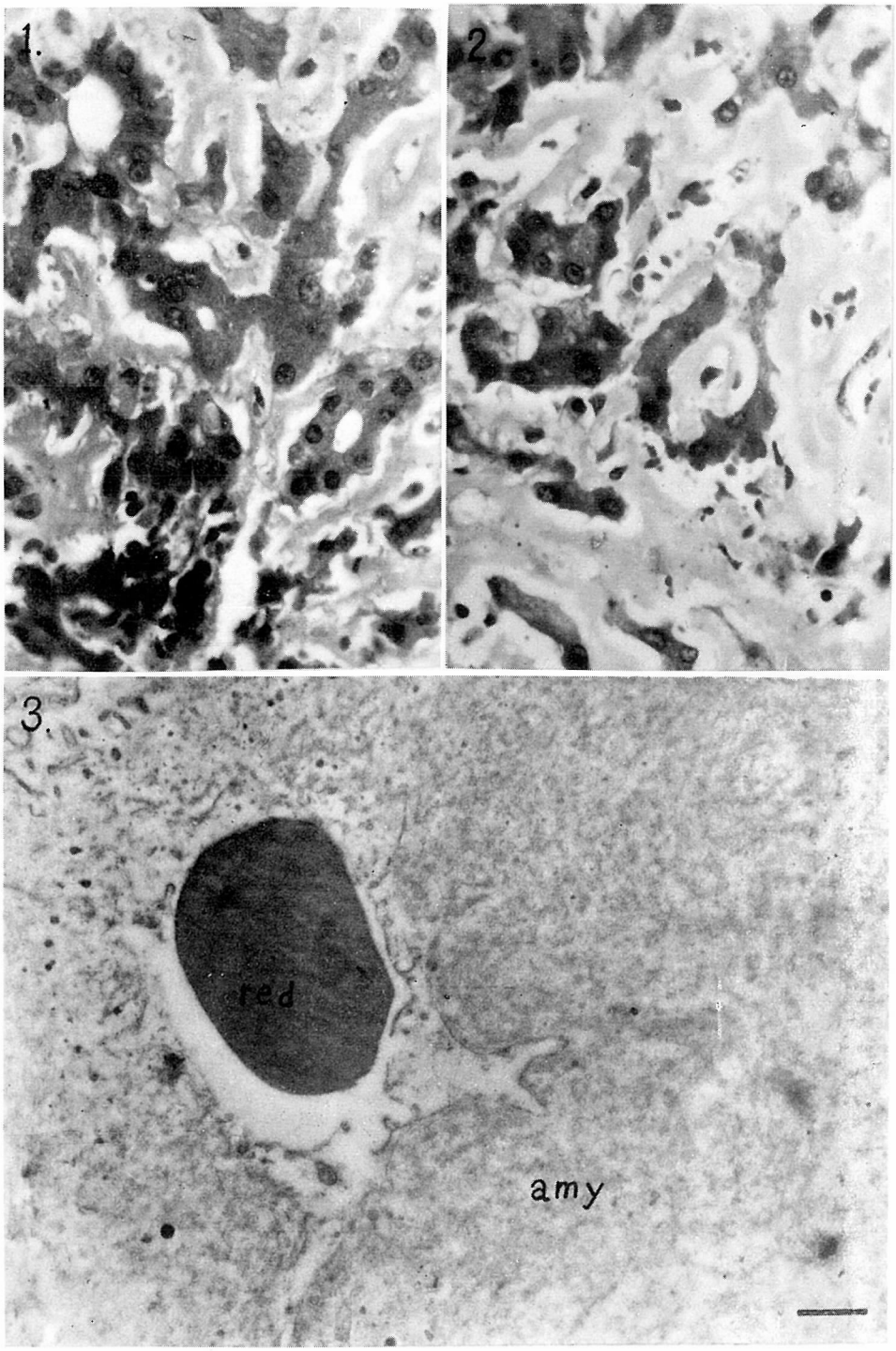
Fig. 7. Electron micrograph of mitochondria of liver parenchymal cell. Some of mitochondria show irregular outline. The mitochondria have moderately dense matrix which contain several extremly dense small granules (mg).

Fig. 8. Electron micrograph of mitochondria and rough surfaced endoplasmic reticulums of parenchymal cell. Rough surfaced endoplasmic reticulums (res) show a dilatation of the space surrounded by limiting membrane. Interruption in the continuity of mitochondrial membrane (↑) can be seen on the right upper areas.

Fig. 9. Electron micrograph of degenerated parenchymal cell. Endoplasmic reticulums have bead-like appearance. The mitochondria show irregular outline. Lipid droplet (lip) can be seen on the upper left corner.

Fig. 10. Electron micrograph of parenchymal cell. Parenchymal cell shows deformation caused by the pressure of deposited amyloid. Artifical gap can be seen between amyloid and parenchymal cell.

Fig. 11. Electron micrograph of lipofuscin granules. Lipofuscin granules lie in close proximity to the nucleus (n) of parenchymal cell. Within the granules three electronopacities can be seen: peripheral electron-opaque material, a material of moderate opacity in globular areas and inner vacuole-like electron-lucid regions.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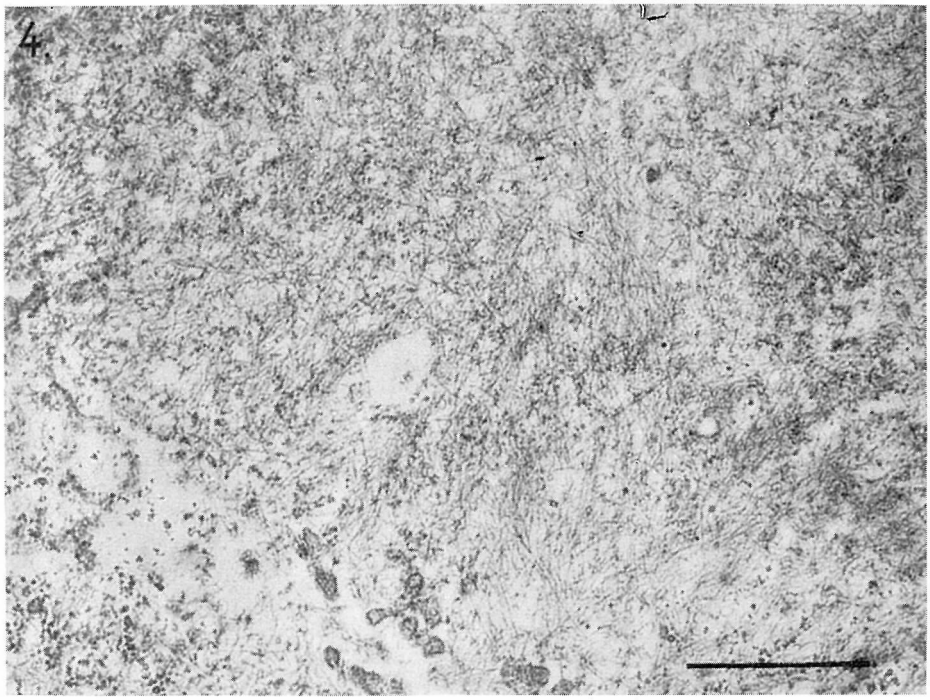
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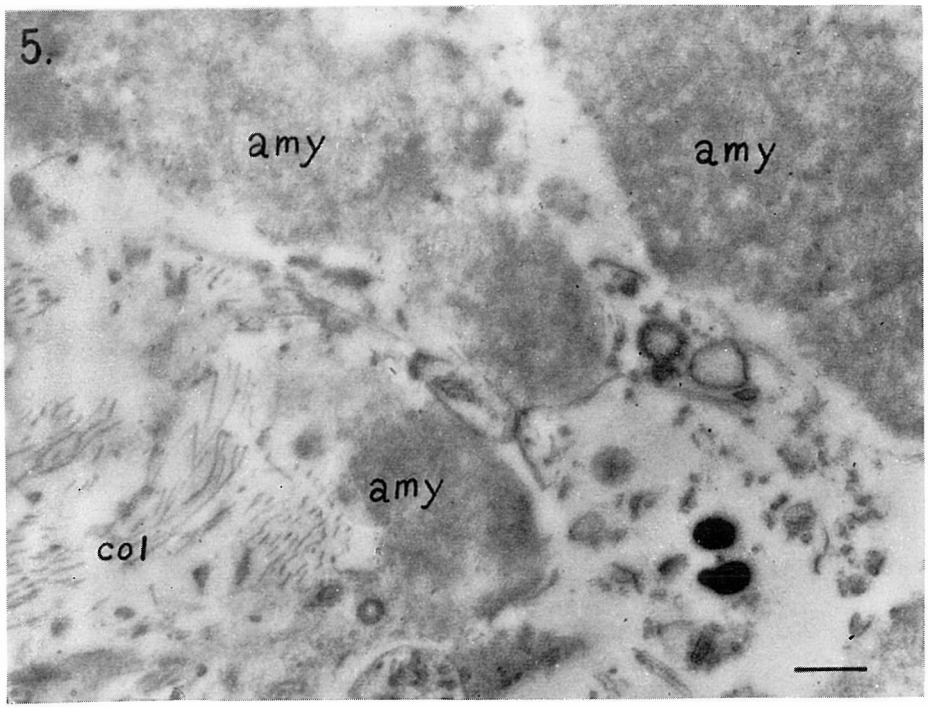
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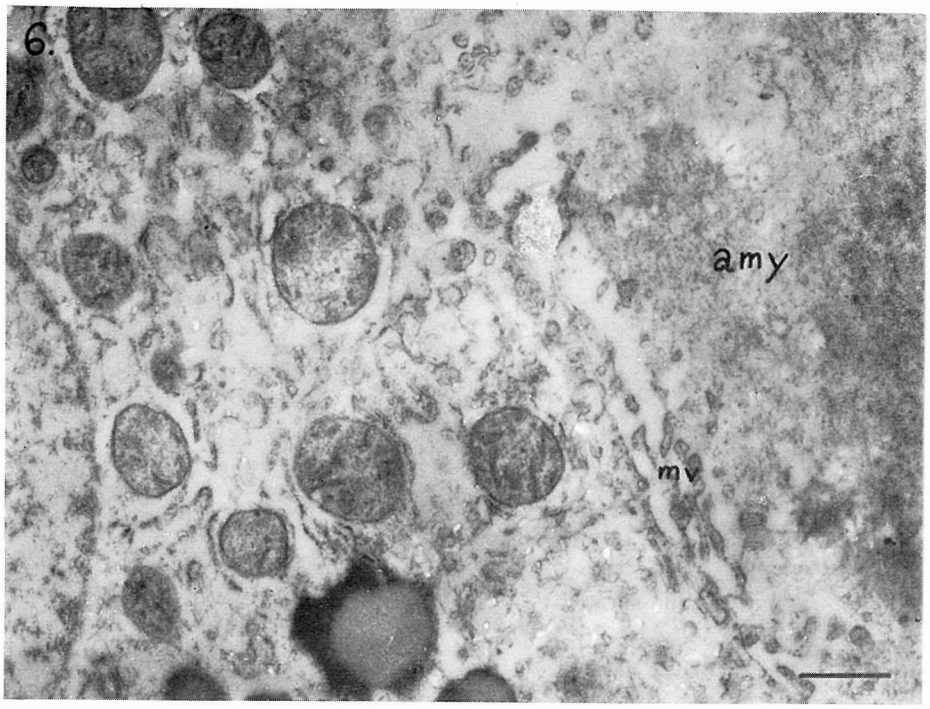
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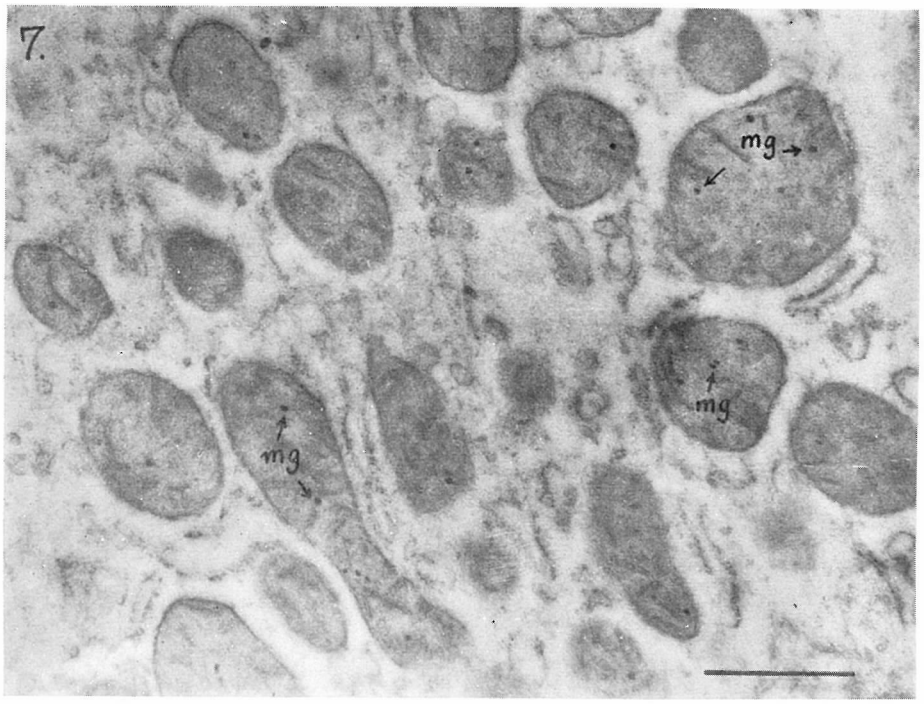
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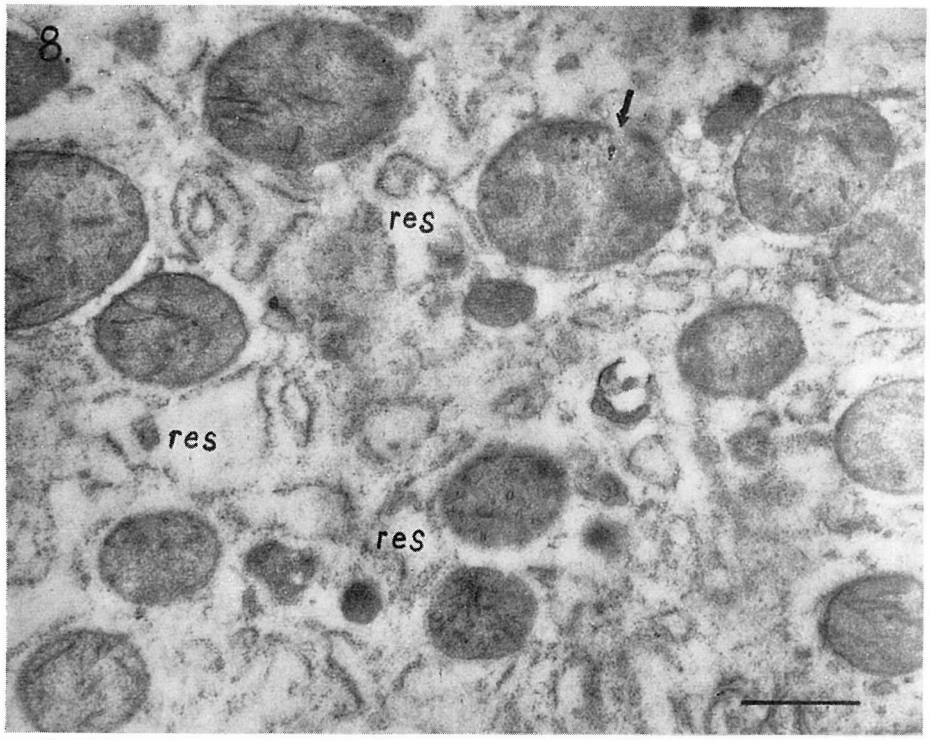
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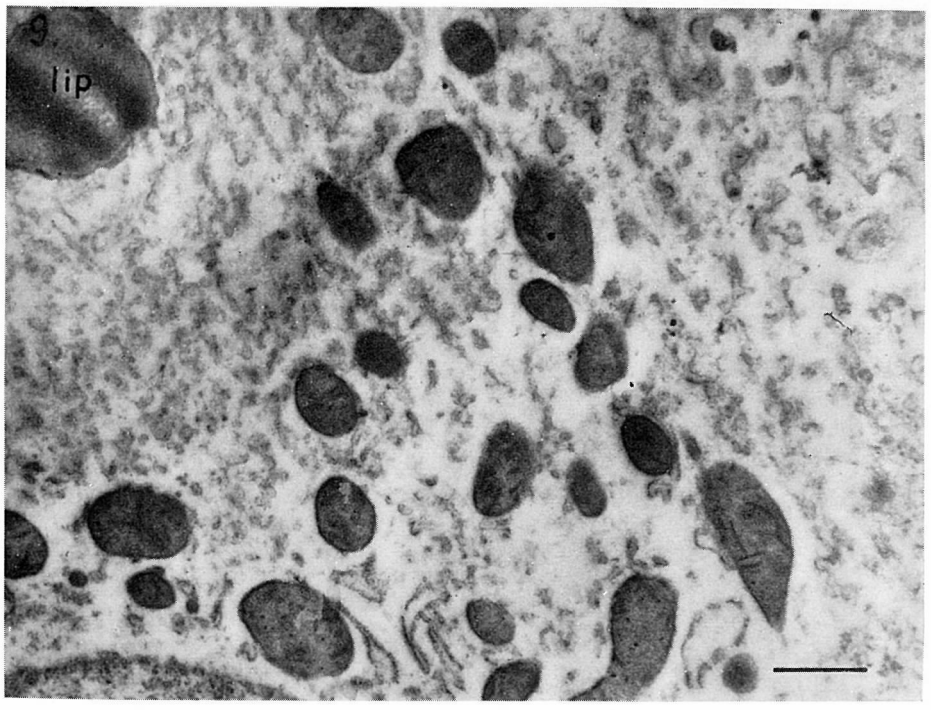
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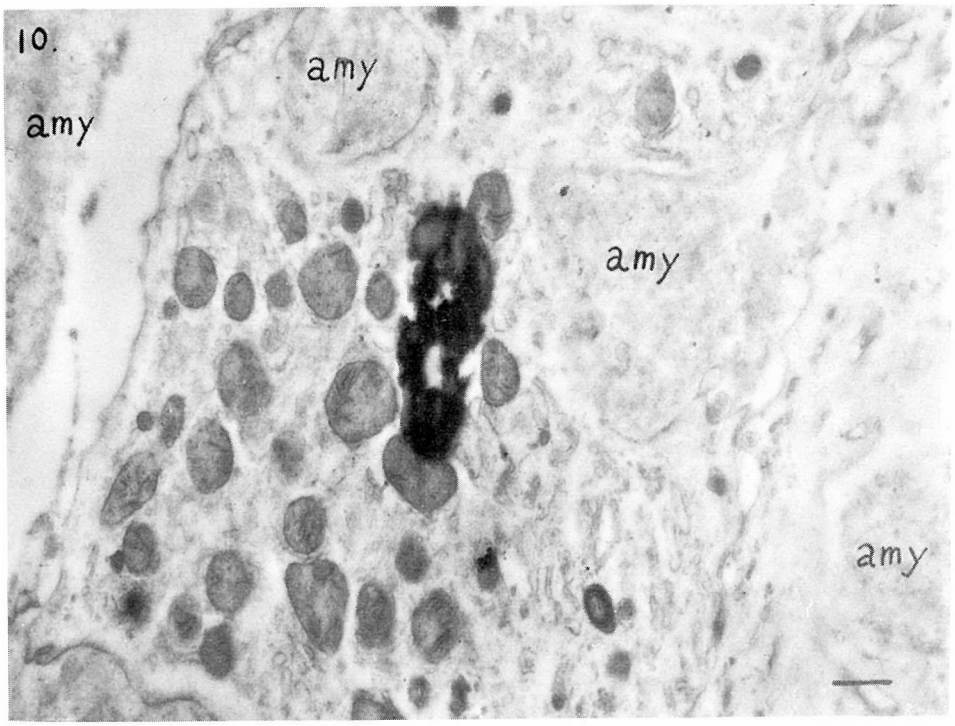
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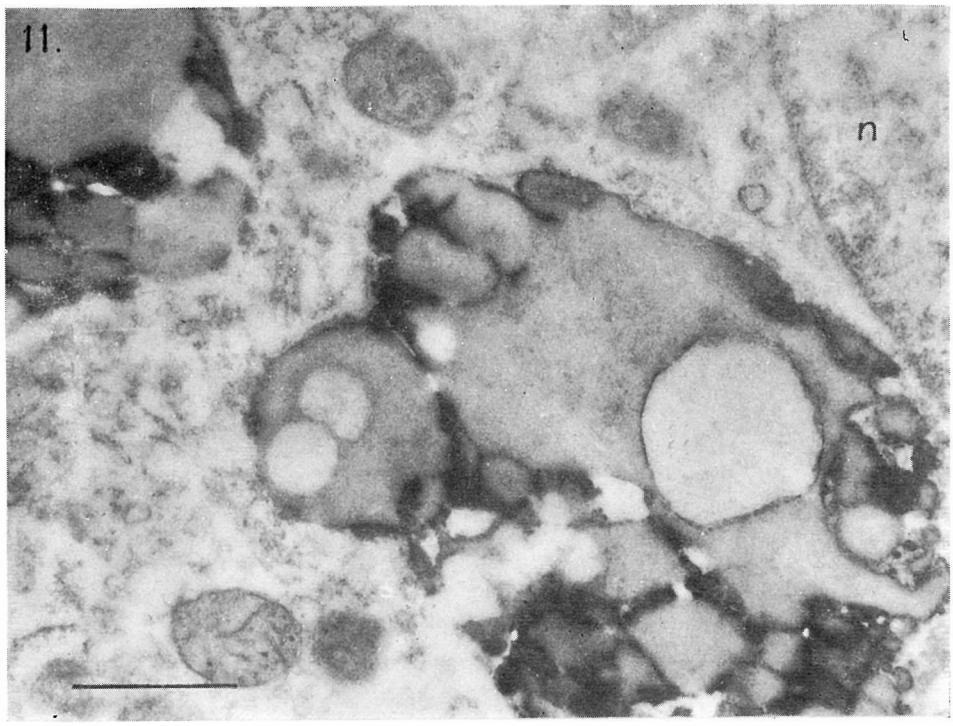
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