Electron Microscopic Observation on Mitochondria in Hepatic Parenchymal Cells of a Case of Primary Intrahepatic Biliary Cirrhosis.

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Electron microscopic observation of the ultrathin sections has demonstrated the ultrastructure of the mitochondria, and its function has also been revealed recently associated with the advance of biochemical studies. Correlation between the biochemical and morphological findings are now under study.

The morphological changes of the mitochondria, such as enlargement, decrease of electron density of the mitochondrial matrix and appearance of dense bodies in the mitochondrial matrix, are recognized in many different cases.

We have made electron microscopical observations of the specimens which were taken by liver biopsy from the patient diagnosed as liver cirrhosis.

In this paper, the process of occurrence of the intramitochondrial vacuoles, which were found abundantly in the liver cells in case of primary intrahepatic biliary cirrhosis, is reported.

CASE REPORT

A 44 year old woman was admitted to the Ist Medicine of Yamaguchi Medical School Hospital with the chief complaint of jaundice.

Approximately 7 months prior to the admission, the patient began to feel fatigue and noticed jaundice at first. Before the admission she has been treated on several occasions, but the treatment was of no effect, and jaundice became increasingly apparent. On physical examination, the liver was palpable 4 finger-breadths below costal margin and its consistency was firm. The results of blood chemistry and urinalyses were as following table I.

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Table I.

Systematic Blood Chemistry

Hemoglobin	9.3	g/dl
Serum Protein	8.2	g/dl
Blood Sugar	92	mg/dl
Alb/Glob. Ratio	0.35	
Icteric Index	10	
Total Bilirubin	3.65	
Alk. Phosphatase	2.6u	
Cholinestrase	0.32	
G P T	21.3u	

Examination of Peripheral Blood

RBC : 370×10^4
WBC : 8600

N. band.	3%
N. segmented.	65%
Eosinophil.	0%
Lymphocyte	28%
Monocyte	4%

Thrombocytes 70 per 10 oil-immersion fields

Examination of Urine

Protein	(+)	10mg/dl
Suger	(−)	
Sediment		
RBC	(1-2)	
WBC	(1-2)	
Bilirugin	(++)	
UrobiIinogen	(+)	
Urobilin	(−)	

MATERIAL AND METHODS

The needle biopsy was performed after the admission. Removed liver tissue was divided into two blocks of a few millimeters. One of them was cut immediately into small pieces of about 0.5–1mm, and these were immersed in cold 1% OsO_4 buffered to pH 7.4 with a veronal-acetate buffer.[1] Following 2 hours fixation OsO_4, the pieces of tissue were washed with physiologic saline solution, dehydrated in graded ethanols, and embedded in a 1:5 mixture of methyl and buthyl methacrylate. Thin sections were cut on a Hitachi UM-3 ultra-microtome and some sections were stained with lead citrate.[2] The unstained and stained sections were

examined with a JAPAN ELECTRON OPTICS LAB. JEM-5HS electron microscope. Another block was fixed in Carnoy's fluid, embedded in paraffin, sectioned and stained with hematoxylin and eosin stain for examination in light microscope.

OBSEVATIONS

Light microscopy

The most outstanding pathological change was the proliferation of periportal connective tissue accompanied by slight inflammatory cell reaction. Macrophages containing bile pigment were sometimes present in the periportal space. Accumulation of bile was recognized in cholangioles in the proliferated periportal connective tissue. There was a shelflike indentation between the periportal connective tissue and the liver lobules. At the periphery of the lobules, bile accumulation was observed in the bile canaliculi, but not in the central zone of the lobule. The peripheral hepatic cells showed a foamy appearance and occasionally contained bile pigment. The central hepatic cells revealed no remarkable changes. From the above findings, primary intrahepatic biliary cirrhosis was diagnosed. Later, operation was performed and no obstruction of extrahepatic bile duct was recognized.

Electron microscopy

In electron microscopic examination, pathological changes were observed in the hepatic parenchymal cells, intercellular space and Disse's space. The present description is concerned only with the morphological changes of the mitochondria in the parenchymal cell.

The morphological changes of the mitochondria were varied in each cells.

According to the severity of injury, the mitochondria showed such abnormal findings as follow:

1) The outline of the mitochondria became irregular and its matrix was denser than normal. The mitochondrial crests showed localized vescle-like distension.

2) The mitochondria appeared markedly enlarged and the matrix was significantly paler than in the normal, but the matrix at the vicinity to the crests maintained almost the normal density. Interruption in the continuity of the outer mitochondrial membrane was occasionally seen. Their crests showed disarrangement and shorter than normal. Accompanied with the enlargement of the mitochondria, intramitochondrial granules were enlarged and density of these granules was slightly decreased.

3) Vacuoles of various size were seen in the mitochondria and the content of these vacuoles revealed irregular reticular pattern. These vacuoles were clearly demarcated from the mitochondrial matrix by the limiting membrane. Because of the large vacuoles, the mitochondrial matrix was rather scanty. The density of the mitochondrial matrix was various in degree; some were paler, and others denser

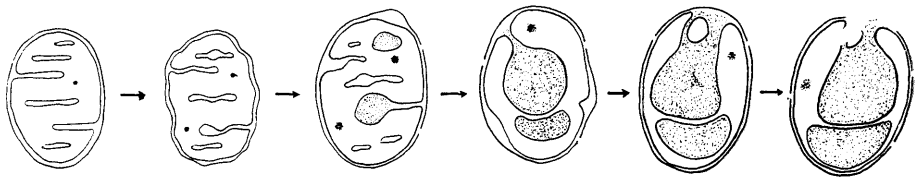
than normal.

4) Some pictures indicated that the limiting membrane of the intramitochondrial vacuoles turned into the inner mitochondrial membrane. At that portion, the outer mitochondrial membrane was interrupted and cytoplasmic matrix was communicated with intramitochondrial vacuole directly.

The process of above mentioned mitochondrial changes is shown in Table II.

Table II

Process of Formation of Intramitochondrial Vacuole



DISCUSSION

As the slightest mitochondrial change, irregularity of the outline of the mitochondria and slight increase of the matrix density were demonstrated.

ASHWORTH et al[3] observed these mitochondrial changes of the rat liver cell in 1 hour after the administration of CCl_4 and described as the small collapsed-appearing, and dense mitochondria. It may be presumed that such mitochondrial changes are the result of concentration of the mitochondrial matrix which is caused by the escape of fluid.

Vesicle-like distension of the mitochondrial crests is the circumscribed wide separation of the crests, so it differs from the wide separation of crest which was recognized in the autolysed hepatic parenchymal cell by TRUMP et al[4]. This vesicle-like distension gradually enlarges and becomes intramitochondrial vacuoles.

Enlargement of the mitochondria of the hepatic parenchymal cell is reported to be one of its morphological changes which are induced under various conditions. We[5] observed such mitochondrial changes in the hepatic parenchymal cell of the mouse which were administered with cortisone.

As TRUMP et al reported, it is impossible to determine precisely the degree of the mitochondrial enlargement, for the size of the mitochondria differs in regard to the peripheral and central zone of the lobule. So, when the mitochondria is round in shape with smooth outline and its matrix is pale, we regard such mitochondria to be enlarged.

Shorter and much widely spaced disorganization of the crests, as well as the interruption of the outer mitochondrial membrane, is reported to appear accompanying with the mitochondrial enlargement.

Compared with biochemical studies, the alteration of the structure of the mitochondrial membrane as mentioned before does not necessarily reflect the decrease of the matrix density is the result of either dilution or disappearance of structural or enzymatic protein and lipid; or both mechanism may be responsible.

Intramitochondrial granules were observed even in the severely injured mitochondria which contained large mitochondrial vacuoles. These granules were also seen in the mitochondria of the hepatic parencymal cells of the rat which was treated with carbon tetrachloride[6] or 3'-methyl 4-dimethyl-aminoazobenzene[7] or put in the state of hypoxia.[8] However, according to TRUMP et al, disappearance of the intramitochondrial dense granules is the earliest change in case of autolysis of the hepatic parenchymal cells. The properties of these granules are still unknown, so the meaning of disappearance or existence of these granules is not clear.

Dense intramitochondrial bodies were observed as the intramitochondrial inclusion in the uterine epithelium,[9] colon mucoid cells, gastric mucoid cell of the mouse,[10] LEYDIG'S cells of the opossum,[11] hyperplastic epidermis,[12] the muscle from the hypermetabolic human,[13] the corneal epithelium of the vitamin A deficient mouse[14] and during skin carcinogenesis of the mouse.[15] And it is considered that the occurrence of the intramitochondrial bodies may be related to a particular phase of the cell metabolism.

On the other hand, intramitochondrial vacuoles are scarcely reported in any papers. These vacuoles are different from cytolysome, which was described by Novikoff and Essnen[16] (1962) and ASHFORD and PORTER[17] (1962), and as such named by NOVIKOFF.[18] The cytolysome is considered to be one of the inclusion bodies which contains mitochondria and observed in the autolysed or degenerated cells. We assume that these intramitocondrial vacuoles were not revealed in spite of severe changes of the organellae of the hepatic parenchymal cells. These vacuoles may be developed as the result of the influence of accumulated bile to the hepatic parenchymal cells.

SUMMARY

1.) Light and electron microscopic examination of the biopsy specimen of the liver taken from 44 year old woman suffering from continuous jaundice was done.

2.) The diagnosis of primary cholangiolitic cirrhosis was made by light microscopical examination.

3.) In electron microscopic observation, intramitochondrial vacuoles were recognized and the process of occurrence of these vacuoles was pursued.

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EXPLANATION OF PLATES

Fig. 1. The micrograph indicates masses of bile (bp) and dilated intercellular space. In hepatic parenchymal cells many glycogen granules are observed. The mitochondrial show slight decrease in density of its matrix.

Fig. 2. This micrograph shows cytoplasm of hepatic parenchymal cell and Disse's space (S). Intramitochondrial vacuoles (↑) can be seen in parenchymal cell. In Disse's space,

somewhat dense amorphous structure and a part of fibroblastic cytoplasm (f) are recognized.

Fig. 3. The mitochondria show enlargement and its matrix is rather pale. Intramitochondrial vacuoles are seen (↑).

Fig. 4. The outline of mitochondria are irregular and the mitochondrial crests show circumscribed distension (vesicle-like distension) in some areas. The mitochondrial matrix is denser than normal.

Fig. 5. The micrograph shows mitochondria and microbodies (mb).
The crest of mitochondrion shows vesicle-like distension (↑).

Fig. 6. The mitochondria are enlarged and pale, the crests are shorter and more widely spaced transformation. The intramitochondrial granules (mg) were enlarged.

Fig. 7. Two mitochondria with abnormal crests. Two are arch-shaped (↑) and seem to implantate against in the inner membrane of mitochondrial wall.

Fig. 8. The mitochondrial matrix became pale, but the part surrounded by mitochondrial crest maintains the same density as normal (↑).

Fig. 9. The mitochondrial matrix, except the vicinity of mitochondrial crests, are pale. The mitochondrial granules (mg) are slightly enlarged. Disappearance of outer mitochondrial membrane (↑) is observed.

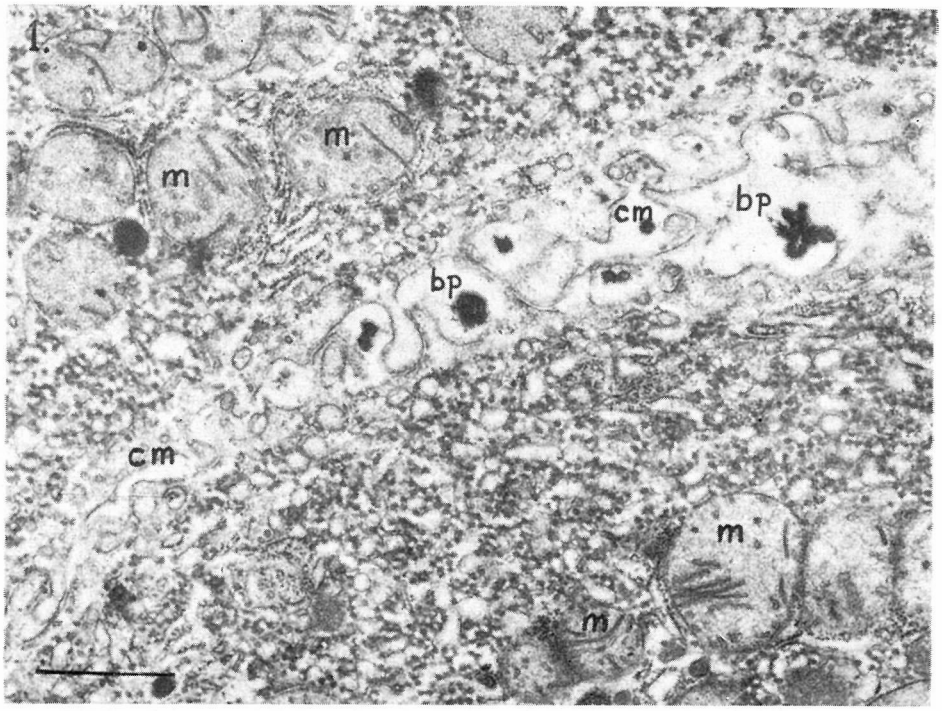
Fig. 10. The continuity of outer mitochondrial membrane is disappeared in some places (↑). The mitochondrial matrix is pale expect the vicinity of crests.

Fig. 11. The mitochondrial matrix is pale in all mitochondria. Mitochondrial matrix, which contains vacuoles (↑), is remarkably pale.

Fig. 12. The mitochondria, including vacuoles (↑), are seen. In these vacuoles contains mesh-work structure.

Fig. 13. The mitochondria which have vacuoles are observed. These vacuoles are clearly defined from mitochondrial matrix by limiting membrane. These vacuoles are electron lucid and contained dense mesh-work structure. The density of mitochondrial matrix is rather increased than normal. The vacuole in the huge mitochondrion, which is seen in the central protion of this micrograph, has connection with hyaloplasm (↑).

Fig. 14-15. The mitochondria which contain vacuoles are seen.
The mitochondrial matrix is scanty because of large vacuoles.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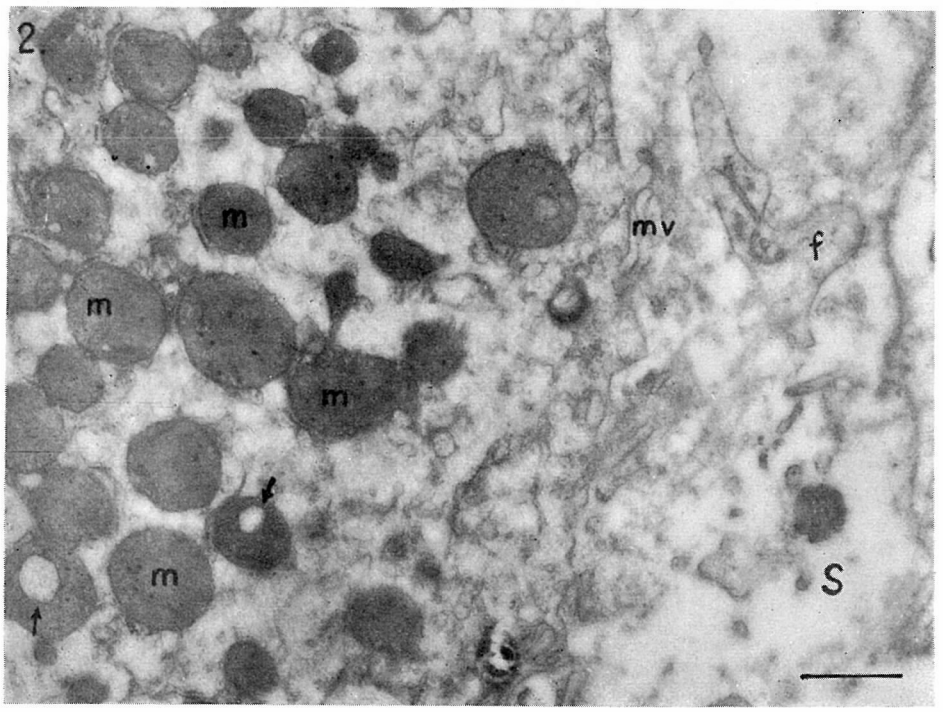
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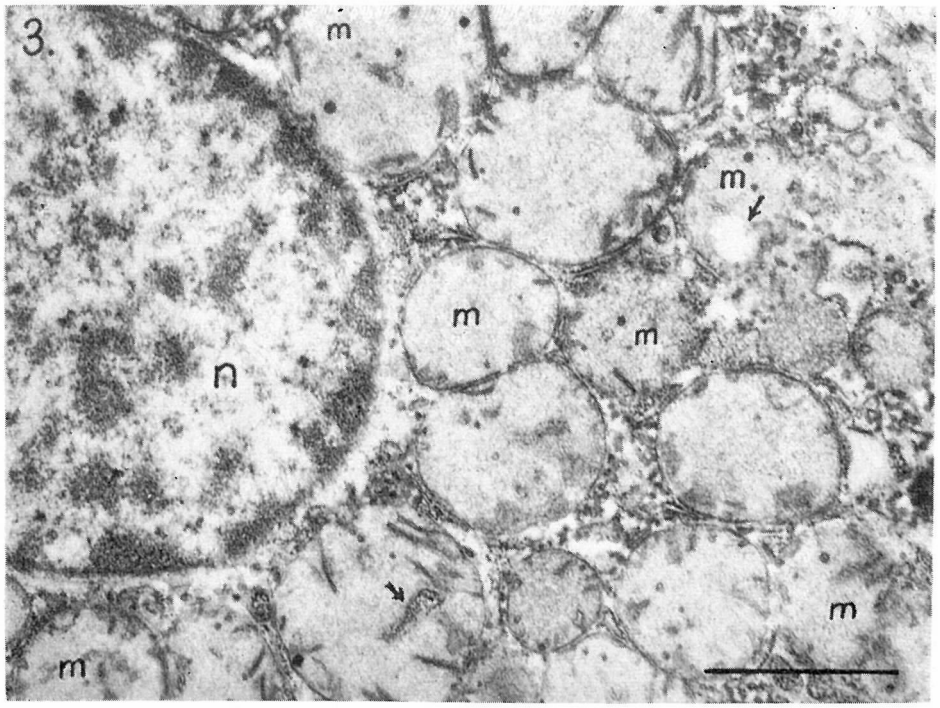
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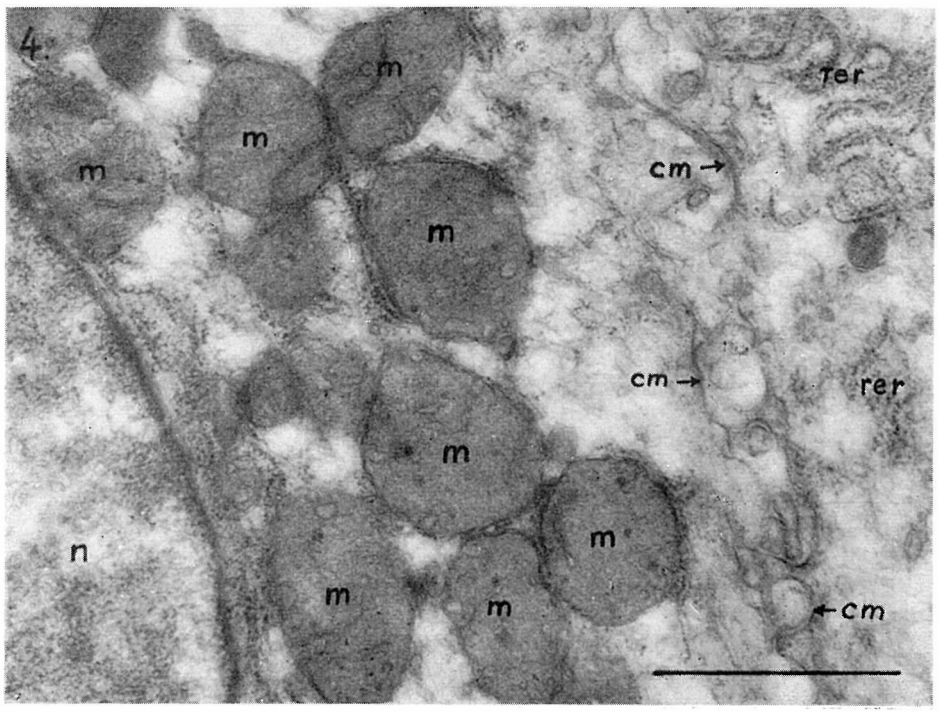
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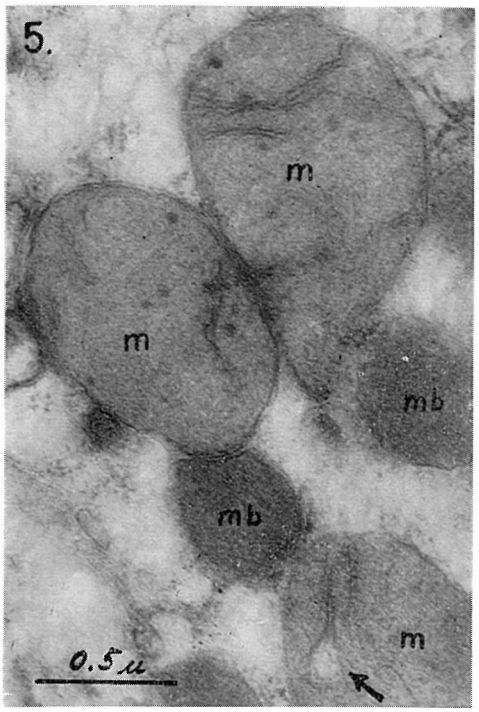
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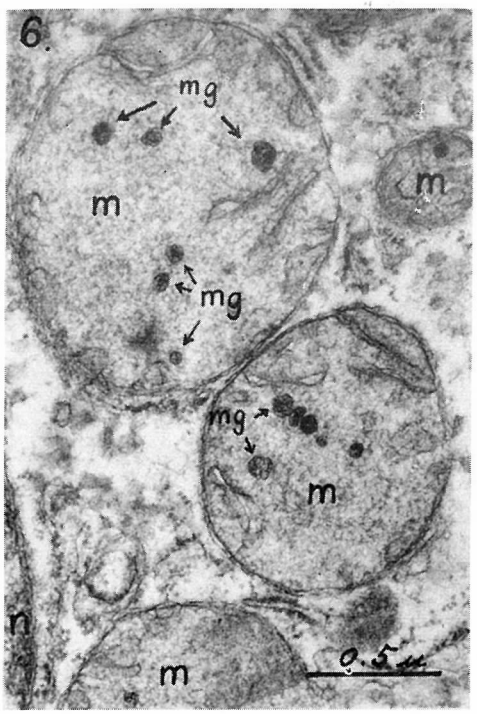
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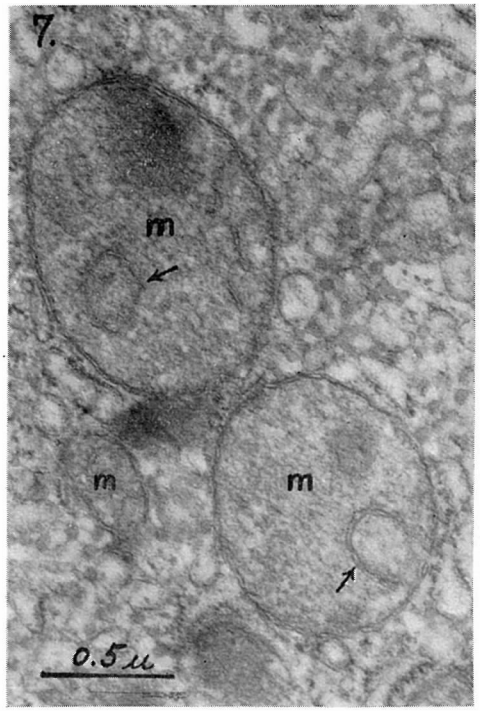
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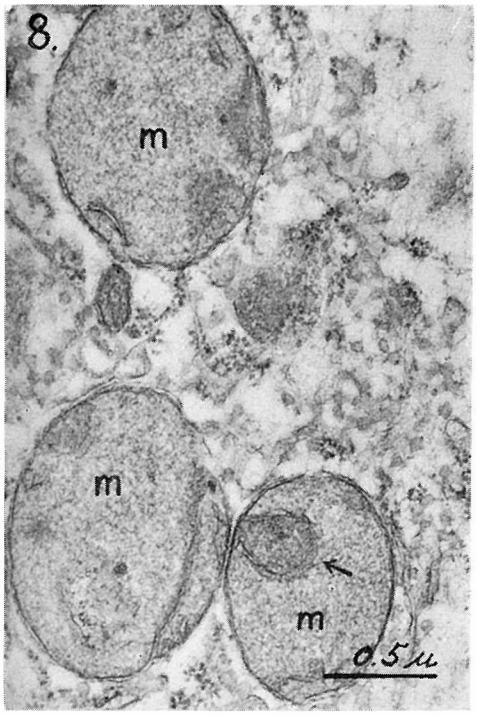
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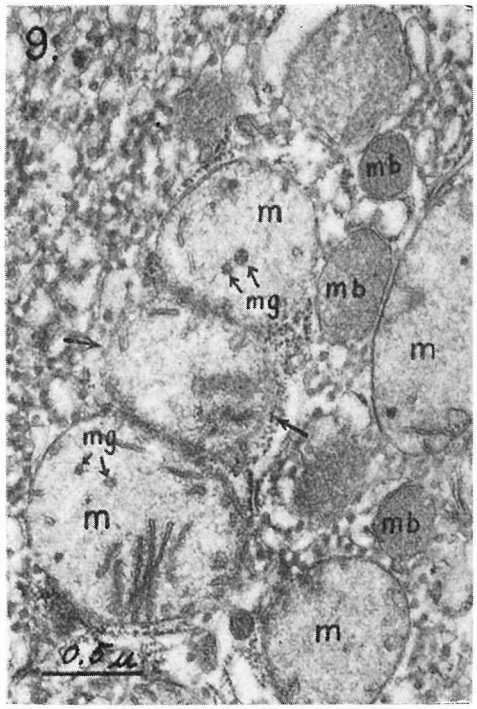
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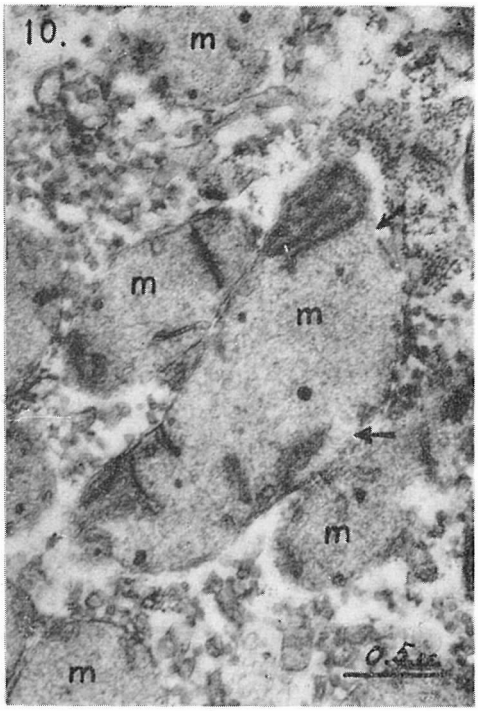
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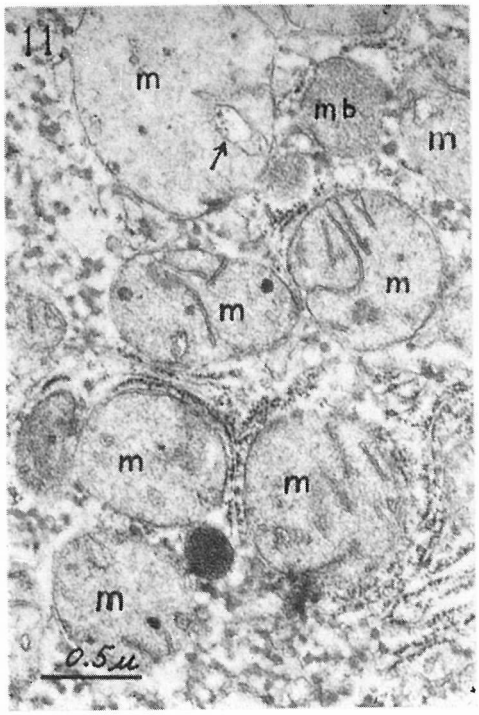
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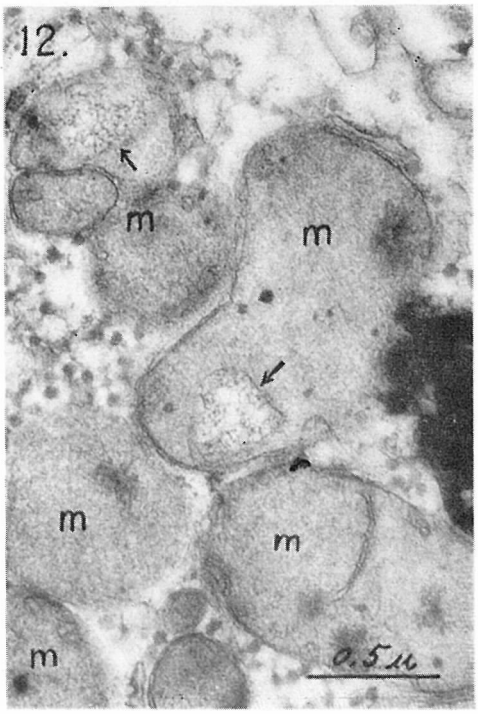
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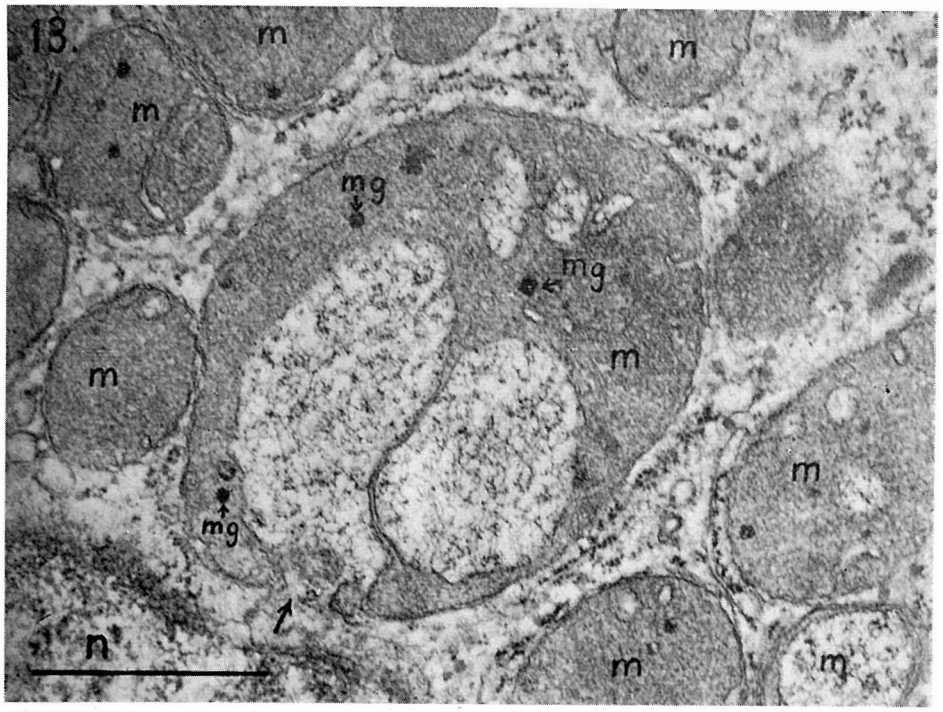
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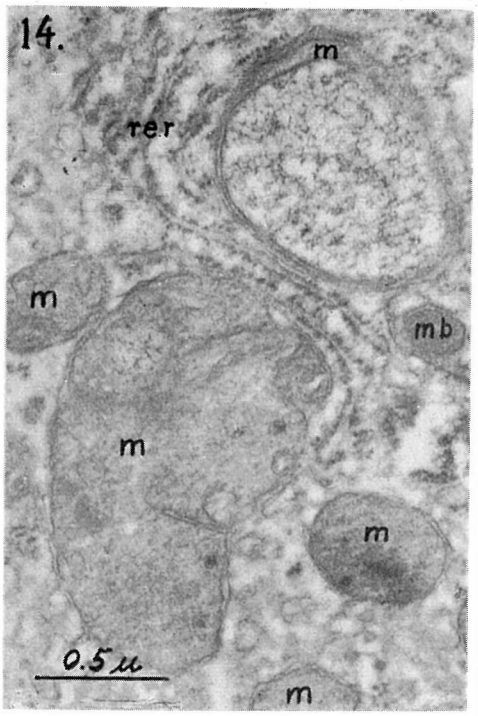
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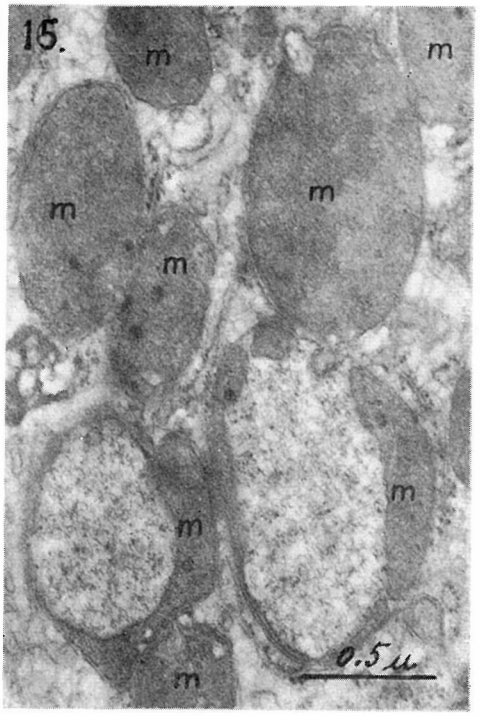
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