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Electron Microscopic Study on the Endocrine Cells in the Stomach and Duodenum of the Pond Tortoise(*Amyda sinensis*)

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자라 위 및 십이지장 내분비세포에 관한 전자현미경적 연구

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抄 錄

자라의 위 및 십이지장에 출현하는 내분비세포의 형태를 알아보기 위하여 전자현미경으로 관찰을 행하였던 바, 과립의 크기, 형태, 전자밀도 및 세포의 형태 등에 의해 다음과 같이 6종의 세포형으로 구분할 수 있었다.

Ⅰ형 : 직경 100~430 nm(평균 290 nm)로서 전자밀도가 낮고 많은 수의 다양한 형태의 과립을 가진 것.

Ⅱ형 : 직경 190~430 nm(평균 280 nm)로서 높은 전자밀도를 나타내며 다양한 형태의 과립을 가진 것.

Ⅲ형 : 직경 170~650 nm(평균 430 nm)로서 전자밀도가 높고 과립막과 내용물 사이에 넓은 halo를 형성하는 과립을 가진 것.

Ⅳ형 : 직경 140~370 nm(평균 240 nm)로서 전자밀도가 대체로 낮고 과립막과 내용물이 밀착된 과립을 가진 것.

Ⅴ형 : 직경 300~600 nm(평균 410 nm)로서 전자밀도는 낮거나 중등도이며, 과립은 과립막과 내용물 사이에 명확한 halo를 형성하고, 세포질내 미세섬유를 가지는 것.

Ⅵ형 : 직경 100~220 nm(평균 160 nm)로서 대체로 소형의 과립을 가지며, 전자밀도는 다양하고 과립은 과립막과 내용물 사이에 halo를 형성하는 것, 밀착된 것 또는 내용물이 결정구조를 나타내는 것 등으로 다양하며 세포질내 풍부한 미세섬유를 가지는 것.

Introduction

Progress in the field of the gastrointestinal endocrine cells, synthesizing such gastrointestinal hormones as serotonin, gastrin and secretin, has made remarkably during the past decade with the improvements in electron microscopic and immunohistochemical technics. As yet more

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than 16 different endocrine cell types have been described in the gastrointestinal tract(Solcia *et al.*, 1975, 1981 ; Grube and Forssmann, 1979).

Although many reports have been made on the histochemical properties and the structure of the endocrine cells in the gastrointestinal mucosa of many mammals(Kawano *et al.*, 1983; Sato *et al.*, 1978 ; Kitamura *et al.*, 1982 ; Solcia *et al.*, 1980 ; Comori, 1983) and birds(Yamada *et al.*, 1979, 1980 ; Okamoto, 1980), only a few papers have been published on the other animals.

In the lower animals endocrine cells were reported, at least two types in lancelet mid-gut (Katakoka and Fujita, 1974), five types in frog gastric mucosa(Chung and Kwun, 1983). In insects, six types of endocrine cells were identified in the midgut and enteric caeca of the adult cockroach(Nishiitsutsuji-uwo and Endo, 1981).

In reptilia, however, one paper has been published by Kobayashi(1967) on the argentaffin cells of the snake intestine. The present report describes the ultrastructure of six types of endocrine cells in the stomach and duodenum of the pond tortoise.

Materials and Methods

Eight pond tortoises(200~250 g), *Amyda sinensis*, of both sexes were killed by exanguation. For electron microscopy, small tissue blocks were taken from four regions respectively, thus, cardia, fundus, pylorus and duodenum. The tissue blocks were immediately fixed in 2% paraformaldehyde—2.5% glutaraldehyde mixture buffered with 0.1M cacodylate(pH 7.4) for 2 hrs at 0~4°C, washed in the same buffer, post-fixed in 1% osmium tetroxide in the same buffer for 2 hrs at 0~4°C, dehydrated in graded ethanol solutions, and embedded in Epon-812. Ultrathin sections, made by Porter-Blum Ultramicrotome MT2-B type, were contrasted with uranyl acetate and lead citrate, and examined under JEOL-100CX type electron microscope. The size of the endocrine granules were established by measuring more than 15 granules in electron micrographs.

Results

The gastrointestinal endocrine cells observed by electron microscope had characteristic secretory granules and these granules showed different electron densities and structures according to each cell types. Moreover, the development of the cell organelles in the cytoplasm were different from cell types. At least six types of endocrine cells, namely, type I, II, III, IV, V and VI were classified on the base of the size, shape and electron density of secretory granules and ultrastructures of the cell organelles(Table 1).

Type I cell(Fig. 1, 1-a) ; This cell showed closed type and mainly observed in the cardiac and pyloric glands. The numerous secretory granules were mostly round and oval or pleomorphic. The mean diameter of these granules was 290 nm, ranging from 100 to 430 nm. The granules showed moderate in electron density and the limiting membrane was closely attached to the cores of the granules or showed a very thin halo. The development of the cell organelles was poor, and a small amount of round mitochondria and r-ER were scattered throughout the cytoplasm.

Type II cell(Fig. 2, 2-a) ; This cell was mainly observed in the duodenal glands, and moderate number of granules showing high in electron density were round or oval in shape. The mean diameter of the granules was 280 nm, ranging from 190 to 430 nm, and the limiting membrane was closely attached to the solid

Table 1. Ultrastructure of the secretory granules in the endocrine cells of the stomach and duodenum of the pond tortoise(*Amyda sinensis*)

Cell type	Granules				Cell organelles
	Shape	Number	Size in nm (mean)	Density	
I	pleomorphic with closely attached membrane	numerous	100~430(290)	moderate	poorly developed
II	pleomorphic with closely attached membrane	moderate	190~430(280)	high	moderately developed
III	oval, irregular with wide vesicular halo	numerous	170~650(430)	high	relatively poor
IV	round with closely applied membrane or often a very thin halo	numerous	140~370(240)	low to moderate	well developed
V	round, oval with relatively wide clear halo	numerous	300~600(410)	low to moderate	poorly developed, a small amount of microfilaments
VI	round with closely applied membrane or often a very thin halo	numerous	100~220(160)	low to moderate	well developed a number of microfilaments and crystalline structure

and dense core contents. Moderately developed r-ER, Golgi complex, a small amount of mitochondria, somewhat indented nuclear membrane, a clear nucleolus and a small amount of chromatin associated with the nuclear membrane were observed.

Type III cell(Fig. 3, 3-a) ; This cell was characterized by having numerous large oval or irregular granules with a high electron density. The dense core was located eccentrically in a limiting membrane with a wide clear space or a halo filled with low electron dense materials. These cells were mainly found in the cardiac and pyloric glands, and the mean diameter of the granules was 430 nm, ranging from 170 to 650 nm. Dilated r-ER and a small amount of round or oval mitochondria were observed, and the cytoplasm was somewhat clear.

Type IV cell (Fig. 4, 4-a) ; This cell was mainly observed in the fundic and duodenal glands and the granules were numerous and mostly round or oval in shape. The granules showed low to moderate electron density and the limiting membrane was closely applied to the cores of the granules or showed a very thin halo. The mean diameter of the granules was 240 nm, ranging from 140 to 370 nm. The cell organelles were poorly developed except a few round mitochondria.

Type V cell(Fig. 5, 5-a) ; This cell was mainly observed in the pyloric glands. The cell membrane was almost smooth but the nuclear membrane was somewhat indented. The secretory granules, low to moderate in electron density, were round or oval in shape with a relatively wide clear halo. The mean diameter of the granules was 410 nm, ranging from 300 to 600 nm. In the cytoplasm a small amount of mitochondria, dilated cisternae of r-ER and a small amount of microfilaments were observed around the nucleus. This type was spindle-shaped.

Type VI cell (Fig. 6, 6-a) ; This cell, mainly observed in the pyloric and duodenal glands,

was spindle-shaped and the cell membrane was highly indented. This cell was characterized by having numerous small round granules, and the limiting membrane was closely applied to the cores of the granules. Some of the granules showed a very thin halo, and contained crystalline core structure. The mean diameter of the granules was 160 nm, ranging from 100 to 220 nm, and showed low to moderate in electron density. Many microfilament bundles, dilated cisternae of r-ER, ribosomes and lipid droplets were observed in the cytoplasm of this cell type.

Discussion

Only one paper has been published on the endocrine cells of reptilia. Kobayashi(1967) described briefly on the argentaffin cells in the intestinal mucosa of the snake which were quite similar in structural feature to those described in mammalian and avian gastrointestinal argentaffin cells. In addition to argentaffin cell, at least two to three or six to seven endocrine cells have been identified in mammals(Sato *et al.*, 1978 ; Oomori, 1983), aves (Yamada *et al.*, 1978 ; Okamoto, 1980), amphibia (Chung and Kwun, 1983) and pisces(Chung *et al.*, 1980). The main criteria for the subdivision of endocrine cells are size, shape and electron density of their secretory granules(Grube and Forssmann, 1979).

In present study, according to the granular size, number, electron density, cell organelles and cell shape, six types of endocrine cells could be identified in the stomach and duodenum of the pond tortoise by electron microscopy. Type I cell was characterized by having numerous pleomorphic granules which were moderate in electron density and 100∼430 nm in diameter. Type II cell was characterized by having pleomorphic granules which were high in electron density and 190∼430 nm in diameter. Type III cell was characterized by having numerous oval or irregular granules which were high in electron density and 170 ∼650 nm in diameter and had wide vesicular halo between the limiting membrane and the dense core. Type IV cell was characterized by having numerous round or oval granules which were low to moderate in electron density and 140∼370 nm in diameter. Type V cell was characterized by having numerous round or oval granules which were low to moderate in electron density and 300∼600 nm in diameter and had relatively wide clear halo and a small amount of microfilaments. Type VI cell was characterized by having numerous small granules which were low to moderate in electron-density and 100∼220 nm in diameter. The granules had closely applied membrane or showed a very thin halo. A number of microfilaments and crystalline structures could be seen.

The ultrastructures of these six types could not directly compare with reptilia due to lack of reports on those of reptilia, however, according to Santa Monica 1980 classificatoin of human gastroenteropancreatic endocrine-paracrine cells(Solcia *et al.*, 1981), the type I, II, III, IV and V cells of the pond tortoise demonstrated in the stomach and duodenum in this study may corresponded to the human EC, EC1, ECL, D and G cells, respectively, and type IV cell was somewhat similar to that of bombesin-producing cell in amphibia. Yamada *et al.*(1980) demonstrated remarkable differences between mammals and birds in the ultrastructure of the gastrin granules and suggested that ultrastructural classification of the gastrointestinal endocrine cells in mammals could not be applied directly to the gastroin-

testinal endocrine cells of birds. Therefore, further study is necessary to identify the precise cell type and the functional roles of the endocrine cells.

Previous immunohistochemical studies have identified the polypeptide hormones of some endocrine cell types in the gastrointestinal mucosa of various mammals; A cells producing glucagon, D cells producing somatostatin, EC cells producing serotonin, ECL cells producing histamin, G cells producing gastrin, I cells producing cholecystokinin, K cells producing GIP, S cells producing secretin and X cells producing enteroglucagon (Grube and Forssmann, 1979 ; Solcia *et al.*, 1980).

The present study demonstrated six types of endocrine cells in the stomach and duodenum of the pond tortoise and it remained to be elucidated as to what and how many endocrine cell types are distributed in the other intestinal regions in this species.

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Figure Legends

Fig. 1. Electron micrograph of Type I cell. Numerous pleomorphic secretory granules whose contents are moderate in electron density are found. N; nucleus, M; mitochondria, bm; basement membrane. Bar=1μm. ×16,000.

Fig. 1-a. High magnification of Fig. 1. Numerous pleomorphic granules with closely attached membrane are shown here. ×40,000.

Fig. 2. A Type II cell having pleomorphic and medium-sized granules whose contents are high in electron density. Dilated cisternae of rough endoplasmic reticulum(rER) and well-developed Golgi apparatus(G) are found. ×16,000.

Fig. 2-a. High magnification of Fig. 2. ×40,000.

Fig. 3. A Type III cell having large oval or irregular granules with wide vesicular halo whose contents are high in electron density. Oval mitochondria(M) are found. ×20,000.

Fig. 3-a. High magnification of Fig. 3. ×40,000.

Fig. 4. A Type IV cell having round and medium-sized granules whose contents are low to moderate in electron density. A few round mitochondria(M) are also found. bm; basement membrane. ×20,000.

Fig. 4-a. High magnification of Fig. 4. The granules possess a very thin halo. ×30,000.

Fig. 5. A Type V cell having large round or oval granules whose contents are low to moderate in electron density. Microfilaments(arrow) are found around the nucleus(N). ×20,000.

Fig. 5-a. High magnification of Fig. 5. The granules possess a dense core and a wide clear halo. ×40,000.

Fig. 6. A Type VI cell having round and small granules whose contents are low to moderate in electron density and possess a very thin halo or crystalline structure(arrow) of the contents. Many microfilament(mf) bundles are also found in the cytoplasm. ×30,000.

Fig. 6-a. High magnification of Fig. 6. ×40,000.

Abstract

The endocrine cells of the stomach and duodenum of the pond tortoise (*Amyda sinensis*) have been studied by electron microscopy. At least six types of endocrine cells could be identified in these regions.

Type I cells were characterized by having pleomorphic or medium sized granules (100~430 nm in diameter, mean; 290 nm) which were moderate in electron density.

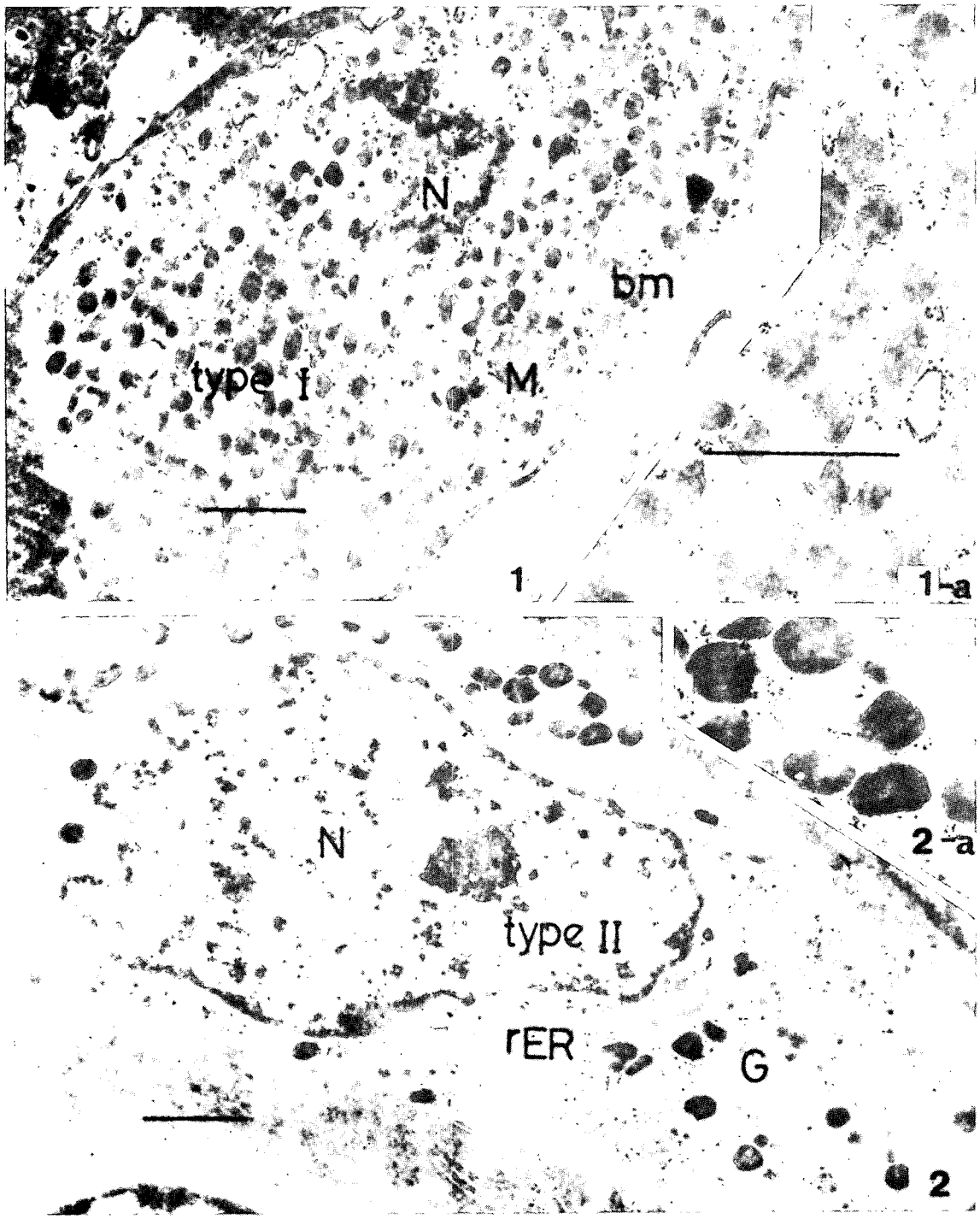
Type II cells were characterized by having pleomorphic or medium sized granules (190~430 nm in diameter, mean; 280 nm) which were high in electron density.

Type III cells were characterized by having large oval or irregular granules(170~650 nm, mean: 430 nm) with wide vesicular halo which were high in electron density.

Type IV cells were characterized by having round or medium sized granules(140~370 nm, mean; 240 nm) which were low to moderate in electron density and showed a very thin halo.

Type V cells were characterized by having large round or oval granules(300~600 nm, mean; 410 nm) which were low to moderate in electron density and showed a dense core and a wide clear halo. Microfilaments were also found in the cytoplasm.

Type VI cells were characterized by having round and small granules(100~220 nm, mean; 160 nm) which were low to moderate in electron density and showed a very thin halo or crystalline structure of the contents. Many microfilament bundles were also found in the cytoplasm.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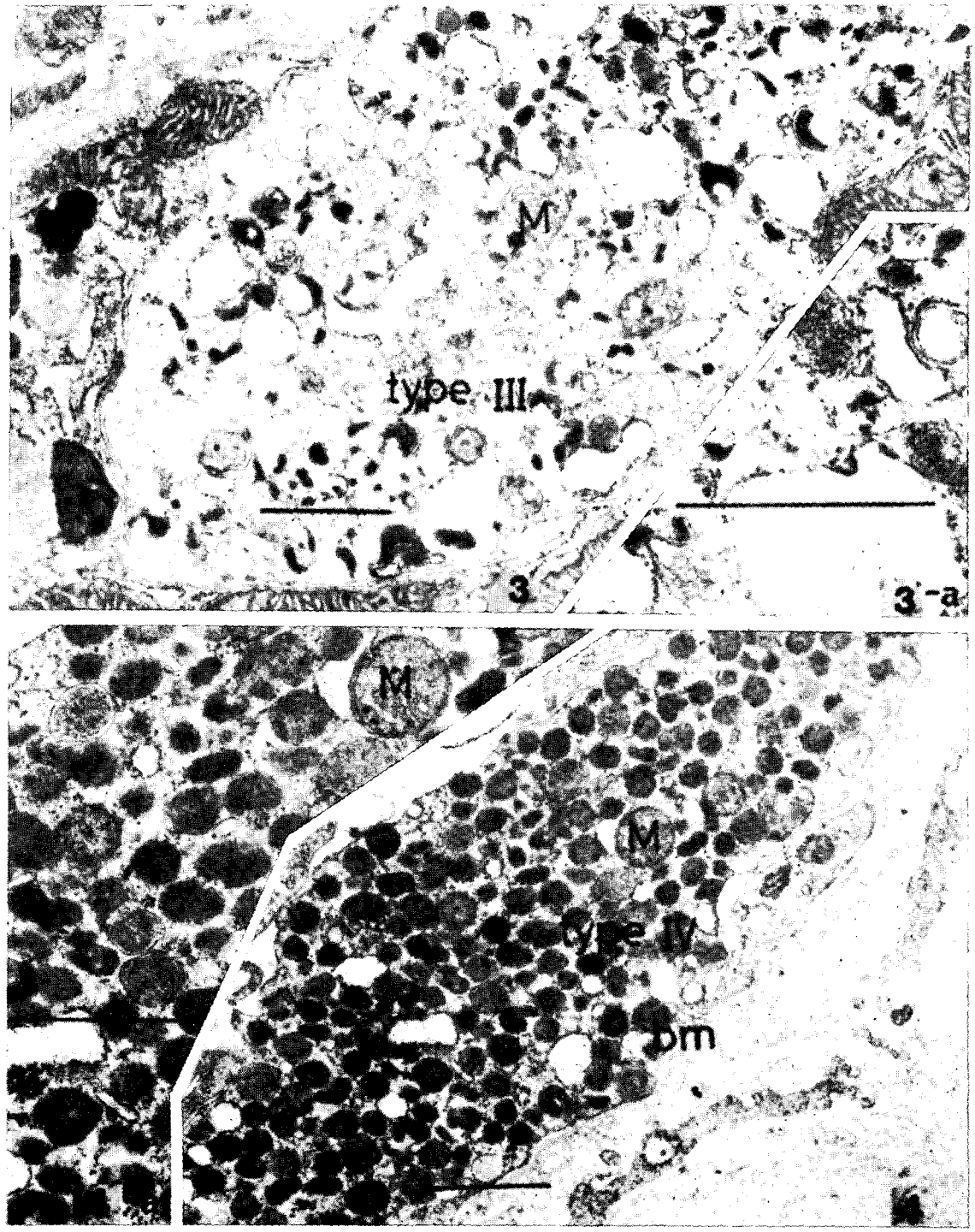
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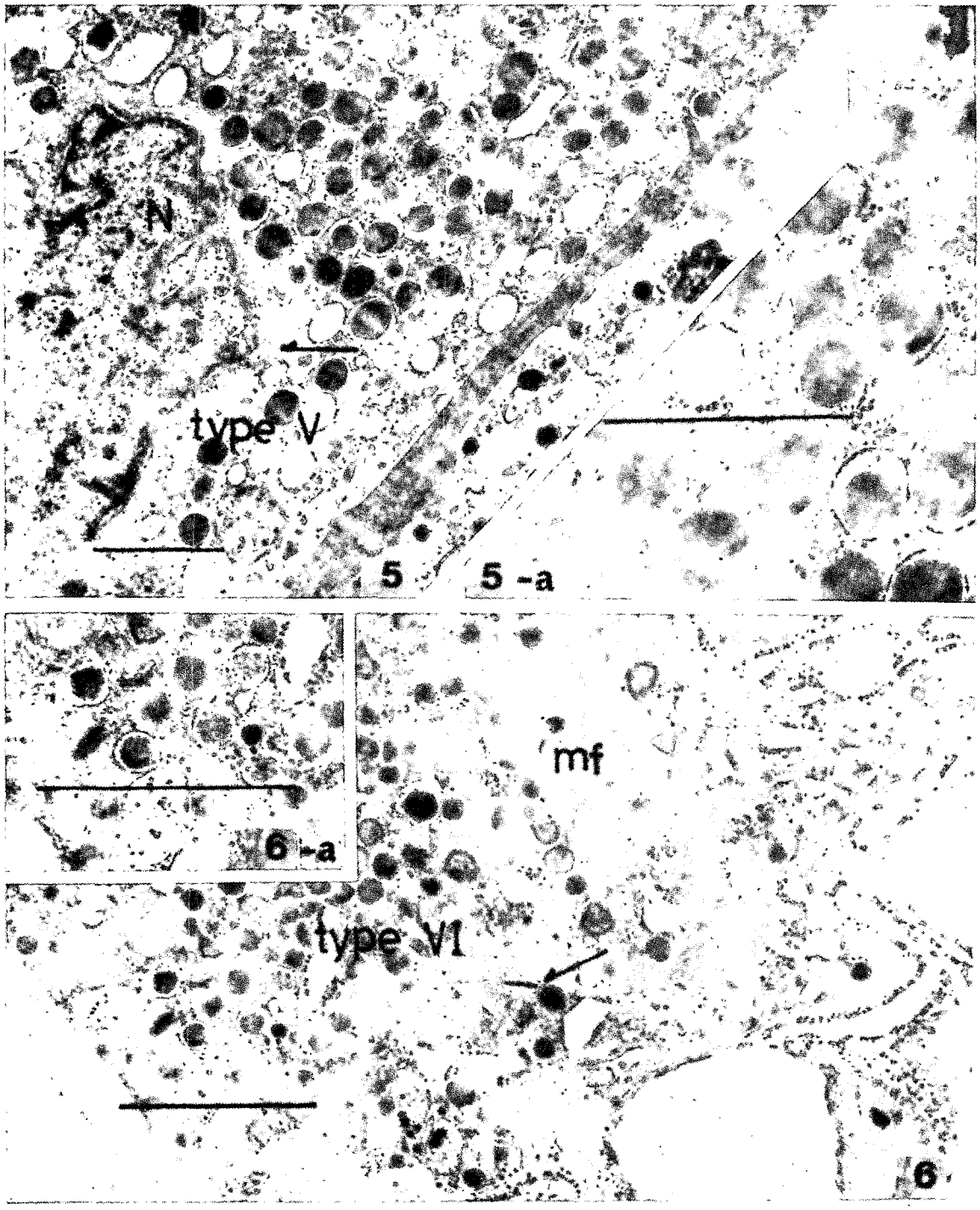
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