Light and Electron Microscopic Study of the Endocrine Cells in the Pyloric Mucosa of Pre- and Postnatal Rats

Mineo YOSHINO, Tadayuki YAMASHITA,
Junzo YAMADA and Mikio MISU

(Department of Veterinary Anatomy, Obihiro University of Agriculture and Veterinary Midicine, Obihiro, Hokkaido, Japan 080)

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ラット胎児および生後発育過程の幽門腺部粘膜における内分泌細胞の光学顕微鏡的および電子顕微鏡的研究

吉野峰生*1・山下忠幸*・山田純三*・三須幹男*

Introduction

Investigations regarding the origin of the gastrointestinal endocrine cells have been performed by some previous researchers, and developmental origins have been postulated, namely, in the endoderm lining the gut, and in the underlying mesenchyme or the neural crest.

Studies of the ontogenesis of gastro-entero-pancreatic endocrine cells are few. MATSUMOTO (1973) studied the fundus of rats, DE LEMOS (1976) worked on the corpus of the stomach of human fetus, while DORN *et al.* (1976) described the pancreas of rats.

This study, therefore, deals with the differentiation and development of the endocrine cells by examining the pyloric mucosa of pre and postnatal rats through light and electron microscopy.

Materials and Methods

The materials used in this research were the pyloric mucosa taken from the 16, 18, 20 and 22 day-old embryos and also in 1, 5, 10, 20 and 30 day-old Wister-Imamichi rats.

Materials for light microscopy were fixed with 10% formalin (0.1 M phosphate buffer, pH 7.3), BOUIN and formalin-ethanol-acetic acid fluids and embedded in paraffin. Sections of 4–6 μ were cut and stained with different staining technics like hematoxylin-eosin (H-E),

* 帯広畜産大学家畜解剖学教室
*1 Present address: National Research Institute of Police Science, Chiyoda-Ku, Tokyo 102.

PAS, alcian blue (AB), AB-PAS, MASSON-HAMPERL modified method (SINGH, 1964), SEVIER-MUNGER's silver impregnation (SEVIER & MUNGER, 1965), GRIMELIUS's silver impregnation (GRIMELIUS, 1968) and DAVENPORT's silver impregnation (HELLERSTRÖM & HELLMAN, 1960). Further, enzyme-antibody method for gastrin was performed in BOUIN's fixed paraffin sections of 16, 18 and 20 day-old embryos.

For electron microscopy, embryos were fixed in 1.5% paraformaldehyde and 0.5% glutaraldehyde mixture (0.1 M phosphate buffer, pH 7.3), while postnatal rats were fixed in 2.6% glutaraldehyde (0.1 M phosphate buffer, pH 7.3). Then, they were post fixed with 1% Osmic acid (0.1 M phosphate buffer, pH 7.3) and were embedded in SPURR epoxy resin. Ultra-thin sections were stained with uranyl acetate and lead citrate (RAYNOLDS, 1963) and examined under a JEM-7 electron microscope.

Results

1) General features of the tissue

The formation of gastric pit was found earliest in about 18 day-old embryos, while the differentiations of the gastric pit, pyloric gland and lamina propria were observed in 20 day-old embryos (Figs. 1, 2).

PAS reactive substances were found in the epithelial cells of mucosal surface, gastric pit and pyloric gland in 20 day-old embryos (Fig. 2). A few possitive cells were observed at the base of the pyloric glands of 1 day-old rats (Fig. 3). From there on PAS and AB reactive cells showed a progressive increase with age. In 30 day-old rats, the structure of the pyloric mucosa was similar to that of the adult pylorus. Gastric pit was straight and entered deeply into the lamina propria. Pyloric gland curved slightly at the base of the gland. The epithelial cells of the mucosal surface and gastric pit contained only PAS reactive substances. The cells from the depth of gastric pit to the body of pyloric gland had both PAS and AB reactive substances, while the cells at the base of the gland had only AB substances (Fig. 4).

2) Light microscopy (Table 1)

For the first time cells that reacted to MASSON, SEVIER-MUNGER, GRIMELIUS and DAVENPORT were rarely observed in 1 day-old rats. MASSON reactive cells increased slightly at the base of gland in 10 day-old rats with almost equal number in 20 day-old rats and increased significantly in 30 day-old rats (Fig. 5).

SEVIER-MUNGER reactive cells were rarely observed in the depth of gastric pit and in the neck of pyloric gland in 5 and 10 day-old rats. However, they showed a remarkable increase at the base of gland in 20 day-old rats (Fig. 6) and found rather frequent from the gastric pit to the base of the pyloric gland in 30 day-old rats.

A few of the cells showed argyrophil when using of GRIMELIUS stain and were observed from the depth of the gastric pit to the base of the pyloric gland of 5 and 10 day-old rats. At 20 days, the cells developed black and brown granules and the frequency

Table 1. Approximate time of appearance and frequency of endocrine cells in pyloric mucosa of pre- and postnatal rats by various staining methods under light microscopy

Age	MASSON	SEVIER-MUNGER	GRIMELIUS	DAVENPORT	Enzyme-antibody
16 E*	−	−	−	−	−
18 E	−	−	−	−	+
20 E	−	−	−	−	+
22 E	−	−	−	−	
1 D**	+	+	+	+	
5 D	+	+	++	+	
10 D	++	+	++	+	
20 D	++	+++	+++	++	
30 D	+++	++++	++++	++	

*: day-old embryo; **: day-old rat (day after birth), −: Absent, +: Rare, ++: Few, +++: Frequent, ++++: Very frequent

became higher. Many similar argyrophil cells were found below the depth of the gastric pit in 30 day-old rats (Fig. 7).

DAVENPORT reacting cells were rare in 10 day-old rats and a few argyrophil cells were observed below the neck of the gastric pit in 20 day-old (Fig. 8) and 30 day-old rats.

Gastrin cells were first found at the base of the mucosa in 18 day-old embryos by the enzyme-antibody method (Fig. 9).

3) Electron microscopy (Tables 2, 3)

The main parameter for the identification of the endocrine cells was the ultrastructure study, especially the morphology of the granules.

According to granular morphology, four kinds of cell types are found in the pyloric mucosa in 5 day-old rats, they are EC (Enterochromaffin) (Figs. 10, 11), G (Gastrin) (Figs. 12, 13), A (Pancreatic A like) (Figs. 14, 15) and D_1 (Pancreatic D_1 like) (Figs. 16, 17) cells.

However, in 16 day-old embryos although the four kinds of endocrine cells were not

Table 2. Approximate time of appearance and frequency of endocrine cells in pyloric mucosa of pre- and postnatal rats by electron microscope

Age	NCE cell	UDE cell	EC cell	G cell	A cell	D_1 cell
16 E	+	−	−	−	−	−
18 E	−	−	+	+	−	−
20 E	−	+	+	+	−	−
22 E	−	−	+	+	−	−
1 D	−	−	+	++	+	−
5 D	−	−	+	++	+	+
10 D	−	−	++	+++	+	+
20 D	−	−	++	+++	++	++
30 D	−	−	+++	++++	++	++

−: Absent, +: Rare, ++: Few, +++: Frequent, ++++: Very frequent

Table 3. Ultrastructure of secretory granules in endocrine cells of the pyloric mucosa of pre- and postnatal rats

Cell types	Granular size (nm)	Shape	Contents
EC	100–350	Polymorphous	High density
G	250–400	Round, sometimes oval	Various structure
A	200–300	Round	High dense core and clear halo
D_1	180–250	Round, sometimes oval	Low to moderate density

present yet, the cells that contained both a small number of polymorphous granules resembling EC granules and round granules with low to moderate density about 150 nm in diameter resembling G granules were already observed in the mucosa (Figs. 18, 19). These cells were called non-classifiable endocrine cells (NCE cell). In 18 day-old embryos, cells identified as EC cells because they had a few polymorphous granules with high density, were observed (Fig. 20). The cells that contained various granule contents as well as G granules were identified as G cells in 18 day-old embryos (Fig. 21). In 20 day-old embryos, a few clear cells were found on the basal membrane. They had many polysomes and a few secretory granules in cytoplasm (Figs. 22, 23). These cells were called undifferentiated endocrine cells (UDE cell). The same cell was observed only in one case instead of the lamina propria (Fig. 24). In 1 day-old rats, in addition to EC and G cells, the cells contained granules with high density about 250 nm in diameter were identified as A cells because their granules were similar to pancreatic A granules (Fig. 25). In 5 day-old rats, the cells had some round and a few oval granules with moderate density of about 200 nm in diameter. These were identified as D_1 cells because the structure of the granules was similar to pancreatic D_1 granules (Fig. 26).

The endocrine cells showed a progressive increase with age. In 30 day-old rats, the frequency of the endocrine cells reached that of adult rats. G cells are more frequent than EC cells while A and D_1 cells are the least frequent.

Discussion

Although the four kinds of silver stains for endocrine cells in prenatal rats were performed, none was demonstrated positive in all cases. Through electron microscopy, however, NCE cells were already observed in the pyloric mucosa in 16 day-old embryos, but these cells contained only a few granules, thus making it hard for recognition especially under light microscopy only.

MASSON reacting cells have been regarded as argentaffin, enterochromaffin cells (SINGH, 1964) secreting serotonin (5-hydroxytryptamine) (ERSPAMER & ASERO, 1952). SEVIER-MUNGER method stains argyrophil cells and has been considered staining EC, ECL (SEVIER & MUNGER, 1965) and D_1 cells (SOLCIA *et al.*, 1915). GRIMELIUS method has primarily been used in staining EC, ECL, G, D_1 and A cells (GRIMELIUS, 1968; SOLCIA *et al.*, 1975).

The argyrophil cells by this method are recognized as black or brown granules. These two kinds of granules have been thought to be due to the difference of cell types or functional levels in the cells. Because the DAVENPORT method has been used to stain D cells in the pancreatic islets selectively (HELLERSTRÖM & HELLMAN, 1960), this was performed to identify D and D_1 cells in the pyloric mucosa (SOLCIA *et al.*, 1975). DAVENPORT reacting cells were especially remarkable in 20 day-old rats, indicating that D_1 cells show progressive increase at this stage. This is supported also by the results of SEVIER-MUNGER method and electron microscopy. The approximate time of apperrance of D_1 cells, however was slower than those of other endocrine cells but showed a remarkable increase at about the weaning period. These results might support the idea that D_1 cells were gastric inhibitory peptide (GIP) cells (PEARSE, 1974) that control the mechanism of other endocrine cells.

LARSSON *et al.* (1974) have shown by immunofluorescent study that G cells in the pyloric mucosa of rats were first observed 2 days after birth. In this study, G cells were first identified in 18 day-old embryos by the enzyme-antibody method and it was considered that G cells were already differentiated at this stage.

The four kinds of cell types, EC, G, A and D_1 cells were identified in 5 day-old rats by electron microscopy. These names of endocrine cells were based on the classification of Bologna in 1973 (SOLCIA *et al.*, 1973). NCE and UDE cells were found in the early period embryos and were regarded as early undifferentiated endocrine cells or endocrine cells in the process of differentiation.

MATSUMOTO (1973) has found cells which do not contain secretory granules and has clear cytoplasm in the depth of the gastric pit of fundus in 20 day-old rat embryos. These cells are considered to be UDE cells in this study.

EC and G cells were differentiated in 18 day-old embryos through electron microscopy and the result agreed with that of the inzyme-antibody method.

By electron microscopic observation, UDE cell was found in lamina propria of the 20 day-old embryos. But, this cell was located near the basal membrane and it was not established whether this cell migrated from lamina propria to epithelium or from epithelium to lamina propria. NCE cells on the other hand were already found in the pyloric mucosa of 16 day-old embryos without any NCE and UDE cells in submucosal tissues.

It might be considered that the endocrine cells have originated from endodermal epithelium. If the endocrine cells migrated from the neural crest or from the mesenchyme to the epithelium, the cells must have been found in lamina propria and submucosal tissue in the early period of embryos. However, the authors do not deny the hypothesis that the endocrine cells originate from the neural crest or mesenchyme.

It is difficult to justify the existence of undifferentiated endocrine cells in the lamina propria or submucosal tissue because endocrine cells are unable to be distinguished from other undifferentiated cells in early embryos. Though DE LEMOS (1976) found endocrine cells in the lamina propria, she has described how these cells were usually a short distance

from the epithelium completely surrounded by thin processes of adjacent cells or in part, by basal membrane. She declared that their appearance was therefore due to the plane of section. The authors believe that UDE and NCE cells in early embryos are stem cells in a broad sense and that these cells will be the future endocrine cells containing special granules.

Summary

Endocrine cells were investigated in the pyloric mucosa of 16, 18, 20 and 22 day-old embryos, and in 1, 5, 10, 20 and 30 day-old Wister-Imamichi rats by light and electron microscopy.

The results obtained were as follows:

1) The formation in the gastric pit was found earliest in 18 day-old embryos and the differentiations of gastric pit, pyloric gland and lamina propria were observed in 20 day-old embryos through light microscopy. Cells having PAS positive secretory granules were observed in the pyloric mucosa of 20 day-old embryos and the cells possessing AB positive secretory granules were observed in the pyloric gland of 1 day-old rats by light microscopy.

2) MASSON, SEVIER-MUNGER, GRIMELIUS, and DAVENPORT reacting cells were found in the pyloric mucosa begining in 1 day-old rats. Moreover, G cells were identified in as early as 18 day-old embryos by the enzyme-antibody method.

3) Four kinds of cell types, EC (Enterochromaffin), G (Gastrin), A (Pancreatic A like) and D_1 (Pancreatic D_1 like) cells were all identified through electron microscopy in the pyloric mucosa starting in 5 day-old rats.

4) Non-classifiable endocrine cells were found in 16 day-old embryos through electron microscopy. The differentiations of EC and G cells were observed in 18 day-old embryos.

5) The differentiations of A and D_1 cells were slower than those of EC and G cells. A cells were identified first in 1 day-old rats and D_1 cells were identified in 5 day-old rats through electron microscopy.

6) Endocrine cells of the pyloric mucosa of pre- and postnatal rats showed a progressive increase with age. In 30 day-old rats, the frequency of endocrine cells reached that of adult rats. G cells appeared more frequent than EC cells while A and D_1 cells were least frequent in occurrence.

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摘　　要

16, 18, 20, 22 日齢胎児と生後 1, 5, 10, 20 および 30 日齢のウィスター・今道ラットの幽門部粘膜を用い，内分泌細胞を光顕的および電顕的に観察し，次の成績を得た。

1) 光顕的に，18 日齢胎児頃から胃小窩の形成が始まり，20 日齢胎児において，胃小窩および幽門腺が分化し，粘膜固有層も明らかになった。PAS 陽性細胞が 20 日齢胎児の幽門腺部粘膜上皮において，AB に陽性を示す細胞が 1 日齢ラットの幽門腺底部において認められた。

2) 光顕的に，1 日齢ラットで初めて，MASSON 法，SEVIER-MUNGER 法，GRIMELIUS 法および DAVENPORT 法に陽性を示す細胞が認められた。さらに，酵素抗体法により G 細胞は 18 日齢胎児において認められた。

3) 電顕的に，5 日齢ラットにおいて，EC, G, A, D_1 細胞の 4 種の内分泌細胞の幽門腺部粘膜において認められた。

4) 電顕的に，16 日齢胎児において NCE 細胞が認められ，EC および G 細胞は分化が早く，18 日齢胎児において観察された。

5) A 細胞および D_1 細胞は，EC 細胞および G 細胞に比べ分化が遅く，電顕的に，A 細胞は 1 日齢ラットにおいて，D_1 細胞は 5 日齢ラットにおいて認められた。

6) 内分泌細胞は，胎齢および生後日齢が進むに従って漸次増加を示し，30 日齢ラットにおいては，成体とほぼ同程度の出現頻度に達した。すなわち，内分泌細胞の型別の出現頻度は，G 細胞が最も高く，ついで EC 細胞で，A 細胞と D_1 細胞は低かった。

Explanation of Plates

Plate I

Fig. 1 The formation of gastric pit (arrow) in the pyloric mucosa in a 18 day-old embryo. FEA, H. -E, ×465.

Fig. 2 Gastric pit, pyloric gland, lamina propria and PAS reactive substances in the epithelial cells of mucosal surface, gastric pit and pyloric gland in a 20 day-old embryo. FEA, AB-PAS, ×280.

Fig. 3 A few AB positive cells (arrows) at the base of the pyloric gland in a 1 day-old rat. formalin, AB, ×465.

Fig. 4 This shows the pyloric mucosa in a 30 day-old rat. FEA, AB-PAS, ×240.

Fig. 5 Seven argentaffin cells at the base of the pyloric gland in a 30 day-old rat. formalin, MASSON, ×465.

Fig. 6 Five argyrophil cells at the base of the pyloric gland in a 20 day-old rat. formalin, SEVIER-MUNGER, ×465.

Fig. 7 Black (long arrow) and brown (short arrow) argyrophil cells from the depth of gastric pit to the base of gland in a 30 day-old rat. BOUIN, GRIMELIUS, ×465.

Fig. 8 Three argyrophil cells (arrows) below the neck of gastric pit in a 20 day-old rat. BOUIN, DAVENPORT, ×465.

Plate II

Fig. 9 G cell at the base of the pyloric mucosa in a 18 day-old embryo. BOUIN, Enzyme-antibody method, ×1,500.

Fig. 10 Open type of EC cell in the pyloric mucosa of a 20 day-old rat. glutaraldehyde, Ur & Pb, ×7,600.

Fig. 11 Enlargement of Fig. 10. ×20,000.

Fig. 12 G cell in the pyloric mucosa in a 10 day-old rat. glutaraldehyde, Ur & Pb, ×6,000.

Fig. 13 Enlargement of Fig. 12. ×20,000.

Fig. 14 A cell at the base of the pyloric gland in a 20 day-old rat. glutaraldehyde, Ur & Pb, ×6,000.

Plate III

Fig. 15 Enlargement of Fig. 14. ×20,000.

Fig. 16 D_1 cell in the pyloric mucosa in a 10 day-old rat. glutaraldehyde, Ur & Pb, ×6,000.

Fig. 17 Enlargement of Fig. 16. ×20,000.

Fig. 18 NCE cell in the pyloric mucosa of 16 day-old embryo. paraformaldehyde & glutaldehyde, Ur & Pb, ×8,000.

Fig. 19 Enlargement of Fig. 18. ×20,000.

Fig. 20 EC cell in the pyloric mucosa of a 18 day-old embryo. paraformaldehyde & glutaraldehyde, Ur & Pb, ×8,000.

Plate IV

Fig. 21 G cell in the pyloric mucosa of a 18 day-old embryo. paraformaldehyde & glutaraldehyde, Ur & Pb, 20,000.

Fig. 22 UDE cell at the base of the pyloric gland of a 20 day-old embryo. paraformaldehyde, Ur & Pb, ×5,250.

Fig. 23 Enlargement of Fig. 22. ×20,000.

Fig. 24 UDE cell in the lamina propria of a 20 day-old embryo. paraformaldehyde & glutaraldehyde, Ur & Pb, ×6,000.

Fig. 25 A cell in the pyloric mucosa of a 1 day-old rat. glutaraldehyde, Ur & Pb, ×20,000.

Fig. 26 D_1 cell in the pyloric mucosa of a 5 day-old rat. glutaraldehyde, Ur & Pb, ×20,000.

Plate I YOSHINO, M. *et al.*

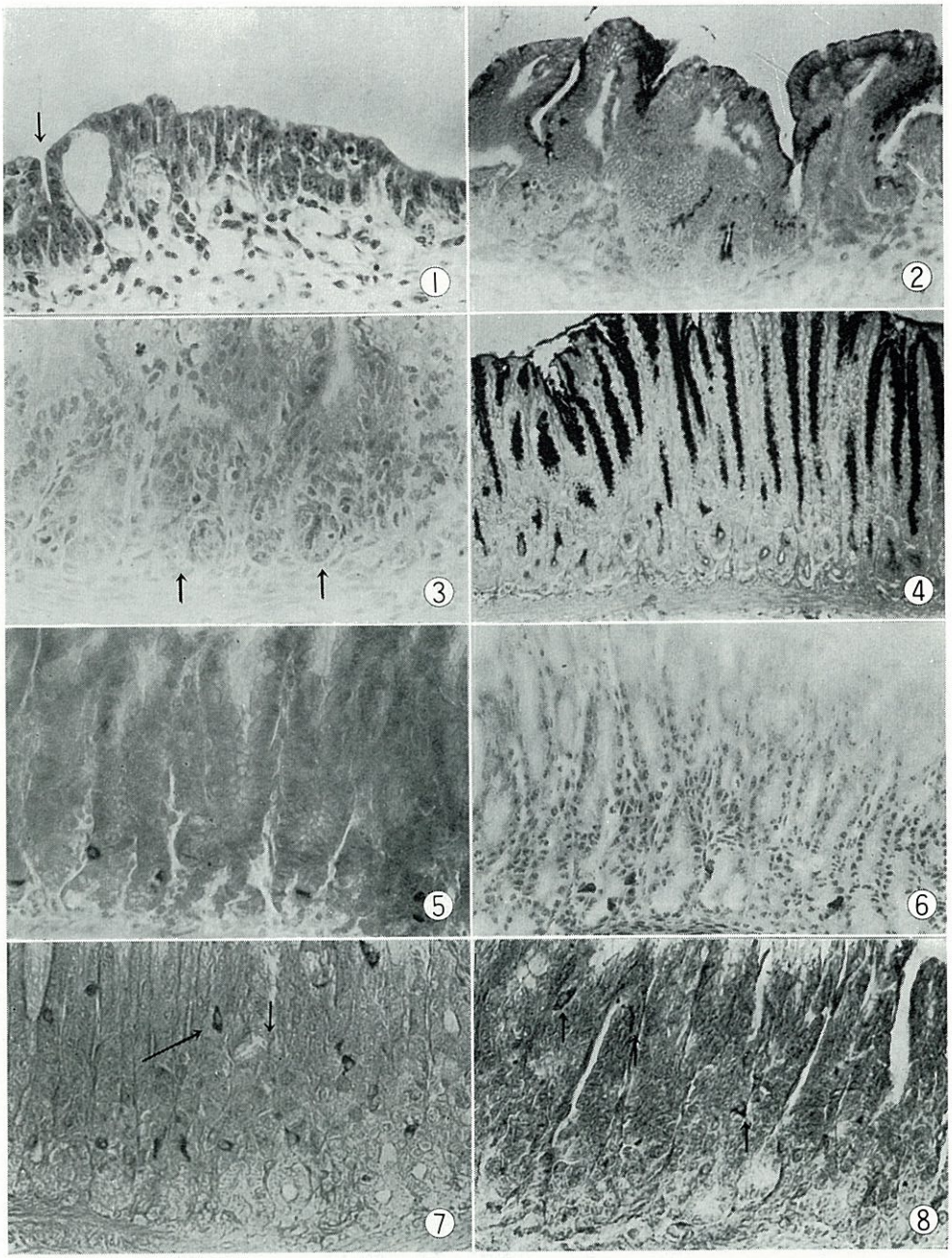

Plate II YOSHINO, M. *et al.*

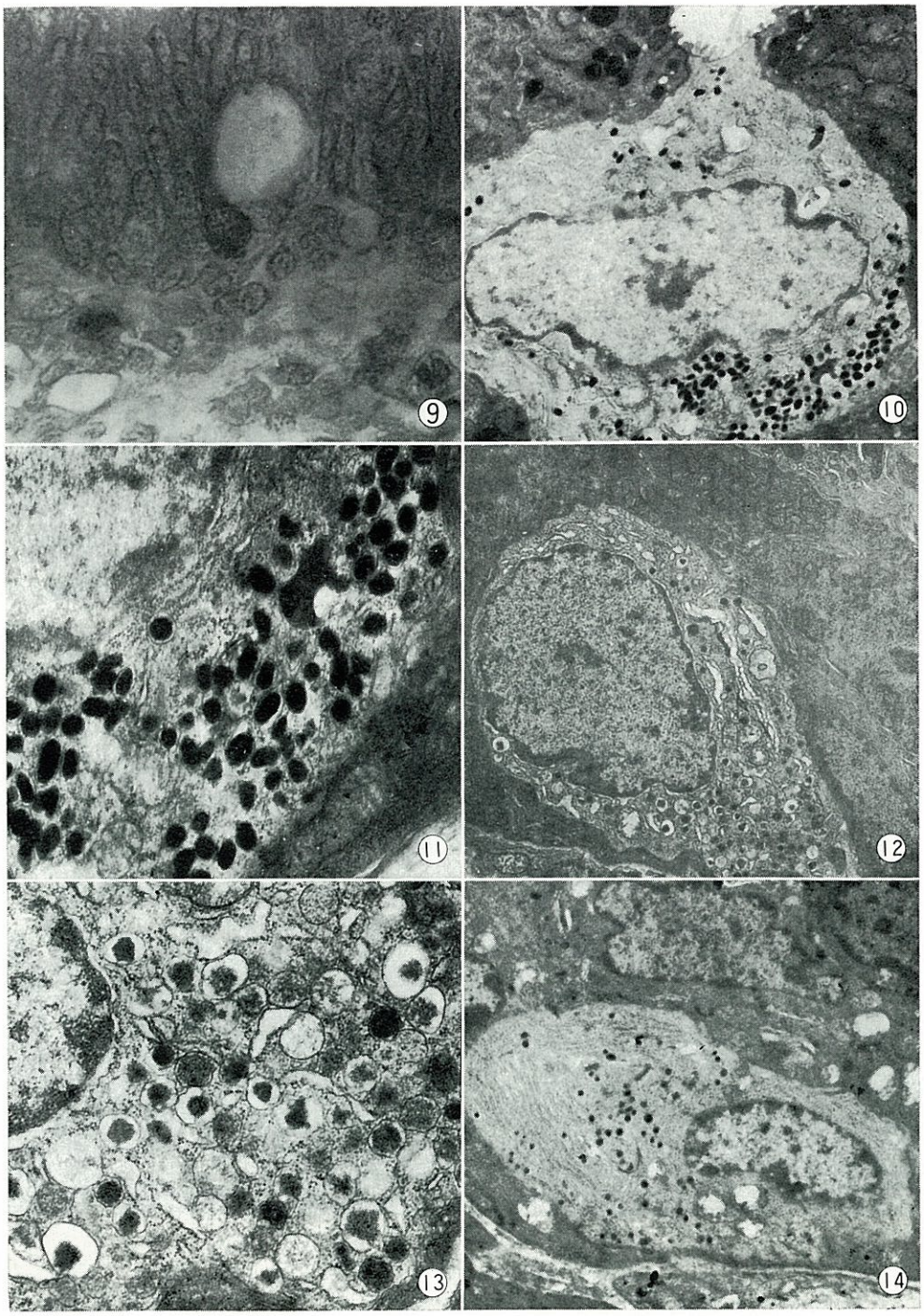

Plate III YOSHINO, M. *et al.*

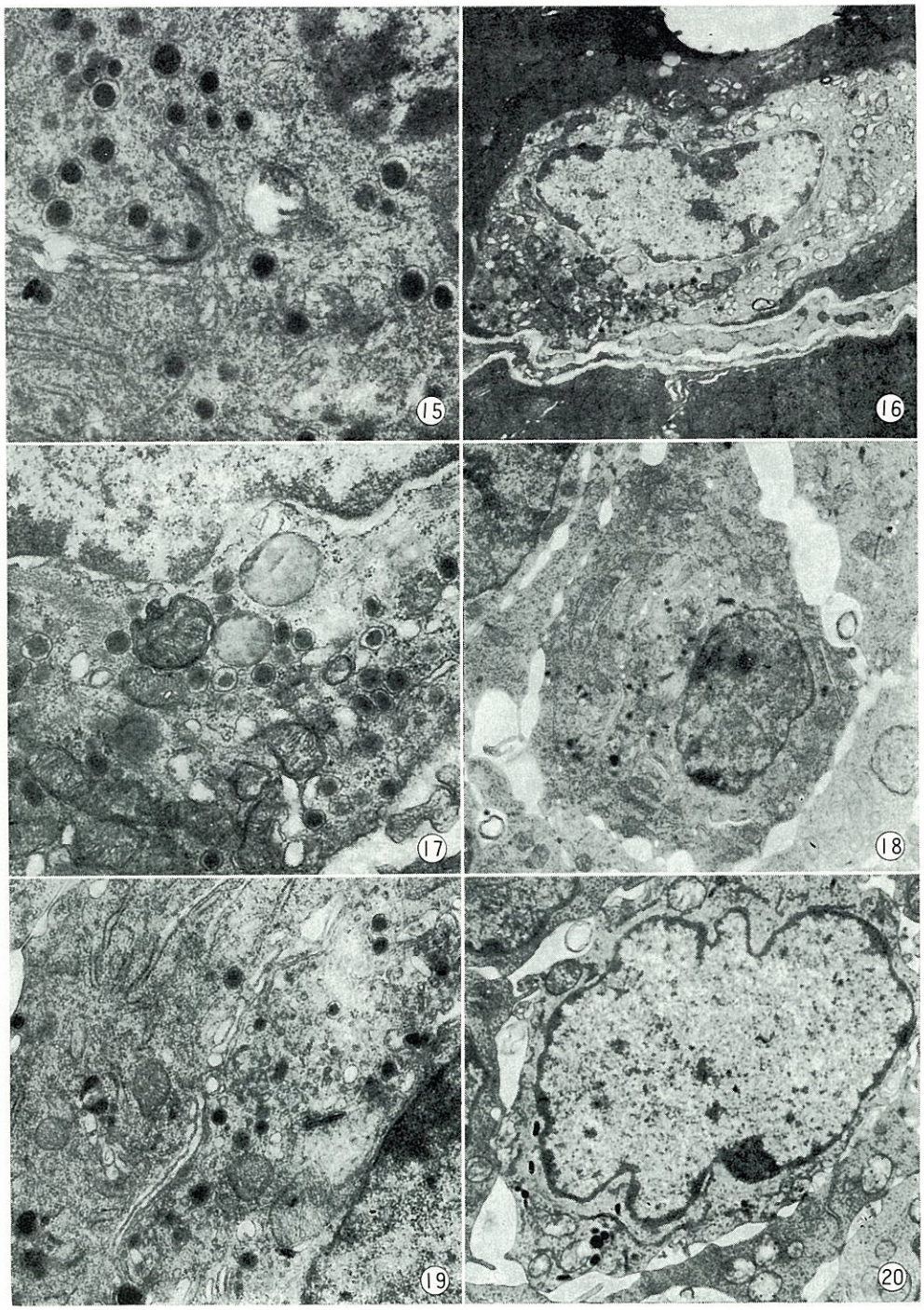

Plate IV YOSHINO, M. *et al.*