Ontogeny of the Endocrine Cells in the Pyloric Region of the Japanese Quail (*Coturnix coturnix japonica*)

Takehito KAYAMORI, Junzo YAMADA, Tadayuki YAMASHITA and Mikio MISU

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

Received May 31, 1978

ウズラ幽門領域における内分泌細胞の個体発生について

栢森武等*・山田純三*・山下忠幸*・三須幹男*

Introduction

Endocrine cells in the adult digestive tract have been studied and elucidated by many investigators. But the information concerning the differentiation and development of the endocrine cells in the fetal gastrointestinal tract is scant. (PENTILLÄ, 1968 a, b; OSAKA, 1975; HAGE *et al.*, 1972; DE LEMOS, 1976).

The present correlative investigation was undertaken under light and electron microscopy to study the development and maturation of the endocrine cells in the pyloric region of the Japanese quail.

Materials and Methods

For these experiments, the pyloric region of the Japanese quail from the 8th day of incubation up to one day after hatching were used. Postnatal pyloric mucosae were also taken from one-week and three-week old quails. For light microscopic study, BOUIN's solution and neutral buffered formaldehyde solution were used as fixatives. Staining methods used in this study were as follows: alcian blue(AB)-periodic acid shiff (PAS) staining method, Diazonium reaction (SOLCIA *et al.* 1969 a), MASSON-HAMPERL silver method (SINGH, 1964), GRIMELIUS silver method (GRIMELIUS 1968), DAVENPORT silver method (HELLERSTRÖM and HELLMAN, 1960), Lead-haematoxylin method (SOLCIA *et al.* 1969 b).

For electron microscopy, tissues were fixed in 2.66% glutaraldehyde in 0.1 M phosphate

* 帯広畜産大学獣医学科家畜解剖学教室

buffer (pH 7.3), and postfixed in 1% osmium tetroxide in the same buffer. Sections about 1μ thick were stained with 1% toluidine blue (pH 7.0–8.0). In addition, ultrathin sections were stained with uranil acetate and lead nitrate solution.

Results

Light microscopic observations: On the 14th day of incubation, exocrine granules weakly stained with alcian blue were seen on the surface of the epithelium (Fig. 1). GRIMELIUS positive cells and DAVENPORT positive cells first appeared in the epithelium on the 13th day of incubation (Figs. 3 and 7). No endocrine cells were seen in any tissues except the epithelium. The lamina muscularis mucosae appeared thick at this stage.

GRIMELIUS silver impregnated granules appeared to be weakly brown-stained (Fig. 4), while DAVENPORT silver impregnated granules were clearly black-stained (Fig. 8) on the 14th day of incubation. The pyloric glands appeared to develop into the branching duct system at about this stage. Exocrine granules reactive to alcian blue were seen restricted in the upper portion of the gland after this stage.

Lead-haematoxylin positive cells occurred in the epithelium on the 15th day of incubation (Fig. 11). Exocrine cells in the upper portion of the gland appeared to be gradually increasing, with the endocrine cells showing the same parallel increase in the lower portion of the gland during the development (Figs. 2 and 9).

It seems that from one week after hatching, the frequency of occurrence and distribution of endocrine cells are similar to that of the adult (Figs. 6, 10 and 12). All the endocrine cells seen in this study were restricted to the epithelium. Argentaffin cells and enterochromaffin cells were not found in any tissues of the pyloric region.

Electron microscopic observations: There were few remarkable differences in the endocrine cells of the embryo and the adult. Only small, round mitochondria and poorly developed Golgi apparatus were recognized in the earlier stages.

The granulated cells first appeared in the epithelium on the 11th day of incubation (Fig. 13), with granules (280–520 nm) having a lucent halo and high dense core (190–330 nm). The morphology of these granules appeared somewhat similar although a little different from the II-type cells (YAMADA *et al.*, 1978).

Some immature cells having a few and small granules (190–260 nm) were seen on the 12th day of incubation (Figs. 16 and 17), some of which already showed microvilli projecting into the glandular lumen (Fig. 17).

Three types of endocrine cells first appeared in the epithelium on the 13th day of incubation (Figs. 18, 20 and 23). Type I cell granules were 280–340 nm in diameter, whereas granules of type II cell were 230–330 nm in diameter. The granules of type III cells were smaller (140–280 nm). At this stage, the frequency of the three cell types appeared to be low.

The same endocrine cells seen on the 11th day of incubation occurred also in the

lamina propria mucosa on the 13th day of incubation (Fig. 14), and in the epithelium on the 16th day of incubation (Fig. 15). These cells, however, did not show a direct connection with the nervous elements.

The appearance of the emiocytotic figure present in the II-type cell occurred on the 14th day of incubation (Fig. 21). Granules larger than 400 nm were observed in I-and II-type cells.

Endocrine cells on the day after hatching appeared to have the frequency and morphology of that of the adult (Figs. 19, 22 and 24).

Discussion

These results suggest that the endocrine cells differentiate in the epithelium, as found by MASSON (1914), MONESI (1960), SINGH (1964), PENTTILÄ (1968a), DE LEMOS (1976) and not in the connective tissue (KULL, 1925) or in the nervous tissue (DANISH, 1924; PEARSE, 1969, 1973; OSAKA, 1975). The final differentiation, *ie.*, the development of the function to produce specific endocrine granules, at least seems to occur in the epithelium. This study, however, can not give specific evidence as to the origin of the endocrine cells in the pyloric region. Much more intensive investigations are needed for elucidating this problem.

No argentaffin cell or enterochromaffin cell was observed in this study. All endocrine cells found during embryonic development were identified as argyrophil cells. This finding suggests that the argentaffin or enterochromaffin cells never occur in the pyloric region throughout the individual development and that this region of the digestive tract is largely different from the pyloric antrum of the mammal.

The granulated cells first found in the epithelium on the 11th day of incubation appeared to be a little different from the earlier classified II-type cells in the pyloric region (YAMADA *et al.*, 1978). The absence of reactivity to silver methods and Lead-haematoxylin staining method before the 13th day of incubation possibly suggests that these cells seem to be classified into a different cell type. Their granules, however, have some similarlities with II-type cells. In addition, the morphological changes caused by the fixation (McC MORTENSEN *et al.*, 1977) can be another factor for consideration.

Endocrine cells in the corpus of the fetal human were considered to be closed-type (DE LEMOS, 1976). It was reported, however, that the basal-granulated cells in the duodenum of the human fetus are at first the closed-type, but later reach the lumen to become open-type (OSAKA, 1975). In this study, the unclassified and likely undifferentiated endocrine cells did not necessarily show a closed-type in the pyloric region of the quail embryo.

The concommitant appearance of argyrophil cells and the ultrastructurally identified endocrine cells on the 13th day of incubation suggests that the development of specific endocrine granules is connected with the beginning of the synthesis and storage of argyrophil materials. Earlier reports of the occurrence of 5-hydroxytryptamine fluorescence and enterochromaffin granules at the same time (PENTTILÄ, 1968 a, b) supports this parallel

maturation.

In the rat pyloric antrum, argyrophil cells and argentaffin cells occurred on the day after birth (YOSHINO *et al.*, 1978). However, argyrophil cells in the pyloric region of the quail appeared on the 13th day of incubation. This remarkable difference may be due to species variation and eating habits.

The emiocytotic release of granules of the II-type cell on the 14th day of incubation supported an earlier report that endocrine cells are not only functioning in the embryonic stage, but also may be releasing hormones without any luminal stimulation by the administration of some chemicals (OSAKA, 1975).

In the quail, the epithelium of the pyloric region seems to be similar to the adult ultrastructurally as early as one day after hatching. Histochemically, the similarity exists one week after hatching.

Summary

The development and maturation of the endocrine cells in the pyloric region of the Japanese quail were examined under light and electron microscope from the 8th day of incubation to 3 weeks after hatching. The results obtained are summarized as follows:

1) Histochemically, the alcian blue reaction was first seen along the crypts on the 11–12th day of incubation. From the 14th day of incubation, the gland appeared to show a branching duct system and two poorly defined layers, one of exocrine cells in the upper portion and the other of endocrine cells in the lower portion.

2) Argyrophil cells positive to the GRIMELIUS silver method and the DAVENPORT silver method were first seen in the epithelium on the 13th day of incubation, although still few in number. Lead-haematoxylin positive cells were never found before the 15th day of incubation.

3) Argentaffin cells and enterochromaffin cells were never seen in any tissues of the pyloric region during development.

4) Ultrastructurally, unclassified endocrine cells with many granules showing specific morphology were found only at three stages: in the epithelium on the 11th day, in the connective tissue on the 13th day and in the epithelium on the 16th day of incubation. Their frequency of occurrence was very low.

5) On the 12th day of incubation, undifferentiated cells were seen in the epithelium, some of which showed the open-type with short microvilli.

6) The emiocytotic figure was found on the 14th day of incubation.

7) On the 13th day of incubation, I-, II- and III-type cells were first found in the epithelium.

8) Histochemically, endocrine cells in the pyloric region are similar to that of the adult at one week after hatching, but ultrastructurally, the morphology and frequency are similar as early as one day after hatching.

Literature

1) DANISH, F.: Breitr. Pathol. Anat. **72**: 687-709 (1924).
2) DE LEMOS, C.: Am. J. Anat. **148**: 359-384 (1976).
3) ERSPAMER, V.: Z. Anat. Entwgesch. **107**: 574-619 (1937). cited by MONESI (1960).
4) GRIMELIUS, L.: Acta. Soc. Med. Upsal. **73**: 243-298 (1968).
5) HAGE, E. and J. KJARAAD: Arch. histol. jap. **35**: 31-36 (1972).
6) HELLERSTRÖM, C. and B. HELLMAN: Acta Endocrinologia **35**: 518-532 (1960).
7) KULL, H.: Z. mikr. -anat. Forsch. **2**: 163-200 (1925).
8) MASSON, P.: C. R. Acad. Sci., Paris, **158**: 59-61 (1914) cited by SINGH (1963).
9) MCC MORTENSEN, N. J.: Cell Tiss. Res. **176**: 251-263 (1977).
10) MONESI, V.: Acta anat. (Basal) **41**: 97-114 (1960).
11) OSAKA, M.: Arch. histol. jap. **38**: 307-319 (1975).
12) PEARSE, A. G. E.: J. Histochem. Cytochem. **17**: 303-313 (1969).
13) PEARSE, A. G. E.: Digestion, **8**: 372-385 (1973).
14) PENTTILÄ, A.: Z. Zellforsch. **91**: 380-390 (1968a).
15) PENTTILÄ, A.: Acta histochem. **31**: 1-13 (1968b).
16) SEVIER, A. G. and B. L. MUNGER: J. neuropath. exp. neuro. **24**: 130-135 (1965).
17) SINGH, I.: J. Anat. **97**: 377-387 (1963).
18) SINGH, I.: Anat. Anz. Bd. 81-82 (1964).
19) SOLCIA, E., R. SAMPIETRO and C. CAPELLA: Histochemie, **17**: 273-283 (1969 a).
20) SOLCIA, E., C. CAPELLA and G. VASSALO: Histochemie, **20**: 116-126 (1969 b).
21) WESTON, J. A.: Adv. Morphogenes. **8**: 41-108 (1970).
22) YAMADA, J., T. KAYAMORI, T. OKAMOTO, T. YAMASHITA and M. MISU: Arch. histol. jap. **41**: 41-52 (1978).
23) YOSHINO, M., T. YAMASHITA, J. YAMADA and M. MISU: Res. Bull. Obihiro Univ. **11**: 13-24 (1978).

摘　　要

孵卵8日目から孵化後3週齢までのニホンウズラ (*Coturnix coturnix japonica*) の幽門領域における内分泌細胞の個体発生を光学および電子顕微鏡的に観察し，次のごとき所見を得た。

1) 孵卵11日目から12日目にかけて，浅く発達し始めた幽門腺の辺縁に沿ってAB弱陽性の外分泌顆粒を認めた。孵卵14日目から，幽門腺は分岐管状腺構造を示し始め，上層の外分泌細胞層と下層の内分泌細胞層とが不明瞭ながら分かれる傾向が認められた。

2) 光顕的に，孵卵13日目で初めて，GRIMELIUS 弱陽性細胞と DAVENFORT 弱陽性細胞とを上皮中に観察した。鉛ヘマトキシリン陽性細胞は，孵卵15日目で初めて認められた。

3) 銀親和性細胞や腸クロム親和性細胞はいずれの時期においても観察できなかった。

4) 微細構造的に，特異的な形態を示す顆粒細胞を孵卵11日目に観察したが，この細胞とほぼ同形態の顆粒をもつ細胞を，孵卵13日目では粘膜固有層中で，孵卵16日目では上皮中で開放型細胞としてそれぞれ認めた。

5) 孵卵12日目に，未発達な顆粒細胞を上皮中に観察した。それらのなかには短い微絨毛をそなえた開放型の細胞もあった。

6) Ω型の顆粒放出像を孵卵14日目に観察した。

7) 粘膜固有層中に1例だけ内分泌細胞と考えられる細胞を認めたが，神経要素との密接な関係は観察できなかった。

8) 光顕的には孵化後1週齢で，電顕的には孵化日で，成体で観察される内分泌細胞とほぼ同様の頻度や分布状態あるいは形態を示していた。

Explanation of Plates

Plate I

Fig. 1 Alcian blue reaction found along the surface of the epithelium. 14th day of incubation. BOUIN fixation. AB-PAS. ×300

Fig. 2 Exocrine cell layer containing considerable of exocrine material strongly positive to alcian blue. 1-week old. BOUIN fixation, AB-PAS. ×300

Fig. 3 Two argyrophil cells (arrows) in the epithelium. Positive granules in the cytoplasm are quite few in number. BOUIN fixation. GRIMELIUS. ×900

Fig. 4 Argyrophil cell (arrow) in the epithelium. The reactivity appeared to be increased. 14th day of incubation. BOUIN fixation. GRIMELIUS. ×900

Fig. 5 Argyrophil cells in the epithelium. 16th day of incubation. BOUIN fixation. GRIMELIUS. ×450

Fig. 6 Argyrophil Cells showing almost the adult frequency and distribution. Insert: a cell containing plenty of positive granules (×900). 1-week old. BOUIN fixation. GRIMELIUS. ×450

Plate II

Fig. 7 A cell (arrow) showing poor reactivity in the epithelium. 13th day of incubation. BOUIN fixation. DAVENPORT. ×900

Fig. 8 Two argyrophil cells (arrows) stained clearly in the epithelium. 14th day of incubation. BOUIN fixation. DAVENPORT. ×300

Fig. 9 Strongly positive cells, but still few in number, are seen in the epithelium. 16th day of incubation. BOUIN fixation. DAVENPORT. ×320

Fig. 10 Many argyrophil cells showing almost the adult frequency and distribution. 1-week old. BOUIN fixation. DAVENPORT. ×600

Fig. 11 Lead-haematoxylin positive cells appeared in the basal portion of the epithelium. 15th day of incubation. Formalin fixation. Lead-haematoxylin. ×450

Fig. 12 Endocrine cells histochemically showing almost the same frequency and distribution as the adult. 1-week old. Formalin fixation. Lead-haematoxylin. ×600

Plate III

Fig. 13 Unclassified granulated cell in the epithelium showing the long bundle of microfilaments and many granules. 11th day of incubation. ×5600

Fig. 14 A cell with the same type of granules as Fig. 15 is seen in the connective tissue. 13th day of incubation. ×5600

Fig. 15 The peculiar basal-granulated cell showing the open type. 16th day of incubation. ×5600

Fig. 16 An undifferentiated cell in the epithelium showing poor developed. 12th day of incubation. ×5600

Fig. 17 An undifferntiated cell showing the open-type. 12th day of incubation. ×5600

Fig. 18 A I-type cell in the epithelium. 13th day of incubation. ×5600

Plate IV

Fig. 19 A I-type cell of the open-type having large granules. One day after hatching. ×5600

Fig. 20 A II-type cell in the epithelium showing the closed-type. 13th day of incubation. ×5600

Fig. 21 The emiocytotic figure (arrow) is seen in the type II cell. 14th day of incubation. ×30000

Fig. 22 II-type cells (II) and a III-type cell (III) in the epithelium. ×5600

Fig. 23 A III-type cell showing very small granules in the epithelium. 13th day of incubation. ×5600

Fig. 24 A III-type cell showing an undistinguishable change compared with the granule of III-type cells found on the 13th day of incubation. One day after hatching. ×5600

Plate I T. KAYAMORI, *et al.*

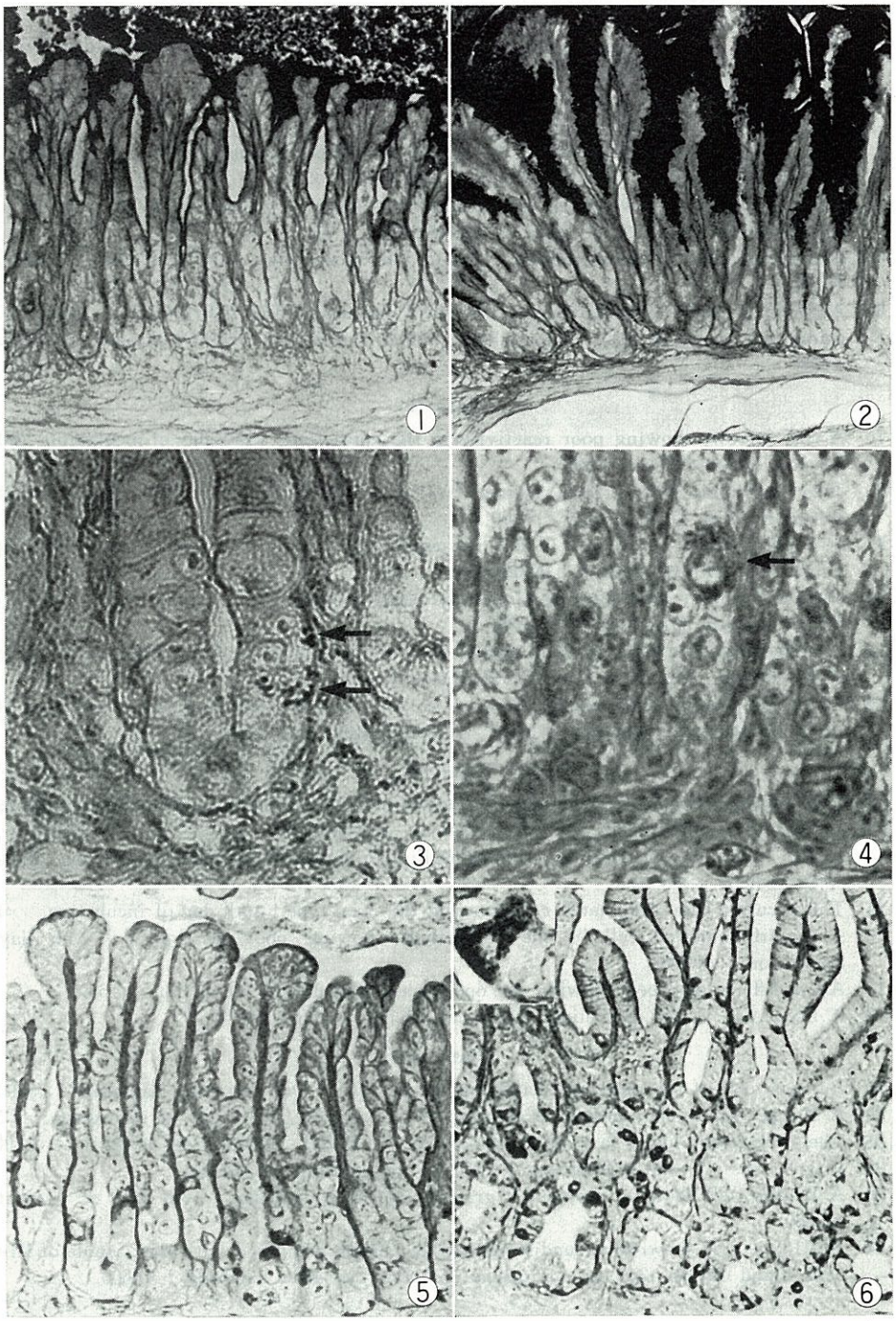

Plate II T. KAYAMORI, *et al.*

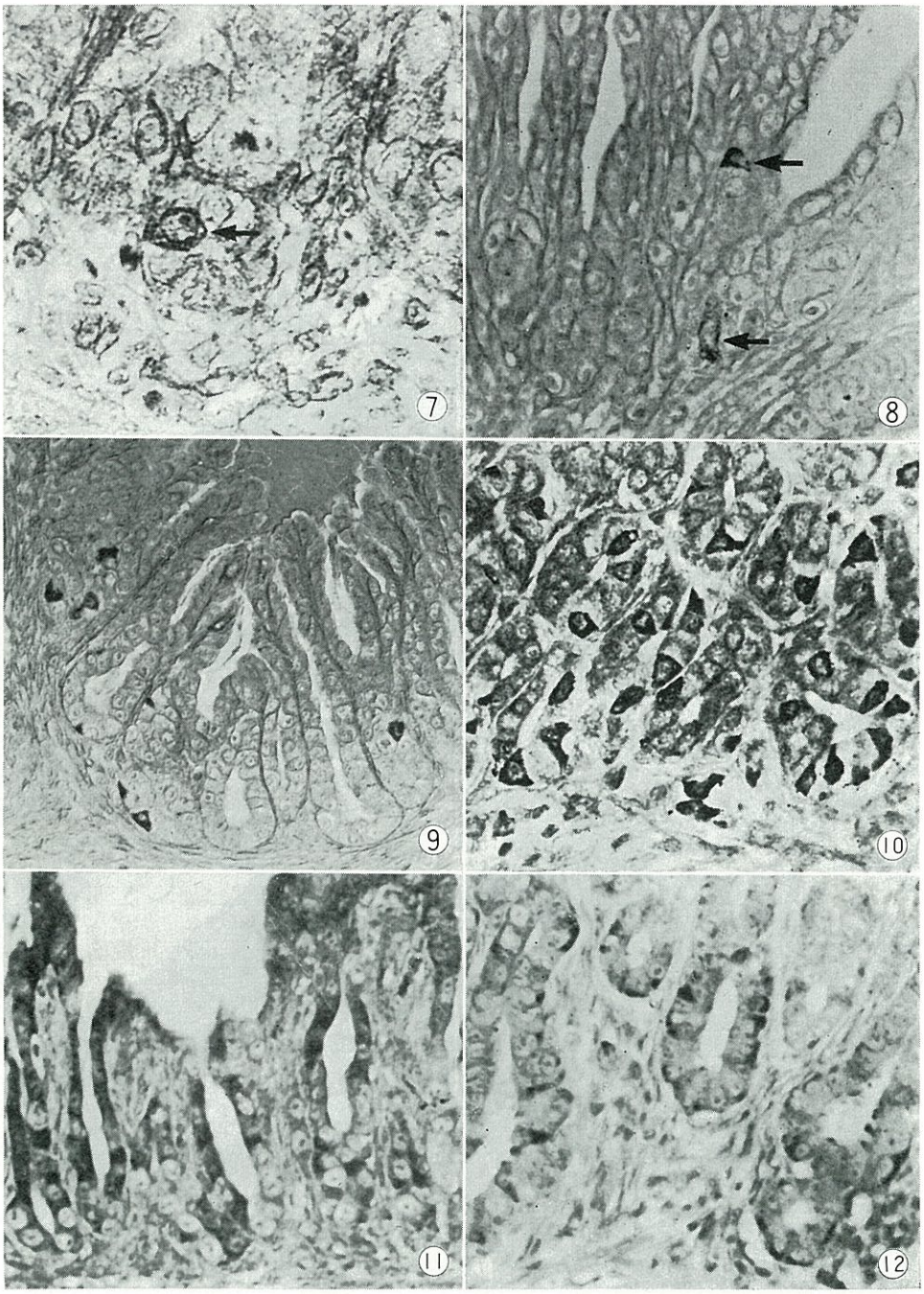

Plate III T. KAYAMORI, *et al.*

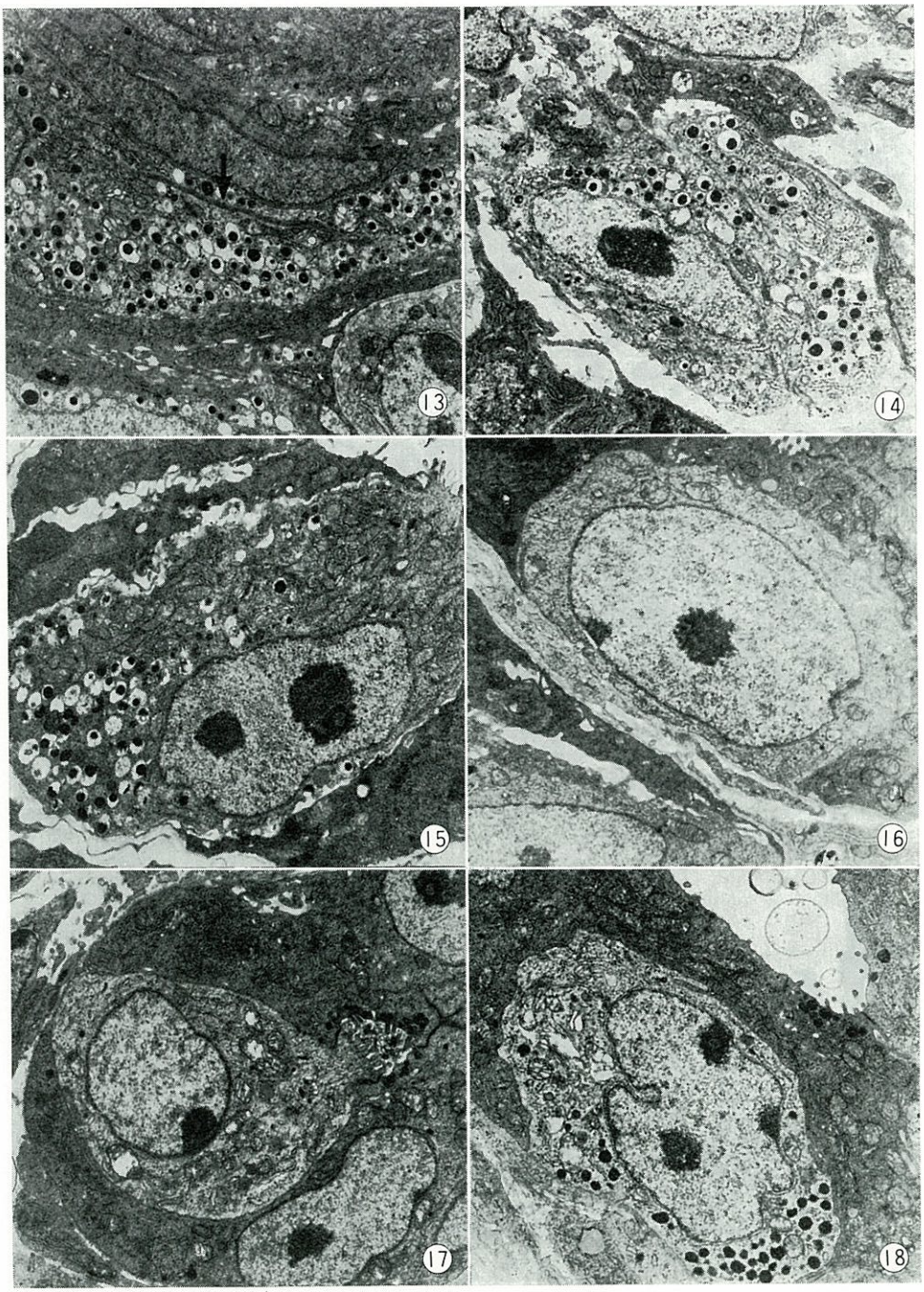

Plate IV T. KAYAMORI, *et al.*