Histological Studies in the Adrenal Gland of Teleosts

Part I. Optical and Electron Microscopic Observations on the Adrenal Tissue of Rainbow Trout

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INTRODUCTION

Concerning the cytology and histology of the adrenal tissue there have been a large number of investigations, including elaborate ones recently achieved through the electron microscopy by Lever (1955 a, b, 1956 a), Belt (1956, 1957), Sjöstrand and Wetzstein (1956), Wetzstein (1957), Leut and Hechter (1957), etc. All of them, however, were carried out with the tissue of mammals, and came under our notice no works done with lower vertebrates, except those on domestic fowl by Kano (1956) and Fujita et al. (1959), bullfrog by Fujimura et al. (1959), domestic duck by Hirata (1961) and toad by Kamizono (1961).

According to these studies, a certain amount of variation in structure of the adrenal tissue as well as in ultrastructure of its component cells seemed to be recognized with different species of animals. With the intention of making histological comparison of the structure of adrenal of the teleost, to which little attention has been paid, with that of other various animals, the present author has conducted observations on the adrenal tissue of rainbow trout by means of both optical and electron microscopes.

It is alredy known that the adrenal gland does exist among teleosts; in this connection the corpuscles of Stannius described for the first time by Stannius has been said to correspond to the adrenal cortex of mammals. However, Giacomini found that on the wall of veins in the lymphoid tissue of the cephalic part of kidney (head kidney) there existed chromaffin cells which contained adrenalin granules, and they were regarded as being homologous to the cells of adrenal medulla of mammals. Giacomini also considered the acidophile cells found around veins in the head kidney to be the interrenal cells, which homologized to the cells of adrenal cortex of mammals. Thus the chromaffin cells and the acidophile cells of head kidney of the teleost are at present understood to be respectively homologous to the medulla and the cortex of adrenal gland of the mammals.

From this standpoint an attempt was made by the author to study the chromaffin and acidophile cells observed in the head kidney of rainbow trout by comparing

them with the medullary and cortical cells of adrenal tissue of other animals.

The author here wishes to express his deepest gratitude to Prof. Gako Jimbo of Yamaguchi Medical School for his kind guidance rendered throughout the present study.

MATERIAL AND METHOD

The rainbow trout (*Salmo irideus* Gibbons) used for study was obtained from the Trout-Breeding Farm at Beppu, Schuho-cho, Yamaguchi-ken, where they had been incubated and bred. The water of the breeding farm is directly laid on from Benten-ike Spring welling out of the Akiyoshi Karst Hydrography, and contains calcium rather in great quantity, though not enough to affect the physiology of the trout. The result of hydrologic analysis of the water was published in another report (Wakisaka et al., 1962).

All the individuals of the trout used were approximately one year old, measuring ca. 20 cm in body-length.

The kidney of this species is an unpaired slender body situated on the dorsal wall of the abdominal cavity, just beneath the vertebral column, and is reddish-purple in color. The individuals of more than sixth months old show no variation notwithstanding their age or body-length, and the kidney of the individuals used for observation measured ca. 10 cm in length. In the rainbow trout the head and body kidneys are not definitely distinguishable anatomically from each other, and the triangular cephalic part of the kidney was used as representing the head kidney. The habitus of the entire kidney is shown in Fig. 1.

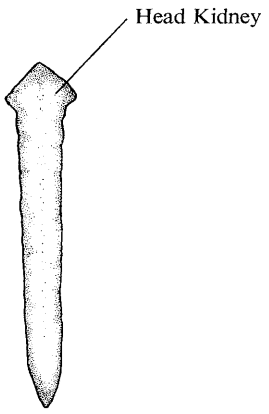

Fig. 1. Ventral view of the kidney of Salmo irideus.

Tissues for optical microscopic observations were fixed in 10 per cent formalin, Bouin's fluid, Orth's fluid or Zenker-formol solution, and imbedded in paraffin for serial sections, which were then stained with Mayer's acid hemalum and eosin.

For electron microscopy the polystyren-resin imbedding method developed by Shinagawa and Ogura (1960) was employed.

The customary methacryl-resin imbedding has been pointed out to involve certain disadvantages for electron microscopy. Ogura and Shinagawa (1960) pointed out the deformation of specimens through the bombardment of electron rays. This deformation derives, according to Ogura and Shinagawa, from the depolymerization of the methacryl-resin by high-energy radiant rays. In order to obviate this defect Maalϕe and Birch-Andersen devised the epoxy-resin imbedding

method, which has been later improved by Glauert, Rogers and Glauert (1956) and Kushida (1959). More recently, Ogura and Shinagawa (1960) recommended the method for imbedding tissues in polystyren-resin which is more stable against radiation than is the epoxy-resin.

In the present study the preparations were made through the following procedure.

Marketed styren-monomer, added with $\frac{1}{4}$ volume of 20 per cent potassium hydroxide solution, was violently agitated, and rinsed several times. Subsequently it was repeatedly washed with distilled water until the washings were no longer alkaline, and then dehydrated for use with the aid of calcium chloride for more than 24 hours. Tissues were cut with a razor into pieces of approximately 1 mm, and fixed for an hour in 1 per cent osmium tetroxide solution buffered at pH 7.4 with the same amount of sodium acetate-veronal buffer. After fixation the specimens were washed several times with distilled water, and then dehydrated through the routine alcohol series. The dehydrated tissues were submerged for permeation first in a 1 : 1 mixture of ethanol and styren-monomer for 30 minutes, then in pure styren-monomer for an hour; during the treatment the specimens were kept in an ice-chamber. Then the tissues were transferred into the styren-monomer containing 2 per cent benzoil peroxide and allowed to stand for 24 hours. Polymerization was performed in gelatine capsules irradiated with ultraviolet rays from 5 Madzuda sterilamps set in a semicircular metal box of 25 cm in radius. It took 5 or 6 days for each polymerization at a temperature between 55 and 63°C in the irradiating apparatus.

The tissues thus imbedded in polystyren-resin were sliced into ultra-thin sections with the Shimadzu Ultramicrotome furnished with the glass knife, then stained with potassium permanganate according to Lawn's (1961) method.

Observations as well as photomicrographing were made with a JEM-5HS type electron microscope of the Nippon Denshi-Kogaku Kenkyusho.

RESULT OF OPTICAL MICROSCOPIC OBSERVATIONS AND THE DISCUSSION ON THE SUBJECT

General Structure:

In the optical microscopic picture, the head kidney of rainbow trout seems to consist of a wholly lymphoid tissue, in which no renal tubules nor glomeruli are recognizable. Around the cardinal vein and its branches penetrating the head kidney are found large epithelial cells in clusters or in rows. When these cells are fixed in Orth's fluid or Zenker-formol solution and stained with Mayer's acid hemalum and eosin, they are shown to be differentiated into two groups: one comprises the chromaffin cells of which the cytoplasm is filled with fine brownish granules showing a positive chromaffin reaction, and the other consists of the so-called acidophile cells

containing the cytoplasm evenly stained with eosin. The cells of the former group are referable to the medullary cells, and those of the latter to the cortical cells of adrenal tissue.

Medullary Cells:

The nucleus of the medullary cells is considerably large as compared with that of either lymphoid cells or blood corpuscles in veins or cortical cells which shall be later described. It is oblong or irregular in profile, with smaller amount of chromatin than in lymphoid cells. The nucleolus is indistinct.

The cytoplasm stains brown as described above, when fixed either in Orth's fluid or in Zenker-formol solution, whereas it does not stain in that manner after the fixation in 10 per cent formalin or in Bouin's fluid.

The medullary cells adhere to the walls of veins in the head kidney; in some cases they may be found even projecting into the vessel of veins. They constitute such a group of cells that is quite distinct from that of cortical cells.

Cortical Cells:

The nucleus of the cortical cells is somewhat smaller than that of the medullary cells, and larger than that of the lymphoid cells. It is oblong in profile, and contains large amount of chromatin and the nucleolus rather distinctly visible. The cytoplasm evenly stains with eosin. There are vacuoles in the peripheral portion of the cells.

The cortical cells occur around the veins as a group of those cells which are distinct from and more developed than the medullary cells. These two groups of cells never manifest themselves in intermingling occurrence, and are readily distinguishable from each other on account of their peculiarities mentioned above.

Discussion:

According to Suehiro (1960) the head kidney of teleosts is pronephrotic in ontogenetic origin, and the renal tubules and the glomeruli degenerate to be replaced by the lymphoid tissue as the mesonephros comes to function. It is natural, therefore, that the head kidney observed in rainbow trout should be a lymphoid tissue without renal tubules or glomeruli.

It has been well known since Henle (1865) that the medullary cells of adrenal of mammals show a positive chromaffin reaction, which has been ascribed by Ogata and Ogata (1917), Verne (1923), Gerard, Cordier et Lison (1930), Lison (1953), etc. to adrenalin oxidized by potassium bichromate involved in fixatives.

From this reaction the presence in various animals of adrenalin-secreting cells which are homologous to the adrenal medulla of mammals may be inferred. Thus the existence of the chromaffin cells in the kidney of certain teleosts was confirmed by Giacomini.

Based upon their optical microscopic observations on the adrenal gland with 15

species of teleosts, Oguri and Hibiya (1957 a, b) briefly reported an interesting fact that the chromaffin cells of rainbow trout differed from those of other species studied in projecting into the vessel of veins of the head kidney. The chromaffin cells projecting into the venous vessel was also found by the author in the present observation, as already similarly shown by Oguri and Hibiya. It is of paticular interest that a similar disposition of the chromaffin cells was recognized by Krause in the Cyclostomata. In rainbow trout these cells are disposed in 1 or 2 rows around the venous wall or developed into a layer of 3 or 4 rows of cells to project into the vessel of veins.

With respect to the arrangement of the medullary and cortical cells of the head kidney, the result of the author's observations perfectly agrees with that obtained by Oguri and Hibiya, that is, in rainbow trout the medullary cells and the cortical cells are by no means in juxtaposition, always occurring as two discrete cell groups.

In teleosts, the acidophile cells of the head kidney have been taken for the homologue to the adrenal cortex of mammals since Giacomini. Nevertheless, the homology between the adrenal cortex and the acidophile cells is nothing but a presumptive one through cell morphology, and there is as yet no histochemical evidence to support the view, in contrast with the histochemically well-established homology existing between the adrenal medulla and the chromaffin cells. On the other hand, the medulla and the cortex of the adrenal are the two cell groups of different embryological origin, and are known to be definitely separated in certain primitive vertebrates, whereas in higher vertebrates they join together to constitute a single organ such as seen in mammals. Taking these points into account, the author is inclined to support the opinion that the adrenal cortex of mammals is homologous to the acidophile cells which are juxtaposed or intermingle with the medullary chromaffin cells in the head kidney of teleosts.

As for the structure of the acidophile cells—cortical cells—in the head kidney of rainbow trout and other teleosts as well, there are no available works except for those by Oguri and Hibiya above referred to. Meanwhile, the cells of bullfrog have been described in detail by Fujimura et al., who claimed that the cell containing numerous acidophile granules was the so-called summer cell, which was once regarded by Naruse (1938) as belonging to the adrenal cortex. Yoshimura (1951, 1952) reasoned out that the acidophile granules in the summer cell might have been synthesized in the cytoplasm in consequence of a mitochondrial function.

The acidophile cells, as observed by the author in rainbow trout, are present in 2 to 5 rows surrounding the veins, and are more strongly developed than the chromaffin cells mentioned above. They may be found on almost entire surfaces of the veins in the head kidney.

RESULT OF ELECTRON MICROSCOPIC OBSERVATIONS AND THE DISCUSSION ON THE SUBJECT

Medullary Cells:

Throughout the cytoplasm of the medullary cells are recognized a lot of small granules measuring 30 to 60 mμ in diameter and of larger structure ranging from 120 to 250 mμ in diameter. The granule is circular or oblong in profile, and its limiting membrane is clear, whereas the larger structure contains an aggregation of small particles. The mitochondria are oblong or rod-like in profile, and smaller than those of the cortical cells in both number and size. As to the mitochondrial inner membrane two types are recognizable in structure: one is cristae and the other is vesicular in profile. Some mitochondria are observed to contain but an aggregation of dense particles. A few vacuoles as well as the rather clear Golgi apparatus are found in the cytoplasm. In the nucleus the chromatin is nearly evenly distributed, except for a partial granular agglomeration shown in some cases.

Cortical Cells:

In the cytoplasm are seen numerous vacuoles, which give, when closely packed, the appearance of a honeycomb. The vacuole is irregular in shape and surrounded by a clear limiting membrane, which is lower in density than the mitochondrial limiting membrane. The mitochondria are large and abundant as compared with those in the medullary cells. They are oblong or irregular in profile, having a clear limiting membrane. Their inner membrane appears as a vesicle, and are not detected the cristae ones which are generally seen in the medullary cells. The matrix of the mitochondria exhibits rather a high density. No particular structures are perceptible in the nucleus.

Apart from the mitochondria and the vacuoles the dense granules are also contained in the cytoplasm of the cortical cells. The granule, in which the density is much higher than that in other particles contained in the cells, possesses a double limiting membrane, and is oblong in profile and smaller in size than the mitochondrion, though rather large as compared with the minute granule in the medullary cells. The interior thereof is not always homogeneous, but shows aggregation of tiny particles.

Discussion:

The dense granules staining positively with osmium tetroxide have been observed in the cells of the adrenal medulla of rat by Lever, mouse and guinea-pig by Wetzstein, bull-frog by Fujimura et al., domestic fowl by Fujita et al., domestic duck by Hirata, and toad by Kamizono, and have been regarded as adrenalin granules.

In the author's observations on rainbow trout, similar dense granules are found in abundance in the cytoplasm. They are assumed to be the same cytoplasmic

granules that are well stained with chromic salt in the optical microscopic preparations. This assumption would be supported by the histological disposition of the cells that they occur on the inner wall of veins.

The fact that the mitochondria of the medullary cells are smaller in size than those of the cortical cells has already been noticed by Wetzstein, Hirata, Kamizono etc. Of the two types in structure of the mitochondrial inner membrane, the one assuming a vesicular profile may probably be tubular in its tri-dimentional structure. With reference to this, it should be pointed out that the mitochondria in a "pipe-shaped" internal structure have been found abundant in the adrenal medulla of toad by Kamizono. The dense particles found in the mitochondria seem to be vestiges of the inner membrane that mayhave degenerated.

The finding that in the cytoplasm of the cortical cells there are many vacuoles which appear to be a honeycomb when closely packed, is compatible with the description for toad by Kamizono; this fact serves as a criterion for distinguishing the cortical cells from other cells. Arima (1960) and Lever et al. mentioned the vacuoles in the cortical cells to be the reduced mitochondria, and Kamizono expressed the same opinion. The result obtained by the present author would also support the view of vacuolization of the mitochondria.

In respect of the mitochondria in the cortical cells the result of the author's observation is consistent with Lever's description that they are extremely large and their inner membrane is of tubular shape. Fujimura et al. regarded the bullfrog summer cell which contained numerous acidophile granules as belonging to the adrenal cortex, and the cytoplasmic dense granule in the cell as being the correspondant to the acidophile granule found in the optical microscopic picture. Also in the author's observations the dense granule with clear limiting membrane is found in the cytoplasm of the cortical cells, although no such zonal structure in the granule as was observed by Watanabe (1956) and Sugioka (1958) in eosinophile leucocytes is not recognized, as stated by Kamizono. Consequently it is possible that the acidophile granules of the cortical cells may be different from those of the leucocytes of rainbow trout.

SUMMARY AND CONCLUSION

With both the optical and the electron microscopes, histological observations were made on the head kidney of rainbow trout, *Salmo irideus* Gibbons, which belongs to the Teleostei, from the viewpoint of regarding the head kidney as the adrenal gland. The results obtained may be summarized as follows:

1) In the optical microscopic picture, the head kidney of rainbow trout is shown to consist of a wholly lymphoid tissue, with the chromaffin-positive and the eosinophile cells occurring around the veins. It seem probable that the former cells re-

present the medullary cells, and the latter the cortical cells of the adrenal tissue.

2) The medullary cells are observed in 1 to 4 rows forming a layer on the inner wall of the veins, and partially in a cluster projecting into the vessel of the veins. The cortical cells never intermingle with the medullary cells, constituting a well-developed group of cells surrounding the veins.

3) In the electron microscopic examination, the medullary cells proved to contain in the cytoplasm a lot of minute granules which deeply stain with osmium tetroxide solution, and are presumed to be the adrenalin granules. The mitochondria are not abundant, having the inner membrane of cristae-type or of vesicle-type. There occurs another kind of mitochondria which are devoid of the inner membrane but contain minute dense granules.

4) Throughout the cytoplasm of the cortical cells are found lots of vacuoles and rather large dense granules. The granule possesses a clear limiting membrane, and may be regarded as acidophile granule. The mitochondria occur among the vacuoles, and are large in both size and number, and the inner membrane of the mitochondria is suggestive of a tubular construction.

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EXPLANATION OF PLATES

PLATE 1

Fig. 2. Photomicrograph of medullary cells, or chromaffin cells (C), projecting into the vessel of veins. Stained with Mayer's acid hemalum and eosin after fixation in Orth's fluid.

Fig. 3. Photomicrograph of cortical cells, or acidophile cells (A), occurring around veins. Stained with Mayer's acid hemalum and eosin after fixation in Orth's fluid.

PLATE 2

Fig. 4. Electron micrograph of medullary cells. Magnification 17,000.

Fig. 5. Electron micrograph of cortical cells. Magnification 10,000.

PLATE 3

Fig. 6. Medullary cells. In the cytoplasm are seen the minute granules and the larger structures. Magnification 24,000.

PLATE 4

Fig. 7. Medullary cells. The cytoplasm contains the mitochondria with the inner membrane of vesicle-type. Magnification 33,000.

PLATE 5

Fig. 8. Cortical cells. In the cytoplasm are seen the vacuoles and the large-sized mitochondria exhibiting a vesicular profile in internal structure. Magnification 24,000.

PLATE 1

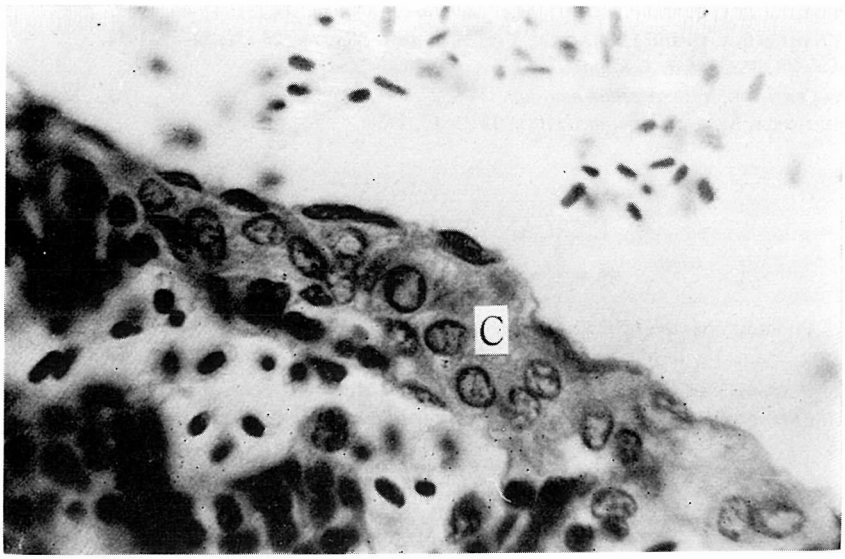

Fig. 2

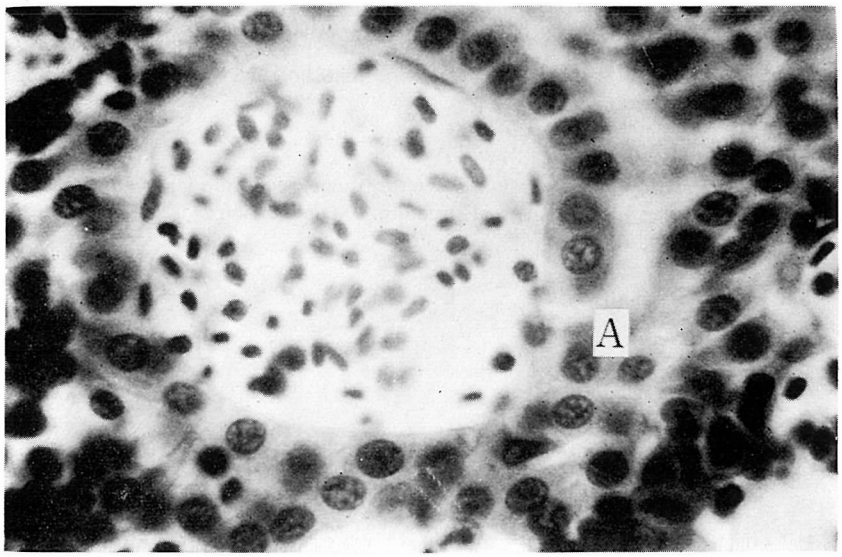

Fig. 3

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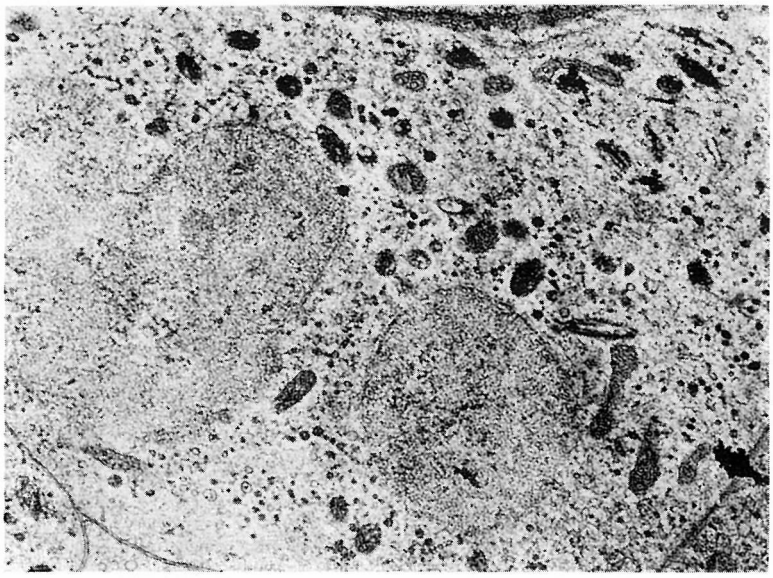

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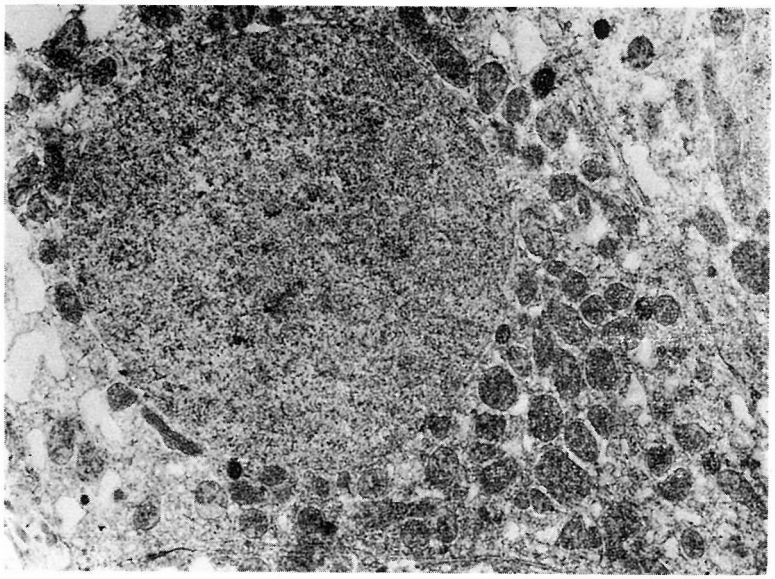

Fig. 5

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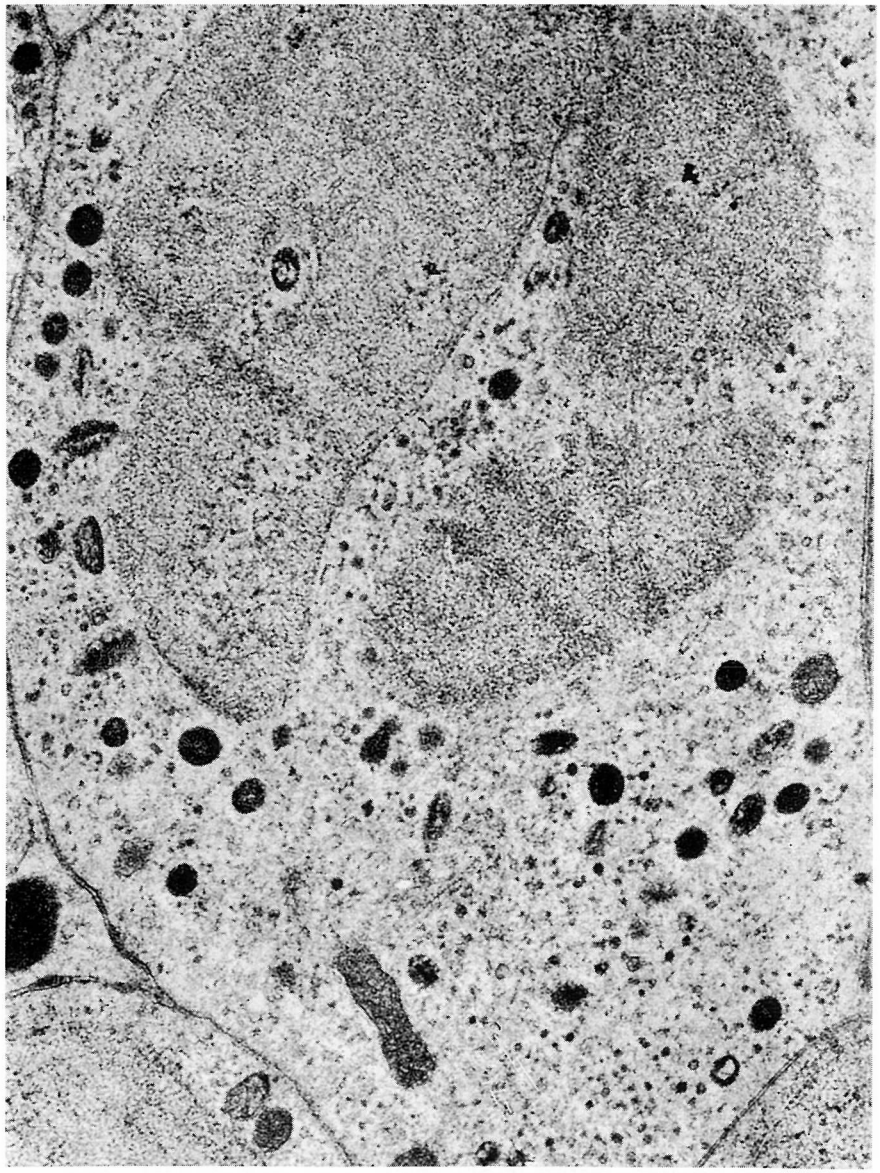

Fig. 6

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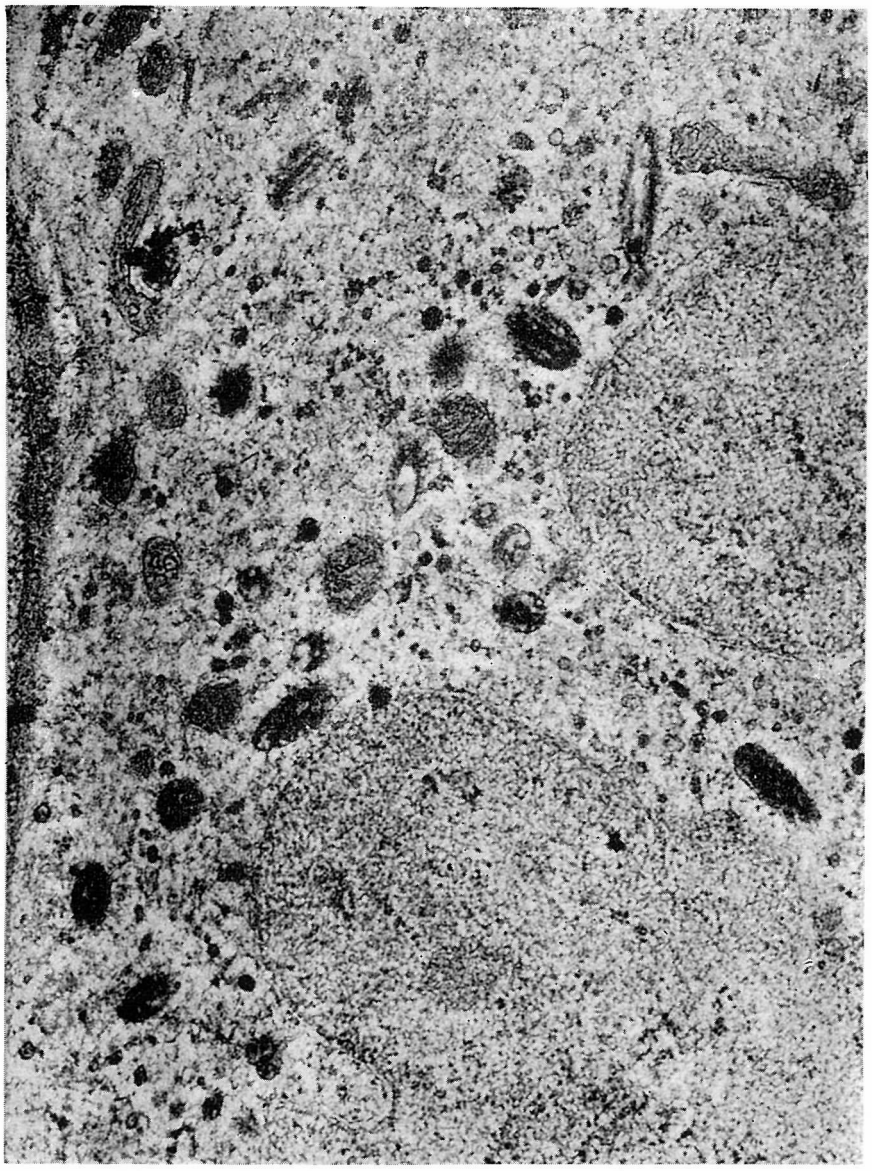

Fig. 7

PLATE 5

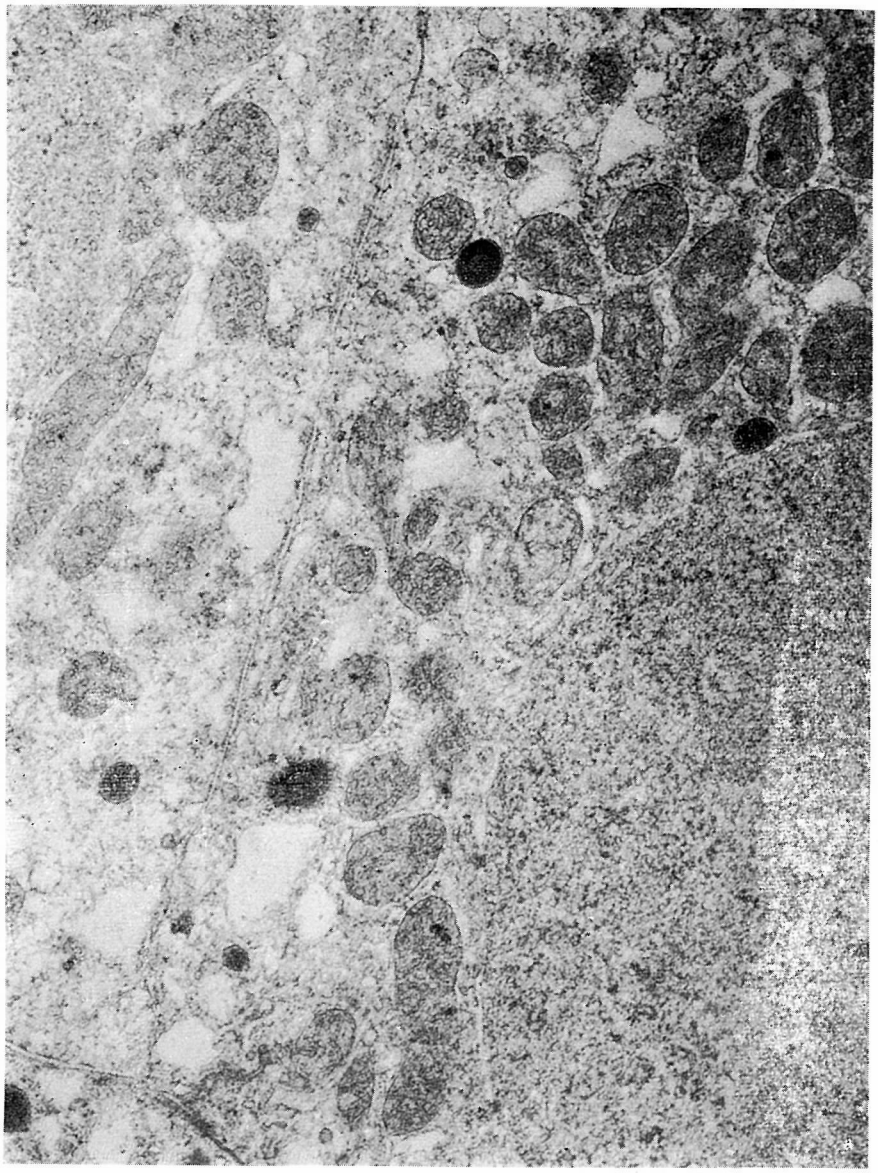

Fig. 8