

Histological Studies in the Adrenal Gland of Teleosts

Part II. Optical and Electron Microscopic Observations on the Adrenal Tissue of Carp

Nobuhisa WAKISAKA

From the Second Department of Anatomy,

(Head: Prof. G. Jinbo)

Yamaguchi Medical School, Ube, Japan

(Received April 28, 1963)

INTRODUCTION

In the preceding work of this series, an investigation was done by comparing critically the result obtained by the author from his optical and electron microscopic examinations in the structure of the chromaffin and acidophile cells in the head kidney of rainbow trout, *Salmo irideus* Gibbons, with the results of previous investigations on the adrenal cells of other various vertebrates by Lever (1955 a, b, 1956 a), Belt (1956, 1957), Sjöstrand and Wetzstein (1956), Wetzstein (1957), Leut and Hechter (1957), Kano (1959), Fujita et al. (1959), Fujimura et al. (1959), Hirata (1961) and Kamizono (1961). The comparison was carried out on the ground that the chromaffin and acidophile cells of teleosts should be respectively homologous to the adrenal medulla and cortex of mammals, which had been previously investigated by Henle (1865), Ogata and Ogata (1923), Verne (1923), Gerard (1930), Lison (1953), and Oguri and Hibiya (1957 a, b).

As regards the arrangement of the adrenal medulla (chromaffin cells) and the cortex (acidophile cells) in the head kidney of teleosts six types in pattern of disposition were distinguished by Oguri and Hibiya, who found that the rainbow trout was peculiar in respect of the medullary cells occurring free from the cortical cells, instead of being in juxtaposition or intermingling with the latter cells as in carp, crucian, loach etc.

In the present article, based upon the optical and electron microscopic observations on the adrenal cells of carp which is known, as mentioned above, to possess the tissue different in structure from that of rainbow trout, are dealt with the result of critical comparison in cellular structure of the adrenal between carp and rainbow trout as well as other vertebrates.

MATERIAL AND METHOD

The carp used for study belongs to *Cyprinus carpio* Linnaeus, which has been

bred for about one year after incubation at the Ino Sewage Plant of Ube, and averages 20 cm in body-length. In the carp the kidney is situated on the dorsal

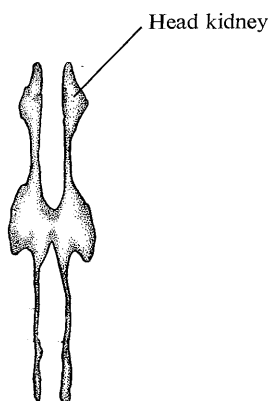


Fig. 1. Ventral view of the Kidney of *Cyprinus carpio*

wall of the abdominal cavity, and is an unpaired rufous body forked anteriorly as well as posteriorly, with the surface frequently covered in parts with fat body. The head kidney is not definitely distinguishable from the body kidney, and the cephalic part was used for observation. The kidney is illustrated in Fig. 1.

For optical microscopy the tissues were fixed in 10 per cent formalin or Orth's fluid, and then imbedded in paraffin for serial sections, which were stained with Mayer's acid hemalum and eosin.

Tissues for electron microscopy were imbedded in polystyren-resin as in the previous study, and the ultrathin sections thus made were stained with potassium permanganate for observation, during which photomicrographs were taken as desired.

RESULT OF OPTICAL MICROSCOPIC OBSERVATIONS AND THE DISCUSSION ON THE SUBJECT

General Structure:

According to the optical microscopic observation, the head kidney of carp consists of a wholly lymphoid tissue, in which no renal structures such as uriniferous tubules are found.

Around the veins in the head kidney there occur the deeply eosin-stained, stratified epithelial cells, with which the chromaffin cells intermingle in the specimens fixed in Orth's fluid. These two kinds of cells are regarded respectively homologous to the medullary and the cortical cells of the adrenal of mammals.

Medullary Cells:

The cells contain a large-sized oblong nucleus, with the cytoplasm stained brown in Orth's fluid fixation followed by staining with Mayer's acid hemalum. Such a staining reaction is not observed in the specimens fixed in 10 per cent formalin.

Cortical Cells:

The cells are subequal in size to the medullary cells just described and contain an oblong nucleus. The cytoplasm is well stained with eosin when fixed either in 10 per cent formalin or in Orth's fluid before staining with Mayer's acid hemalum and eosin, demonstrating itself to be acidophilous in cytochemical nature.

Discussion :

Judging from the result of the optical microscopic observations, which reveal the head kidney of carp to be a lymphoid tissue without renal tubules or glomeruli, it seems probable that the head kidney may have originated, as in rainbow trout, from the pronephors that had lost the renal function as the body kidney had become functional in its turn.

As already mentioned in the previous paper, the medullary cells of the adrenal of mammals are chromaffin, and there exist the cells showing identical reaction with those of certain teleosts. This is also the case with carp, in which are found the cells exhibiting the reaction when fixed in Orth's fluid containing potassium bichromate. Notwithstanding, the cells decidedly differ from those of rainbow trout in the arrangement in the head kidney: The medullary cells, which in rainbow trout were found projecting into the vessel from the inner wall of the veins in the head kidney, are scattered individually or in small clusters among the cortical cells around the veins in the case of carp. In spite of being so disposed, however, two kinds of cells may be quite readily distinguishable by the difference in staining reaction. Similar pattern of distribution of the chromaffin cells in the head kidney is found, according to Oguri and Hibiya, also in such species of teleosts as *Carassius auratus*, *Misgurnus anguillicaudatus*, *Channa argus*, *Plotosus anguillar* etc.

Considering that the adrenal medulla and cortex are united together to constitute a single organ in the case of vertebrates higher than the Amphibia inclusive, it does not seem unaccountable that the chromaffin and acidophile cells intermingle in the head kidney of certain teleosts. Thus an interesting problem for further study is suggested by this finding combined with the fact that the chromaffin and acidophile cells are developed into two separate cell groups in rainbow trout which belongs to the same taxon Teleostei as does carp.

RESULT OF ELECTRON MICROSCOPIC OBSERVATIONS AND THE DISCUSSION ON THE SUBJECT

General Structure:

In the electron microscopic picture, the adrenal tissue of carp is shown to be differentiated into two kinds of cells: the darker cells with the cytoplasm containing the numerous granules and the lighter cells with the cytoplasm rich in vacuoles. The former cells may be identical with the adrenal medullary cells, and the latter with the cortical cells, both encountered in the optical microscopic picture.

Medullary Cells:

The nucleus of the medullary cells possesses a double nuclear membrane scatter-

ing nuclear membrane pores; the karyoplasm consists almost homogeneously of minute particles; the nucleolus is recognizable as particle darker than the karyoplasm. In the cytoplasm are found in abundance the circular or oblong dense granules of 30 to 100 m μ in diameter. In addition, there occur larger, oblong or irregular-shaped granules, which range from 200 to 400 m μ in diameter, exhibit a high density and contain granular particles of 40 m μ in diameter. The mitochondrion possesses a clear double limiting membrane, and is rod-like or oblong in profile, with the inner membrane of cristae-type. Aside from these components there exist the rough-surfaced varieties of endoplasmic reticulum on which Palade's granules are attached. They are of flattened construction measuring ca. 50 m μ in thickness.

Cortical Cells:

The nucleus of the adrenal cortical cells possesses a double nuclear membrane with distinct nuclear membrane pores; the karyoplasm is seen to consist almost homogeneously of minute particles. Throughout the cytoplasm are found numerous vacuoles, which are large, reaching 100 to 800 m μ in diameter, and assume a honeycomb in appearance when closely packed. The vacuole is surrounded by a dense limiting membrane, and is seen in some cases to comprise a granular construction. The mitochondria, which are circular or oblong in profile, occur among the vacuoles with the inner membrane of cristae-type. In the Golgi apparatus the Golgi vesicles as well as the Golgi membrane are recognized.

Discussion:

On the subject of the electron microscopic studies on the structure of the tissues of vertebrates, mammals, birds and amphibians have been the objects of many works, while the teleosts have attracted little attention. Kimura (1961) and Matsuura (1961) made their observations on various tissues of *Parasilurus asotus* and *Sebastiscus marmoratus*, respectively, but mentioned nothing of the adrenal of these species.

The occurrence of the adrenalin granules in the adrenal medullary cells was observed with the mammals by Lever and Wetzstein, further with the birds and amphibians as well by Kano, Fujita et al., Hirata, Fujimura et al., Kamizono etc. The present author reported in the preceding article the presence of similar granules in rainbow trout, and herein-before in carp also. The granules are assumed to be identical with the chromaffin-positive granules staining deeply with chromic salts in the optical microscopic preparations.

The mitochondria of the medullary cells possess the inner membrane of cristae-type, and there is no such inner membrane assuming a vesicular profile or tubular construction, as was found in rainbow trout. The large-sized granules which are found subequal in size to the mitochondria and containing a minute granular con-

struction seem to correspond to the degenerated mitochondria in rainbow trout, though it is not clear why they exhibit a much higher density.

The fact that the adrenal cortical cells contain numerous vacuoles which, when closely packed, take the appearance of honeycomb is in agreement with the findings for rainbow trout as well as with those for toad by Kamizono. On account of their clear limiting membrane and of the internal granular construction, these vacuoles found in abundance are assumed to be the vestiges of the mitochondria. The mitochondria are circular or rod-like in profile; in respect of having exclusively the inner membrane of cristae-type they differ from those in rainbow trout which demonstrate therein a vesicular profile suggestive of the tubular-shaped inner membrane in tri-dimensional structure. The tubular construction of the inner mitochondrial membrane in the adrenal cortical cells was reported by Lever (1956 b) and Belt and Pease (1956); it is interesting that the inner mitochondrial membrane is tubular in rainbow trout whereas it is of cristae-type in carp.

In rainbow trout there are seemingly acidophile granules in the adrenal cortical cells, while no such granules are found in carp. Still in carp abundant vesicular granules are recognizable throughout the cytoplasm.

SUMMARY AND CONCLUSION

Observations were made on the chromaffin and acidophile cells in the head kidney of a carp, *Cyprinus carpio* Linnaeus, on the basis that the former cells may be homologue to the medullary cells and the latter to the cortical cells of the adrenal of mammals. The findings were examined in comparison with the results obtained from the author's previous observations on the adrenal gland of rainbow trout, *Salmo irideus* Gibbons, and from other investigator's studies on the tissue of various vertebrates. Conclusions may be summarized as follows:

- 1) The head kidney of the carp, according to the optical microscopic observation, consists of a wholly lymphoid tissue, as in other teleosts including rainbow trout.

- 2) The medullary cells of the adrenal occur as chromaffin cells intermingling with the acidophile cells which are present around the veins in the head kidney. The pattern of their disposition is quite different from that of rainbow trout.

- 3) In the electron microscopic picture, the medullary cells are recognized to contain in the cytoplasm minute granules staining deeply with osmium tetroxide solution and measuring 50 to 100 $m\mu$ in diameter. It seems probable that the granules may represent the adrenalin granules. The inner membrane of mitochondria is of cristae-type. The cytoplasm contains dense granules subequal in size to the mitochondria.

- 4) Throughout the cytoplasm of the adrenal cortical cells, there are abundant vacuoles varying from 100 to 800 $m\mu$ in diameter; in the vacuole a minute granular

construction showing a high density is recognized. The inner membrane of mitochondria is of cristae-type, never exhibiting a vesicular profile such as is found in rainbow trout.

REFERENCES

- 1) WAKISAKA, N.: *Bull. Yamaguchi Med. Sch.*, **10**: 99-112, 1964.
- 2) LEVER, J. D.: *Endocr.* **57**: 621-635, 1955 a.
- 3) LEVER, J. D.: *Amer. J. Anat.* **97**: 409-429, 1955 b.
- 4) LEVER, J. D.: *Endocr.* **58**: 163-180, 1956 a.
- 5) BELT, W. D.: *Anat. Rec.* **124**: 249-500, 1956.
- 6) BELT, W. D.: *Anat. Rec.* **127**: 264, 1957.
- 7) SJÖSTRAND, F. S. u. WETZSTEIN, R.: *Experientia* (Basel) **12**: 196-199, 1956.
- 8) WETZSTEIN, R.: *Z. Zellforsch.* **46**: 517-576, 1957.
- 9) LEUT, J. and HECHTER, O.: *J. biophys. biochem. Cytol.* **3**: 615-620, 1957.
- 10) KANO, M.: *Arch. histol. jap.* **18**: 25-56, 1956.
- 11) FUJITA, T., KANO, M., KIDO, T. and KUNISHIMA, I.: *Acta anat. Nippon.* **34**: 41-42, 1959.
- 12) FUJIMURA, W., NISHIKI, T., UEYAMA, S., KOISHI, T. and MORI, K.: *J. Nara med. Ass.* **10**: 203-209, 1959.
- 13) HIRATA, M.: *Acta med.* (Japan) **31**: 109-123, 1960.
- 14) KAMIZONO, N.: *Med. J. Kagoshima Univ.* **13**: 77-95, 1961.
- 15) HENLE, J.: *Z. ration. Med.* **24**: 143, 1865.
- 16) OGATA, T. and OGATA, A.: *J. exp. Med.* **25**: 807, 1917.
- 17) VRNE, J.: *Bull. Soc. chim. biol.* **5**: 227, 1923.
- 18) GERALD, P., CORDIER, R. et LISON, L.: *Bull. histol. Appl.* **7**: 133, 1930.
- 19) LISON, L.: *Histochimie et Cytochimie Animales*. 2 ed., 1953, Gauthier-Villara, Paris.
- 20) OGURI, M. and HIBIYA, T.: *Bull. Jap. Soc. Sci. Fis.* **22**: 621-625, 1957 a.
- 21) OGURI, M. and HIBIYA, T.: *Bull. Jap. Soc. Sci. Fis.* **23**: 144-149, 1957 b.
- 22) KIMURA, K.: *Bull. exp. biol.* **11**: 67-71, 1961.
- 23) MATSUURA, F.: *Bull. exp. biol.* **11**: 129-139, 1961.
- 24) LEVER, J. D.: *J. biophys. biochem. Cytol.* **2** Suppl.: 313, 1956 b.
- 25) BELT, W. D. and PEASE, D. C.: *J. biophys. biochem. Cytol.* **2** Suppl.: 369, 1956.

EXPLANATION OF PLATES

PLATE 1

Fig. 2. Electron micrograph of medullary cells. In the cytoplasm are seen the minute granules, the mitochondria with the inner membrane of cristae-type, and the larger structures. Magnification 30,000.

PLATE 2

Fig. 3. Medullary cells. In the cytoplasm are seen the rough-surfaced varieties of endoplasmic reticulum on which Palade's granules are attached. Magnification 30,000.

PLATE 3

Fig. 4. Cortical cells. The cytoplasm contains the numerous vacuoles and the mitochondria with the inner membrane of cristae-type. Magnification 10,000.

PLATE 4

Fig. 5. Medullary and cortical cells of adrenal.

PLATE 1

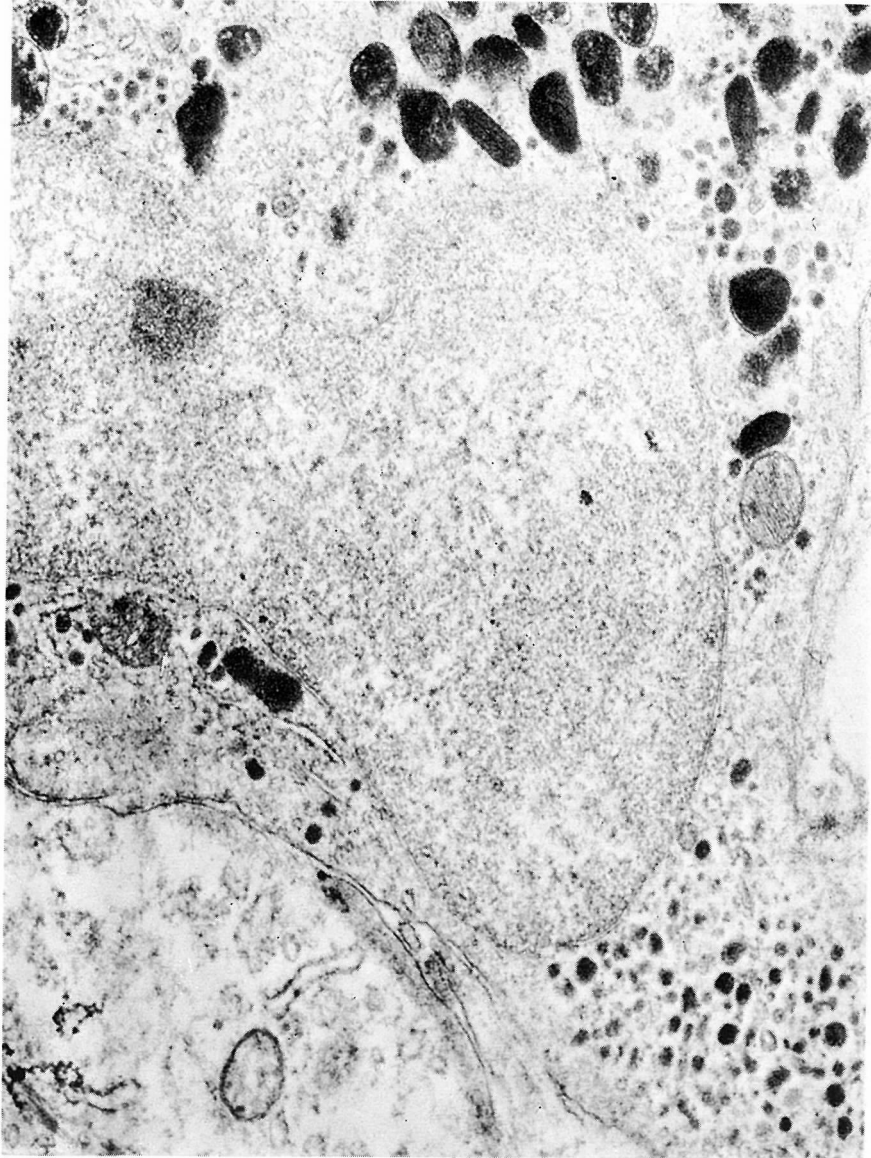


Fig. 2

PLATE 2

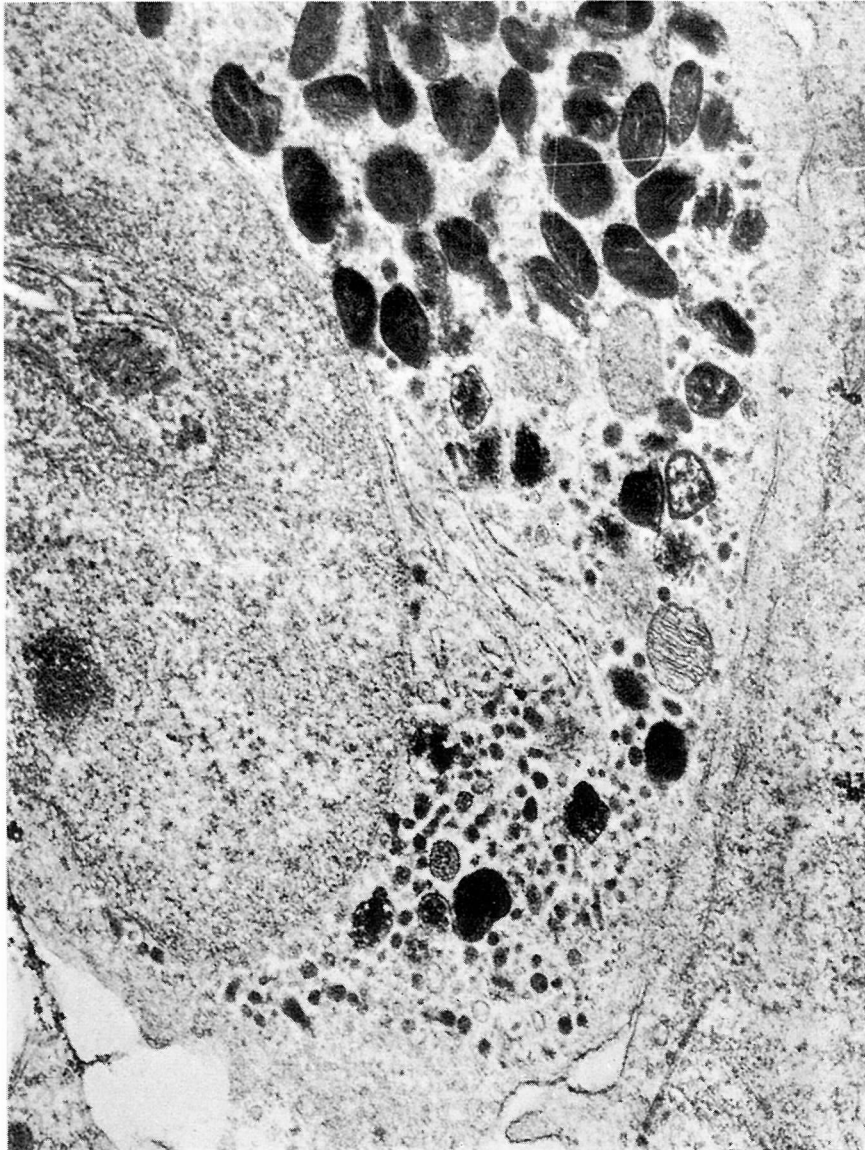


Fig. 3

PLATE 3

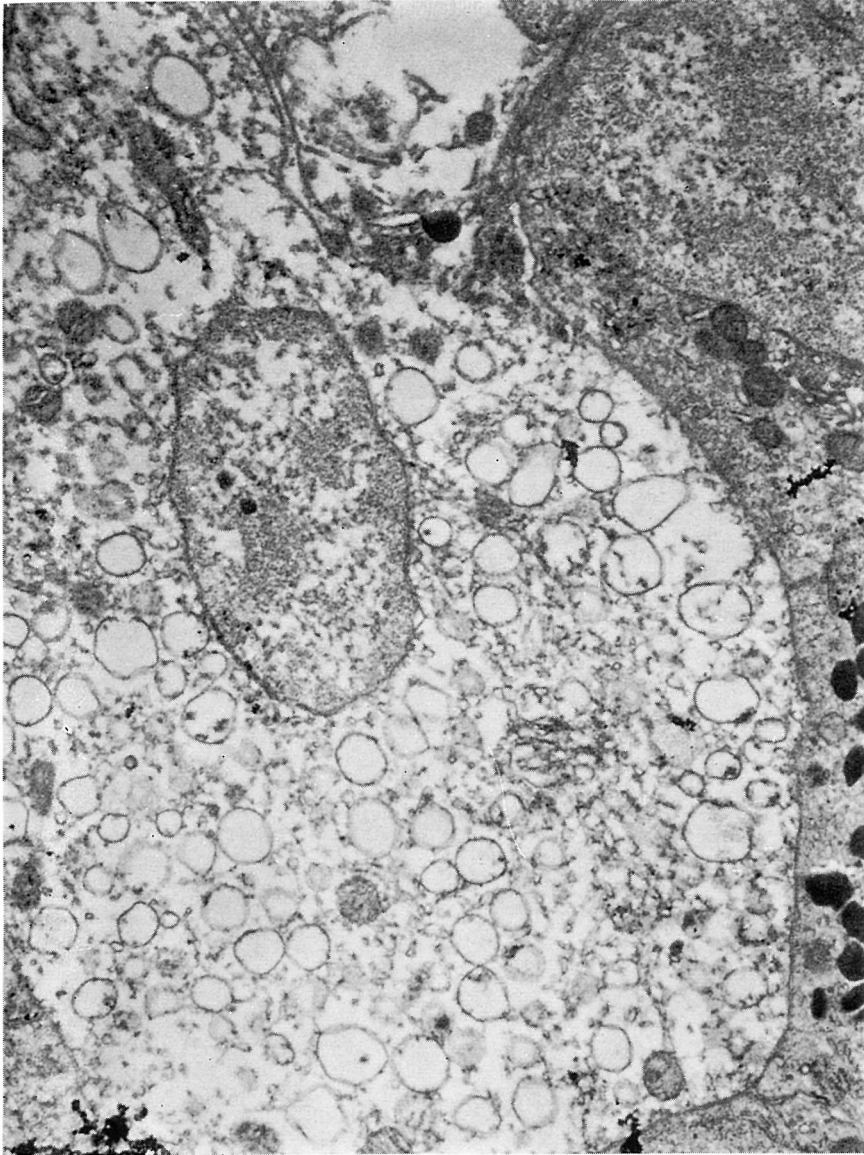


Fig. 4

PLATE 4

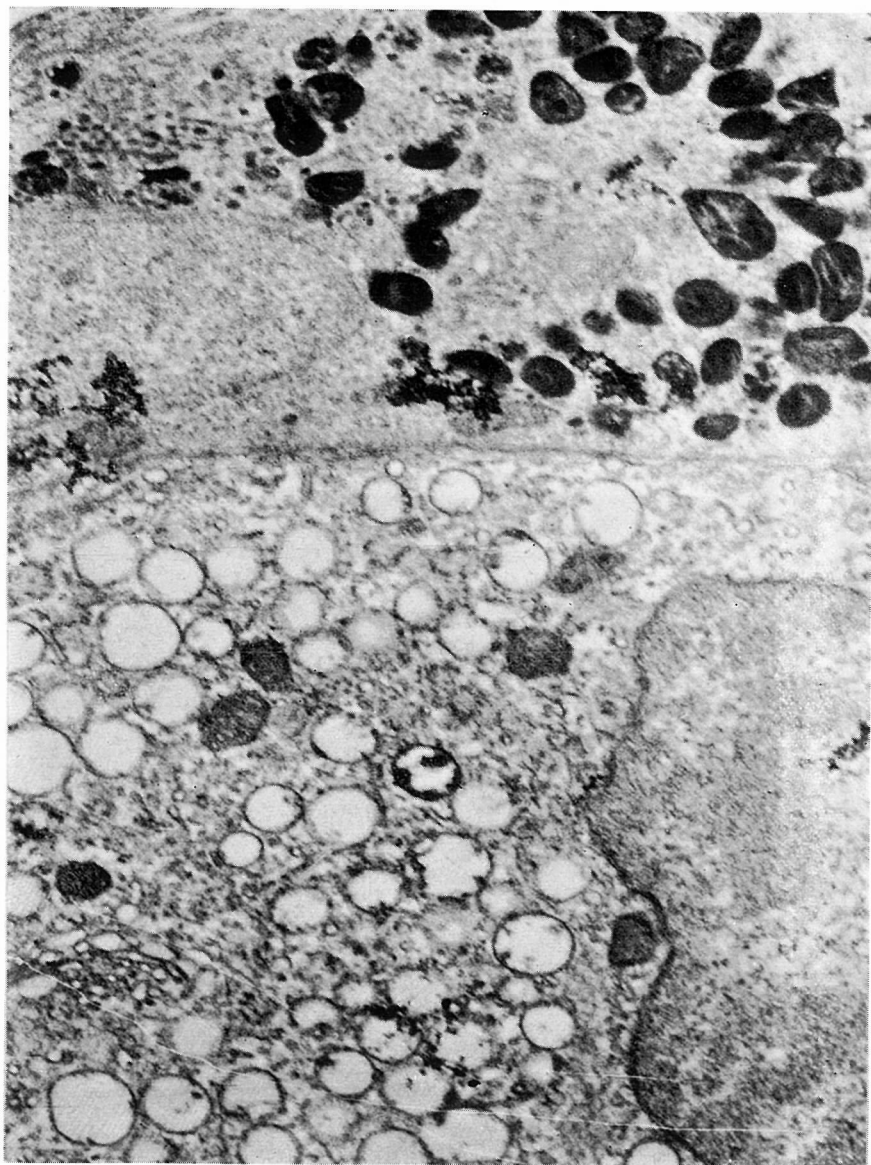


Fig. 5