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دراسات على تأثير ميثا سركاريا الدودة الورقية ايوكلينوستوم ارد يولى على كلية سمكة البلطي النيلي

عزت يواقيم ، محمد النفار

يتضمن هذا البحث دراسات هستولوجية وهستوباثولوجية وهستوكيميائية على كلية سمكة البلطي النيلي المصابة بميثا سركاريا الدودة الورقية ايوكلينوستوم ارد يولى وقد وجد أن جميع مكونات الكلية المصابة ولا سيما في حالة الاصابات الشديدة - قد تأثرت بدرجة واضحة عند مقارنتها بمثلتها من الكليات غير المصابة وتشمل هذه التغيرات مايلي :

- احتقان الجُمع وتهتكه في بعض الاحيان وتمزق محفظة بومان .
- ظهور الانتفاخ السحابي (Gloudy_ swelling) وفجوات متنوعة الاشكال وتهتك الاطراف الحرة لمعظم خلايا الانيبويات .
- يزداد سمك المحفظة الليفية الغراونيه التي تحيط بالطفيل ، كذلك تزداد كمية النسيج الضام بين الانيبويات بزيادة شدة الاصابة .
- تظهر في النسيج الضام بين الانيبويات خلايا التهابية وصيغ بني صدئي وتزداد هذه المكونات بزيادة شدة الاصابة .
- في حالة الاصابة الشديدة توجد في النسيج الضام بين الانيبويات خراجات محاطة بخلايا التهابيه وكرات دمويه حمراء .
- تقل محتويات الكليه من المواد الكربوهيدارتيه والمواد البروتينية بدرجة ملحوظة بينما يكون الطفيل غنيا بهذه المكونات .

بسم الله الرحمن الرحيم
الحمد لله رب العالمين
والصلاة والسلام على سيدنا محمد وآله

والله اعلم بالصواب
فمنعنا من الله ما نريد

هذا ما كتبه في يوم الجمعة
العاشر من شهر ربيع الثاني
سنة ١٢٤٥ هـ الموافق
للعاشور من سنة ١٩٢٤ م
في مدينة مكة المكرمة

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**STUDIES ON THE EFFECTS OF METACERCARIA OF THE PARASITIC
TREMATODE EUCLINOSTOMUM ARDEOLAE ON THE KIDNEYS OF
THE NILE CICHLID FISH TILAPIA NILOTICA**
(With 4 Plates)

By
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SUMMARY

This investigation is mainly concerned with the histopathological effects of the metacercaria of the parasitic trematode Euclinostomum ardeolae (EL-NAFFAR and KHALIFA, in press) on the kidney of the Nile cichlid fish Tilapia nilotica. Fifteen fishes, including normal, slightly infected and heavily infected ones were investigated. The glomeruli, proximal and distal convoluted tubules, collecting tubules and intertubular connective tissue displayed clear signs of degeneration, the extent of which depends on the intensity of infection.

INTRODUCTION

Studies on the histopathological effects of the parasitic adult and larval stages of trematodes on the kidneys of submammalian vertebrates are rather scanty. Only few reports are available in this connection including those of TIMON-DAVID (1933 and 1952), WRIGHT (1956), RILEY and OWEN (1972) and EL-NAFFAR et al. (1977).

RECENTLY, EL-NAFFAR and KHALIFA (in press) reported that the metacercariae of the trematode parasite Euclinostomum ardeolae sp. nov. were found to be encysted in the kidneys of the Nile cichlid fish, Tilapia nilotica. A maximum of five metacercariae per one kidney has been recorded. In the present investigation it is intended to give an account on the histopathological effects of the aforementioned metacercaria on the kidneys of Tilapia nilotica which is one of the most important economic fish species of the Nile.

MATERIALS and METHODS

Live specimens of Tilapia nilotica were collected from the Nile River at Assiut governorate during the period March to June, 1977. The kidneys were dissected out in physiological saline solution and the number of metacercariae, if present, was recorded for each infected fish. The latter were classified into two categories according to the number of metacercariae encountered in their kidneys: slight (1 - 2 metacercariae) and heavy (3 - 5 metacercariae). The kidneys of five specimens of each category and those of five normal specimens were cut into representative pieces and fixed in Bouin's Zenker's formal, Carnoy's

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or acetic acid formalin fluids. Paraffin sections, 5 - 7 μ thick, were cut and stained in either Harris's haematoxylin and eosin or Mallary's triple stains.

PAS technique (McMANUS, 1948) was used for the demonstration of 1 : 2 glycol linkage of carbohydrates. Control slides were incubated in saliva at 37°C for one hour prior to PAS staining. Best's carmine was used for the detection of glycogen; control slides were treated in the same way as those in the PAS technique. Mercury bromophenol blue as recommended by MAZIA, BREWER and ALFERT (Pearse, 1960) was used for the detection of proteins.

RESULTS

1. Normal kidney

The kidney of *Tilapia nilotica* has no zones of cortex and medulla as in mammals. Rather it consists of renal units in no specific pattern. Each renal unit is composed of a glomerulus and a uriniferous tubule which is differentiated into the neck segment, proximal convoluted tubule and the distal convoluted tubule. The latter is connected with the collecting tubule. The intertubular connective tissue contains blood vessels and few collagenous fibres.

The glomerulus is well developed; it is made up of Bowman's capsule embracing a tuft of capillaries. The simple squamous cells of the Bowman's capsule blend directly with the low cuboidal cells lining the neck segment (Fig. 1). The proximal convoluted tubule is differentiated histologically into two segments. The first segment, which is of a larger diameter than the second, is composed of columnar cells the cytoplasm of which stains lightly with eosin and their oval nuclei are basally located. The second segment is formed of cuboidal cells having their cytoplasm more eosinophilic than that of the first segment and their nuclei are centrally located. The distal convoluted tubule is made up of high columnar cells which have faintly stained eosinophilic cytoplasm and oval basal nuclei (Fig. 2). The collecting tubule is lined by pseudostratified epithelium and it is surrounded by a rather thick layer of collagenous fibres (Fig. 3).

Histochemically, the glomerular tuft reveals a faint PAS - positive reaction, whereas the brush border of the proximal convoluted tubule shows a conspicuous PAS - positive reaction. No change in the intensity of PAS reaction has been revealed by control sections. Moreover, Best's carmine gives a negative reaction. These results indicate the presence of carbohydrate material other than glycogen.

All the components of the renal units react positively towards mercury bromophenol blue, indicating their protein content; however, the first segment of the proximal convoluted tubule is less stainable (Fig. 5).

2- The infected kidney

In slightly infected kidneys, the parasite is surrounded by a capsule of collagenous fibres, the average thickness of which is 36 μ (Fig. 6). Some glomeruli show signs of congestion; the glomerular tufts are dilated and reveal a large number of red blood corpuscles as compared with the normals. However, the Bowman's capsules are intact (Fig. 7).

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The cells of most proximal convoluted tubules, distal convoluted tubules and collecting tubules reveal signs of cloudy swelling. Thus, the cells are enlarged, the cytoplasm is granular and the nuclei are swollen and have few chromatin granules. The enlargement of the cells lining the proximal convoluted tubules usually results in a marked reduction or even obliteration of their lumina (Fig. 7).

As compared with the normal kidney, the intertubular connective tissue of the slightly infected kidney slightly increases and is infiltrated, especially near the fibrous capsule surrounding the parasite, by extravasated red blood corpuscles and inflammatory cells. The latter included granular leucocytes, lymphocytes and macrophages. Clusters of rusty brown particles are occasionally encountered in the intertubular connective tissue or intermingled with the collagenous fibres surrounding the collecting tubules. Such rusty brown particles are sometimes seen in the cytoplasm of macrophages (Figs. 7, 8 a & b).

In the heavily infected kidneys, the fibrous capsule surrounding the parasite increases markedly in thickness which measures on the average 61 μ . Congested glomeruli, as described in the slightly infected kidneys, are of frequent occurrence. Many glomeruli have ruptured Bowman's capsules and glomerular tufts (Fig. 9). Some glomeruli are greatly affected, thus their glomerular tufts shrink forming structureless deeply eosinophilic masses (Fig. 10).

Few proximal and distal convoluted tubules show hydropic degeneration; the cells are swollen and have faintly stained vacuolated cytoplasm and pyknotic nuclei. The cells of most proximal and distal convoluted tubules have highly vacuolated cytoplasm, ruptured luminal ends and pyknotic nuclei (Fig. 11). Occasionally, the cells of the proximal and distal convoluted tubules are totally detached from the basement membrane forming groups of scattered cells, or they may collapse to form compact masses of cells with pyknotic nuclei (Fig. 12). The aforementioned histopathological changes are also revealed by the collecting tubules (Figs. 13 a & b).

The intertubular connective tissue of the heavily infected kidney, as compared with that of the normal one, is markedly increased. Such a tissue reveals the presence of inflammatory cells, extravasated red blood corpuscles, clusters of brown particles similar to those described in that of the slightly infected kidney but in a more pronounced manner (Fig. 14). Moreover, the intertubular connective tissue contains several abscesses. Each abscess is formed of a cavity surrounded by numerous inflammatory cells and extravasated red blood corpuscles (Fig. 15).

As compared with the normal kidney, both the slightly and heavily infected kidneys have many of their glomerular tufts intensely stained with PAS reaction, whereas, the brush border of the proximal convoluted tubules is negatively stained with that reaction (Fig. 16). Best's carmine reveals the presence of glycogen in the intercellular substance of the parenchymatous tissue of the parasite and its absence in the renal tissue (Fig. 17). Mercury bromophenol blue shows that the protein content of the tubules is greatly diminished; however, the parasite is rich in protein (Fig. 18).

DISCUSSION

The kidney of *Tilapia nilotica* has been found to contain well developed vascularized glomeruli. This agrees with the generalization postulated by SOMAYAJULU and KAMAT (1972) that the glomeruli are well developed in fresh water fishes as there is a need for the elimination of water, whereas they are illdeveloped in marine fishes as there is a necessity for retention of water.

In Tilapia nilotica the uriniferous tubule is differentiated into, neck segment, proximal convoluted tubule and distal convoluted tubule. The neck segment lacks cilia; in this respect it resembles that of Xiphophorus helleri but differs from that of Danio malabaticus (EDWARDS, 1935). As in Ictalurus punctatus (KENDALL and HINTON, 1974), the proximal convoluted tubule in Tilapia nilotica is differentiated into two segments.

To the best of our knowledge, the histopathological effects of trematodes on the kidneys of fishes are lacking. Only few reports on the histopathologic effects of trematode parasites belonging to the genus Renicola on the kidneys of some birds are available. TIMON - DAVID (1933) stated that the kidneys of Larus argentatus infected with Renicola lari showed little or no host tissue reaction. Conspicuous renal lesions were reported by TIMON - DAVID (1952) in Pica pica infected with Renicola bretensis. WRIGHT (1956), noticed a complete lack of host tissue reaction in the kidneys of Puffinus puffinus infected with unnamed species of Renicola. RILEY & OWEN (1972) came to the conclusion that recently established Renicola glacialis in the kidneys of Fulmarus glacialis elicited only a slight host tissue response, but older infections were accompanied by pronounced eosinophilia and a thick connective tissue sheath investing the parasite. EL-NAFFAR *et al.* (1977) reported that the effects of Renicola ardeolae on the kidney tubules of the buff-backed heron, Ardeolae ibis include cloudy swelling, pyknosis of nuclei and necrosis.

Results of the present investigation have revealed that the metacercaria of the parasitic trematode, Euclinostomum ardeolae markedly affected the histological structure of the kidney of Tilapia nilotica, especially in the heavily infected specimens. The host surrounded the parasite by a capsule of collagenous fibres, the thickness of which was considerably increased in heavily infected specimens. The glomeruli were found to be either congested, ruptured or with shrunken deeply eosinophilic glomerular tufts. The cells of the kidney tubules showed signs of degeneration, high vacuolation of cytoplasm, pyknosis of nuclei and detachment from the basement membrane forming scattered groups or compact masses of cells. In heavily infected kidneys, the intertubular connective tissue was markedly increased and revealed the presence of inflammatory cells, extravasated red blood corpuscles, clusters of rusty brown particles and abscesses. The presence of extravasated red blood corpuscles were due to lesions in the blood vessels. The rusty brown particles were mostly haemosiderin resulting from destruction of haemoglobin of the red blood corpuscles. This was more justified by the fact that such particles were frequently seen phagocytosed by the macrophages.

As compared with the normal kidney, the tubules of the infected kidney showed a marked decrease in proteins and carbohydrates other than glycogen. On the other hand, the tissues of the parasite were found to be rich in proteins and carbohydrates including glycogen. These results indicate that the parasite has got ample supply of these vital compounds at the expense of the host.

REFERENCES

- Edwards, J.G. (1935): The epithelium of the renal tubule of bony fish. *Anat. Rec.*, 63: 263 - 279.
- El-Naffar, M.K.; Anwar, I.M. and Khalifa, R. (1977): Studies on the histopathology of Renicola ardeolae sp. nov. (Trematoda : Renicolinae). *J. Egypt Soc. Parasit.*, 7 (1): 35 - 44.
- El-Naffar, M.K. and Khalifa, R. (in press): Euclinostomum ardeolae sp. nov. (Trematoda : Clinostomatidae) with the description of its life cycle. *ibid.* (in press).

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- Kendall, M.W. and Hinton, D.E. (1974): Renal tubular morphology in the channel catfish, *Ictalurus punctatus* kidney. J. Fish. Res. Board Can., 31 (3): 346 - 347.
- McManus, J.F.A. (1948): Histological and histochemical uses of periodic acid. Stain Technol., 23: 99 - 108.
- Pearse, A. (1960): Histochemistry, Theoretical and Applied. Churchill, London.
- Riley, J. and Owen, R.W. (1972): *Renicola glacialis* sp. nov., a new trematode from the north sea Fulmar, *Fulmaris glacialis* (L.), with observations on its pathology. J. Helmenth., XLVI: 63 - 72.
- Somayajulu, R.S.N. and Kamat, D.N. (1972): Comparative histology of the kidney of fishes from diverse habitats. J. Anat. Soc. India, 21 (3): 113 - 116.
- Timon-David, J. (1933): Sur une nouvelle espece de *Renicola* trematode parasite du rein des larides. Bull. Inst. Oceanogr. Monaco, 616: 1 - 16.
- (1952): Une *Renicola* nouvelle chez la pie. bull. Soc. Zool. Fr., 77: 504 - 511.
- Wright, C.A. (1956): Studies on the life history and ecology of the trematode genus *Renicola* Cohn 1904. Proc. Zool. Lond., 126: 1 - 48.

LEGENDS

Plate I :

- Fig. (1):** T.S. of the normal kidney of *T.nilotica*, showing the structure of the glomerulus and the neck segment. X 475.
- Fig. (2):** T.S. of the normal kidney of *T.nilotica*, showing the structure of the proximal and distal convoluted tubules. X 230.
- Fig. (3):** T.S. of the normal kidney of *T.nilotica*, showing the structure of the collecting tubule. X 475.
- Fig. (4):** T.S. of the normal kidney of *T.nilotica*, showing the reaction of the constituents of the renal units towards the PAS reaction. X 120.
- Fig. (5):** T.S. of the normal kidney of *T.nilotica*, showing the reaction of the constituents of the renal units towards mercury bromophenol blue. X 190.

Plate II :

- Fig. (6):** T.S. of the slightly infected kidney of *T.nilotica* showing the parasite surrounded by a collagenous connective tissue capsule. X 43.
- Fig. (7):** T.S. of the slightly infected kidney of *T.nilotica*, showing congested glomeruli and cloudy swelling of the kidney tubules. X 175.
- Fig. (8 a):** T.S. of the slightly infected kidney of *T.nilotica* showing the intertubular connective tissue infiltrated by extravasated red blood corpuscles, inflammatory cells and clusters of rusty brown particles. X 425.
- Fig. (8 b):** T. S. of the slightly infected kidney of *T.nilotica* showing rusty brown particles phagocytosed by the macrophages. X 450.

Plate III :

- Fig. (9):** T.S. of the heavily infected kidney of T.nilotica, showing glomeruli with ruptured Bowman's capsule and glomerular tufts. X 380.
- Fig. (10):** T.S. of the heavily infected kidney of T.nilotica, showing a shrunken deeply eosinophilic glomerular tuft. X 380.
- Fig. (11):** T.S. of the heavily infected kidney of T.nilotica, showing hydropic degeneration, high vacuolation of the cytoplasm, pyknotic nuclei and ruptured luminal ends of the proximal and distal convoluted tubular cells. X 380.
- Fig. (12):** T.S. of the heavily infected kidney of T.nilotica, showing the proximal and distal convoluted cells are totally detached from the basement membrane forming groups of scattered cells. Some such tubules collapse to form compact masses of cells with pyknotic nuclei. X 380.
- Fig. (13 a & b):** Transverse sections of the heavily infected kidney of T.nilotica, showing the histopathological effects revealed by the collecting tubule. X 230 and 190 respectively.
- Fig. (14):** T.S. of the heavily infected kidney of T.nilotica, showing the marked increase of the intertubular connective tissue and its infiltration with extravasated red blood corpuscles, inflammatory cells and rusty brown particles. X 152.
- Fig. (15):** T.S. of the heavily infected kidney of T.nilotica, showing an abscess surrounded by inflammatory cells and red blood corpuscles. X 380.

Plate IV :

- Fig. (16):** T.S. of the slightly infected kidney of T.nilotica, showing the reaction of the glomeruli and proximal convoluted tubules towards the PAS reaction. X 380.
- Fig. (17):** T.S. of the slightly infected kidney of T.nilotica, showing the reaction of the parasite and the renal tissue towards Best's carmine. X 152.
- Fig. (18):** T.S. of the slightly infected kidney of T.nilotica, showing the reaction of the parasite and the kidney tubules towards bromophenol blue. X 95.

PLATE I

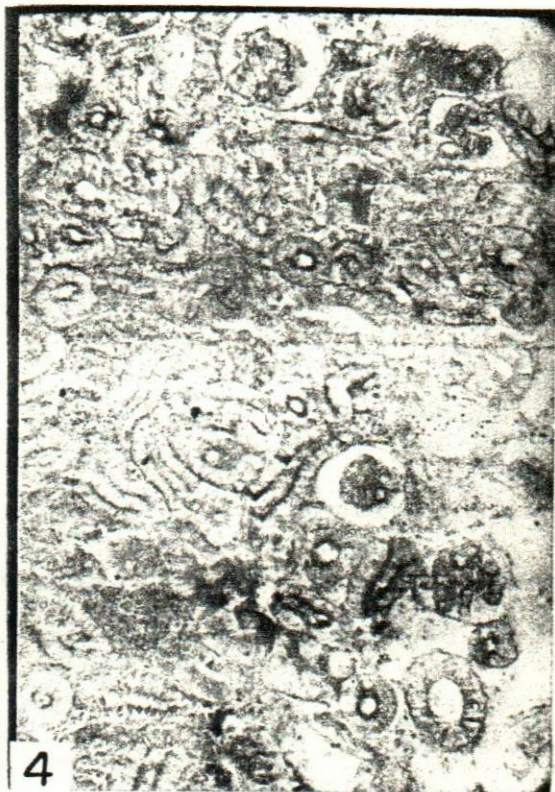
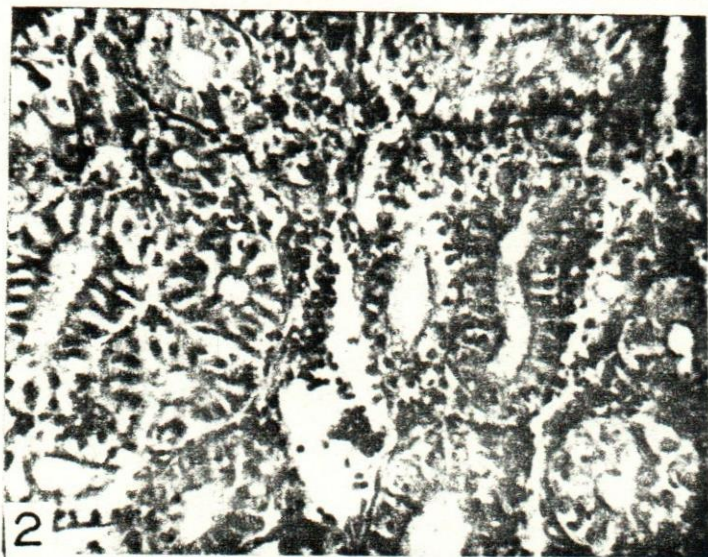
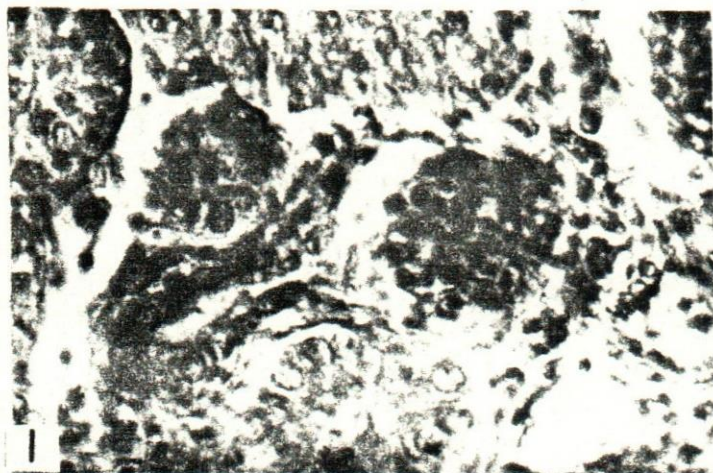


PLATE II

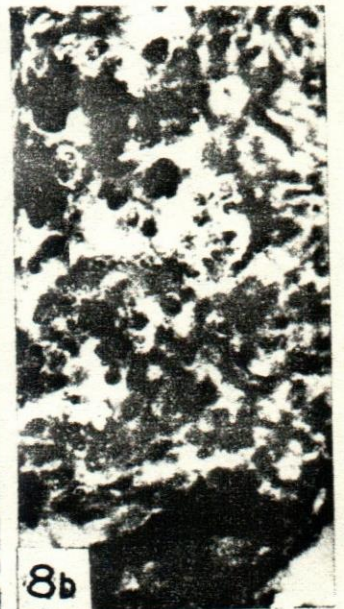
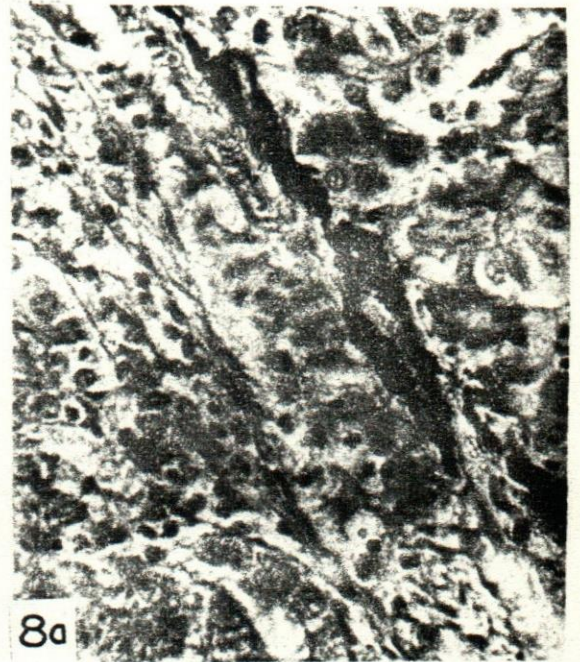
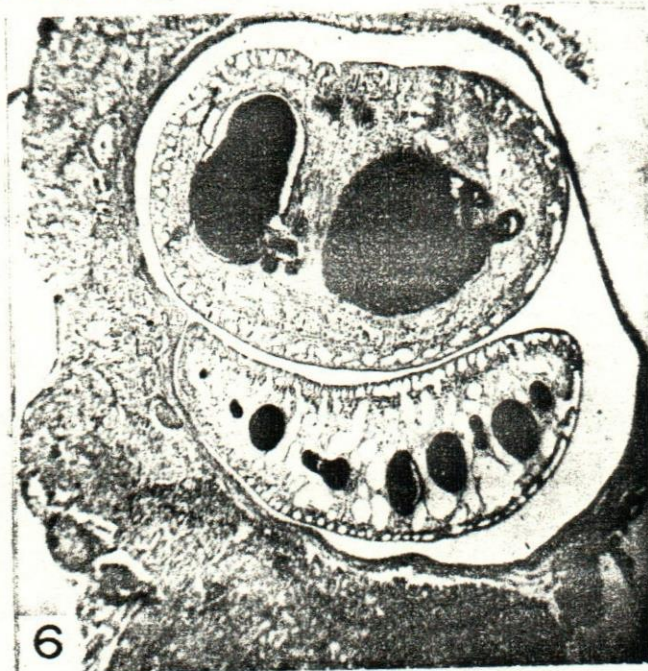


PLATE III

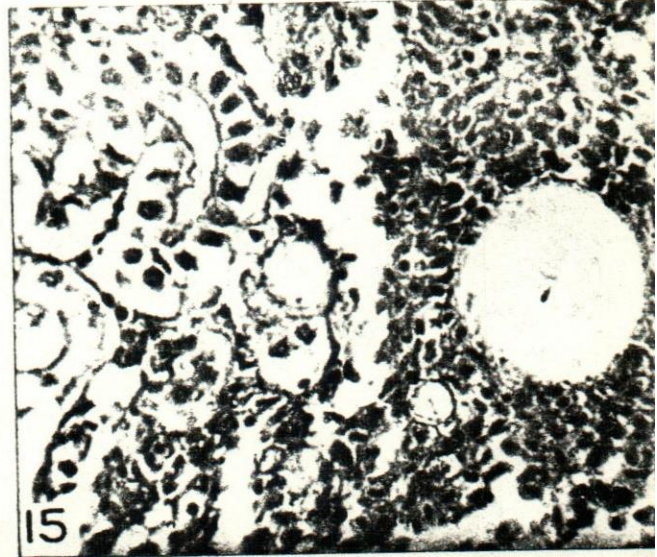
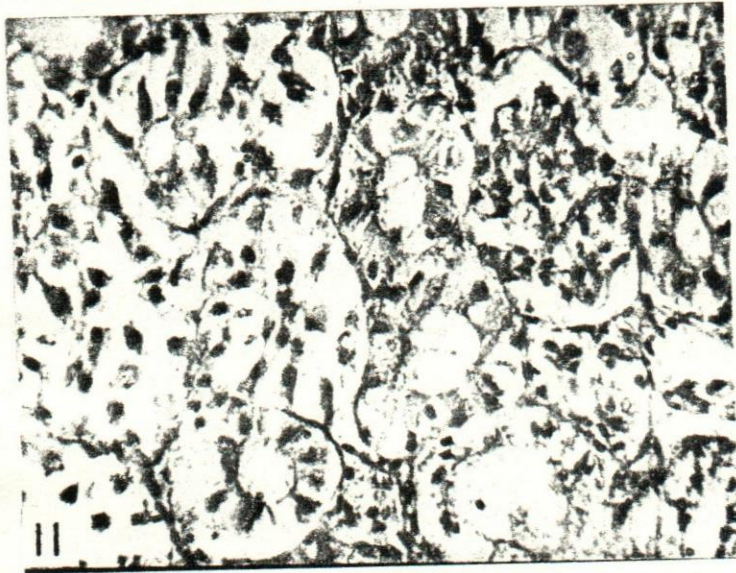
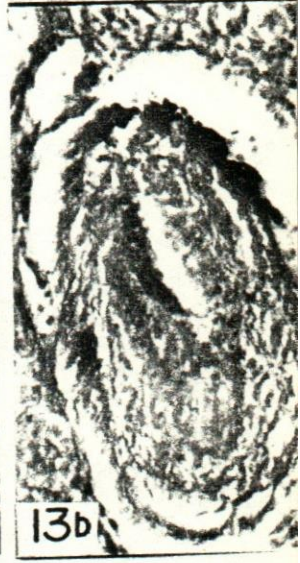
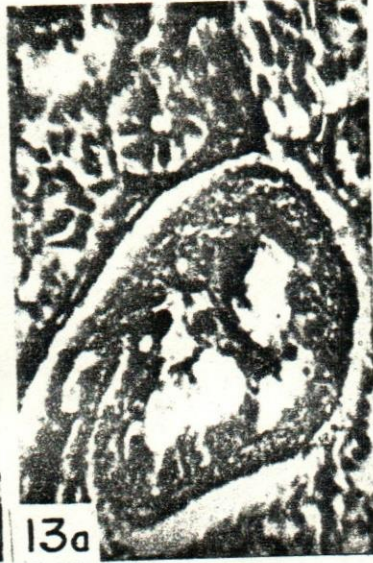


PLATE IV

