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DISTRIBUTION OF SOME HEAVY METALS IN TISSUES OF *OREOCHROMIS NILOTICUS*, *TILAPIA ZILLII* AND *CLARIAS LAZERA* FROM ABU ZA'BAAL LAKES AND THEIR IMPACTS ON SOME BIOCHEMICAL PARAMETERS AND ON THE HISTOLOGICAL STRUCTURES OF SOME ORGANS

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ABSTRACT

Abu Za'baal Lakes are man-made lakes, formed by the fracture and extract of rocks. The lakes, as other inland closed basins, receive their water from the ground and seepage waters. They consist of three lakes and another filling-phase lake. In the present study, the concentrations of some heavy metals (Fe, Zn, Mn, Pb, Cu and Cd) in water and organs (muscles, gills, liver and kidney) of *O. niloticus*, *T. zillii* and *C. lazera* collected from Abu Za'baal Lakes were measured during winter, 2004. The effects of the tested metals on the total protein and total lipid contents in the muscle and liver and on the histological structures of the gills, liver and kidneys of fish were studied.

The present results showed that the levels of Fe, Zn, Mn, Pb, Cu and Cd in the water of Abu Za'baal lakes ranged between 3.22-6.01, 0.21-1.50, 0.67-1.64, 0.62-1.67, 0.13-0.20 and 0.03-0.06 mg/l, respectively. The highest values of metals were reported in the third lake, while their lowest values were in the first one.

It was found that the metals were accumulated in different organs of the studied fish by various levels. The concentration of the tested metals in different organs of *O. niloticus* and *T. zillii* followed a sequence of: Fe, Zn, Mn, Pb and Cd in muscles < gills < liver < kidneys, however, Cu in muscles < gills < kidneys < liver for *O. niloticus* and in gills < muscles < kidneys < liver for *T. zillii*. On the other hand, in *C. lazera*, Fe and Cu were in muscles < gills < kidneys < liver, Zn, Pb and Cd in muscles < gills < liver < kidneys and Mn in muscles < liver < kidneys < gills. In general, the concentrations of

the tested metals in different organs of the studied fish were in the following order. Fe > Zn > Pb > Mn > Cu > Cd. Zn, Cu and Cd concentrations in the fish muscles (edible parts) (except Cd in the muscles of *T. zillii*) were within the maximum permissible limit. The concentrations of Fe and Pb in the muscles exceeded the permissible limit.

The total protein content in the muscle and liver of *O. niloticus*, *T. zillii* and *C. lazera* showed non significant decrease. Otherwise, the muscle lipid content showed highly significant increases in *O. niloticus* and *T. zillii* and non significant decrease in *C. lazera*. In contrast, the liver lipid content showed non significant increase in *O. niloticus* and *T. zillii* and a highly significant increase in *C. lazera*.

Several histopathological alterations were observed in the gills, liver and kidneys of the studied fish collected from Abu Za'baal Lakes.

INTRODUCTION

Abu Za'baal Lakes are, circuitous man-made basins, located in the north of Al Qalyobiyah Governorate (Egypt). The lakes were formed, during last century, probably due to the fracture and extract of the basalt rocks. They are inland closed basins, gradually filled up by seepage and ground waters. The lakes were formed and filled as follows: in the fifth decade (the first lake), in the eighth decade (the second lake) and ninth decade (the third lake), while the small lake is still in the filling phase at present. Abu Za'baal Lakes occupy the area between Latitudes 30° 16.62\' and 30° 17.58\' N and Longitudes 31° 20.90\' and 31° 21.69\' E. The surface areas of the lakes are 375.816 x 10^3, 151.848 x 10^3 and 80.386 x 10^3 m^2 for the first, second and third lake, respectively. The water storage in the lakes is 5234.075 x 10^3 m^3. The highest surface area, water depth and water storage are in the first lake, while the lowest values are in the third lake (Abd Ellah, 2003). Abu Za'baal Lakes are brackish waters (3.408 - 4.933 psu). The pH values of the lake's water lie in the alkaline side (pH: 7.8 - 8.1). The higher Hydrogen ion concentration recorded in the third lake (Abd Ellah, 2003).

Heavy metals represent part of the major aquatic pollutants, since they are present throughout the ecosystem and are detectable in critical amounts. Not only environmental organizations, such as EPA and UNEP but also the public communities are concerned about the possible adverse consequences of such pollutants to the aquatic biota

and, indirectly, to humans. Heavy metals are toxic and tend to accumulate in the body organs (El-Ezaby, 1994).

It is estimated that fish can act as front- line indicators of suspected aquatic pollutants such as metals (Bailey *et al.*, 1996). Fish may absorb dissolved elements and heavy metals from surrounding water and food, which may accumulate in various tissues in significant amounts (Eiman and Zamzam, 1996) and are eliciting toxicological effects at critical targets. However, fish may accumulate significant concentrations of metals even in waters in which those metals are below the limit of detection in routine water samples (Barak and Mason, 1990). Fish might, therefore, prove a better material for detecting heavy metals contaminating the freshwater ecosystems.

Many studies were previously carried out on the level of heavy metals in water (El-Rafei, 1991; Abdel-Shafy *et al.*, 1995; Khallaf *et al.*, 1998; Radwan, 2000; Bahnasawy, 2001 ; Sabae and Abdel-Satar, 2001). The accumulation of heavy metals in fish organs has been studied by several investigators (Ghazaly *et al.*, 1992; Khallaf *et al.*, 1998; El-Moselhy, 1999; Abdel-Baky, 2001; Bahnasawy, 2001; Heba *et al.*, 2001.Gutleb *et al.*, 2002).

Accumulation of metals may lead to high mortality rate or cause many biochemical and histological alterations in the survived members. Several investigations had concerned with the effect of metals on the levels of the tissue protein and lipid. Reduction in protein levels was noticed in the muscle and liver of *Sarotherodon mossambicus* exposed to mercury (Ramalingam and Ramalingam, 1982); in the liver of *Clarias lazera* exposed to mercury (Ibrahim *et al.*, 1991) and in the muscle and liver of grass carp exposed to lethal concentration of cadmium (Salah El-Deen *et al.*, 1996). Similarly, a decrease of lipid levels was observed in the muscle and liver of grass carp exposed to lethal and sublethal concentrations of cadmium (Salah El-Deen *et al.*, 1996). However, Bahnasawy (2001) showed an increase in the protein content of different tissues of fish exposed to heavy metals.

Several histopathological alterations have been reported in the gills, liver and kidneys of fish as a result of exposure to heavy metals. Histopathological changes in the gills were observed in *Archosargus probatocephalus* exposed to copper (Cardeilhac *et al.*, 1979); in *Tilapia nilotica* exposed to lead acetate, mercuric chloride

and cadmium chloride (Balah *et al.*, 1993); in *Cyprinion mhalensis* exposed to copper (Ghazaly *et al.*, 1994); in *Macropsobrycon uruguayanae* exposed to cadmium (Randi *et al.*, 1996) and in *Salmo trutta* exposed to iron sulphate (Dalzell and Macfarlane, 1999). The histopathological effects of metals on the liver of fish were studied by many authors: in *Ictalurus nebulosus* exposed to copper (Beneditti *et al.*, 1989), in *Heteropneustes fossilis* exposed to mercury and cadmium (Bano and Hassan, 1990; Ghosh and Chakrabarti, 1993) and in rainbow trout exposed to $CdCl_2$ (Iliopoulou- Georgudaki and Kotsanis, 2001). Histopathological changes were seen in the kidneys of *Heteropneustes fossilis* exposed to mercury and cadmium (Bano and Hassan, 1990; Ghosh and Chakrabarti, 1993), *Cyprinus carpio* exposed to cadmium (Singhal and Jain, 1997) and rainbow trout exposed to $CdCl_2$ (Iliopoulou- Georgudaki and Kotsanis, 2001).

The aim of the present study was to assess the level of some heavy metals (Fe, Zn, Mn, Pb, Cu and Cd) in water and several organs (muscles, gills, liver and kidneys) of *O. niloticus*, *T. zillii* and *C. lazera* collected from Abu Za'baal Lakes. Moreover, the study tends to evaluate the impact of such metals on the muscle and liver total protein and lipid contents and on the histological features of some organs (gills, liver and kidneys) of fish.

MATERIAL AND METHODS

1. Collection of samples:

1.1- Water samples:

Samples of water were collected from the three lakes (Abu Za'baal Lakes) during winter 2004, in polyethylene bottles. They were preserved with conc. nitric acid, transferred to the laboratory and kept refrigerated for later analysis.

1.2- Fish samples:

Samples of *O. niloticus*, *T. zillii* and *C. lazera* were collected from the three lakes (Abu Za'baal Lakes) during winter 2004. The collected fish were measured to the nearest cm and weighed to the nearest g. The fish specimens used in this study ranged between 15.4 to 20.0, 13.5 to 20.0 and 25.5 to 50.0 cm in total length for *O. niloticus*, *T. zillii* and *C. lazera*, respectively, and 66.0 to 146.5, 46.0 to 160.5 and 145.0 to 776.5g in weight for the same species, respectively. After the dissection, muscles, gills, liver and kidneys of fish were carefully removed.

Other fish samples were collected from Al-Kanater Al-Khairya fish farm to be used as a control group for the biochemical and histological studies.

2. Preparation of samples for heavy metals analysis:

2.1 Water samples:

Five hundred ml of the water sample was acidified with 5ml of conc. HNO_3 and heated on a hot plate. Heating and addition of conc. HNO_3 was continued to complete digestion. The sample was then cooled, filtered and completed to a final volume of 100ml by deionized distilled water as described by Parker (1972) and APHA (1992). This solution was used for the determination of the metals. The concentrations of Fe, Zn, Mn, Pb, Cu and Cd were measured by Perkin Elmer 3110 Atomic Absorption Spectrophotometer and the results were expressed in mg/l.

2.2. Tissue samples:

Tissue samples (muscles, gills, liver and kidneys) were dried at 105°C for 48 hours and then grounded to a fine powder. The dried samples were digested according to the method described by Ghazaly (1988) in which 1g (dry powder) was digested in a solution of nitric acid (HNO_3 – AR grade) and perchloric acid ($HCLO_4$ – AR grade) (5ml + 5ml) on a hot plate at 80-90°C until the sample becomes clear. After cooling, the solution was filtered and the filtrate was made up to a known volume (25ml) with deionized distilled water. The concentrations of Fe, Zn, Mn, Pb, Cu and Cd in the muscles, gills, liver and kidneys were measured by Perkin Elmer 3110 Atomic Absorption Spectrophotometer and the results were expressed in µg/g of the dry weight of the tissue.

3. Biochemical analysis:

3.1. Total protein content in tissues:

Sample of 0.1g of muscle or liver was homogenized in a glass homogenizer for 3 minutes in 5ml saline then centrifuged at 3000 r.p.m for 10 minutes. The supernatant was used for determination of total protein content. Total protein content was determined according to the method described by Doughaday *et al.* (1952).

3.2. Total lipid content in tissues:

Sample of 0.1g of muscle or liver was homogenized in a glass homogenizer for 3 minutes in 5ml saline then centrifuged at 3000 r.p.m for 10 minutes. The supernatant was discarded and the pellet obtained was washed with ice cold 10% trichloroacetic acid (TCA).

The mixture was centrifuged for 10 minutes at 600 r.p.m and the supernatant discarded. This step was repeated twice with ice cold 5% TCA. The dry pellet obtained was extracted 3 times with a mixture of chloroform: ethanol: ether (1: 2: 2 [v/v/v]) (Little Field *et al.*, 1955). The combined extract was used for determination of total lipid content. Total lipid content was determined according to the method described by knight *et al.* (1972).

4. Histological investigations:

Immediately after isolation from the fish, the gills, liver and kidneys were fixed in Bouin's fluid for 24-48 hrs. The fixed samples were washed several times in 70% ethyl alcohol and then dehydrated in ascending series of ethyl alcohol. The specimens were cleared in xylene for 15-20 min and then embedded in paraffin wax. Sections of 4-6μm thickness were cut, mounted on glass slides and stained with Harris' haematoxylin and eosin.

5- Statistical analyses:

Values were expressed as means (M) ± standard deviation (SD). Data were analysed using t-test (Snedecor, 1962). The values were considered highly significant at $P \leq 0.01$.

RESULTS

I. Concentration of heavy metals in water:

The results given in Table (1), revealed that the concentrations of Fe, Zn, Mn, Pb, Cu and Cd in the water of lakes ranged between 3.22-6.01, 0.21- 1.50, 0.67-1.64, 0.62-1.67, 0.13-0.20 and 0.03-0.06 mg/l, respectively. The third lake had the highest levels of metals, while the first lake had the lowest values. Moreover, the results showed that Fe recorded the highest concentration (3.22-6.01 mg/l) among the tested metals, while Cd recorded the lowest ones (0.03-0.06mg/l).

II. Accumulation of heavy metals in fish organs:

Iron (Fe):

The concentrations of Fe in the muscles, gills, liver and kidneys of *O. niloticus*, *T. zillii* and *C. lazera* collected from Abu Za'baal Lakes are given in Table (2). The results indicated that the mean Fe concentrations in organs of the studied fish ranged between 45.20-761.92, 75.09-2945.00 and 54.41-1548.78μg/g dry wt. for *O. niloticus*, *T. zillii* and *C. lazera*, respectively. Fe accumulation in the

studied organs was in the following order: muscle < gills < liver < kidneys for *O. niloticus* and *T. zillii*, but it was in muscle < gills < kidneys < liver for *C. lazera*. The muscles (edible parts) of *O. niloticus* contained the lowest concentration of Fe (45.20 µg/g dry wt.), followed by *C. lazera* (54.41 µg/g dry wt.) and *T. zillii* (75.09 µg/g dry wt.).

Zinc (zn):

The concentrations of Zn in the studied organs of *O. niloticus*, *T. zillii* and *C. lazera* collected from Abu Za'baal Lakes are given in Table (3). The results showed that Zn concentration in organs of the studied fish ranged between 21.06- 81.67, 38.30-170.83 and 17.83-62.06 µg/g dry wt. for *O. niloticus*, *T. zillii* and *C. lazera*, respectively. The concentrations of Zn in the studied organs were in the following order: muscle < gills < liver < kidneys for the three studied fish. The muscles of *C. lazera* contained the lowest concentration of Zn (17.83 µg/g dry wt.), followed by *O. niloticus* (21.06 µg/g dry wt.) and *T. zillii* (38.30µg/g dry wt.).

Manganese (Mn):

The concentrations of Mn in the studied organs of *O. niloticus*, *T. zillii* and *C. lazera* collected from Abu Za'baal Lakes are given in Table(4). The concentration of Mn in organs of the studied fish ranged between 4.61-150.25, 0.00-133.33 and 2.60-13.56 µg/g dry wt. for *O. niloticus*, *T. zillii* and *C. lazera*, respectively. Mn concentrations in the studied organs were in the following order: muscle < gills < liver < kidneys for *O. niloticus* and *T. zillii* and muscle < liver < kidneys < gills for *C. lazera*. The results showed that Mn was not detected in the muscles of *T. zillii*.

Lead (Pb):

The concentrations of Pb in the studied organs of *O. niloticus*, *T. zillii* and *C. lazera* collected from Abu Za'baal Lakes are given in Table (5). The results indicated that the concentration of Pb in organs of the studied fish ranged between 4.81-107.50. 17.58-629.17 and 8.17-49.79 µg/g dry wt. for *O. niloticus*, *T. zillii* and *C. lazera*, respectively. Pb accumulations in the studied organs were in the following order: muscle < gills < liver < kidneys for the three studied fish. The muscles of *O. niloticus* contained the lowest concentration of Pb (4.81 µg/g dry wt.), followed by *C. lazera* (8.17 µg/g dry wt.) and *T. zillii* (17.58µg/g dry wt.).

Copper (Cu):

The concentrations of Cu in the studied organs of *O. niloticus*, *T. zillii* and *C. lazera* collected from Abu Za'baal Lakes are given in Table (6). The results showed that the concentration of Cu in organs of the studied fish ranged between 1.50- 320.57, 2.35-25.25 and 2.32- 17.20 µg/g dry wt. for *O. niloticus*, *T. zillii* and *C. lazera*, respectively. The concentrations of Cu in the studied organs were in the following order: muscles < gills < kidneys < liver for *O. niloticus* and *C. lazera* and gills < muscles < kidneys < liver for *T. zillii*. The present results indicated that the liver accumulated higher amounts of copper. The muscles of *O. niloticus* contained the lowest concentration of Cu (1.50 µg/g dry wt.), followed by *C. lazera* (2.32 µg/g dry wt.) and *T. zillii* (8.84 µg/g dry wt.).

Cadmium (Cd):

The concentrations of Cd in the studied organs of *O. niloticus*, *T. zillii* and *C. lazera* collected from Abu Za'baal Lakes are given in Table (7). The concentration of Cd in organs of the studied fish ranged between 1.73-24.17, 3.19- 92.92 and 1.46-12.65µg/g dry wt. for *O. niloticus*, *T. zillii* and *C. lazera*, respectively. Cd concentrations in the studied organs were in the following order: muscles < gills < liver < kidneys for the three studied fish. The muscles of *C. lazera* contained the lowest concentration of Cd (1.46 µg/g dry wt.), followed by *O. niloticus* (1.73 µg/g dry wt.) and *T. zillii* (3.19µg/g dry wt.).

III. Biochemical parameters:

All biochemical data of *O. niloticus*, *T. zillii* and *C. lazera* are presented in Table (8). The total protein and lipid contents in the muscle and liver of control fish collected from Al-Kanater Al-Khairya fish farm are 16.77 ± 0,81, 10.03 ± 0.71, 0.67 ± 0.06 and 5.08 ± 0.54 g/100g wet weight, respectively for *O. niloticus*, 16.95 ± 0.86, 9.80 ± 0.84, 0.70 ± 0.08 and 5.13 ± 0.64 g/100g wet weight, respectively for *T. zillii* and 17.40 ± 1.08, 10.20 ± 0.67, 1.10 ± 0.15 and 6.35 ± 0.62 g/100g wet weight, respectively for *C. lazera*.

The total protein content in the muscle and liver of the three studied fish species collected from Abu Za'baal Lakes showed a slight non significant ($P > 0.05$) decrease as compared with the control values. Meanwhile, the muscle lipid content showed highly significant ($P \leq 0.01$) increases in *O. niloticus* (43.28%) and *T. zillii* (34.29%), but it did not display any significant difference in *C. lazera*. In contrast, the liver lipid content showed a slight non

significant increase in *O. niloticus* and *T. zillii* and a highly significant ($P \leq 0.01$) (16.22%) increase in *C. lazera*.

IV. Histopathological alterations:

Several histopathological changes were observed in the gills, liver and kidneys of the three studied fish collected from Abu Za'baal Lakes during winter 2004.

Gills:

The gills of the control fish collected from Al-Kanater Al-Khairya fish farm showed normal histological features (Fig. 1A).

The gills of the three studied fish collected from Abu Za'baal Lakes showed proliferative changes in the epithelium of gill filaments and secondary lamellae (Figs.1B and 3A&C) and degenerative and necrotic changes in gill filaments and secondary lamellae (Fig. 1C&D, 2H and 3C&D). Besides, inflammatory cells infiltration was noticed among the proliferated epithelial cells (Figs.1B&3B). Moreover, in the gills of *O. niloticus*, severe curling of secondary lamellae (Figs.1D &E), severe atrophy of secondary lamellae (Fig. 1G) and dilation and congestion in the blood vessels of gill filaments (Fig. 1H) were seen. Collapse of the epithelium of gill filaments (Fig. 1F) was observed. Bulging with blood at the tips of lamellae (Fig. 2A) was observed. Dark deposits were seen on the surface of gill epithelia (Fig.2B). Nodular proliferation was also observed in some gill filaments (Fig. 2C).

Also, in the gills of *T. zillii*, severe edema in secondary lamellae and separation of the epithelium of secondary lamellae from the lamellar supporting cells (Fig. 2G) were seen. In some gill filaments, nodular proliferation was seen (Fig. 2D). Moreover, haemorrhage in secondary lamellae (Fig. 2F) and dilation in the blood vessels of gill filaments (Fig. 2E) were observed.

The gills of *C. lazera* showed curling of secondary lamellae (Fig. 3D). Besides, bulging was seen at the end of secondary lamellae (Figs.3E&F).

Liver:

The liver from the control fish collected from Al-Kanater Al-Khairya fish farm showed normal histological features (Fig. 4A).

In the liver of the three studied fish collected from Abu Za'baal Lakes, vacuolar degeneration in the hepatocytes (Figs. 4B, 6A&7A), focal areas of necrosis (Figs.4C&7B) and haemorrhage and haemosiderin between the hepatocytes (Figs.4D&E and 6B) were

seen. Moreover, in the liver of *O. niloticus*, severe dilation and congestion in hepatic (Fig. 5C) and hepatoportal (Fig. 5A) blood vessels were observed. Haemorrhage and haemosidérin were noticed around the hepatoportal blood vessels (Figs.4H.5A&B). Severe aggregations of inflammatory cells were seen between the hepatocytes (Fig. 4F). Dilation was observed in central veins (Fig. 4G). Edema around hepatic blood vessels (Fig. 5C) and thrombosis formation in hepatic (Fig. 5C) and hepatoportal (Fig. 5B) blood vessels were observed.

Also, in the liver of *T. zillii*, dilation and congestion were seen in central veins (Fig. 6C). Dilation and intravascular haemolysis were observed in hepatoportal blood vessels (Fig. 6F). Thrombosis formation in hepatoportal blood vessels and haemosiderin and fibrosis around them were observed (Fig. 6D). Degeneration was seen in the wall of hepatoportal blood vessels (Fig. 6E).

In *C. lazera*, the liver showed dilation and congestion in hepatic blood vessels and haemorrhage and haemosiderin around them (Fig. 7C). Thrombosis formation in other hepatic blood vessels and edema around them were observed (Fig. 7D).

Kidney:

The kidney from the control fish collected from Al-Kanater Al-Khairya fish farm showed normal histological features (Fig. 7E).

In the kidneys of *O. niloticus* collected from Abu Za'baal Lakes, vacuolar degeneration in the epithelium of renal tubules (Fig. 7F), focal areas of necrosis (Fig. 8A) and haemorrhage (Fig. 7G) and haemolysis (Fig. 7H) between the renal tubules were observed. Moreover, depletion in the haemopoietic areas was seen (Fig. 8A). Besides, edema in Bowman's capsules with atrophy in the glomeruli was observed (Figs.8C&D). The kidneys of *T. zillii* showed the same histopathological changes observed in the kidneys of *O. niloticus*.

Similarly, in the kidneys of *C. lazera*, vacuolar degeneration in the epithelium of renal tubules, focal areas of necrosis (Fig. 8B) and severe haemorrhage, haemolysis (Figs.8E&F) and haemosiderin (Fig. 8G) between the renal tubules were observed. Moreover, dilation and haemolysis were seen in renal blood vessels (Fig. 8H).

DISCUSSION

The present study showed that the concentrations of Fe, Zn, Mn, Pb, Cu and Cd in water of Abu Za'baal Lakes ranged between 3.22-6.01, 0.21-1.50, 0.67-1.64, 0.62-1.67, 0.13-0.20 and 0.03-0.06

mg/l, respectively. By comparing these levels with the levels of the same metals in different Egyptian water bodies, the present ones appear higher than them (Table 9).

Fish are notorious for their ability to concentrate heavy metals in their tissues. The metals exist most probably as cationic form in water (Fe, Zn, Mn, Pb, etc.) and tend to form ionic complexes and accumulate in the internal organs of fish (Mears and Eister, 1977). The present results showed that the concentrations of Fe in the studied organs of the fish ranged between 45.20-761.92, 75.09-2945.00 and 54.41-1548.78 µg/g dry wt. for *O. niloticus*, *T. zillii* and *C. lazera*, respectively. It was found that Fe concentration in the muscle < gills < liver < kidneys for *O. niloticus* and *T. zillii*, however, its level in *C. lazera* followed the following manner: muscle < gills < kidneys < liver. The high accumulation of Fe in different fish organs can be attributed to the large quantities of Fe in water, this agree with the findings of Ghazaly *et al.* (1992), Tariq *et al.* (1993) and Bahnasawy (2001). The present results of higher Fe content in kidneys of *O. niloticus* and *T. zillii* agree with the findings of Latif (1982). On the other hand, the present data for *C. lazera* agree with those of El-Moselhy (1999) and Yacoub (1999) who reported that Fe concentrations exhibited their highest levels in the liver. The present study showed that iron concentrations in the studied organs of the fish were more than US maximum permissible level for Fe (5.0 µg/g) cited by Adeyeye (1993b).

Zinc is an essential element and a common pollutant as well. It is taken up by fish directly from water especially by mucus and gills (Skidmore, 1964). The present study showed that Zn concentrations in organs of the studied fish ranged between 21.06-81.67, 38.30-170.83 and 17.83-62.06 µg/g dry wt. for *O. niloticus*, *T. zillii* and *C. lazera*, respectively. Such results indicated that Zn concentrations were in the following order: muscles < gills < liver < kidneys for the three studied fish. The present results are in agreement with those obtained by Shenouda *et al.* (1992), Gomaa *et al.* (1995) and Khallaf *et al.* (1998). Western Australian Food and Drink Regulations recommended a level of 40 mg/kg Zn for human consumption (Marks *et al.*, 1980). Accordingly, the concentrations of Zn in the muscles of the studied fish are still below the permissible level.

The present study indicated that Mn concentrations in organs of the studied fish ranged between 4.61-150.25, 0.00-133.33 and

2.60-13.56µg/g dry wt. for *O. niloticus*, *T. zillii* and *C. lazera*, respectively. Mn concentrations in the studied organs were in the following order: muscle < gills < liver < kidneys for *O. niloticus* and *T. zillii*. The present results agree with the findings of Gomaa *et al.* (1995) and Khallaf *et al.* (1998). On the other hand, Mn concentrations in the studied organs of *C. lazera* were in the following order: muscle < liver < kidneys < gills, which agree with the results of Abdel-Baky (2001). The high accumulation of Mn in the gills of *C. lazera* (13.56 ± 0.60 µg/g dry wt.) may be attributed to the complex formation between both metal ions and the proteins in gills which contain nitrogen, oxygen and/or sulfur as previously reported by Cotton and Wilkinson (1980) and Abdel-Baky (2001).

Lead is toxic even at low concentrations and has no known function in biochemical processes. The present results showed that Pb concentrations in organs of the fish ranged between 4.81- 107.50, 17.58-629.17 and 8.17-49.79 µg/g dry wt. for *O. niloticus*, *T. zillii* and *C. lazera*, respectively. Pb accumulation in the studied organs was in the following order: muscle < gills < liver < kidneys for the three studied fish. The present results agree with the results of Barak and Mason (1990), Ghazaly *et al.* (1992), Gomaa *et al.* (1995) and Khallaf *et al.* (1998). The present study revealed that Pb concentrations in organs of the studied fish were more than US FDA maximum permissible level for Pb (2.0µg/g) cited by Adeyeye (1993a).

Cu is an essential element for all living organisms. It is among the most toxic metals. The present study showed that Cu concentrations in organs of the studied fish ranged between 1.50-320.57, 2.35-25.25 and 2.32-17.20µg/g dry wt. for *O. niloticus*, *T. zillii* and *C. lazera*, respectively. Cu concentrations were in the following order: muscles < gills < kidneys < liver for *O. niloticus* and *C. lazera*, however, gills < muscles < kidneys < liver for *T. zillii*. The present results agree with the results of Khallaf *et al.* (1998) and Abdel-Baky (2001). Also, Benedetti *et al.* (1989), Gomaa *et al.* (1995) and El-Moselhy (1999) found that Cu exhibited its highest levels in the liver and the lowest values in the muscles. The high accumulation of Cu in the liver could be certainly attributed to the specific metabolic processes and enzyme catalyzed reaction involving Cu taking place in the liver. The sulfur legends in liver also have a great tendency to co-ordinate with Cu *via* oxygen carboxylate amino group nitrogen and/or sulfur of the mercapto group in the

metalothionin protein which is in the highest concentration in liver (El-Shahawi and Yousuf, 1998; Abdel-Baky, 2001). The concentrations of Cu in the muscles of the studied fish are still blow the permissible level for Cu (30 mg/kg) recommended by the National Health and Medical Research Council (NHMRC) (Marks *et al.*, 1980).

Cd is a highly toxic to aquatic organisms and accumulates in liver and kidney inducing hepatic and renal injury (Kjellstrom and Nordberg, 1985). The present results showed that Cd concentrations in organs of the studied fish ranged between 1.73-24.17, 3.19-92.92 and 1.46-12.65 µg/g dry wt. for *O. niloticus*, *T. zillii* and *C. lazera*, respectively. Cd concentration was in the following order: muscle < gills < liver < kidney for the three studied fish. Kidneys are known to play an important role in the detoxification and excretion of toxicants and this explains the high levels of Cd in this organ. Generally, kidney accumulated highest amount of Cd as compared with other organs (Allen, 1995). The concentrations of Cd in the muscles of the studied fish (except the muscles of *T. zillii*) are still below WHO permissible level for Cd (2.0mg/kg) reported by FAO (1992).

It is clear that gills, liver and/or kidneys had higher tendency to accumulate heavy metals more than muscles. Moreover, the present results indicated that the order of metal distribution in fish organs followed the concentration pattern: Fe > Zn > Pb > Mn > Cu > Cd. It could be concluded that the concentrations of heavy metals in organs of the studied fish depended mainly on the metal, organ and species. This is in agreement with that reported by Abdel-Moneim and Iskander (1995).

The control values of the muscle and liver protein and lipid contents obtained in the present study for *O. niloticus*, *T. zillii* and *C. lazera* are within the same range for *Tilapia zillii* (Gad, 1999), *Oreochromis niloticus* (Mechail, 1999) and *Labeo rohita* (Das and Mukherjee, 2003). In the present study, the total protein content in the muscle and liver of the fish collected from Abu Za'baal Lakes showed non significant decrease as compared with the control values. The results indicated that the present levels of the tested metal in the water and organs of the studied fish from Abu Za'baal Lakes did not exhibit any effect on the muscle and liver protein contents.

The muscle lipid content showed highly significant increases in *O. niloticus* and *T. zillii* collected from Abu Za'baal Lakes and non significant decrease in *C. lazera*. In contrast, the liver lipid content showed non significant increase in *O. niloticus* and *T. zillii* and a highly significant increase in *C. lazera*. The elevation in the lipid content observed in the muscles of *O. niloticus* and *T. zillii* could be the result of enhanced lipid synthesis and/or reduced utilization (Woo and Tong, 1982). The increase in the lipid content observed in the liver of *C. lazera* may be attributed to increased lipid synthesis, decreased lipid catabolism and/or decreased lipid extrusion from the liver (Woo and Tong, 1982; El-Beih *et al.*, 1991; Mohamed and Gad, 2004). The present results agree with the findings of Gupta and Sastry (1981).

In the present study, examination of the gills of the fish collected from Abu Za'baal Lakes showed marked histopathological changes. These changes included proliferative, degenerative and necrotic changes in the epithelium of gill filaments and secondary lamellae, edema in secondary lamellae, separation of the epithelium of the secondary lamellae from the lamellar supporting cells, dilation and congestion in the blood vessels of gill filaments, atrophy in secondary lamellae, bulging at the tips of secondary lamellae and dark deposits on the surface of gill epithelia. The observed proliferative changes in the respiratory lamellar epithelium may increase the epithelial thickness which retard or prevent the entry of toxic metals into the blood stream (Laurent, 1984). Such proliferative changes may lead to a great disturbance of gas exchange. Ionic regulation might also be seriously affected, since fish gills are involved in ion exchange for osmoregulatory purposes (Eckert *et al.*, 1990).

According to Balah *et al.* (1993), the observed dilation of the lamellar blood vessels and the presence of edematous fluid in the secondary lamellae may be due to increased permeability induced by the prolonged exposure to the metals. This edematous fluid separated the respiratory epithelium from the underlying tissue and led to its desquamation as well as necrosis. The dark deposits observed on the surface of gill epithelia were most probably of heavy metals as reported by Peuranen *et al.* (1994).

The histological changes observed in the gills of the studied fish are in agreement with those observed by Cardeilhac *et al.*

(1979). Ferreira and Wolke (1979). Naidu *et al.* (1983a). Balah *et al.*(1993) and Dalzell and Macfarlane (1999).

The present study showed several histopathological alterations in the liver of the studied fish. These alterations included vacuolar degeneration in the hepatocytes. focal areas of necrosis. severe haemorrhage and haemosiderin between the hepatocytes and around hepatic and hepatoportal blood vessels and dilation and congestion in hepatic and hepatoportal blood vessels. The observed degeneration in the liver may be attributed to disruption in the lysosomal membrane. which is very sensitive to toxicants as heavy metals. and thus their enzymes released and caused degeneration and vacuolation of cytoplasm of hepatocytes (Yacoub. 1999). The histological changes observed in the liver of the studied fish agree with those observed by Naidu *et al.* (1983b). Ghosh and Chakrabarti (1993) and Iliopoulou-Georgudaki and Kotsanis (2001).

The kidneys of the studied fish revealed vacuolar degeneration in the epithelium of renal tubules. focal areas of necrosis. depletion in the haemopoietic areas. haemorrhage and haemosiderin between the renal tubules. edema in Bowman's capsules and dilation and haemolysis in renal blood vessels. The present results are in agreement with those observed by many investigators who have studied the effects of different metals on fish (Bano and Hassan. 1990; Ghosh and Chakrabarti. 1993; Singhal and Jain. 1997; Iliopoulou-Georgudaki and Kotsanis. 2001).

In conclusion. the results revealed that the concentrations of Fe. Zn. Mn. Pb. Cu and Cd in Abu Za'baal Lakes were high. These metals accumulated in different organs (muscle. gills. liver and kidney) of *O. niloticus*. *T. zillii* and *C. lazera*. The levels of the metals in the fish organs were depended on the metal. organ and species. The results indicated that Zn. Cu and Cd concentrations in the muscles (except Cd in the muscles of *T. zillii*) were within the maximum permissible limit. however. Fe and Pb levels in the muscles exceeded the permissible level. It was found that these levels of metals induced changes in the total lipid content. while. they had not any effect on the total protein content in the muscle and liver of the studied fish. Moreover. these metals caused several histological alterations in the gills. liver and kidney of the studied fish.

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Table (1): Concentration of heavy metals (mg/l) in Abu Za'baal Lakes.

	First Lake	Second Lake	Third Lake
Fe	3.80	3.22	6.01
Zn	0.21	0.29	1.50
Mn	0.67	1.40	1.64
Pb	0.62	1.67	1.19
Cu	0.13	0.17	0.20
Cd	0.03	0.04	0.06

Table (2): Iron concentrations (µg/g dry weight) in different organs of *O. niloticus*, *T. zillii* and *C. lazera* collected from Abu Za'baal Lakes.

Organs	Fish		
	O. niloticus M ± SD	*T. zillii* M ± SD	*C. lazera* M ± SD
Muscles	45.20 ± 9.69	75.09 ± 3.20	54.41 ± 10.78
Gills	208.80 ± 28.11	140.51 ± 17.13	73.85 ± 5.99
Liver	720.56 ± 26.34	523.29 ± 21.35	1548.78 ± 96.31
Kidneys	761.92 ± 33.91	2945.00 ± 163.13	930.05 ± 35.11

Table (3): Zinc concentrations (µg/g dry weight) in different organs of *O. niloticus*, *T. zillii* and *C. lazera* collected from Abu Za'baal Lakes.

Organs	Fish		
	O. niloticus M ± SD	*T. zillii* M ± SD	*C. lazera* M ± SD
Muscles	21.06 ± 4.09	38.30 ± 5.19	17.83 ± 2.89
Gills	41.38 ± 8.84	44.96 ± 4.12	32.15 ± 10.92
Liver	58.71 ± 1.04	61.92 ± 7.51	59.71 ± 10.54
Kidneys	81.67 ± 8.93	170.83 ± 21.32	62.06 ± 8.28

M ± SD = Mean ± Standard Deviation.

Number of fish used (n) = 6

Table (4): Manganese concentrations (µg/g dry weight) in different organs of *O. niloticus*, *T. zillii* and *C. lazera* collected from Abu Za'baal Lakes.

Organs	Fish		
	O. niloticus M ± SD	*T. zillii* M ± SD	*C. lazera* M ± SD
Muscles	4.61 ± 2.17	0.00 ± 0.00	2.60 ± 0.50
Gills	74.74 ± 13.34	16.74 ± 3.48	13.56 ± 0.60
Liver	114.59 ± 6.20	21.63 ± 5.22	8.33 ± 2.50
Kidneys	150.25 ± 13.13	133.33 ± 23.01	12.29 ± 3.07

Table (5): Lead concentrations (µg/g dry weight) in different organs of *O. niloticus*, *T. zillii* and *C. lazera* collected from Abu Za'baal Lakes.

Organs	Fish		
	O. niloticus M ± SD	*T. zillii* M ± SD	*C. lazera* M ± SD
Muscles	4.81 ± 1.51	17.58 ± 3.27	8.17 ± 1.65
Gills	13.38 ± 5.48	18.33 ± 2.19	17.30 ± 2.25
Liver	47.40 ± 12.47	84.17 ± 12.39	25.55 ± 6.07
Kidneys	107.50 ± 23.17	629.17 ± 31.20	49.79 ± 12.08

M ± SD = Mean ± Standard Deviation.
Number of fish used (n) = 6

Table (6): Copper concentrations (µg/g dry weight) in different organs of *O. niloticus*, *T. zillii* and *C. lazera* collected from Abu Za'baal Lakes.

Organs	Fish		
	O. niloticus M ± SD	*T. zillii* M ± SD	*C. lazera* M ± SD
Muscles	1.50 ± 0.10	8.84 ± 1.57	2.32 ± 0.82
Gills	2.08 ± 1.20	2.35 ± 0.04	4.57 ± 0.52
Liver	320.57 ± 76.49	25.25 ± 3.21	17.20 ± 0.49
Kidneys	43.42 ± 5.11	24.17 ± 5.32	10.37 ± 0.49

Table (7): Cadmium concentrations (µg/g dry weight) in different organs of *O. niloticus*, *T. zillii* and *C. lazera* collected from Abu Za'baal Lakes.

Organs	Fish		
	O. niloticus M ± SD	*T. zillii* M ± SD	*C. lazera* M ± SD
Muscles	1.73 ± 0.43	3.19 ± 0.57	1.46 ± 0.68
Gills	2.11 ± 0.64	6.38 ± 1.56	3.25 ± 0.18
Liver	10.15 ± 2.28	15.92 ± 2.12	4.16 ± 1.50
Kidneys	24.17 ± 4.90	92.92 ± 13.94	12.65 ± 4.10

M ± SD = Mean ± Standard Deviation.

Number of fish used (n) = 6

Table (8): Total protein and lipid contents (g/100g wet weight) in the tissues of *Oreochromis niloticus*, *Tilapia zillii* and *Clarias lazera* collected from Abu Za'baal Lakes.

Fish	Site	Muscle protein content (g/100g wet weight)		Liver protein content (g/100g wet weight)		Muscle lipid content (g/100g wet weight)		Liver lipid content (g/100g wet weight)	
		M ± SD	t-value	M ± SD	t-value	M ± SD	t-value	M ± SD	t-value
O. niloticus	Control	16.77 ± 0.81	-----	10.03 ± 0.71	-----	0.67 ± 0.06	-----	5.08 ± 0.54	-----
	Abu Za'baal Lakes	15.95 ± 0.79 (- 4.89)	1.75	9.88 ± 0.88 (-1.50)	0.33	0.96 ± 0.10 (+43.28)	6.25**	5.85 ± 0.73 (+15.16)	2.06
T. zillii	Control	16.95 ± 0.86	-----	9.80 ± 0.84	-----	0.70 ± 0.08	-----	5.13 ± 0.64	-----
	Abu Za'baal Lakes	16.60 ± 1.20 (-2. 07)	0.58	8.92 ± 0.66 (-8.98)	2.02	0.94 ± 0.11 (+34.29)	4.31**	5.67 ± 0.39 (+ 10.53)	1.71
C. lazera	Control	17.40 ± 1.08	-----	10.20 ± 0.67	-----	1.10 ± 0.15	-----	6.35 ± 0.62	-----
	Abu Za'baal Lakes	15.87 ± 1.37 (-8.79)	2.14	10.08 ± 0.88 (-1.18)	0.26	0.99 ± 0.07 (-10.00)	1.62	7.38 ± 0.46 (+16.22)	3.26**

M ± SD = Mean ± Standard Deviation. Number of fish used (n) = 6

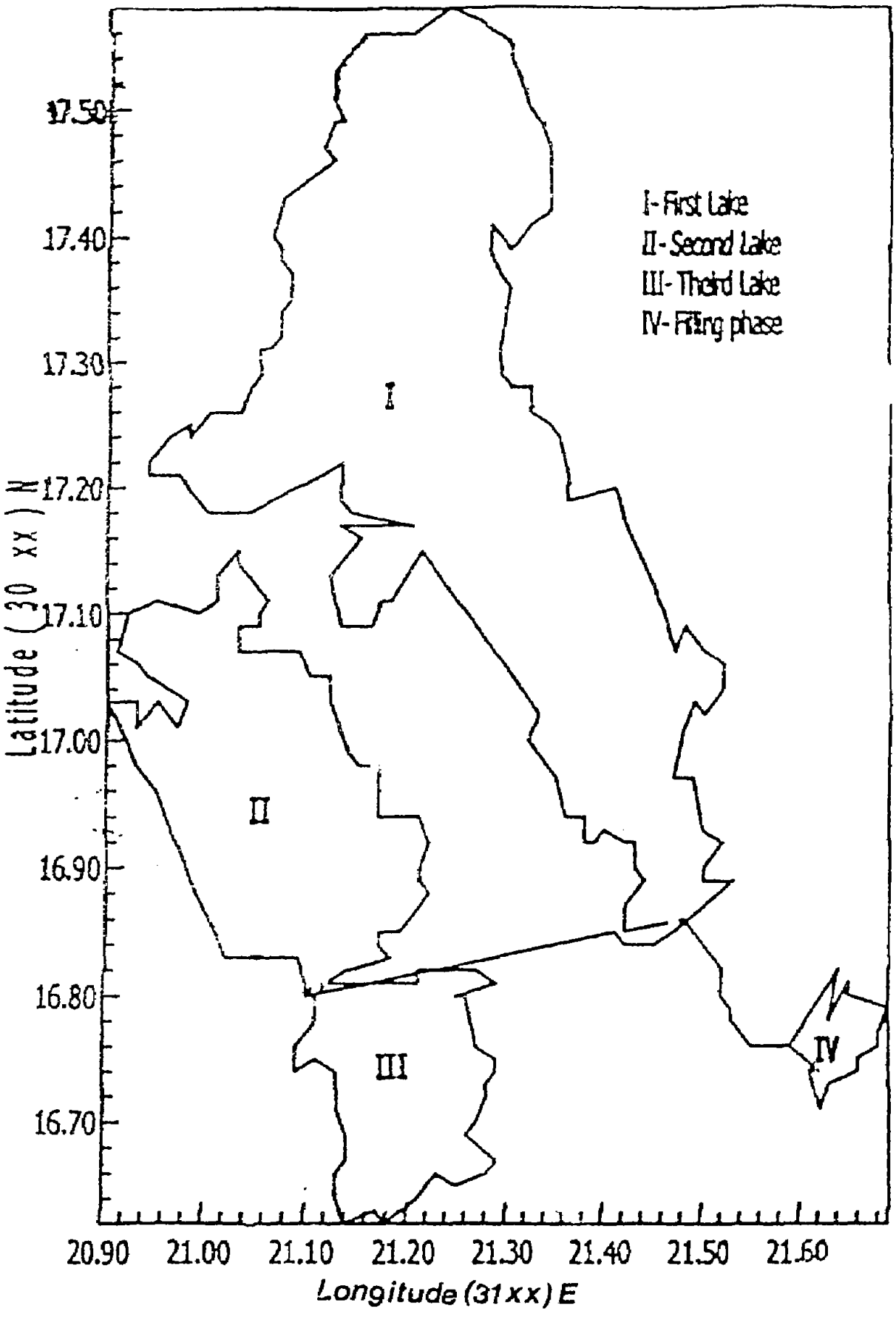
Figures between brackets are % change from control value.

t- value = between control and Abu Za'baal Lakes values.

** Highly significant differences at $P \leq 0.01$

Table (9): Mean metals concentrations (mg/l) in different Egyptian water bodies and the present study.

Location	Fe	Zn	Mn	Pb	Cu	Cd	References
River Nile (Meridian site)	0.47	0.07	0.04	0.06	0.13	0.00	El-Rafei (1991)
River Nile (Giza)	0.06	0.24	0.08	0.04	0.06	0.004	Abdel-Shafy *et al.* (1995)
Lake Burullos	0.003	0.007	-	0.003	0.004	0.002	Radwan (2000)
Lake Manzala	-	0.11-0.51	0.07-0.24	0.05-0.29	0.02-0.14	-	Bahnasawy (2001)
El-Salam Canal	-	0.15	0.09	0.09	0.04	-	Bahnasawy (2001)
River Nile	-	0.21	0.10	0.11	0.06	-	Bahnasawy (2001)
El-Salam Canal	0.33-4.76	0.01-0.06	0.03-0.22	0.01-0.05	0.003-0.055	-	Sabae and Abdel-Satar (2001)
Abu Za'baal Lakes	3.22-6.01	0.21-1.50	0.67-1.64	0.62-1.67	0.13-0.20	0.03-0.06	Present study



Map showing Abu Za baal Lakes

EXPLANATION OF FIGURES

Fig. (1): Sections of gills of fish showing:

(A): control (X100).

(B): proliferative changes and inflammation in the epithelium of gill filaments and secondary lamellae (*O. niloticus*) (X100).

(C):degenerative and necrotic changes in gill filaments and secondary lamellae (*O. niloticus*) (X100).

(D)&(E): curling of secondary lamellae (*O. niloticus*) (X100 & 400, respectively).

(F): collapse of the epithelium of gill filaments (*O. niloticus*) (X100).

(G): atrophy of secondary lamellae (*O. niloticus*) (X100).

(H): dilation and congestion in the blood vessels of gill filaments (*O. niloticus*) (X400).

Fig. (2): Sections of gills of fish showing:

(A): bulging with blood at the tips of lamellae (*O. niloticus*) (X400).

(B): dark deposits on the surface of gill epithelia (*O. niloticus*) (X400).

(C)&(D): nodular proliferation in gill filaments (*O. niloticus* and *T. zillii*, respectively) (X100).

(E): dilation in the blood vessel of gill filament (*T. zillii*) (X400).

(F): haemorrhage in secondary lamellae (*T. zillii*) (X400).

(G): edema in secondary lamellae (*T. zillii*) (X100).

(H): degenerative and necrotic changes in the epithelium of gill filaments and secondary lamellae (*T. zillii*) (X100).

Fig. (3): Sections of gills of fish showing:

(A)&(C): proliferative changes in the epithelium of gill filaments and secondary lamellae (*T. zillii* and *C. lazera*. respectively) (X100).

(B): inflammatory cells among the proliferated epithelial cells (*T. zillii*) (X400).

(D): degenerative and necrotic changes in the epithelium of gill filaments and secondary amellae and curling of secondary lamellae (*C. lazera*) (X100).

(E)&(F): bulging at the end of secondary lamellae (*C. lazera*) (X100&400, respectively).

Fig. (4): Sections of liver of fish showing:

(A): control liver (X400).
(B): vacuolar degeneration of the hepatocytes (*O. niloticus*) (X400).
(C): necrosis between the hepatocytes (*O. niloticus*)(X400).
(D): haemorrhage between the hepatocytes (*O. niloticus*) (X400).
(E): haemosiderin between the hepatocytes (*O. niloticus*) (X400).
(F): inflammation between the hepatocytes (*O. niloticus*) (X400).
(G): dilation in central vein (*O. niloticus*) (X100).
(H): haemosiderin around hepatoportal blood vessels (*O. niloticus*) (X100).

Fig. (5): Sections of liver of fish showing:

(A): dilation and congestion in hepatoportal blood vessels and haemorrhage around them (*O. niloticus*) (X400).
(B): thrombosis formation in hepatoportal blood vessels and haemorrhage around them (*O. niloticus*) (X400).
(C): thrombosis formation, dilation and congestion in hepatic blood vessels and edema around them (*O. niloticus*) (X100).

Fig. (6): Sections of liver of fish showing:

(A): vacuolar degeneration of the hepatocytes (*T. zillii*) (X400).
(B): haemorrhage between the hepatocytes (*T. zillii*) (X400).
(C): dilation and congestion in central veins (*T. zillii*) (X400).
(D): thrombosis formation in hepatoportal blood vessels and haemosiderin and fibrosis around them (*T. zillii*) (X100).
(E): degeneration in the wall of hepatoportal blood vessels (*T. zillii*) (X400).
(F): dilation and haemolysis in hepatoportal blood vessels (*T. zillii*) (X100).

Fig. (7): Sections of liver and kidneys of fish showing:

(A):vacuolar degeneration of the hepatocytes (*C. lazera*) (X400).
(B): necrosis between the hepatocytes (*C. lazera*)(X400).

(C): dilation and congestion in hepatic blood vessels and haemorrhage and haemosiderin around them (*C. lazera*) (X100).
(D):thrombosis formation in hepatic blood vessels (*C. lazera*) (X100).
(E): control kidney (X400).
(F): vacuolar degeneration in the epithelium of renal tubules (*O. niloticus*) (X400).
(G): haemorrhage between the renal tubules (*O. niloticus*) (X400).
(H): haemolysis between the renal tubules (*O. niloticus*) (X400).

Fig. (8): Sections of kidneys of fish showing:
(A): focal areas of necrosis between renal tubules and depletion in the haemopoietic areas (*O. niloticus*) (X400).
(B): focal areas of necrosis between renal tubules (*C. lazera*) (X400).
(C)&(D): edema in Bowman`s capsules with atrophy in glomeruli (*O. niloticus*) (X400).
(E)&(F): haemolysis and haemorrhage between the renal tubules (*C. lazera*) (X400).
(G): haemosiderin between the renal tubules (*C. lazera*) (X400).
(H): dilation and haemolysis in renal blood vessels (*C. lazera*) (X400).

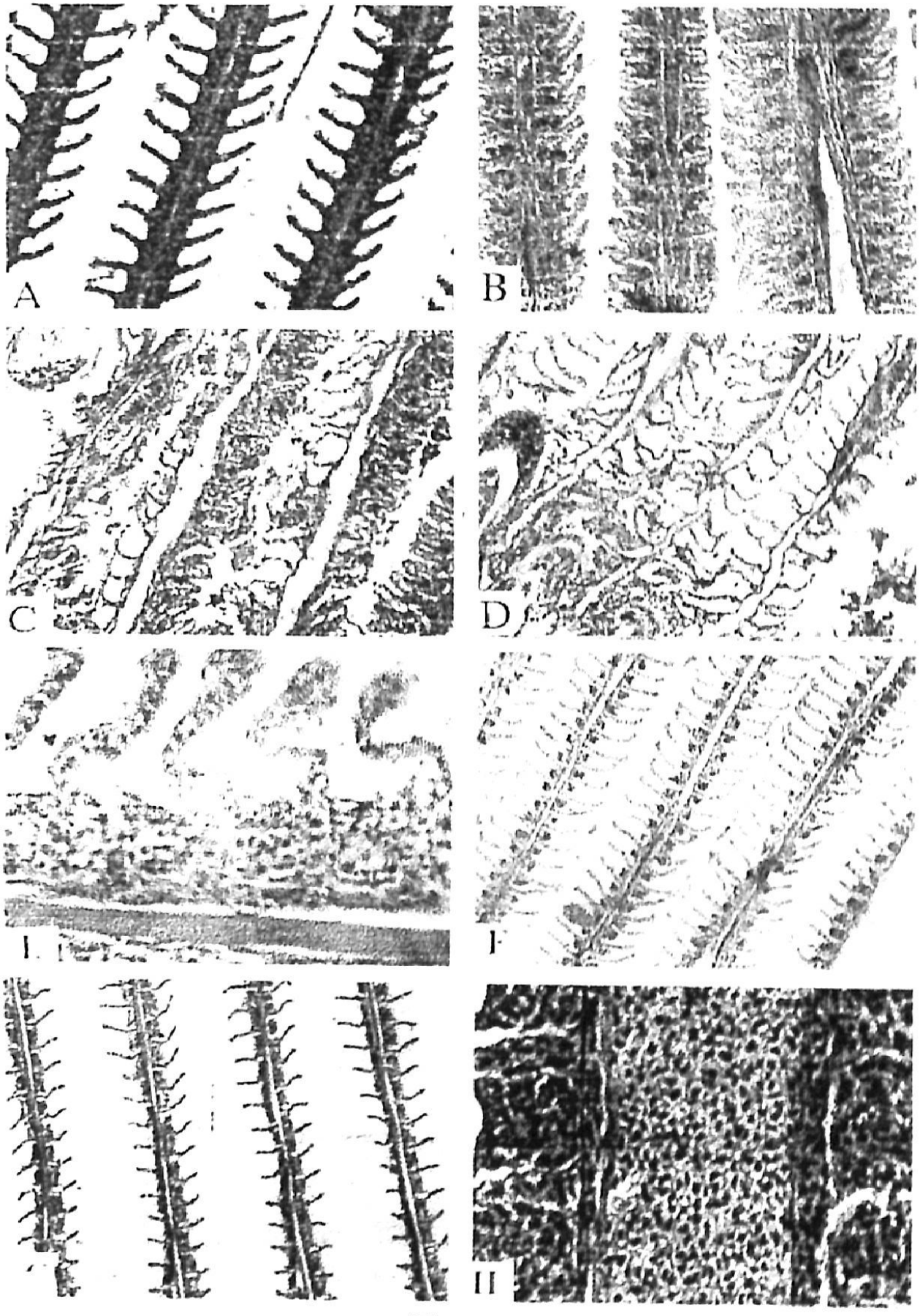


Fig. 1

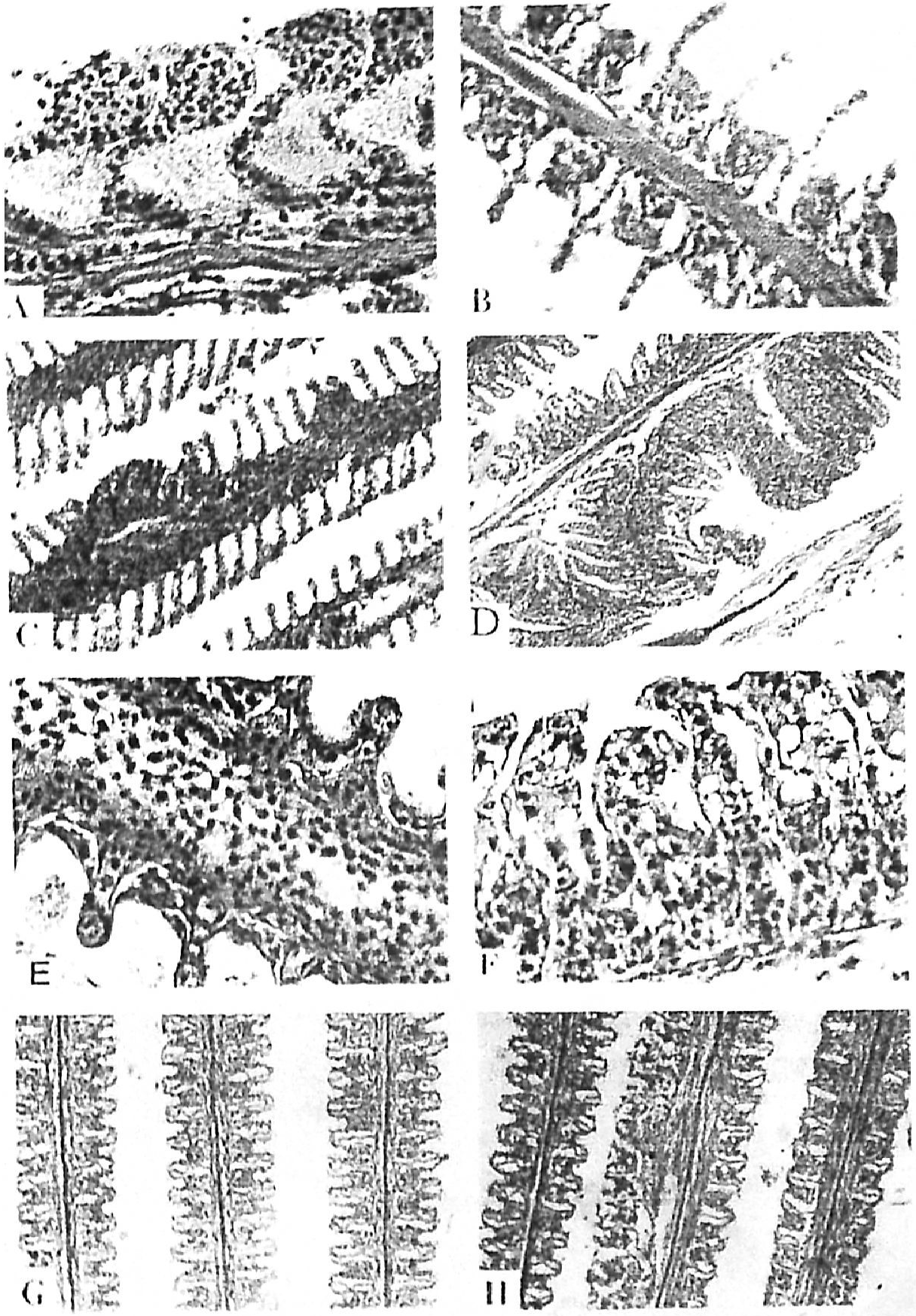


Fig.2

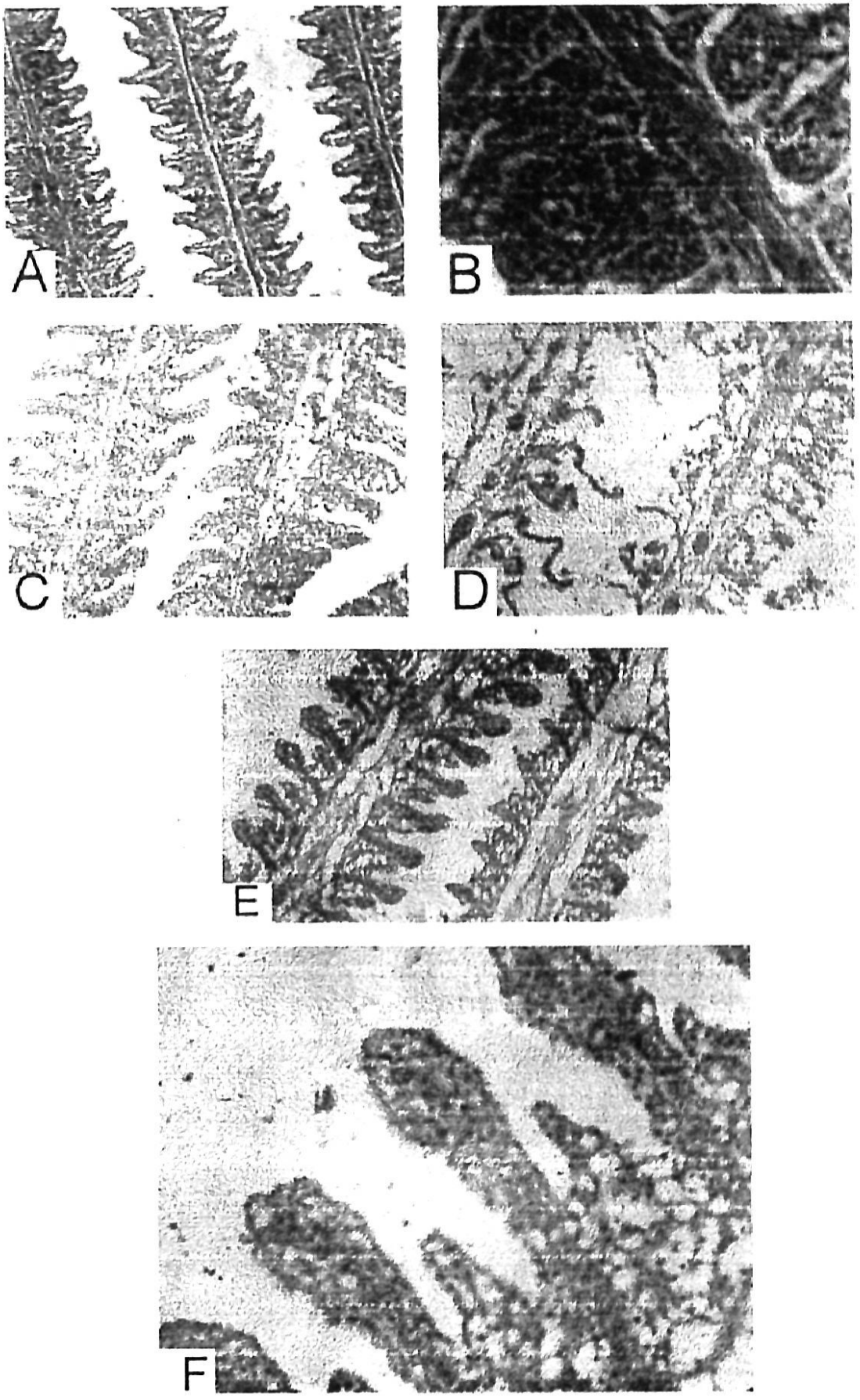


Fig. 3

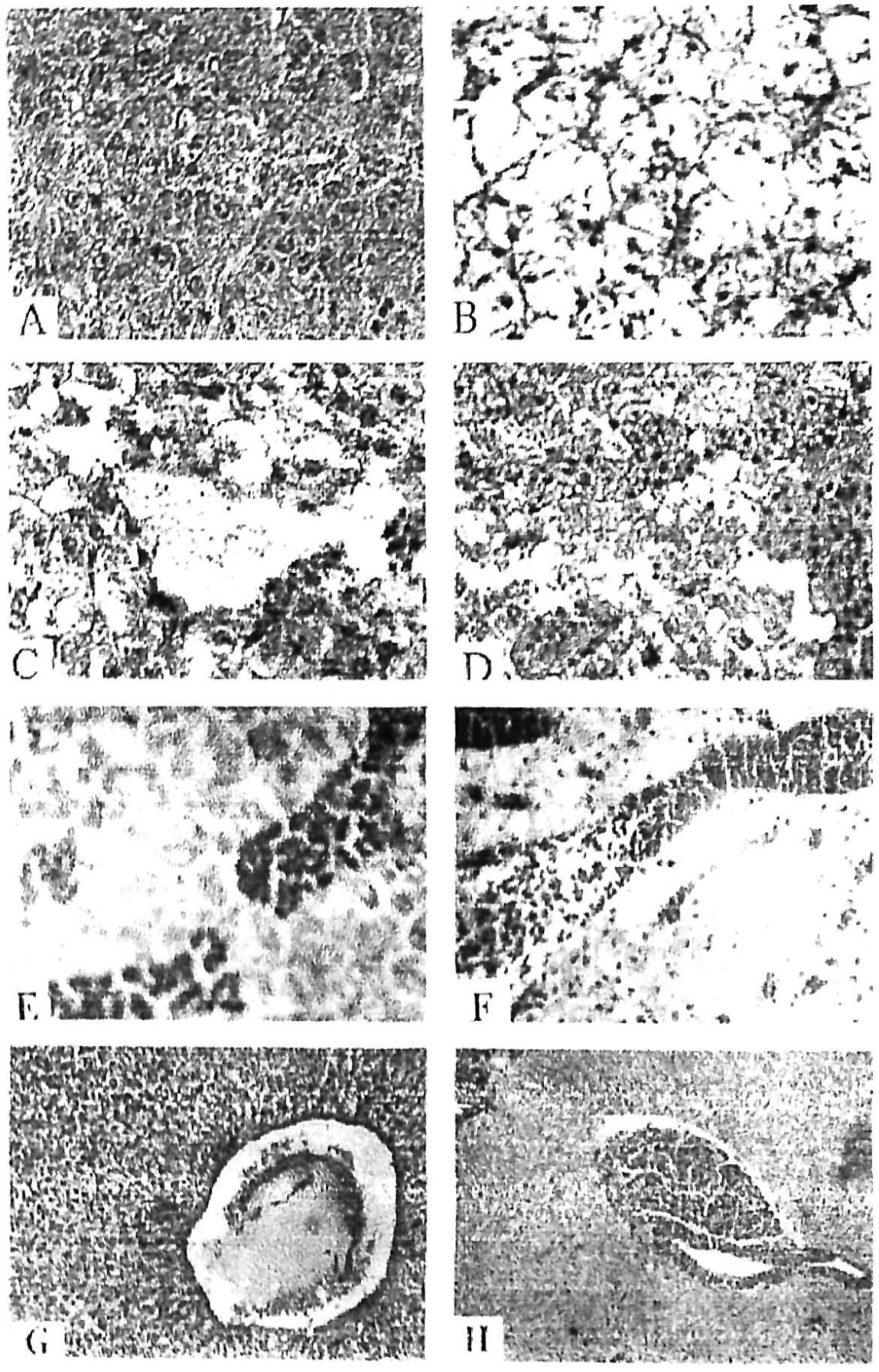


Fig. 4

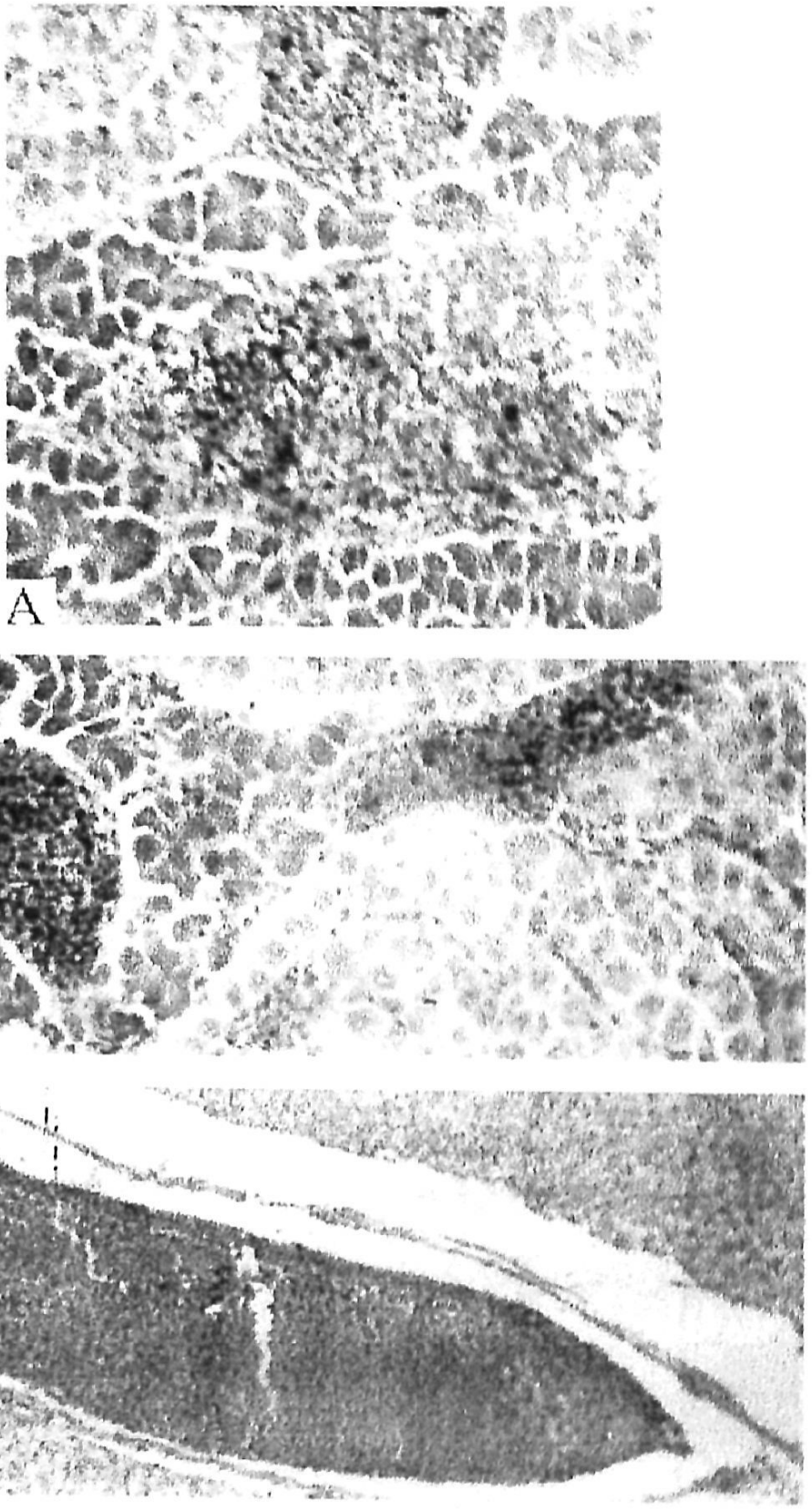


Fig. 5

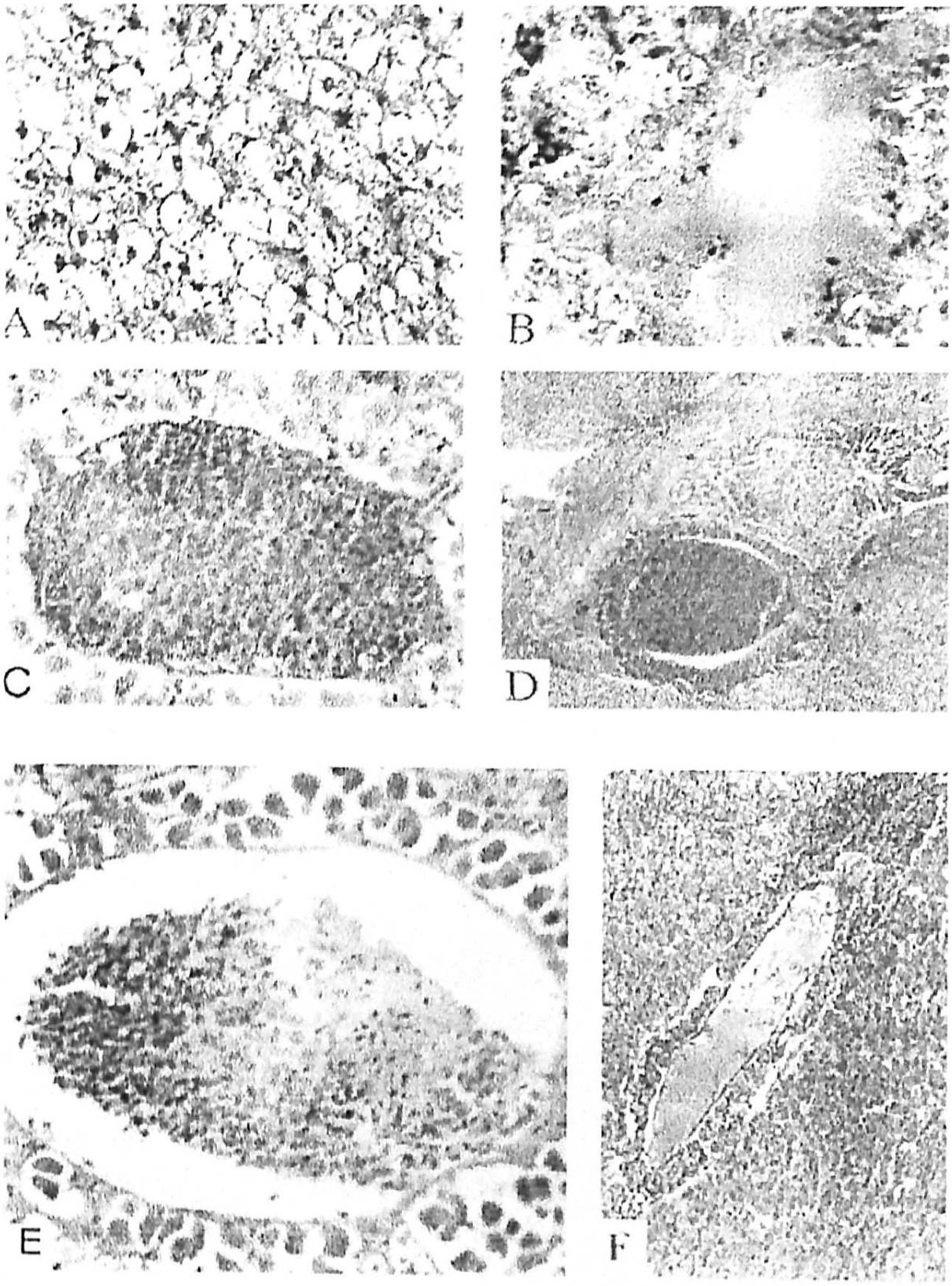


Fig. 6

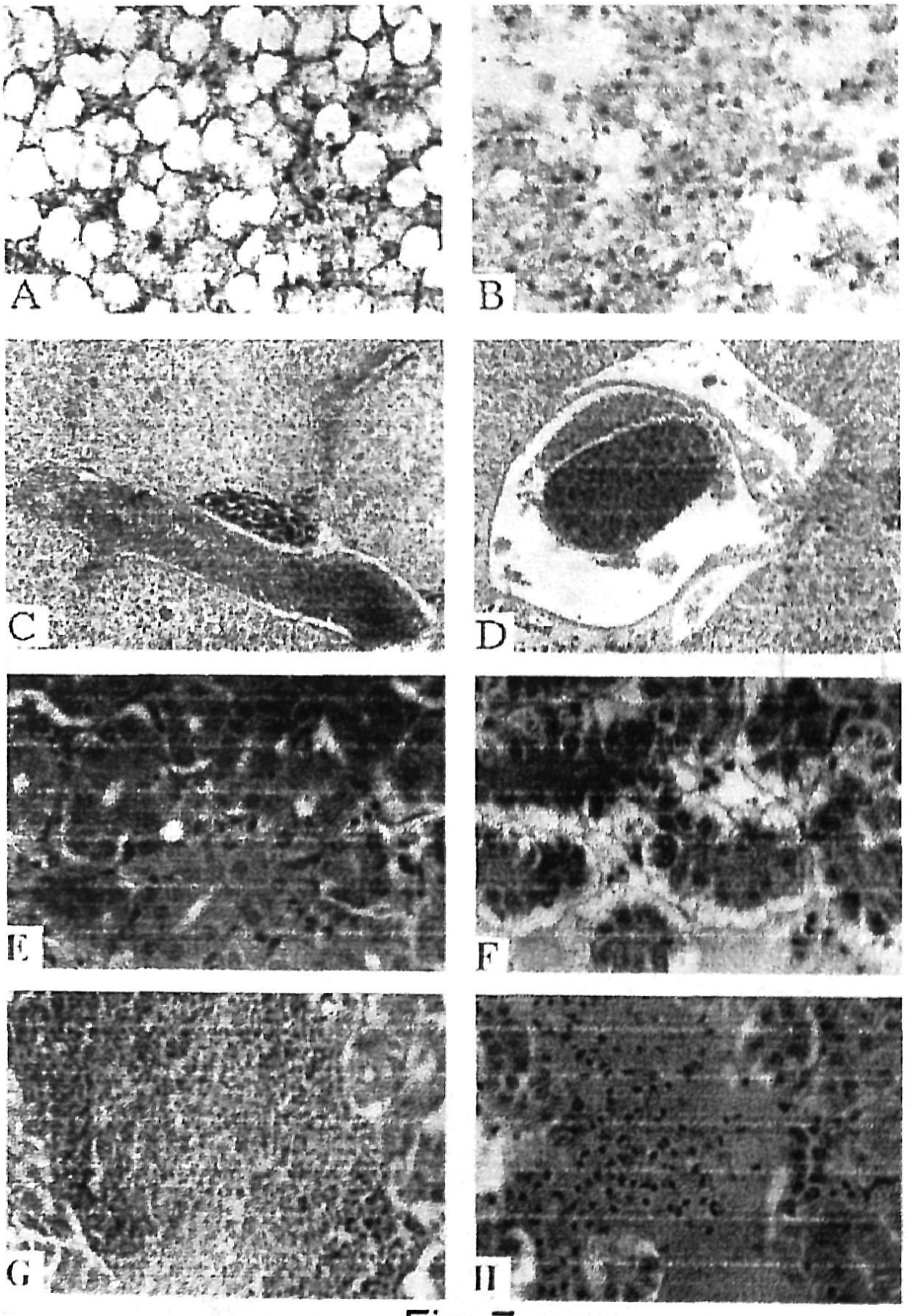


Fig. 7

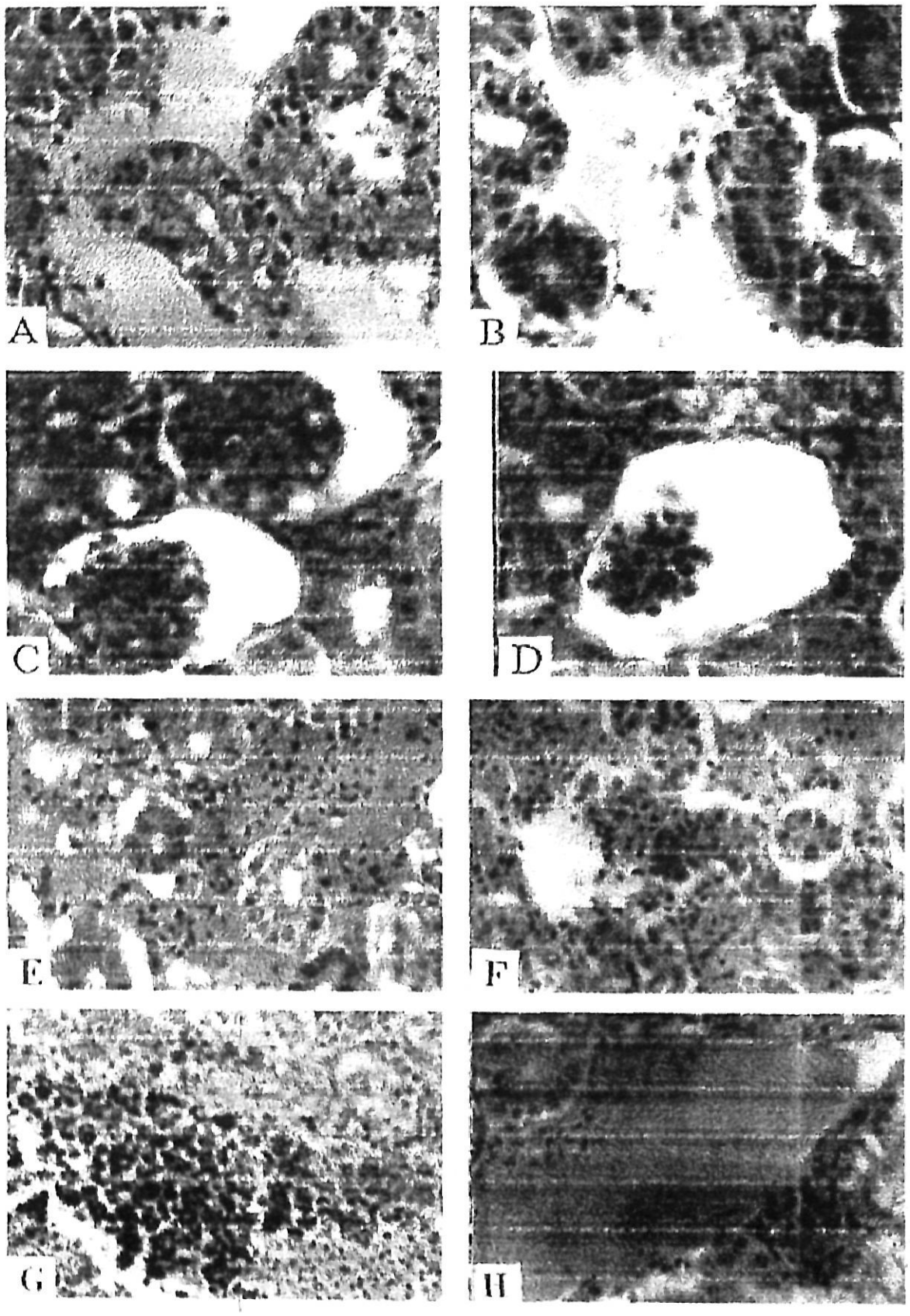


Fig. 8