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EFFECT OF HEAVY METALS ACCUMULATION ON ENZYME ACTIVITY AND HISTOLOGY IN LIVER OF SOME NILE FISH IN EGYPT

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Key words: River Nile, fish, heavy metals, histopathology, liver, GOT, GPT.

ABSTRACT

During spring, 2004, samples of *Tilapia zillii, Clarias gariepinus* and *Mugil cephalus* were collected from Nile water at El- Kanater region in order to study the effect of heavy metals (Fe, Mn, Zn , Cu ,Pb and Cd) accumulation on the enzyme (GOT & GPT) activity as well as livers tissues of the three fish species. The results showed that Fe, Mn, Zn, Cu, Pb and Cd accumulation in the livers of the above three fish species ranged between 802.626 –1021.97, 9.731 – 26.061 and 123.656- 166.364,34.144–116.364, 27.957 – 89.394 , 16.061 – 42.742 µg/g dry wt respectively.

The main observed lesions were (1) dilation and congestion in blood vessel and blood sinusoids (2) hemorrhages in hepatocytes (3) hemolysis and hemosidrine pigment (4) degeneration and necrosis in hepatocytes (5) fatty degeneration in hepaocytes (6) parasite cysts in liver tissues of *Tilapia zillii.*

Increase in GOT and GPT activities was associated with liver cells damage. These changes in enzymes activity and lesions in liver were recorded by the following order: *Tilapia zillii* > *Clarias gariepinus* > *Mugil cephalus.*

INTRODUCTION

The increase of population density with the heavy industrialization and agricultural activities have resulted in more and more wastes entering the River Nile. These wastes lead to changes in the Nile water quality, including the increase of turbidity , suspended solids, total

Salem and Khalifa (2003) reported that several factors affected the disappearance of some fish species from the River Nile. The first was the High Dam construction and the second was the changes in water quality.

Recently, several works were conducted on the effect of water pollutants on fish organs and the estimation of the amount of heavy metals on these organs (Elewa. 1993; Masoud *et al.*, 1994; Abdel Satar. 1998; Sayed. 1998; Yacoub. 1999; Abdel Satar and Shehata. 2000; Elewa *et al.*, 2001 ; Mahmoud. 2002)

Some heavy metals such as Zn, Cu, Mn and Fe are essential for growth and well being of living organisms including man. However. they likely show toxic effects when organisms are exposed to levels higher than normally required. Other elements such as Pb and Cd are not essential for metabolic activities and exhibit toxic properties even with traces levels. Many heavy metals including Hg, Cd, Pb, As and Cu. inhibit photosynthesis and phytoplankton growth. At higher trophic levels, they have delayed embryonic development, causing malformation and reduced growth of adult fishes, molluscs and crustaceans (FAO. 1992).

The present study aimed to evaluate the effect of six heavy metals (Fe. Mn. Zn. Cu. pb and Cd) on the three fish species inhabiting the River Nile. indicating by histological changes in the liver of *Tilapia zillii, Clarias gariepinus* and *Mugil cephalus* as well as the enzyme activity of GOT and GPT in the serum of these fishes.

MATERIAL AND METHODS

1- Fish samples:

Samples of adult healthy *Tilapia zillii. Clarias gariepinus and Mugil cephalus* were collected from the River Nile sector of El-Kanater region (26 km north of Cairo). The weights of specimens ranged between 30-355. 286.5 – 490.0 and 12.0- 530.0 mg/kg. for *Tilapia zillii, Clarias gariepinus* and *Mugil cephalus* respectively.

2- Heavy metals

Liver samples of different fish species were dried in an oven at 105°C for about 24 hour. then ground to fine powder. 0.2 gm dry weight of the liver of each was taken, and digested according to the method described by Goldberg *et al.* (1993). Concentrated nitric and perchloric acids (AR grade) with ratio of 5: 5 were used in teflon beakers on a hot plate, at 50°C for about 5 hours till complete decomposition of organic matter. The digested samples were cooled.

beakers on a hot plate, at 50°C for about 5 hours till complete decomposition of organic matter. The digested samples were cooled, filtered and diluted to a final volume of 50 ml with deionized distilled water. The concentration of iron, manganese, zinc, copper and cadmium were measured by atomic absorption spectrophotometer, Hitachi model, 170-30. Results were expressed in µg/kg dry weight of liver tissue.

3- Biochemical experiments:

Blood samples were taken by severance of the caudal peduncle of fish and collected into two small sterilized vials. The blood was left to clot and then centrifuged at 3000 r.p.m for 10 minutes to obtain serum. Supernatant serum was obtained using micropipette model (Labystems K 33071) for biochemical studies. The levels of serum glutamic oxaloacetic transaminase (GOT), and serum glutamic pyruvic transaminase (GPT) were measured, using kits of Biomericux France.

4- Histopathological examination:

Tissue specimens from the liver of the studied fishes were fixed in 10% phosphate buffer formalin, followed by dehydration through an ascending series of ethyle alc. Finally embedding in paraffin and sections of 5 microns were cut, using Euromex Holand Microtome and stained according to Harris hematoxylin and eosin method (1900). Then the sections were examined histopathologically by light microscope and photographed by a microscopic camera.

RESULTS AND DISCUSSION

The present study was carried out to correlate the accumulation of Fe, Mn, Zn, Cu, Pb and Cd in fish liver with lesions and changes in liver enzymes (GOT and GPT). Mersch *et al.* (1993) stated that fish can accumulate trace metals and act as indicators of pollution. The heavy metals concentrations in liver of fihes cought from EL- Kanater region are presented in Table (1).

The results indicated that the highest value of iron concentrations was recorded in *Tilapia zillii* (1021.97 µg/g dry wt.), followed by *Clarias gariepinus* (899.140 µg/g dry wt) and the lowest value was recorded in *Mugil cephalus* (802.626 µg/g dry wt.).The increase of iron concentration may be related to the increase of total dissolved iron in Nile water and consequently increase the free metal iron concentration and thereby lead to an increase in metal uptake by different organs (Carbonell *et al.*, 1998).

26.061 µg/g dry wt. (Table 1). The accumulated Mn was by the following order: *Tilapia zillii* > *Mugil cephalus* > *Clarias gariepinus*.

Zinc concentration in the liver of the present fish species ranged from 123. 656 to 166.364 µg/g dry wt. (Table 1). Zinc concentrations were recorded in the following order: *Tilapia zillii.* > *Mugil cephalus.* > *Clarias gariepinus.* Mining. smelting and sewage diposal are the major sources of zinc pollution. Koli *et al.* (1978) reported that Zn concentrations in muscle tissue of fish species from non-polluted areas were less than 10 ppm. However. the present study revealed that zinc levels in all fishes species were significally higher than 10 ppm.

Copper is a fundamental micronutrient to all forms of life. in enzyme activity or random rearrangement of natural proteins (Bower. 1979). Copper concentration in the livers of present fish ranged from 37. 424 to 116.364 µg/g dry wt. (Table 1). The concentrations of Cu were recorded in the following order: *Tilapia zillii.* > *Mugi cephalus.*> *Clarias gariepinus.* All examined samples showed higher Cu values than the permissible limit of WHO (10 ppm) reported by Reichenback Klinke (1974). The obtained results were also higher than that recorded by Abdel–Satar and Shehata. (2000) in the River Nile of EL- Kanater region. who showed that copper levels were lower than the permissible level for flesh of *Oreochromis niloticus* and *Tilapia zillii.*

Pb and Cd are known to be toxic at low concentrations. Both metals were reported by the following order: *Tilapia zillii.* > *Mugil cephalus.*> *Clarias gariepinus.* and *Clarias gariepinus.* > *Mugil cephalus.*> *Tilapia zillii.* respectively. The increase of Pb and Cd concentrations in fish tissues may be attributed to the domestic effluents discharged into the Nile water (Moon *et al.*. 1994 : Abdel-Satar and Shehata. 2000). The present study revealed that lead concentrations of the studied fish (Table.1) were more than USFDA maximum permissible level (2.0 µg/g in muscle) cited by Adeyeye (1993). For Cd concentration. the present levels were higher than the permissible limit (0.5µg/g dry wt) recorded by National Academy of Science (1972). On the other hand. the recommendations of National Health and Medical Research council in Australia (NHMRC). imply that the concentration of Cd and Pb in the edible part of fish should not exceed 2.0 µg/g (Bebbington *et al.* 1977).

The present study revealed marked differences among the concentrations of heavy metals in fish liver of the three studied fishes. The fish take the metal directly from water or through their food chain. There were higher levels of all the measured heavy metals in the livers of three species. These results agree with those reported by Yacoub and Abdel-Satar (2003), who showed similar patterns of Fe, Mn, Zn, Cu, Pb and Cd for some fish species in Bardawil lagoon. Also, they showed that these patterns were higher than their permissible limits. The higher concentration of metals in fish liver of *Tilapia zillii* than those of *Clarias gariepinus* and *Mugil cephalus* may be attributed to the higher tolerance of *Tilapia zillii* than the other species (Abdel Satar and Shehata, 2000).

Histological examination of the liver samples of the examined fish species showed dilation and thrombosis in blood vessels (Fig. 1), hemolysis and hemosidrine due to destruction of erythrocytes (Fig.2), hemorrhagic areas and inflammatory hepatic cellular infiltration (Fig.3). In addition, congesion in blood sinusoids appeared in all liver tissue (Fig. 4).

The liver sections of *Tilapia zillii* showed severe degeneration and vacular necrosis in hepatocyts (Fig. 5). Some hepatocytes contained large fat vacoules, fatty degeneration (Fig. 6) as well as odema (Fig. 7). Also, the liver of *Tilapia zillii* contained parasitic cysts in blood vessel walls (Fig. 8).

The lesions in the hepatocytes of *Clarias gariepinus* showed degeneration of nuclei, accumulation of lipid and focal necrosis (Figs. 9 and 10). The liver sections of *Mugil cephalus* showed degeneration in hepatocytes, hemolysis in RbCs, and dilation of blood vessels (Fig. 11).

The present study suggests a strong link between heavy metals and lesions in the liver. Sorensen *et al.* (1980) cited that heavy metals in Elbe water might cause liver damage. Similar results were obtained by Aly *et al.* (2003) after exposure of *Clarias gariepinus* to lead pollution. They found that the vacuolar degeneration and necrosis of hepatocytes may appear after 3 days but after 2 weaks, hemolysis of red blood corpusles and vacuolar degeneration as well as nerosis of hepatocytes were observed.

Similar alterations in the liver of *Tilapia zillii and Clarias gariepinus* were observed in fishes living in Nile water polluted with heavy metals and ammonia (Yacoub, 1999 ; Tayel, 2003).

Yacoub and Abdel-Satar (2003) studied the effect of heavy metals on some fishes inhabiting Bardawil lagoon. They observed degeneration and vacuole necrosis in hepatocytes, with, hemolysis and hemosidrin prigments.

The fatty degenerative changes in *Tilapia zillii* liver, may be due to a decrease in the rate of utilization of energy reserve or pathologically enhance synthesis (Desai *et al.*, 1984). Also, the abnormal accumulation of fats in experimental animals could be due to induced imbalance between fat production and utilization (Kadry, 1997). In general, all fish livers contained hemosidrin pigments that may result from destruction of erythrocytes. Breakdown of hemoglobin converting into hemosidrin was responsible for the brown deposits within hepatic tissues (Fayed, 2004). Several factors have been held responsible for the abnormal accumulation of hemosidrin in the liver tissue. some were named by Strassmann (1974) and Mazhar *et al.* (1986) to be rapid and continue destruction of erythrocytes with increased hemolysis and damage of the iron metabolism.

El-Kanater region was found to contain high level of iron (0.9 mg/l) (Yacoub, 1999 : Mahmoud 2002). This large amount of iron might be absorbed through the intestinal mucosa, leading to abnormal accumulation of hemosidrin in the liver (Yacoub, 1994). Some studies also revealed a link between heaptic alteration and hemosidrin pigments (Khan, 1998 and 1999).

El-Banhawy and El-Ganzuri, (1980) reported that lysosomal membranes which are very sensitive to many pathogenic factors are disrupted and thus their enzymes released and caused degeneration and vacuolation of cytoplasm of liver cells.

The transaminases, GOT and GPT are two key enzymes considered as a sensitive measure to evaluate hepatocellular damage and some hepatic diseases (Todd, 1964). The increased serum aminotransferases might reflect myocardial and hepatic toxicity, leading to extensive liberation of the enzymes into the blood (Heath, 1987 ; Daabess *et al.*, 1992 ; Abo-Hegab *et al.*, 1993).

The present study showed a general trend of increase in GOT and GPT activities in *Tilapia zillii*, followed by *Clarias gariepinus* and *Mugil cephalus*. (Table 2). The present findings coincide with the reported histopathological lesions, which revealed a marked degeneration and necrosis of hepatocytes as the elevation in transaminases activities may be attributed to liver injury (Kristaffersson and Okari, 1974. : Aly *et al.* 2003). The results indicate that the pollutants of Nile water affect the liver cells and GOT and GPT activities.

Finally, it can be concluded that the livers of the fishes *Tilapia zillii, Mugil cephalus and Clarias gariepinus* were contaminated with heavy metals that were elevated by the discharge of different wastes into the River Nile at El-Kanater region. The results indicate that the examined heavy metals caused different degrees of injuries in the fish livers with the increase in GOT and GPT activities.

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LEGEND OF FIGURES

Photomicrographs of:

(Fig. 1): Section of liver of *Tilapia zillii* obtained from EL-Kanater region showing dilation and thrombosis formation in blood vessel (B). (H. & E. X400)

(Fig. 2): Section of liver of *Mugil cephalus* obtained from El-Kanater region showing hemolysis (H) and hemosidrine (S). (H. & E. X400)

(Fig. 3): Section of liver of *Mugil cephalus* obtained from EL-Kanater region showing hemorrhages (R). (H. & E. X400)

(Fig. 4): Section of liver of *Clarias gariepinus* obtained from El-Kanater region showing congesion in blood sinusoid (C). (H. & E. X100)

(Fig. 5): Section of liver of *Tilapia zillii* obtained from El-Kanater region showing severe degeneration (D) and vacuolar necrosis in hepatocyts (n). (H. & E. X400)

(Fig. 6): Section of liver of *Tilapia zillii* obtained from El-Kanater region showing fatty degeneration (F). (H. & E. X400)

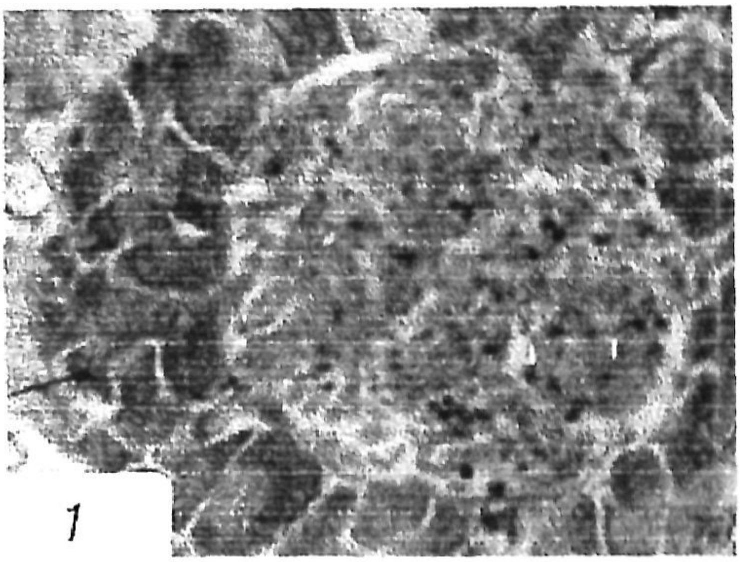
(Fig. 7): Section of liver of *Tilapia zillii* obtained from El-Kanater region showing Odema (O). (H. & E. X400)

(Fig. 8): Section of liver of *Tilapia zillii* obtained from EL-Kanater region showing parasite cysts (P). (H. & E. X400)

(Fig. 9): Section of liver of *Clarias gariepinus* obtained from EL-Kanater region showing degeneration in nuclei (D). focal necrosis (n). (H. & E. X400)

(Fig. 10): Section of liver of *Clarias gariepinus* obtained from EL-Kanater' region showing fatty degeneration (F). (H,& E. X400)

(Fig. 11): Section of liver of *Mugil cephalus* obtained from EL-Kanater region showing degeneration in liver cell (D). hemolysis (H). (H. & E. X400)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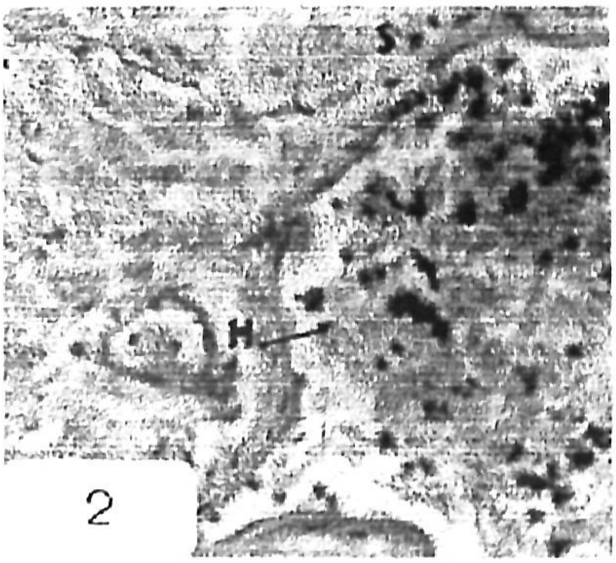
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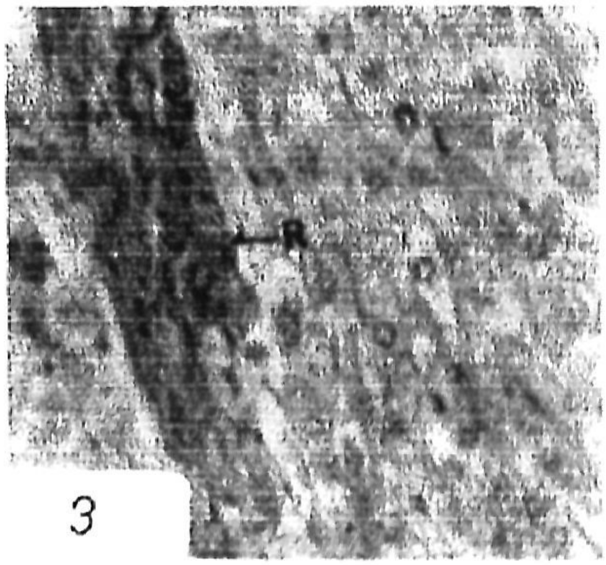
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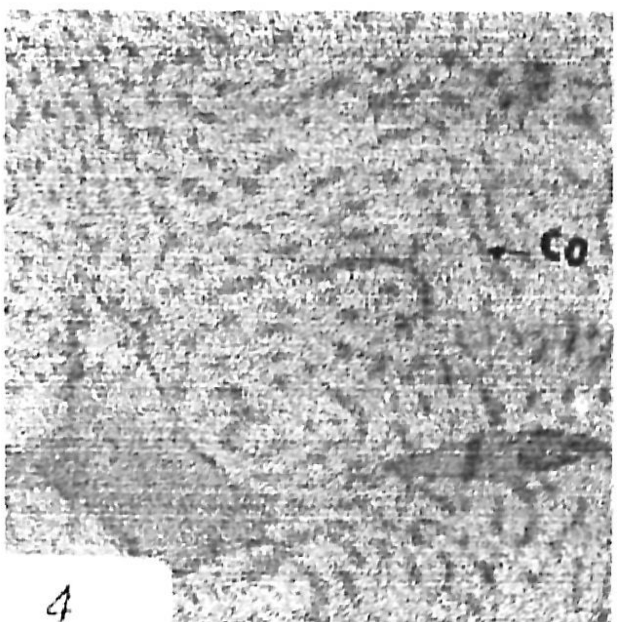
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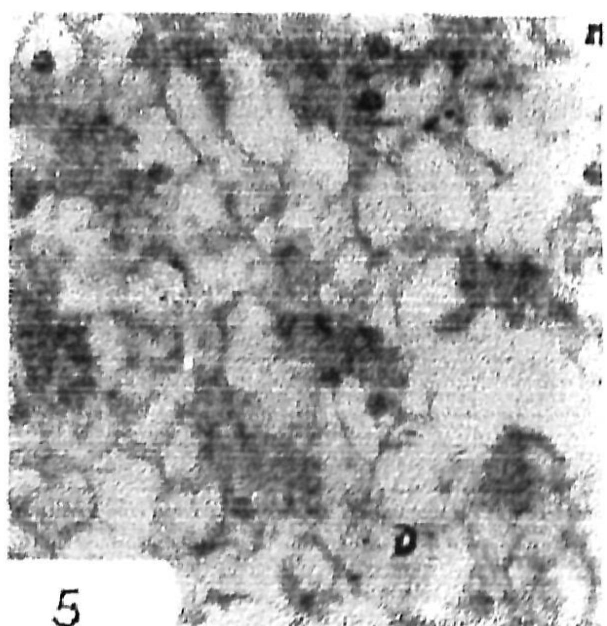
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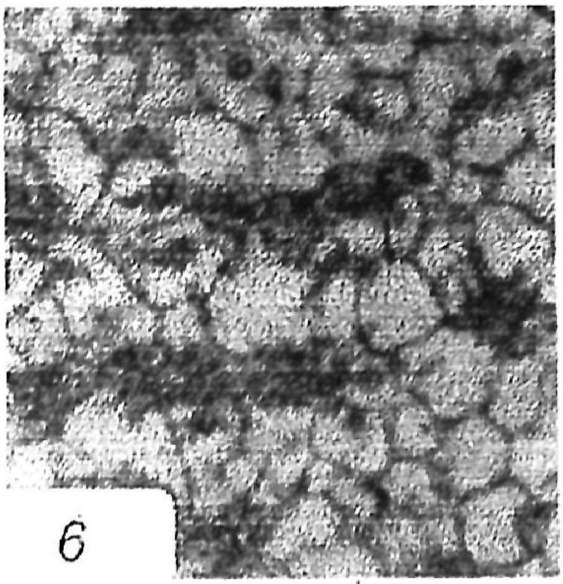

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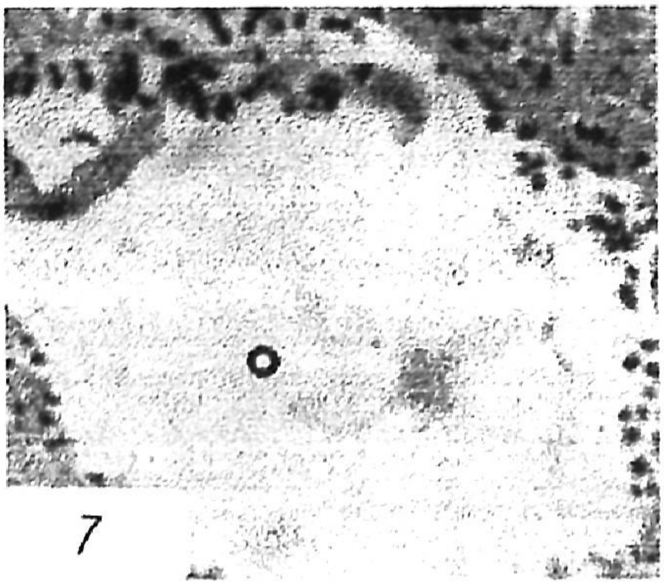

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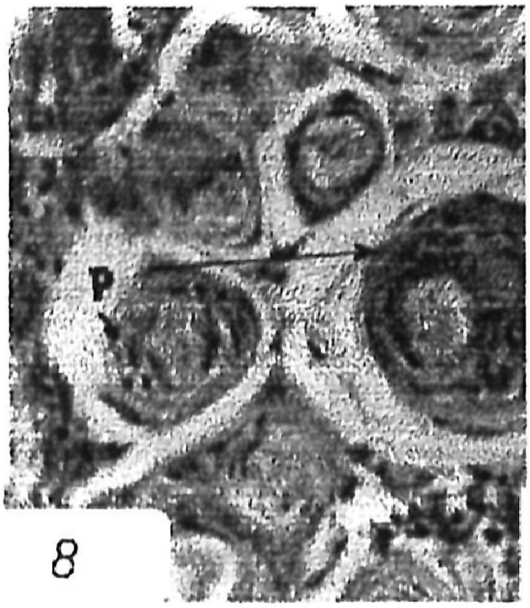

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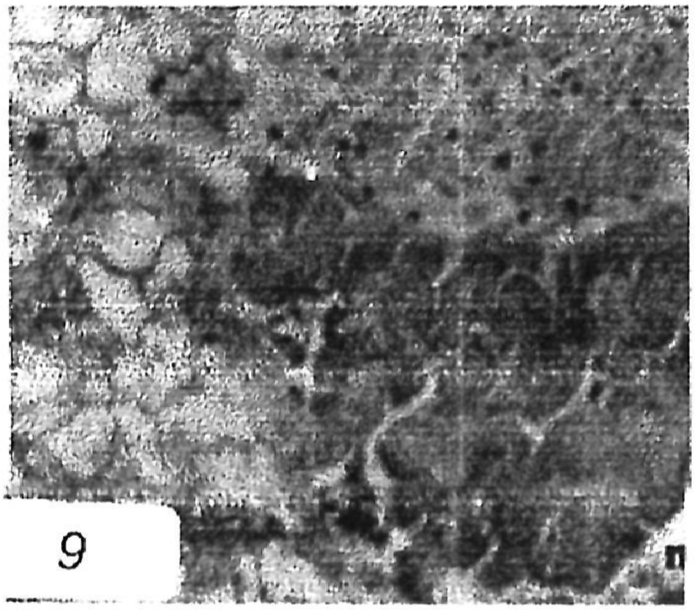

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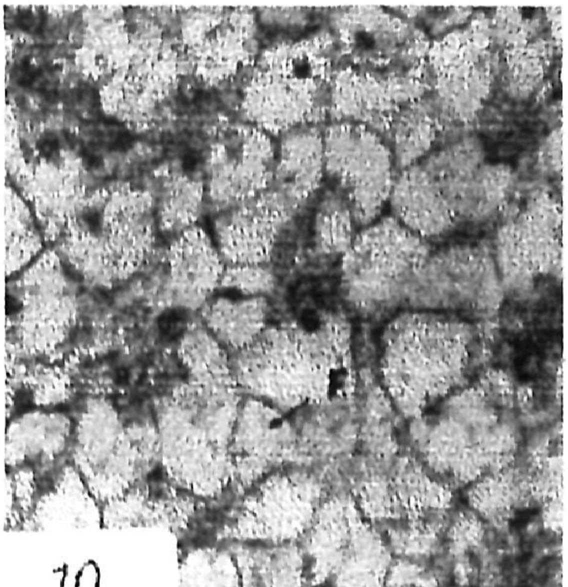

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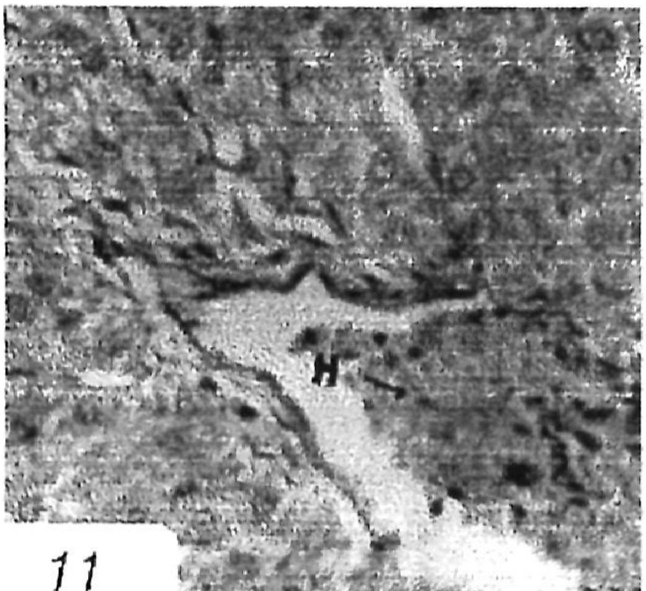

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