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HEAVY METALS ACCUMULATION AND MACRONUTRIENTS IN THE LIVERS OF SOME FISH SPECIES OF BARDAWIL LAGOON AND THEIR HISTOLOGICAL CHANGES

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Key Words: Bardawil Lagoon, fish, liver, heavy metal, Histopatholgy.

ABSTRACT

Fish samples of *Solea vulgaris, Mugil cephalus, Liza ramada* and *Sparus aurata* were collected during November 2001 from El Telol location in Bardawil lagoon, north eastern of Egyptian Mediterranean coast . The study was conducted to assess the effect of the heavy metals (Fe, Mn, Zn, Cu, Pb and Cd) and the macronutrients (Na, K, Ca, and Mg) on livers tissues of the four fish species. The results showed that the concentrations of Fe, Mn, Zn, Cu, Pb, and Cd in the livers of the four fish species ranged between 48.680- 121.940, 1.270- 2.170, 15.220- 32.570, 82.280- 301.830, 1.100- 4.960 and 0.180- 0.300 mg/ Kg respectively. The concentrations of Na, K, Ca and Mg in the livers of the four species ranged between 1131.60- 1702.00, 327.60- 507.00, 74.490- 398.300 and 72.180- 170.590 mg/ Kg respectively. Microscopical examination of livers tissues of *Solea vulgaris, Liza ramada, Mugil cephalus* and *Sparus aurata* showed different degrees of injuries, including fatty degeneration, ballooning degeneration, accumulation of hemosiderin pigment and necrosis.

INTRODUCTION

Bardawil lagoon is a salt water lagoon in the northern coast of Sinai province of Egypt, with an average water spread area of about 1600Km^2. Its average depth is 2.5m. It is connected to the Mediterranean Sea through two Boughases (Fig. 1). The lagoon is subjected to tidal effects, arid conditions and high evaporation. Yitzhak (1971) studied the cations and anions of water samples collected from three parts of the lagoon, also sediment was examined by X Rays. Siliem (1988 a and b) determined the major cations namely Sodium, Potassium, Calcium and Mágnesium as well as the major anions namely sulphate and bromide, with their distribution and chlorinity ratios. The anions chlorinity ratios were higher in the lagoon than in normal seawater. Siliem (1989 a, b and c) studied some limnological characteristics of the lagoon with the distribution of nutrients and the enrichment ratio of the major cations and anions.

The importance of fisheries economy of Egypt depends mainly on the Egyptian lakes. So, it was necessary to make intensive studies on the environment of these lakes to evaluate their productivity with regard to their fish production. The General Authority for Fish Resources Development (2002) reported that production of fishes and crustaceans decreased in Lake Bardawil during the last decade as illustrated in Table (1). The production of the lagoon decreased to the minimum level in 1994.

Fishes often lie at the top of aquatic food chain and may accumulate large amounts of metals. These metals accumulate differently in fish organs (kidney, brain, muscles, gills, gonads and liver)(Van Hassel *et al.*,1980; Moriarty, 1984; Dallinger *et al.*, 1987; Mason,1987; Barak and Mason, *et al.*, 1990; Naqvi and Howel,1993; Abou Arab *et al.*, 1995 ; Gomaa *et al.*, 1995).

Some heavy metals such as Zn, Cu, Mn and Fe are essential for growth and well being of living organisms including man. However, they are likely to 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. At low concentrations, many heavy metals including Hg, Cd, Pb, As and Cu, inhibit photosynthesis and phytoplankton growth. Effects at higher trophic levels include delayed embryonic development, malformation and reduced growth of adult fishes, molluscs and crustaceans (FAO, 1992).

A knowledge of health condition of common fishes of Bardawil lagoon is necessary to explain the decline of fish production. The present study aimed to compare the effect of accumulation of six heavy metals (Zn, Cu, Cd, Pb, Fe and Mn) and four macronutrients (Mg, Ca, Na, and K) on the liver tissue in four common fish species in Bardawil lagoon, namely *Solea vulgaris* (Linnaeus), *Mugil cephalus* (Linnaeus), *Liza ramada* (Risso) and *Sparus aurata* (Linneaus).

MATERIAL AND METHODS

a- Collection of fish samples :

Fish samples of *Solea vulgaris, Mugil cephalus, Liza ramada* and *Sparus aurata* were collected during November, 2001from El Telol location in Bardawil lagoon. The length and weight of each specimen was recorded. Specimens were dissected and their livers were carefully removed and fixed in 10% formalin for histological examination, other liver specimens were kept frozen for analysis of metals.

The weights of specimens ranged between 69.5- 139.6, 102.5-138.2, 110- 168.8 and 115.4- 170 gm for *Solea vulgaris, Sparus aurta, Mugil cephalus* and *Liza ramada* respectively.

b- Method of metals analysis :

Liver tissues of different fish species were dried in an oven at 105°C for about 24 hours, then ground to fine powder. A representative sample of 2 gm dry weight of liver was taken from fish specimens. The samples were digested according to the method described by Goldberg *et al.* (1993) in which concentrated nitric and perchloric acid (AR grade) with ratio of 5 ml +5ml were used in Teflon beakers on a hot plate, at 50°C for about 5 hours till complete decomposition of organic matter. The digested solutions were cooled to room temperature, filtered and diluted to a final volume of 50 ml deionized distilled water. The concentration of Zinc, Copper, Cadmium, Lead, Iron, Manganese, Magnesium, Calcium, Sodium and Potassium were measured by (I. C.P.) Plasma 400. Results were expressed in mg/Kg dry weight of liver tissue .

c- Histological method :

About five fishes were dissected from each species. Liver specimens were carefully removed and immediately fixed in 10% formalin, then dehydrated in ascending grades of alcohol,cleared in xylene. The fixed tissues were embedded in parafin wax, sectioned at 5 microns, stained with haematoxylin and eosin (H &E) and examined microscopically.

RESULTS AND DISCUSSION

The results indicated that iron concentrations in livers of the four fish species ranged from 48.680 to 121.940 mg/Kg dry weight (Table 2). The concentrations of iron were of the following order : *Mugil cephalus* < *Solea vulgaris* < *Liza Ramada*<*Sparus aurta.* U.S.recommended 0.5gm iron daily dietary allowance supplied by 100gm serving of fish muscle as cited by Adeyeye (1993a). The concentrations obtained in the present study were significantly higher than the allowed level in all fish species.

Manganese concentrations in the livers of the four fish species ranged from 1.270 to 2.170 mg/Kg dry weight (Table 2). The concentrations of manganese were detected in the following order : *Mugil cephalus* < *Sparus aurata* < *Liza ramada* < *Solea vulgaris.*

Manganese functions as an essential constituent for bone structure, reproduction and normal functioning of the enzyme System (Fleck,1976). Manganese is toxic only when present in higher amount, but at low levels is considered essential as micronutrient (Sarkka *et al.*,1978).

Zinc concentrations in the livers of the present fish species ranged from 15.220 to 32.570 mg/Kg dry weight (Table2). The concentrations of zinc were recorded in the following order : *Solea vulgaris* < *Mugil cephalus* < *Liza ramada* < *Sparus aurata.* Zinc is an essential element and a common pollutant as well. Mining, smelting and sewage disposal are major sources of zinc pollution. It is taken up by fish directly from water especially by mucous and gills (Skidmore, 1964). Koli *et al.* (1978) reported that Zn concentrations in muscle tissue of fish species from non-polluted areas. were less than 1ppm. In the present study, zinc levels in the four fish species in Bardawil lagoon were significantly higher than in non-polluted areas.

Copper concentrations in livers of the present fish species ranged from 82.280 to 301.830mg / Kg dry weight (Table 2). They were found in the following order : *Sparus aurata* < *Mugil cephalus* < *Solea vulgaris* < *Liza ramada.* Copper is a fundamental micronutrient to all forms of life, in enzyme activity or random rearrangement of natural proteins (Bower, 1979).

National Health and Medical Research Councils, recommended that the Standard Concentration of copper for human consumpution is 30.0mg/Kg (Bernard,1982). The concentrations of copper in the studied fish species in Bardawil lagoon were higher than the Standard Concentrations. Gainey and Kenyom (1990) mentioned that exposure of fishes to sublethal concentrations of copper leading to decrease in cardiac activity and reduction in heart rate.Khallaf *et al.*(1998) indicated that liver accumulated higher amounts of copper and which may be due to its ability to retain copper .The concentrations of lead in the livers of the four fish species ranged from 1.10 to 4.96 mg /Kg dry weight (Table2). They were present in the following order : *Sparus aurta* < *Mugil cephalus* < *Liza ramada* < *Solea vulgaris*.

USFDA (Food and Drug Administration) maximum permissible level for lead as cited by Adeyeye(1993b) is 2.0 mg/Kg in fish muscles. So, the present concentrations were higher than the maximum permissible level (MPL) in all fishes except *Sparus aurata* . Lead is toxic even at low concentrations and has no known function in biochemical processes. It is known to inhibit active transport mechanisms, involving ATP, to depress cellular oxidation reduction reaction and to inhibit protein synthesis (Waldron and Stofen, 1974). The major source of lead is the use of leaded gasoline. The concentrations of cadmium in the livers of the present fish species ranged from 0.18 to 0.3 mg /Kg dry weight (Table 2) .They were determined by the following order : *Solea vulgaris* < *Liza ramada* < *Mugil cephalus and Sparus aurata.* Cadmium levels in the investigated fish livers were found to be lower than the permissible limit (0.5mg /Kg) of National Academy of Science (1972). On the other hand, the recommendations of National Health and Medical Research Council in Australia (NHMRC), showed that the concentrations of Cd and Pb in the edible parts of fish should not exceed 2.0mg /Kg (Bebbington *et al.*,1977) . It is clear from the present study that, bioaccumulation of heavy metals in fish livers varied according to the fish species. Bryan and Uysal(1978) rported that, the feeding type and environmental conditions of the aquatic animals have an effect on their concentrations of elements. In general , animals take the metal directly from water or through their food chain. The levels of bioaccumulation of trace metals in various organs of fish reflect the degree of water pollution in the aquatic environment in which such fish are living.

Portman (1972) indicated that the rate of accumulation of heavy metals in aquatic organisms is positively correlated to their concentrations in the marine environment. Because fish accumulate trace elements from their environment and can act as indicator of such metals in the environment, they are excellent organisms for the study of long term changes in the marine environment (Ghazaly,1992).

The concentrations of macronutrients in livers of the present fishes ranged between 398.3- 74.49, 170.59- 72.18, 1702-1131.6, 507- 327.6mg/Kg dry weight for Ca, Mg, Na and K (Table2). The maximum values of magnesium and sodium were detected in the livers of *Solea vulgaris* while the maximum values of calcium and potassium were detected in *Sparus aurata*. Abdel Satar and Shehata (2000) showed that sodium levels were in the range of 2497- 4713 and 2579-4030mg/Kg for the fish flesh of *Oreochromis niloticus* and *Tilapia zillii* respectively in River Nile. However, potassium levels were in the range of 3042- 4249 and 3042- 4499mg/Kg for *O. niloticus* and *T. zillii* respectively. Calcium levels were in the range of 419- 1240 and 469- 1642mg/Kg and magnesium concentrations ranged between 282.2- 321.4 and 2923- 325.3mg/Kg. In comparison with results of Abdel-Satar and Shehata (2000), muscles of *Oreochromis niloticus* and *Tilapia zillii* were quite richer in macronutrients than livers of *Solea vulgaris, Mugil cephalus, Liza ramada* and *Sparus aurata* in the present study.

Heavy metals pollution induce pathological changes in fishes, as the liver is the main detoxificating organ of the body and the integrator for biochemical and physiological functions. Also this organ carries out key functions in excretion of xenobiotics (Geneser, 1993).

The liver section of *Solea vulgaris* showed dilation and destruction of central vein. The hepatocytes showed fatty degeneration with vacuolar necrosis (Fig.2) and the blood cells were infiltrated within the hepatocytes (Fig. 3). Beside, hemolysis of blood cells appeared (Fig. 4).

The liver sections of *Liza ramada* contained enlarged nuclei (Fig. 5). Some hepatocytes contained eccentrically located nuclei and large fat vacuoles occupied the cytoplasm (fatty degeneration) (Fig. 6). It also showed accumulation of hemosiderin pigment as a result of destruction of erythrocytes (Fig. 7).

Alterations developed to include hemorrhagic lesions accompanid by accumulated hemosiderin pigment. Hemorrhage was abundant most probably due to the inflammation of tissue. The rapid and continued destruction of erythrocytes, breakdown of hemoglobin converted them into hemosiderin. The respiratory activity and iron metabolism of cells were damaged with anaemic anoxia. Large amounts of iron were absorped by the way of intestinal mucosa. This process was responsible for the abnormal accumulation of hemosiderin in the liver (Yacoub, 1994).

The histopathological changes in the liver of *Solea vulgaris* and *Liza ramada* were similar to those observed in other fishes due to chemical toxicant under laboratory conditions. Sastry and Gupta (1979) have reported vacuolation, degeneration of nuclei, accumulation of lipid and focal necrosis in liver cells of *Heteropneustes fossilis* subjected to sublethal concentration of cadmium chloride. They attributed the fatty accumulation to an increased mobilization and transport of fat to the liver. Also, the liver of *Clarias gariepinus* contained fatty degeneration, severe hemorrhage and accumulation of hemosiderin in River Nile water polluted with lead in the area of Greater Cairo (Yacoub, 1999).

Laboratory studies have shown that hemosiderin (ferric iron) concentration increased temporally in fish exposed to petroleum-contaminated sediment and also sediment originating from the vicinty of a pulp and paper mill (Khan and Nag, 1993). Some studies also revealed a link between hepatic lesions and the concentration of hemosiderin (Khan, 1998 and 1999). These results support the view proposed by other studies that hemosiderin concentration can represent a useful tool for monitoring fish health (Bucke *et al.* 1984, 1992; Wolke *et al.* 1985 and Peters *et al.* 1987).

Liver tissue of *Mugil cephalus* demonstrated ballooning degeneration (Fig. 8). The hepatocytes were degenerated and the central vein dilated and invaded with hemolysed blood cells (Fig.9). The hepatic vein appeared congested with blood and the hemoglobin of some erythrocytes converted into hemosiderin (Fig.10). The hepatocytes showed degeneration, necrosis and infiltration of blood cells (Fig.11).

The liver of *Sparus aurata* contained a diffuse arrangement of pancreatic tissue (Fig.14 and 15). Section of liver of *Sparus aurta* exhibited degeneration and necrosis of hepatocytes (Figs.12 and 13). A large necrotic area was occupied by debris of hepatocytes and blood cells. Stagnant blood and degenerated pancreatic acini appeared within the hepatic tissue (Figs.14 and 15). *Sparus aurata* exhibited the most progressive pathological changes among the four examined fish species of Bardawil lagoon. 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 that might cause liver damage, are chiefly responsible for necrotic processes in fish liver.

Sastry and Gupta (1978) studied the effect of lead nitrate on the teolest fish *Channa punctatus*. They reported that the structural damage in the liver may be correlated with alterations in enzyme activities. There was an inhibition in alkaline phosphatase activity indicating that the transphosphorylation reactions were inhibited. There is a slight insignificant increase in acid phosphatase activity, which can be correlated with the degeneration of hepatocytes. Lumlertdacha *et al.*(1995) noticed areas of vacuolated hepatocytes in channel catfish. The vacuoles contain lipid, which is suggestive of impaired lipid metabolism.

Similar results were obtained by Ghosh and Chakarbati(1993) after treatment of *Heteropneustes fossilis* by sublethal concentrations of cadmium and histological changes were examined in liver. Cytoplasmic vacuolations, eccentric nucleus and rupture of cell membranes of hepatocytes were the most conspicuous changes in liver.

Also, similar histopathological lesions were observed in liver of fish under the effect of different toxicants. Degenerative and inflammatory lesions of liver of brown trout *(Salmo trutta)* and rainbow trout *(Oncorhynchus mykiss)* were observed after a long term exposure to diluted sewage plant effluent (Schmidt *et al.*,1999) and vacoulizations and necrosis of hepatocytes of rainbow trout *(Oncorhynchus mykiss)* after feeding with diets rich in oxidized lipids (Daskalov *et al.*,2000)

In conclusion, water level in Bardawil lagoon is low and constant. So, concentrations of heavy metals increased gradually through the years. The accumulations of heavy metals caused different degrees of injuries in the livers of the investigated fish species.

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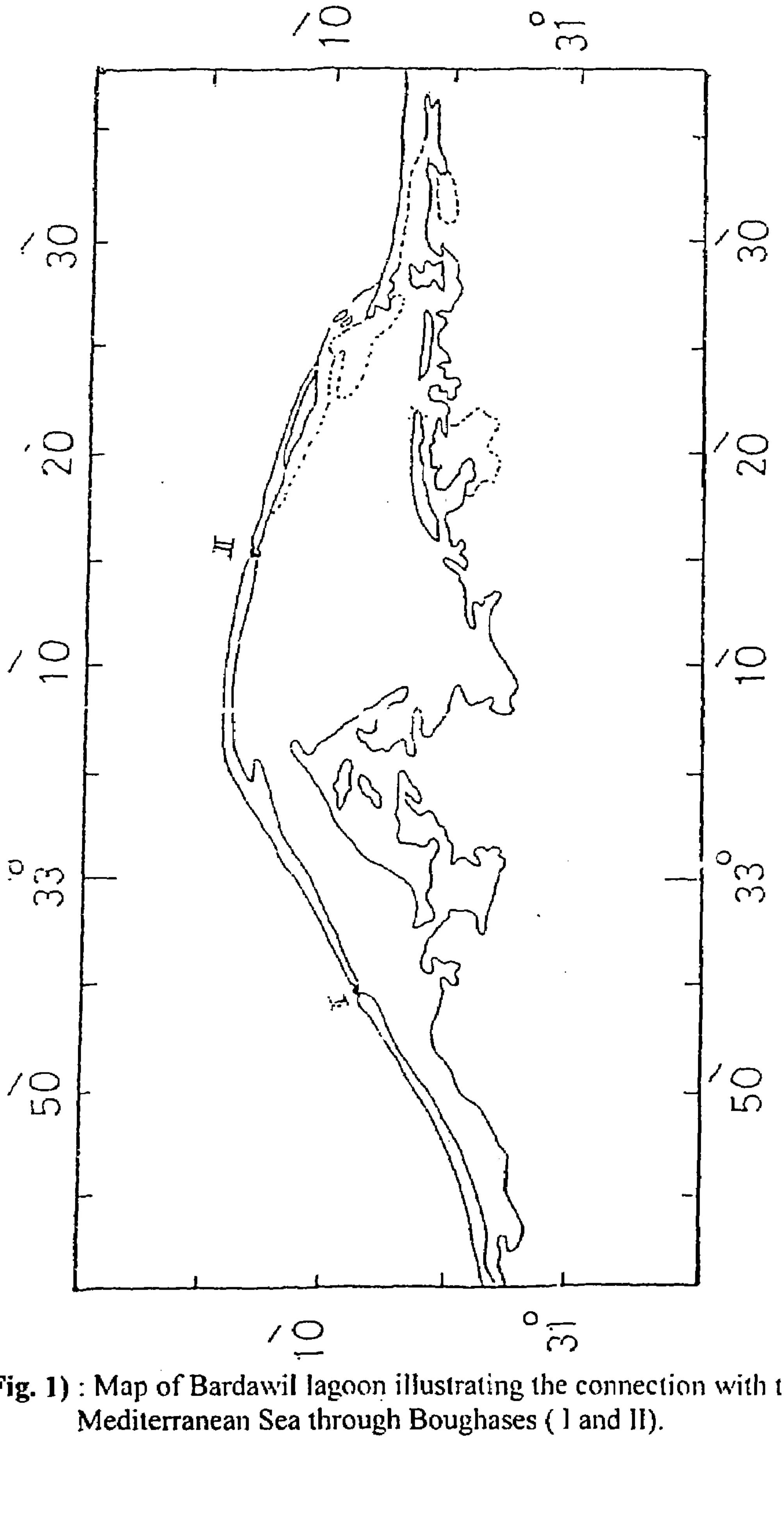


(**Fig. 1**) : Map of Bardawil lagoon illustrating the connection with the Mediterranean Sea through Boughases (I and II).

(Table 1) Production of Bardawil lagoon in the period from 1991 to 2001.

Years	Total production in tons
1991	2988
1992	1838
1993	2205
1994	1575
1995	2172
1996	1627
1997	2230
1998	1937
1999	3890
2000	3300
2001	3146

(Table 2) The concentrations (mg / Kg dry weight) of heavy metals and macronutrients in the liver of the studied fish species in Bardawil lagoon.

Species / Metal	*Solea vulgaris*	*Mugil cephalus*	*Liza ramada*	*Sparus aurata*
Fe	72.95	48.68	97.15	121.94
Mn	2.17	1.27	1.71	1.48
Zn	15.22	18.07	30.80	32.57
Cu	184.25	147.76	301.83	82.28
Pb	4.96	2.32	3.13	1.10
Cd	0.18	0.30	0.28	0.30
Ca	89.70	74.49	112.84	398.30
Mg	170.59	72.18	133.00	94.64
Na	1702.00	1131.60	1177.60	1288.00
K	335.40	358.80	327.60	507.00

EXPLANATION OF FIGURES

(Fig. 2) : Section of liver of *Solea vulgaris* fish obtained from Bardawil lagoon, showing dilation and destruction of central vein (CV). X400

(Fig. 3) : Section of liver of *Solea vulgaris* fish obtained from Bardawil lagoon, showing fatty degeneration of hepatocytes (H) and infiltration of erythrocytes (E). X400

(Fig. 4) : Section of liver of *Solea vulgaris* fish obtained from Bardawil lagoon, showing hemolysis of some erythrocytes and stagnant blood (B). X400

(Fig. 5) : Section of liver of *Liza ramada* fish obtained from Bardawil lagoon, showing degeneration of hepatocytes (H) and enlargement of some nuclei. X400

(Fig. 6) : Section of liver of *Liza ramada* fish obtained from Bardawil lagoon, showing dilation of central vein (CV) and fatty degeneration of hepatocytes (H). X400

(Fig. 7) : Section of liver of *Liza ramada* fish obtained from Bardawil lagoon, showing accumulation of hemosiderin pigment (He). X400

(Fig. 8) : *Section of liver of Mugil cephalus fish obtained from* Bardawil lagoon, showing ballooning (Ba). X400

(Fig. 9) : Section of liver of *Mugil cephalus* fish obtained from Bardawil lagoon, showing dilation of central vein and hemolysis of erythrocytes (E). X400

(Fig. 10) : Section of liver of *Mugil cephalus* fish obtained from Bardawil lagoon, showing congestion with blood and accumulations of hemosiderin (He) inside a hepatic vein. X400

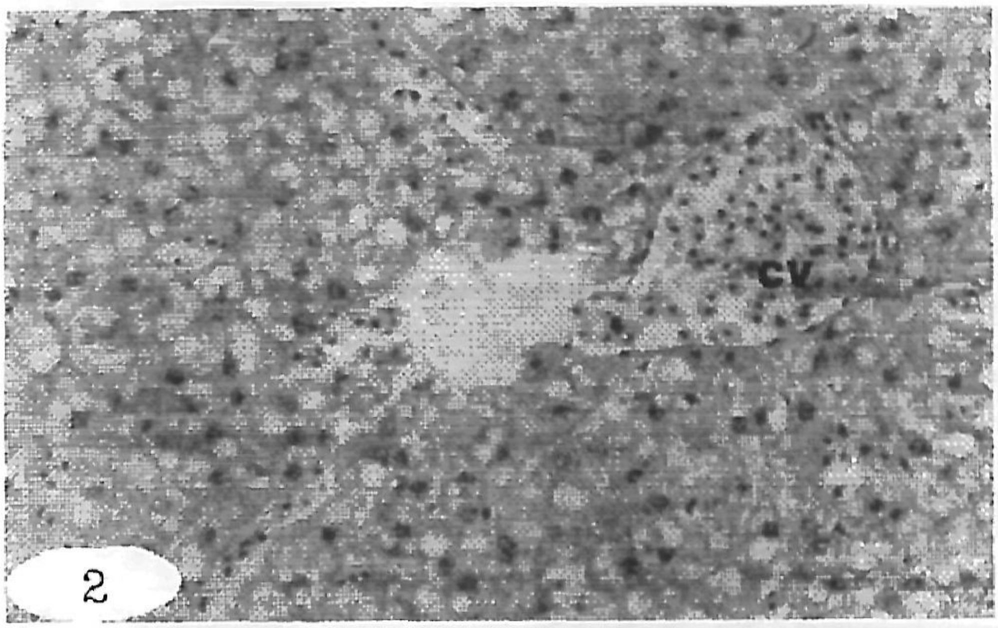
(Fig. 11) : Section of liver of *Mugil cephalus* fish obtained from Bardawil lagoon, showing necrotic area (NA) occupied by blood cells. Notice infiltration of blood cells (BC) within degenerated hepatocytes. X400

(Fig. 12) : Section of liver of *Sparus aurata* fish obtained from Bardawil lagoon, showing lucent necrotic area (NA).X400

(Fig. 13) : Section of liver of *Sparus aurata* fish obtained from Bardawil lagoon, showing severe necrosis. Notice large necrotic area occupied by blood cells (BC) and debris of hepatocytes. X400

(Fig. 14) : Section of liver of *Sparus aurata* fish obtained from Bardawil lagoon, showing stagnant blood (B). Notice diffused arrangement of pancreatic acini within hepatic tissue. X100

(Fig. 15) : Section of liver of *Sparus aurata* fish obtained from Bardawil lagoon, showing degeneration of pancreatic acini(PA). X400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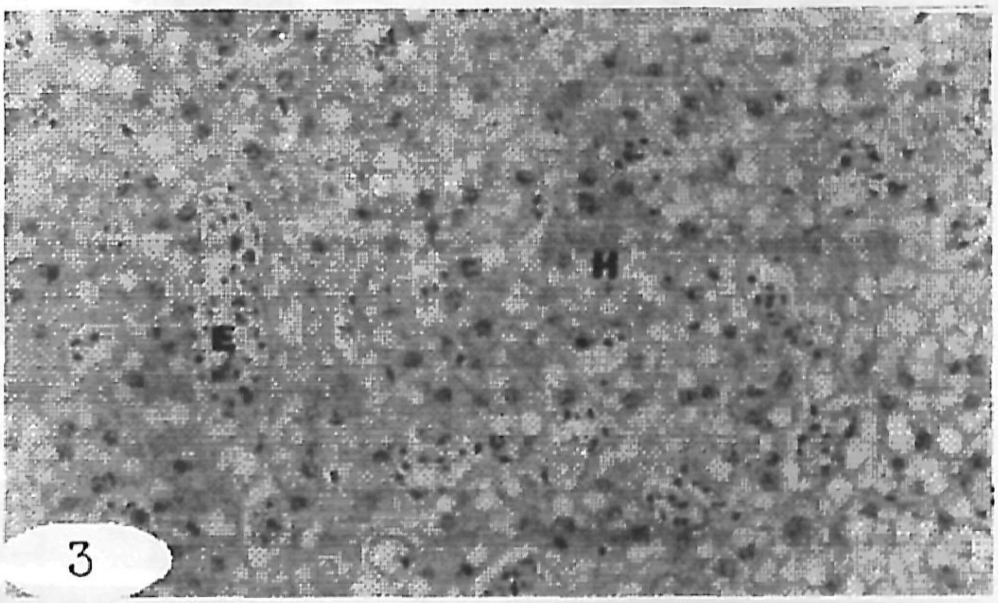
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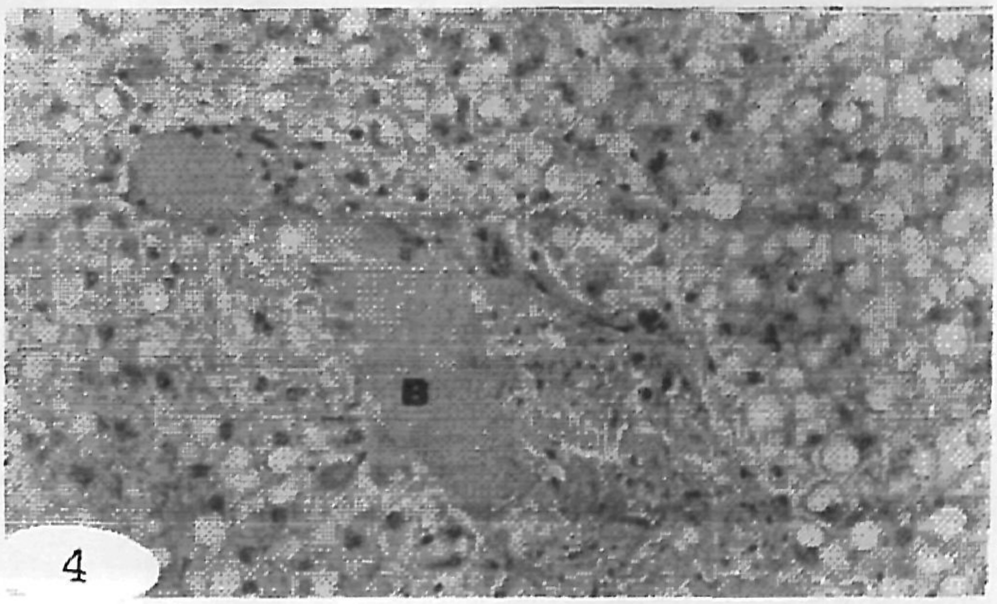
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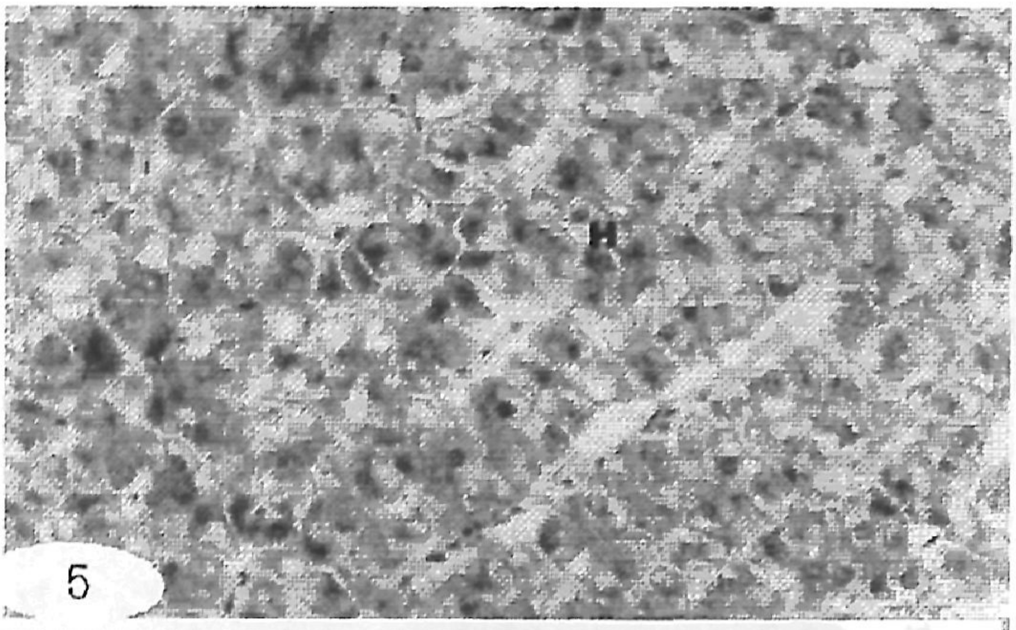
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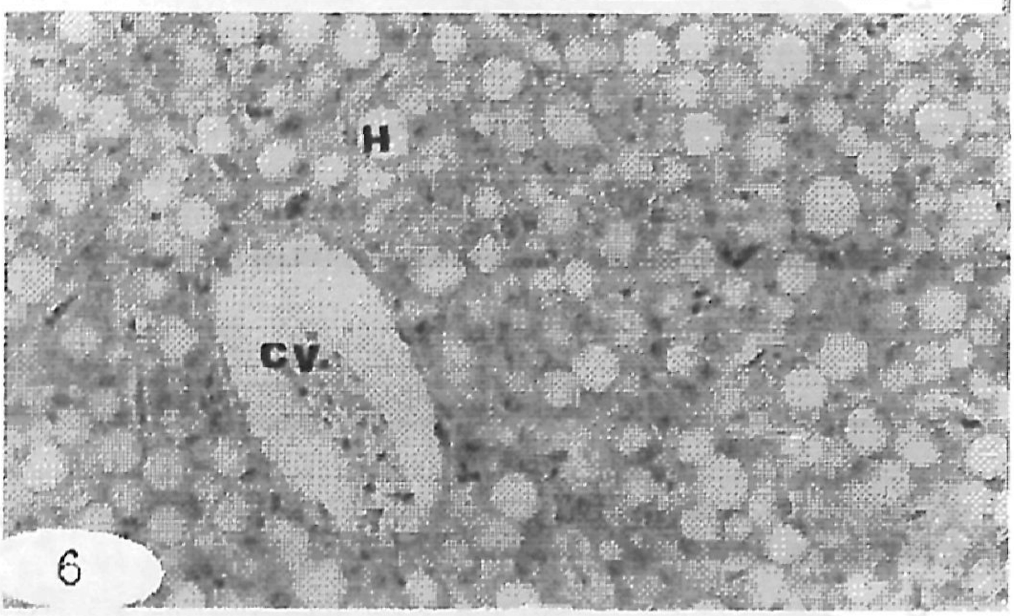
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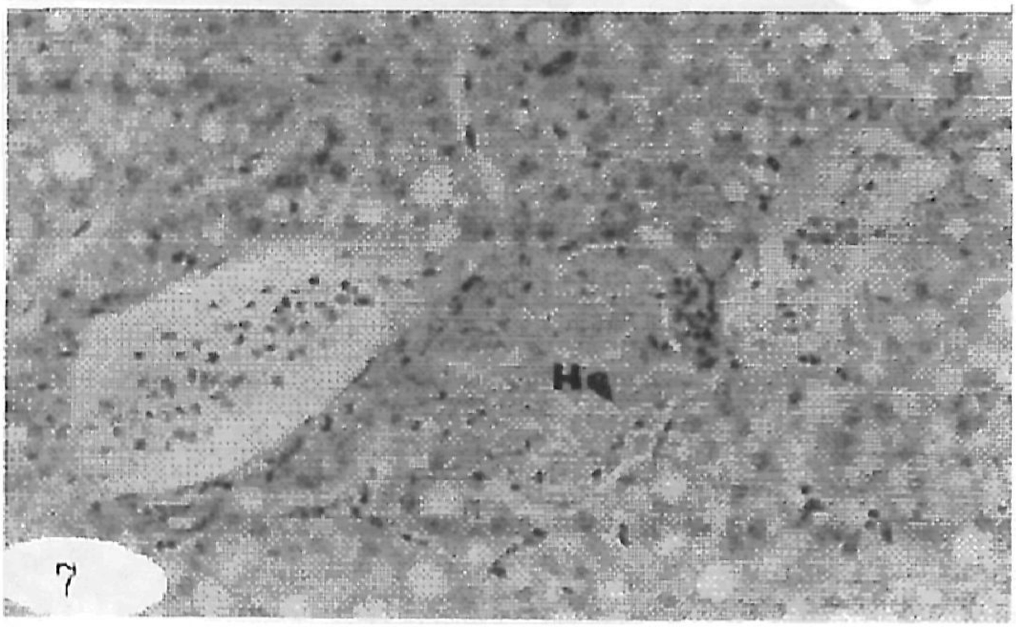
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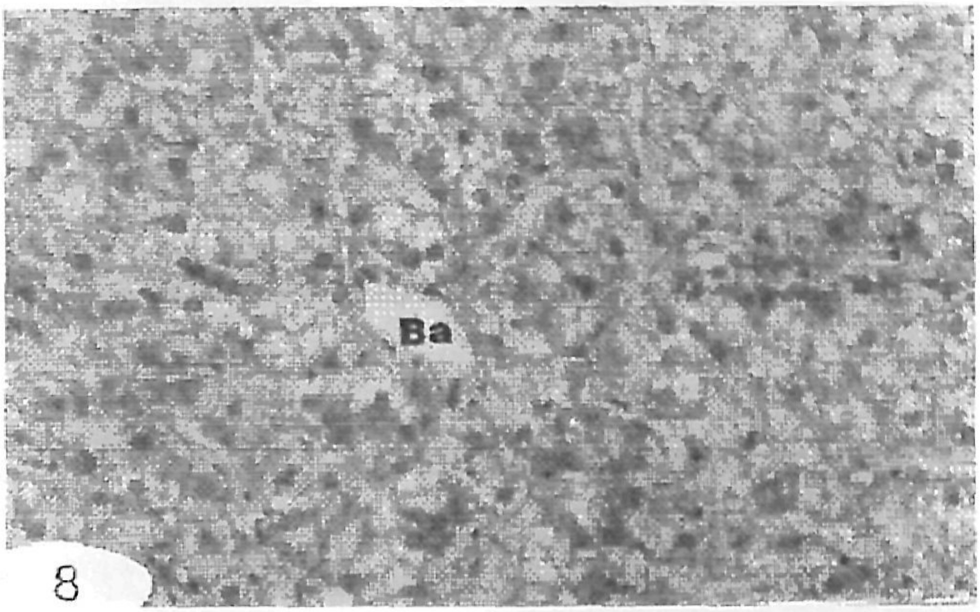
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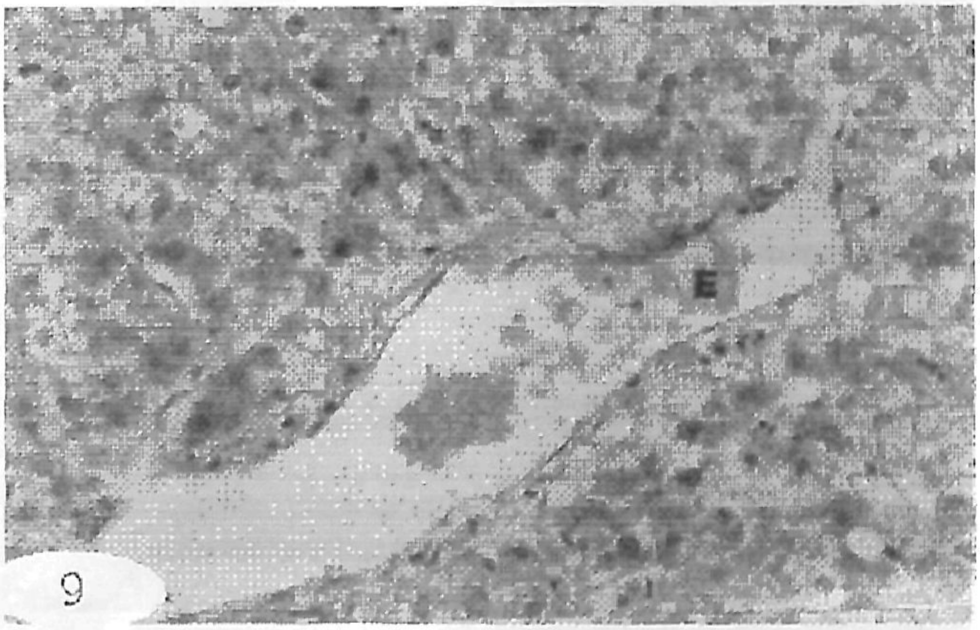
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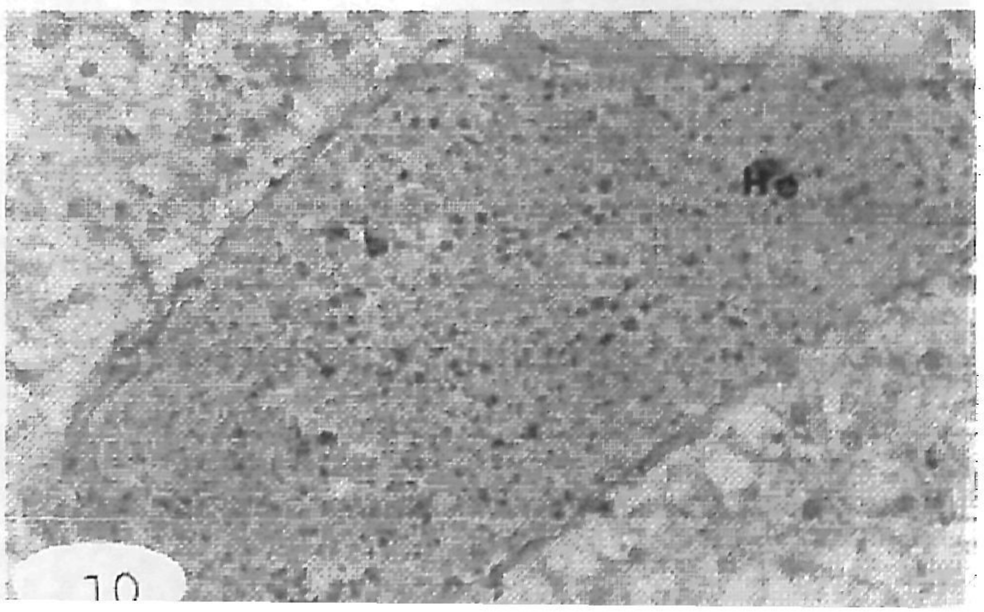
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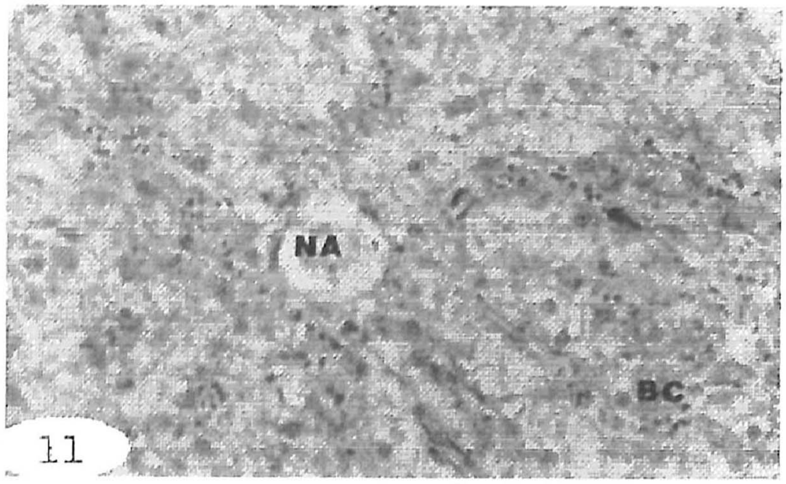
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BC
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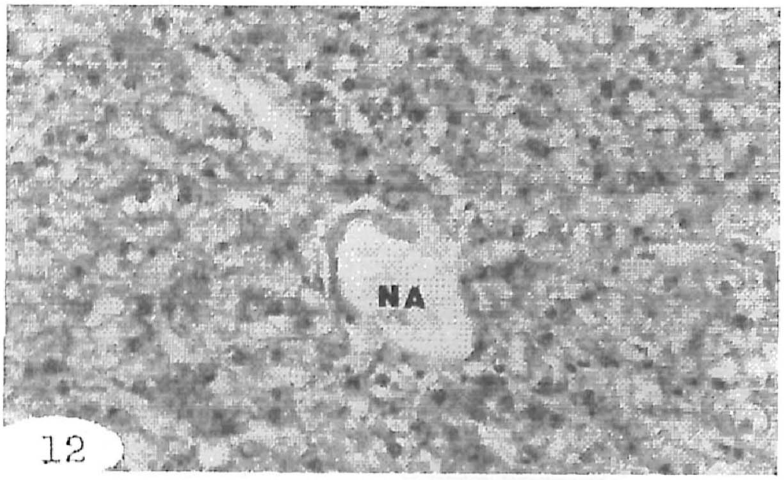
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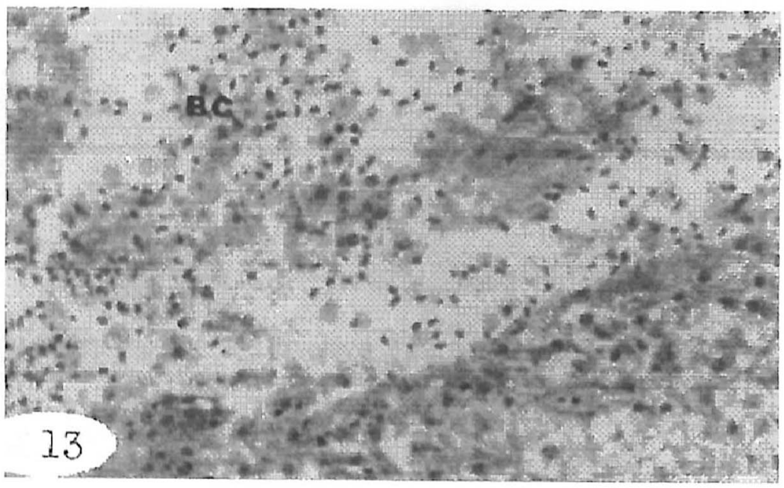
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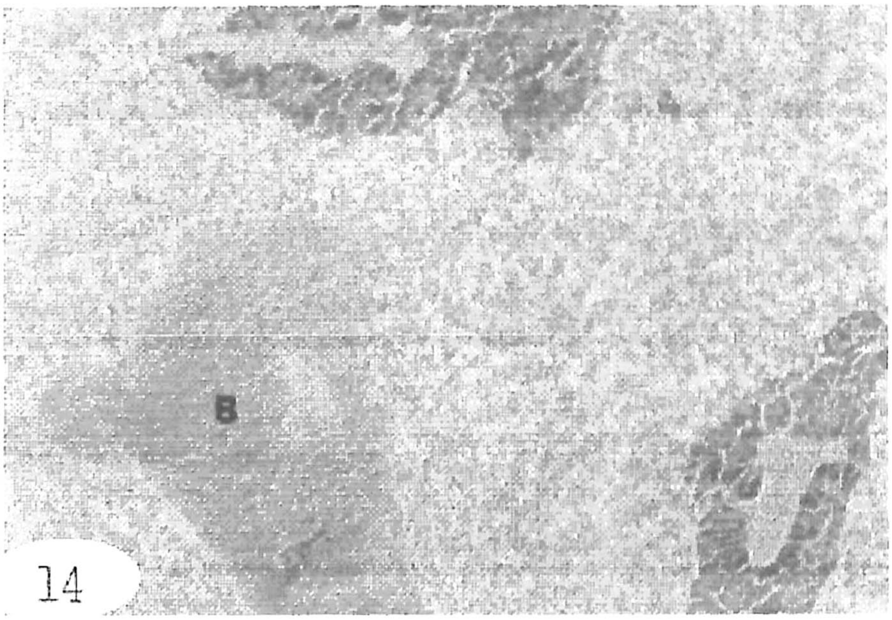
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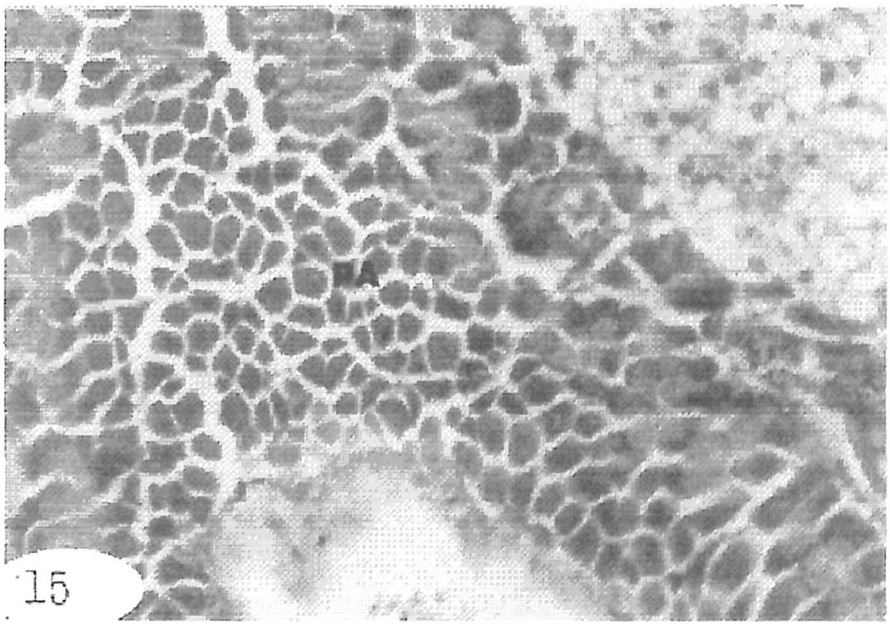
B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BA