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COMPARATIVE STUDY ON SOME HISTOLOGICAL AND BIOCHEMICAL ASPECTS IN FISHES FROM QARUN AND BARDAWIL LAKES

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ABSTRACT

Male specimens of *Tilapia zillii, Mugil cephalus, Liza ramada* and *Solea egyptiaca* were collected from Ayoub and Shakshouk Stations at the west and middle of Qarun Lake. At the same period, other male specimens of *M. cephalus, L. ramada* and *Solea vulgaris* were collected from Bardawil Lake. The histological and biochemical findings can be summarized as follows:

Histological examination of gills, liver and kidney of the four studied fish species in both lakes showed variable pathological changes. Bardawil Lake fish showed more damage. The histopathological features are discussed.

Biochemical significant differences were found between the fishes collected from the two stations of Qarun Lake. *T. zillii* showed significant higher values of liver total proteins (TP) and inorganic phosphorus (P) than the other three species, while *L. ramada* showed significant higher values of liver alkaline phosphatase (ALP) and calcium (Ca). Muscle total cholesterol (TCh), low-density lipoprotein (LDL) and Ca were higher in *M. cephalus* but TP, high-density lipoprotein (HDL) and P in *L. ramada*; ALP and total lipids (TL) in *Solea egyptiaca* and triglycerides (TG) in *T. zillii*.

Mugil cephalus of Bardawil Lake showed significant higher values of muscle TP, ALP, TL, TG, HDL, LDL and Ca than the other two fish species. Muscle TCh and P were significantly higher in *S. vulgaris* and *L. ramada* respectively. Also *S. vulgaris* showed significant higher values of liver TP and P and *L. ramada* showed significant higher values of liver ALP and Ca.

Significant differences were detected between the three fish species collected from both Qarun and Bardawil Lakes. Liver P was higher in the three fish species of Bardawil Lake than Qarun Lake. while liver Ca was higher only in *M. cephalus* and lower in both *L. ramada* and *S. vulgaris*. Liver TP and ALP were lower in *M. cephalus* . and *L. ramada* respectively, while liver TP of *L. ramada* of Bardawil Lake was higher than that of Qarun Lake.

Muscle TL and P in one hand and TG and Ca in the other hand were significantly lower and higher respectively in the three fish species of Bardawil Lake than that of Qarun Lake. The other muscles parameters in the three fish species recorded either significantly higher or lower values between the two lakes.

INTRODUCTION

Lake ecosystens have individuality and peculiarities in physical, chemical and biological features due to their position relative to catchment's drainage and their separation from other standing waters. Fishes lie generally at the upper levels of the aquatic food web and are thus affected directly or indirectly by several levels below them. Fish are mobile, and thus capable of responding to altere biological and/or physical conditions. Due to their mobility, fish populations are relatively hard to sample accurately compared to the other taxonomic groups. Their biology, however, is relatively easy to study for several reasons. They are large, have distinct age classes and growth is temperature-dependent. Fishes are also the only taxa in the aquatic ecosystem directly exploited by man. Their history of exploitation in lakes is often one of changes caused through deliberate or accidental management practices. Over-exploitation may alter the competitive balance between species and widespread introductions of non-native fishes have often had similar competitive-mediated changes. Data from wild fishes provide baseline information on body composition, which is useful in evaluating the quality and physiological condition of cultured ones. Additionally, knowledge of wild fish can elucidate environmental and physiological controls of growth and nutrient utilization that may aid in the enhancement of growth or final product quality of cultured fishes. Thus, in considering lake management, each water body must be viewed individually and the strategy for its management must be based on scientific knowledge.

Qarun Lake is located in the western desert in the lowest part of El-Fayoum depression. between W 30° 29 - E 30° 24 and S 29° 25 - N 29° 34 . its total area is about 54.000 acres. The lake lies at 44.3 – 44.8 m below the sea level. It is a closed basin with no apparent outlets. It represents a reservoir of drainage water of the cultivated lands in El-Fayoum Province via two main drains namely El-Bats (east) and El-Wadi (middle) drains. Lake Qarun has been undergoing a progressive increase of salinity due to the continuous supply of the slightly brackish land-drainage water and the excessive evaporation caused by high temperature in summer. However. Bardawil Lake is situated in the north of Sinai Peninsula. between W 32° 40' - E 33° 30' and S 31° 03' - N 31° 19', its area is about 471.000 acres. The lake is a natural depression separated from Mediterranean Sea by long arrow-shaped sand bar. Two man-made inlets represent the main connections between the sea and lake. They control the salinity of the lake. There is also a third small inlet at the lake eastern most corner. which has little effect on the flooding of the lake or the salinity. Because of the very high rate of evaporation. which is not compensated by fresh water supply. the whole lake is highly saline throughout the year. The physical and chemical characters of Qarun and Bardawil Lakes at summer season were tabulated by Abd El-Hady (2001).

Items	Qarun Lake	Bardawil Lake
Temperature (°C)	34.2 ± 1	33.0 ± 1
Turbidity (N.T.U.)	88.3 ± 0.1	26.3 ± 0.08
pH	7.01	7.43
Salinity ‰ (g l^{-1})	340.9	219.1
PO_4 (µg l^{-1})	171.4 ± 14.26	143.7 ± 0.30
Total organic P (µg l^{-1})	2267.3 ± 11.85	253.3 ± 0.14
NH_4 (µg l^{-1})	1544.4 ± 5.09	126.7 ± 1.02
NO_2 (µg l^{-1})	86.51 ± 0.64	9.9 ± 0.02
NO_3 (µg l^{-1})	178.0 ± 0.23	165.2 ± 1.46
Bicarbonate (mg l^{-1})	1290.0 ± 14.14	233.3 ± 12.21
Carbonate (mg l^{-1})	250.0 ± 0.18	129.6 ± 0.12
Total alkalinity (mg l^{-1})	1540.0 ± 0.15	362.9 ± 0.17
SO_4 (mg l^{-1})	2780 ± 0.19	980 ± 0.09
Ca^{+2} (mg l^{-1})	8281.3 ± 0.21	5458.5 ± 0.13

Now the fish culture ponds are widespread in Egypt and depend in their economical profits on the transporting of saltwater fries such as *Mugil cephalus*. *Liza ramada* and *Solea egyptiaca*. In the present study, the authors try to find the histological and biochemical body composition differences between fish species inhabited two Egyptian salt lakes to increase the scientific knowledge about these species under different environmental conditions. This knowledge of wild species will assess in the management of the same species under the cultured conditions.

MATERIALS AND METHODS

Fish sampling:

Within the summer season (July and August) of 2002, male specimens of *Tilapia zillii* (8.3-10.6 cm), *Mugil cephalus* (30-34 cm). *Liza ramada* (19.5-23.5 cm) and *Solea egyptiaca* (15.8-19.0 cm) were collected from Ayoub and Shakshouk Stations at the west and middle of Qarun Lake. At the same period, other male specimens of *M. cephalus* (31-35 cm), *L. ramada* (22.3-25.8 cm) and *Solea vulgaris* (20.0-25.8 cm) were collected from Bardawil Lake. Each fish was dissected to remove the liver and a sample of dorsal muscle tissue. Samples from each were sealed in a plastic bag and stored frozen at -30 °C for the biochemical analysis.

Histological analysis:

Immediately after dissection, pieces from gills, liver and kidney of each fish were fixed in Bouin's fluid, dehydrated in ethanol, cleared in xylol, embedded in paraffin, sectioned to 5 µm thick and stained by hematoxylin-eosin.

Biochemical analysis:

Samples of liver and muscle tissue were homogenized (Hollongworth and Wekell. 1976) and analyzed for total protein using improved Kjeldahl method (Willits and Ogg. 1950), alkaline phosphatase (Belfiled and Goldberg. 1971), total lipids by the Soxihlet apparatus (Fine. 1955), total cholesterol (Richmond. 1973), triglycerides (Lowell and Ralph. 1973), HDL-cholesterol (Gidez and Miller. 1982), LDL-cholesterol (Lopez *et al.*. 1973), inorganic phosphorus (Fraser *et al.*. 1987) and calcium (Gindler and King. 1972).

Statistical analysis:

The statistical analysis of the results was preformed using t-test, ANOVA test (two way analysis of variance) and $LSD_{0.05}$ (Farver. 1989).

RESULTS

Histological findings:

1] Gills:

1) Qarun Lake:

a. *Tilapia zillii*: From Shakshouk station, histological sections showed oedematous interlamellar epithelial cells; necrosis of epithelial cells surrounding pillar system and epithelial rupture (Fig. 1). From Ayoub station, deformation of pillar system; epithelial lifting with hypertrophid chloride cells as well as mucus secreting cells (Fig. 2).

b. *Mugil cephalus* and *Liza ramada*: In both Ayoub and Shakshouk stations, they showed the same histological features. They had lifting epithelial layer with desquamation of epithelium and necrosis of chloride cells (Fig. 3).

c. *Solea egyptiaca*: In both stations, the same histological lesions are common and represented by histological deformed pillar system with zigzag epithelial lifting and plenty mucus secretion in between the secondary lamellae (Fig. 4).

2) Bardawil Lake:

a. *Mugil cephalus*: The gills showed severe histological degeneration, desquamation of epithelium and the epithelial lifting accompanied with epithelial necrosis. Also, displacement of chloride cells was observed (Fig. 5).

b. *Liza ramada*: All the examined specimens revealed the same lesions with different degrees of severity. The most common features were epithelial lifting and hyperplasia of mucus cells with extensive mucus in the epical part of the secondary filament (Fig. 6).

c. *Solea vulgaris*: The major changes were necrosis and marked epithelial lifting with hyperplasia of mucus cells in-between secondary gill lamellae (Fig. 7).

2] Liver:

1) Qarun Lake:

a. *Tilapia zillii*: In fish taken from Shakshouk station, the hepatic parenchymal cells were highly vacuolated, the cytoplasm became faint with accentric nucleus. Dilated vein with severe congestion, lose of surrounding parenchyma cells and increase of küpffer cells were also observed (Fig. 8). The specimens from Ayoub station showed distortion of hepatic architecture. The individual parenchymal cells were mostly inhibiting degenerative lesions and necrosis areas in between hepatic cells (Fig. 9).

b. *Mugil cephalus* and *Liza ramada*: From Shakshouk station, liver cells showed vacuolar degeneration and granular cytoplasm with pyknotic nuclei (Fig. 10). While the examined liver sections from Ayoub station showed sever degeneration or necrotic hepatic cells with fibrosis and odema (Fig. 11).

c. *Solea egyptiaca*: Section reflects severe vacuolar degeneration with odema and deformed hepatic architecture with decrease of nucleus size (Fig. 12).

2) Bardawil Lake:

a. *Mugil cephalus*: The hepatocytes were extremely swollen and their cytoplasm exhibited a degree of granular degeneration accompanied with the presence of numerous clear vacuoles denoting fatty or hydrobic degeneration. Widened of sinusoids and hepatic cells with pyknotic nuclei were also obvious (Fig. 13).

b. *Liza ramada*: Hepatic tissue showed also hydropic degeneration and presence of focal necrosis. The blood vessel congestion with heamosidrosis (Fig. 14).

c. *Solea vulgaris*: The hepatic cells showed sever vacuolation with pyknotic nuclei, cells infiltration and increase number of küppfer cells (Fig. 15).

3] Kidney:

All fish species in both Qarun and Bardawil Lakes, showed nearly the same histopathological lesions, hydropic degeneration with dilated proximal and distal convoluted tubules. Epithelial cells were flattened and incomplete in some tubules and rounded in other. Cystic appearance of glomerulei, indicated severe renal damage. Many leucocytes and other blood cells were present among the renal tubules (Figs. 16 & 18). Fish specimens of Ayoub station from Qarun Lake showed hemosidrosis in addition to the previous appearance (Fig. 17).

Biochemical findings:

The chemical analysis of livers and muscles of *T. zillii* collected from Qarum Lake and *M. cephalus*, *L. ramada* and *Solea* species collected from both Qarun and Bardawil Lakes are tabulated in Tables (1 – 3).

a. *Tilapia zillii*: *T. zillii* from the Ayoub station of Qarum Lake had significantly higher liver and muscle TP and muscle TL, TCh and ALP than that of the Shakshouk station, while muscle HDL, LDL, Ca and P were significantly lower in the Ayoub station than the other ones.

b. *Mugil cephalus*: Muscle TP, ALP, HDL, LDL and P of Ayoub's fish from Qarun Lake were significantly higher than the Shakshouk's fish. Liver TP, Ca and P and muscle TCh were significantly lower in fish of Ayoub than that of Shakshouk.

M. cephalus of Bardawil Lake showed significantly higher values of muscle ALP, TG and HDL; liver and muscle Ca and liver P than that of fish collected from Qarun Lake. Values of muscle TL, TCh, LDL and P and liver TP were significantly higher in fish of Qarun Lake than that of Bardawil Lake.

c. *Liza ramada*: Liver ALP and P and muscle TP, TL, HDL and LDL of fish collected from Ayoub station of Qarun Lake recorded significant lower values than of the Shakshouk's fish, while muscle TG, TCh and P and muscle and liver Ca showed significant higher values.

Liver P and muscle TG, LDL and Ca values of fish from Bardawil Lake were significantly higher than that of Qarun Lake. On the other hand, Liver ALP and Ca also muscle TP, TL, TCh, HDL and P were significantly low in fish of Bardawil Lake.

d. *Solea* species: Evaluation of muscle TG, TCh, HDL and LDL also liver P as well as liver and muscle Ca of *Solea egyptiaca* of Ayoub station of Qarun Lake showed significant lower values than that of the Shakshouk station. Both muscle ALP and TL values were significantly higher in Ayoub station than Shakshouk station.

S. egyptiaca from Qarun Lake showed significant higher values of muscle ALP, TL, LDL and P also liver Ca than that of *S. vulgaris* from Bardawil Lake. Contrarily, muscle TP, TG, TCh, HDL and Ca also liver P of *S. egyptiaca* recorded significant lower values than that of *S. vulgaris*.

Variation of chemical parameters according to fish species:

All the analyzed items showed significant variations between the four fish species except liver ALP in Ayoub station; both liver and muscle P in Shakshouk station of Qarun Lake; muscle TP and liver P in Bardawil Lake.

1. Qarun Lake:

From both sites of the lake, *S. egyptiaca* and *T. zillii* showed higher values of muscle TL and TG respectively. Also Ca contents were highest in liver and muscle of *L. ramada* and *M. cephalus* respectively.

T. zillii collected from the Ayoub station showed highest values of both liver and muscle TP also liver P. *M. cephalus* recorded highest values of HDL and LDL. Muscle TCh and P were higher in *L. ramada*, while muscle ALP was highest in *S. egyptiaca* from the Ayoub station.

Fishes collected from the Shakshouk station of the lake demonstrated that muscle and liver ALP were higher in *T. zillii* and *L. ramada* respectively, while muscle TP and HDL and liver TP were highest *L. ramada* and *S. egyptiaca* respectively. Also muscle LDL was highest in *S. egyptiaca*, while muscle TCh was highest in *M. cephalus*.

2. Bardawil Lake:

The highest values of muscle TL, TG, HDL, LDL and liver and muscle Ca were detected in *M. cephalus*, whereas highest values of liver TP and muscle TCh were observed in *S. vulgaris*. The highest muscle P value was recorded in *L. ramada*.

DISCUSSION

In bony fishes, the continuous flow of water across the gills depends upon the coordinated action of the mouth and the opercular apparatus. Histological examination of gill tissues revealed that the filament epithelium of the *T. zillii* in lake Qarun differs between Shakshouk and Ayoub stations. Fewer or nearly rare chloride cells were observed in gill epithelium of *T. zillii* of Shakshouk station compared to those of Ayoub station and Bardawil Lake. The chloride or mitochondria-rich cells of the gill play a prominent role in teleostean fish's osmoregulation (Erkmen and Kolankaya, 2000; Khidr *et al.*, 2001 ; Shoman and Gaber, 2003). Furthermore, chloride cell proliferation seems to reflect some role for chloride cells in toxicant extrusion or neutralization (Erkmen and Kolankaya, 2000). Presence of oedematus fluid at the base of secondary gill filament of *T. zillii* (Shakshouk station), could be due to increased permeability induced by the prolonged exposure to the pollutant (Balah *et al.*, 1993). These lesions are also characteristic for fishes exposed to Ni (Sorensen *et al.*, 1984). This oedematus separation led to its desquamation as well as necrosis. Necrosis and rupture of branchial system with mucus secretion of *M. cephalus*, *L. ramada* and *Solea* sp. are believed due to the direct deleterious effects of the irritants (Temmink *et al.*, 1983). Mallatt (1985) found that the most frequent lesions in gills, associated with heavy metals, are necrosis of gill epithelium, hyper secretion of branchial mucus cells and hypertrophy of branchial epithelial cells. Clubbing necrosis gills, the condition most closely resembles gill changes, induced by environmental factors. From these factors acidic water, often with elevated aluminum and/or cadmium levels have been studied (Muller *et al.*, 1991 ; Clark *et al.*, 1997).

Desquamation of the epithelium covering the gill rakers of fish of Bardawil Lake may lead to their dysfunction and loss of appetite with lower productivity of fish in polluted water (Balah *et al.*, 1993). Gill alteration, such as epithelial hyperplasia and separation of the epithelium are associated with asphyxiation, partial or complete loss of secretory or excretory function, impairment of oxygen–carbon dioxide exchange and loss of plasma electrolytes or proteins (Cruz and Sunaz, 1988). Changes in gill morphology in response to particular environment may be instrumental in conserving physiological function. Moreover, these changes in gill morphology may contribute to the death of fish in contaminant water (Erkmen and Kolankaya, 2000).

In most studied fish, histological alterations in the liver as well as changes in the hepatic cells, including vacuolization of cells undergoing necrosis, were observed. Teh *et al.* (1996) confirmed this in feral freshwater fish populations exposed to different types of contaminant stress. In addition to this vaculated hepatocytes, cell necrosis, movement of nuclei to the cell periphery, pyknotic nuclei and cytoplasmic degeneration were shown in *T. zillii* collected from Shakshouk station and *M. cephalus, L. ramada and S. vulgaris* taken from lake Bardawil. The liver exhibited various histopathological changes, including cytoplasmolysis degeneration of proliferated hepatocytes and rupturing of blood sinus, causing invasive infiltration of leucocytes (Balasubramanian *et al.*, 1999). Khan (2000) stated that this hyper-vaculation (steotasis) is indicative of high lipid content, and the degenerative lesions are considered as early indicators of hepatotoxin-carcinogen exposure. Liver vaculization has also been reported to be variable distention of hepatocellular cytoplasm (Walter *et al.*, 2000). Palacios *et al.* (2000) showed a clear increase in protein content of zebrafish correlated to nuceolei enlargement and development of granular reticulum in dark hepatocytes. which are signs of increased protein synthesized. This disagrees with the present histological result.

The kidney of fish from both Qarun and Bardawil Lakes showed similar histological features of swelling with severe vaculation, degeneration and necrosis of the epithelial cells of the proximal and distal convoluted tubules. This was in addition to the presence of glomerular oedema with cystic appearance and hyaline degeneration. Periglumerular fibrosis, reduced cell height and desquamation of tubular epithelium, with severe destruction of epithelium in a number of tubules. These conditions. with tubular vacuolation on numerous occasions, have been previously reported in selenium-exposed vertebrates (Sorensen *et al.*, 1984). The glomerular dilation, degeneration and fibrosis are similar to that reported after exposure to estrogenic compounds in rainbow trout, medaka and carp (Metacalfe *et al.*, 2001 ; Weber *et al.*, 2003). Hyaline eosinophilic or granular casts were recorded in the renal tubular lamina of Atrazine treated fish (Khidr *et al.*, 2001). Chugh (1989) attributed the occurrence of such hyaline casts causes desquamation and detaching of the necrotic tubular epithelial cells from their basement membrane.

The results obtained by Verma and Tonk (1983) and Almeida *et al.* (2001) indicated that the contamination of water by Hg and Cd significantly decreased total protein concentrations in liver and muscles of the freshwater fish. *Notopterus notopterus* and *Oreochromis niloticus* respectively. On the other hand. hepatic protein content of *N. notopterus* exhibited an inverse relationship with ovarian contents (Patil and Kulkarni. 1994). Anwar *et al.* (1998) found that hepatic and muscle total proteins were higher in the cold water fish than those found in the warm water fish of the same age groups. Their results indicated that the accumulation of proteins in livers and muscles of cold water fish is due to the low metabolic rate and less demand for energy. Craig *et al.* (2000) recorded that liver composition of red drum. *Sciaenops ocellatus*. varied dramatically throughout the year. while muscle composition was relatively constant. Liver protein was more stable. Minor differences had been observed by Flick (2002) in the protein component between cultured and wild fish species.

Alkaline phosphatase is a polyfunctional enzyme which hydrolyses a broad class of phosphomonoesters and acts as a transphosphorylase at alkaline pH. Also. it may play an important role in the mineralization of the skeleton of aquatic animals (Blasco *et al.*, 1993). Kothari and Suneeta (1990) reported that Zn induced changes in both the concentration and pattern of distribution of liver alkaline phosphatase of *Heteropneustes fossilis* fish. Lan *et al.* (1995) demonstrated that both water Zn at 100 µg/l and Cu at 20 µg/l could markedly enhance hepatic ALP activity of *Chrysophyrys major*; the effect of water Se (VI) on ALP activity was minimal and could suppress the action of Cu on ALP activity; but water Cr (VI) had little effect on ALP activity. When *Labeo rohita* fish was reared in different concentrations of domestic sewage the muscle and liver alkaline phosphatases decreased significantly with increasing concentrations of domestic sewage. In normal conditions, the maximum alkaline phosphatase activity was found in intestine, decreasing in liver and muscle (Rajan, 1990). Basaglia and Cucchi (1998) found that extended farming of the black bullhead, *Ictalurus melas*, in tanks induced an increase of liver alkaline phosphatase levels. It is rather possible that elevating ALP activity is beneficial to the growth of fish, because it has been shown that this enzyme catalyzes the dephosphorylation of organic-P compounds, permitting utilization of the hydrolyzed PO_4^{3-} (Prakash, 1961), which is vital for the satisfactory skeletal development of fish (Woo *et al.*, 1991).

Lipids have important biological functions, such as membrane constituents, hormone precursors, and are efficient energy reserves, due to their high caloric content. Body composition of fish is well known to change in response to environmental conditions (Tanasichuk and Mackay, 1989). These changes were more evident in liver tissue, particularly with lipid, as compared to muscle (Craig *et al.*, 2000). Verma and Tonk (1983) stated that lipid content increased in muscles of freshwater fish, *Notopterus notopterus*, because of exposure to sublethal concentrations of mercuric chloride. In addition, transfer of brown trout from fresh water to sea water induced an increase of lipid contents in light muscle (Viau *et al.*, 2000). On the other hand, Geri *et al.* (1995) found that cholesterol content of muscle in the mirror carp did not differ, while lipid content decreased according to a seasonal change rather than an age effect. A significant effect of sunshine bass size on lipid content was found only for whole fillets, indicating that dietary energy excess in larger fish is mainly stored as subdermal fat. The dietary fatty acid profile was found to be the major determinant of edible muscle lipid composition (Luzzana *et al.*, 2002), while Viau *et al.* (2000) stated that dietary lipids had no significant effect on lipid contents of muscle. Torstensen (2000) found that Atlantic salmon lipid tissue composition and metabolism may be influenced by several dietary components. Moreover. the dietary influences are modified at several stages in the lipid digestion. uptake and metabolism. Phospholipids. which constitute cell membranes, are generally less influenced by dietary components compared to triglyceride, which predominantly serve as storage of lipids. So. cultured fish have a higher quantity of muscle lipid in the TG fraction than the wild ones. On the same trend, Yean *et al.* (1999) reported that the low density serum lipoprotein (LDL) level was higher in cultured red sea bream *Pagrus major* than in wild Red Sea bream. The levels of triglyceride and cholesterol contents in serum also exhibited a higher tendency in cultured fish. From these findings, they suggested that in cultured fish, the nutritional physiological condition of fat in fish body might be unbalanced toward fat accumulation. Furthermore, they considered that the HDL_2 might not be especially concerned with fat accumulation. This is not surprising, since cultured fish are fed diets higher in saturated fatty acids and are relatively inactive (Flick. 2002). Also. Craig *et al.* (2000) reported that lipid storage in red drum is activated during the summer months. The large range observed for TG suggests further that it is mobilized seasonally. possibly for vitellogenin synthesis or energy for migration and spawning activity.

Several researchers have observed an increase in lipid content in the muscle. liver. viscera and whole body in response to low phosphorus diets (Eya and Lovell. 1997 ; Skonberg *et al.*. 1997). while Sugiura *et al.* (2000) found that concentrations of total lipids and total cholesterol in skeletal muscle of post-juvenile rainbow trout were relatively unchanged by the 24 days of dietary phosphorus restriction. Eya and Lovell (1997) speculated that the reduction in muscle lipid content of the channel catfish (*Ictalurus punctatus*) fed higher levels of phosphorus may be due to enhancement of β-oxidation' of fatty acids. increase in glycogenesis. or some type of repartitioning effect. resulting in increased deposition of protein and reduced deposition of lipid. The decrease in lipid content of the muscle with increasing concentrations of dietary available phosphorus parallels an increase in muscle protein. Increased dietary levels of phosphorus could enhance nitrogen retention and. thus. protein accretion.

Ahlgren *et al.* (1995) indicated that muscle total lipid content could vary considerably. both within and between species. Lipid contents of roach fish from two oligotrophic lakes were significantly higher than that from an eutrophic lake. Differences in basic food webs may be responsible for these results. Ando and Mori (1993) stated that serum HDL was a major lipoprotein among five species (puffer, flounder, Red Sea bream, amberjack, and striped jack) and species specific difference was found in the levels of VLDL. A close relationship was found between lipoprotein and muscle and/or liver lipid levels. Lipoproteins synthesized in the liver seemed to be associated with the storage sites of lipid in fish. The principal lipid storage organ varies greatly among fish species. Some species store most of their triglycerides in muscle, while others use either the liver or adipose tissue as their principal site for lipid (Sheridan, 1988). McClelland *et al.* (1995) showed that both gilt-head sea bream (*Chrysophyrys auratus*) and the European sea bass (*Dicentrarchus labrax*) store lipid primarily outside of muscle (only 3 % in both species) as TG in adipose tissue (about 80 %). Sea bass store 33 % of lipid in liver, while sea bream store only 5 %. They stated that muscle can therefore be a large lipid storage depot when total tissue mass is considered. Soares *et al.* (2001) found the highest values of total lipids in red muscle (about 18 %), liver and gonads (about 16.5 %) of matrinxa (*Brycon cephalus*), but the white muscle had a lower value (2.5 %). It has been suggested that the life history of species can affect the way of stored lipid in the body. Sedentary fish such as red drum and cod tend to store lipid in liver and/or adipose tissue, relying heavily on the circulation for transport, while fish that are more active such as herring or mackerel use muscle for fat storage where it is more readily available to power locomotion (Sheridan, 1988). So, regulation of lipid metabolism could come from many sources. Feeding rate and activity have dramatic impacts on lipid decomposition and/or mobilization, as do the life stage, season of year and other environmental factors such as water temperature, light regime and salinity might also affect energy metabolism and thus the lipid metabolism (Sheridan, 1988 ; Torstensen, 2000).

Phosphorus, a limiting nutrient for algal growth, affects water quality in the natural environment by stimulating eutrophication (Elser *et al.*, 1990). However, phosphorus is also a dietary essential nutrient for fish and has many metabolic roles, for example as a constituent of bone, scales, ATP, cell membranes and nucleic acids. The inorganic phosphorus of water is shown to play an appreciable role in controlling energy metabolism in carp liver and gills mitochondria which are of great importance for fish adaptation to the environment (Arsan *et al.*, 1984). Verma and Tonk (1983) and Shukla *et al.* (2002) found that inorganic phosphate of liver and muscles of the freshwater fish. *Notopterus notopterus* and *Channa punctatus*. decreased due to exposure to mercury and cadmium respectively. Contrarily, Jana *et al.* (1985) showed increased inorganic phosphorus content and decreased alkaline phosphatase activity in the muscle and liver of *Clarias batrachus* after 14 days of incubation with mercury. The effect of mercury was markedly pronounced in the liver followed by muscle of the fish. Trusevich (1974) showed that water temperature determines the characteristics of phosphorus metabolism in the skeletal muscles of the Black Sea scad during activity as well as the duration of the steady state period and the onset of fatigue. Low temperatures considerably reduce the endurance of the fish and sharply increase the rate of utilization of energy-rich phosphates in the tissues during the initial period, which leads to a change in the ATP resynthesis pattern and to a shortening of the steady state period and acceleration of the onset of fatigue. It is concluded that the effect of low temperature is similar to that of increased load intensity. In the muscle of *Clarias batrachus*. variations in total inorganic and acid soluble phosphorus were markedly fall in its relative concentration with the increasing length of the fish. This observation appeared to be related to the metabolic variations occurring during the growth and maturation cycle (Bano, 1978). Phosphorus metabolism in farmed aquatic species has become recently a popular research subject. due to rising concerns about the phosphorus load in aquaculture discharge water (Ketola and Harland. 1993). Intestinal inorganic phosphate transport and its regulation have been studied in rainbow trout (*Oncorhynchus mykiss*) by Avila *et al.* (2000). They suggested that development or size had little effect on intestinal inorganic phosphate transport. Inorganic phosphate uptake was strongly inhibited (75% inhibition) by phosphonoformic acid, a competitive inhibitor of mammalian inorganic phosphate transport. as well as by the absence of Na^{+} (90% inhibition). Intestinal luminal and plasma inorganic phosphate concentrations each increased with dietary P concentrations. Intestinal inorganic phosphate absorption rates decreased with dietary phosphorus concentrations.

In addition to calcium structural functions in bones and scales. Ca is essential for blood clotting, muscle function, proper nerve impulse transmission, osmoregulation and for serving as a cofactor during various enzymatic processes. The calcium content in livers and muscles of the four fish species studied in the present work are within the same range recorded by Sanuki *et al.* (1999). They mentioned that the Ca contents of 21 species of fish muscles were 2.2-39.0 mg/100 g and the contents in white muscles were higher than those in reddish muscles. The contents of water soluble fraction were higher than those of water insoluble fraction except in common mackerel, skipjack, albacore and blue shark. In fish muscle containing high-level calcium, a high Ca^{2+}-binding protein content was found in water soluble fraction. The level of Ca in both liver and muscle of *Notopterus notopterus* and *Channa punctatus* was decreased after Hg and Cd exposure (Verma and Tonk, 1983 ; Shukla *et al.*, 2002). Mumba *et al.* (2001) showed that whilst the concentration of Ca was higher in the water, there were variations from month to month in the fish *Oreochromis shiranus*. Comparison of the overall concentrations in the water and fish Ca have higher concentrations in the water than in the fish and it is more concentrated in the intestine than liver, gills and muscle. Hossain and Furuichi (2000) suggested that redlip mullet, *Liza haematocheila*, do not obtain adequate Ca from sea water. Calcium uptake, both by absorption from water and assimilation from food, depends directly on the metabolic rate of the fish (Phillips *et al.*, 1955) and the amount of phosphorus available to the fish has a considerable effect on the absorption of Ca from water. The higher the P concentration in water or its content in the diet, the higher the absorption of Ca (Watanabe *et al.*, 1980). The concentration of Ca in the muscle tissue of *Cyprinus carpio* is not dependent on temperature and is regulated within defined range (Martem'yanov, 1995). The Ca contents in muscles of small-sized fishes were higher than those in the muscles of lager-sized fishes (Sanuki *et al.*, 1999). Abbas (2002) reported that the correlation of body weight or length of juvenile mangrove red snapper, *Lutjanus argentimaculatus* versus Ca was significant. Calcium appeared to have indirect effect on growth because it is significantly correlated with magnesium and sodium through the protein and fat contents.

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Table (1): Liver and muscle total proteins "T.P." (mg\g) and alkaline phosphatase "ALP" (U/g) of *Tilapia zillii*, *Mugil cephalus*, *Liza ramada* and *Solea* species taken from Qarun (Ayoub "A" and Shakshouk "S" Stations) and Bardawil Lakes.

Lake	Items	Organ	Fish species								ANOVA test P <
			T. zillii		*M. cephalus*		*L. ramada*		*Solea* sp.		
			Mean	S.D. ±	Mean	S.D. ±	Mean	S.D. ±	Mean	S.D. ±	
Qarun	TP	Liver "A"	26.56	3.322	8.078	2.635	9.544	3.017	20.21	4.691	0.000
		"S"	16.36	6.478***	10.12	1.225***	9.835	3.396	18.67	8.672	0.000
		Muscle "A"	7.991	2.792	7.345	3.131	6.445	1.727	7.741	3.091	0.002
		"S"	7.248	2.554**	6.864	2.727*	8.545	3.555***	7.558	3.620	0.000
	ALP	Liver "A"	285.3	85.84	424.2	126.0	377.8	9.000	242.3	44.89	N.S.
		"S"	319.4	114.4	460.3	62.10	800.3	208.3***	221.5	79.07	0.001
		Muscle "A"	58.39	27.70	50.23	39.73	20.88	7.260	69.94	45.99	0.000
		"S"	52.34	25.05*	43.90	22.64**	18.46	11.88	32.94	24.39**	0.000
Bardawil	TP	Liver			9.095	2.176	9.841	3.276	19.05	7.844	0.000
		Muscle			8.985	2.567	6.896	3.388	8.201	3.335	N.S.
	ALP	Liver			443.1	99.62	277.4	92.57	228.6	67.60	0.000
		Muscle			60.43	33.92	21.02	11.06	35.06	28.70	0.000
ANOVA test, P <	TP	Liver			0.003		0.03		N.S.		0.0157
		Muscle			N.S.		0.000		0.000		5.44E-6
	ALP	Liver			N.S.		0.003		N.S.		5.221E-30
		Muscle			0.000		N.S.		0.000		0.0129

Number of samples in each fish species and group = 18   N.S.: Not significant.

Significant comparisons between Ayoub (A) and Shakshouk (S) Stations of Qarun Lake:

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Table (2): Muscle total lipids "TL" (%); triglycerides "TG" (mg\g); total cholesterol "TCh" (mg\g) and high and low density lipoprotein "HDL & LDL" (mg\g) of *Tilapia zillii*, *Mugil cephalus*, *Liza ramada* and *Solea* species taken from Qarun (Ayoub "A" and Shakshouk "S" Stations) and Bardawil Lakes.

Lake	Items	Fish species								ANOVA test P <
		T. zillii		*M. cephalus*		*L. ramada*		*Solea* sp.		
		Mean	S.D. ±	Mean	S.D. ±	Mean	S.D. ±	Mean	S.D. ±	
Qarun	TL "A"	7.332	1.158	14.13	8.655	10.01	5.821	19.56	1.849	0.000
	"S"	5.281	0.519***	15.18	5.432	15.95	7.30***	17.91	10.47***	0.001
	TG "A"	522.5	89.50	193.6	56.52	248.1	123.4	41.62	26.47	0.000
	"S"	1087.5	326.2	193.2	52.49	114.3	39.79***	229.5	151.2***	0.000
	TCh "A"	437.5	82.77	232.1	78.38	616.0	121.1	179.4	17.49	0.000
	"S"	176.7	55.21***	703.7	133.32***	261.3	165.81***	306.5	195.3***	0.000
	HDL "A"	43.83	2.122	91.57	17.99	40.68	19.37	31.06	1.285	0.000
	"S"	51.31	15.83***	35.22	6.120***	106.6	61.10***	74.60	48.95***	0.000
	LDL "A"	308.4	15.04	644.3	126.6	272.7	129.7	218.6	9.038	0.000
	"S"	361.1	111.4***	247.8	43.06***	345.7	90.90***	546.7	176.6***	0.000
Bardawil	TL			6.035	2.154	2.983	0.760	3.279	0.496	0.000
	TG			1079.1	280.4	546.5	234.8	527.3	43.38	0.000
	TCh			474.1	240.1	199.5	81.76	525.9	189.3	0.000
	HDL			121.9	59.35	52.92	11.40	101.3	61.09	0.000
	LDL			442.1	88.48	386.8	66.54	311.6	48.31	0.000
ANOVA test, P <	TL			0.000		0.000		0.001		6.74E-19
	TG			0.000		0.000		0.000		N.S.
	TCh			0.000		0.000		0.000		0.0257
	HDL			0.000		0.000		0.000		0.0005
	LDL			0.000		0.000		0.000		1.66E-20

See Footnote table 1

Table (3): Liver and muscle phosphorus " P" (mg/100 g) and calcium "Ca" (mg/100 g) of *Tilapia zillii*, *Mugil cephalus*, *Liza ramada* and *Solea* species taken from Qarun (Ayoub "A" and Shakshouk "S" Stations) and Bardawil Lakes.

Lake	Items	Organ	Fish species								ANOVA test P<
			T. zillii		*M. cephalus*		*L. ramada*		*Solea* sp.		
			Mean	S.D. ±	Mean	S.D. ±	Mean	S.D. ±	Mean	S.D. ±	
Qarun	P	Liver "A"	5.571	1.842	4.601	2.048	2.483	0.299	3.817	1.162	0.000
		"S"	4.535	2.740	5.180	2.019***	4.363	2.396***	4.810	2.646***	N.S.
		Muscle "A"	6.670	1.202	7.968	1.237	8.025	1.360	7.416	0.803	0.000
		"S"	7.840	1.402***	7.148	1.166***	7.374	1.552***	7.395	1.421	N.S.
	Ca	Liver "A"	8.291	0.319	8.469	0.590	9.703	0.682	8.187	0.352	0.000
		"S"	8.168	0.253	8.890	1.130***	9.329	1.439*	8.506	0.670***	0.000
		Muscle "A"	8.056	0.987	9.455	1.393	9.049	1.080	7.589	0.358	0.000
		"S"	9.198	1.137***	9.453	1.283	8.499	0.785***	8.398	0.980***	0.000
Bardawil	P	Liver			6.954	1.405	7.227	1.444	7.421	1.240	N.S.
		Muscle			3.981	1.030	6.468	1.585	5.177	1.583	0.002
	Ca	Liver			9.315	1.347	8.763	1.010	8.409	0.613	0.000
		Muscle			10.332	0.974	9.779	0.424	8.616	0.619	0.000
ANOVA test, P <	P	Liver			0.000		0.000		0.000		0.0004
		Muscle			0.000		0.001		0.000		N.S.
	Ca	Liver			0.000		0.001		0.018		N.S.
		Muscle			0.015		0.000		0.000		0.0193

See Footnote table 1.

EXPLANATION OF FIGURES

[1] Photomicrographs of transverse sections at the gill of different fish species, H & E stain (x 400).

Qarun Lake:

Fig. (1): Oedematous (O) of interlamellar epithelial cells, *Tilapia zillii* (Shakshouk station).

Fig. (2): Pillar system deformation and hypertrophied chloride cells (cc), *Tilapia zillii* (Ayoub station).

Fig. (3): Desquamation of epithelium (E), *Mugil cephalus* (Shakshouk station).

Fig. (4): Epithelial (E) lifting and necrosis (n), *Solea egyptiaca* (Shakshouk station).

Bardawil Lake:

Fig. (5): Sever desquamation of epithelium and plenty mucus secretion, *Mugil cephalus*.

Fig. (6): Epithelial lifting and hyperplasia of mucus cells (mc) with extensive mucus in apical part of the secondary gill filament, *Liza ramada*.

Fig. (7): Odema and zigzag epithelial lifting, *Solea vulgaris*.

[2] Photomicrographs of the liver of different fish species, H & E stain (x 400).

Qarun Lake:

Fig. (8): Hepatic cells lacking extensive vacuolation (v) with accentric nucleus, *Tilapia zillii* (Shakshouk station).

Fig. (9): Necrotic (n) area in between hepatic cells, *Tilapia zillii* (Ayoub station).

Fig. (10): Vacuolar (v) degeneration, *Mugil cephalus* (Shakshouk station) with pyknotic nuclei (p).

Fig. (11): Hepatic fibrosis (F) and oedema (O) with necrosis, *Mugil cephalus* (Ayoub station).

Fig. (12): Sever hepatic vacuolation, *Solea egyptiaca* (Shakshouk station).

Bardawil Lake:

Fig. (13): Vacuolar degeneration with pyknotic (P) nuclei, Mugil *cephalus*.

Fig. (14): Cloudy swelling and blood vessel congestion with heamosidrosis (hs), *Liza ramada*.

Fig. (15): Vacuolar degeneration with inflammatory cells infiltration (ic), *Solea vulgaris*.

[3] Photomicrograph of the kidney of different fish species. H & E stain (x 400).

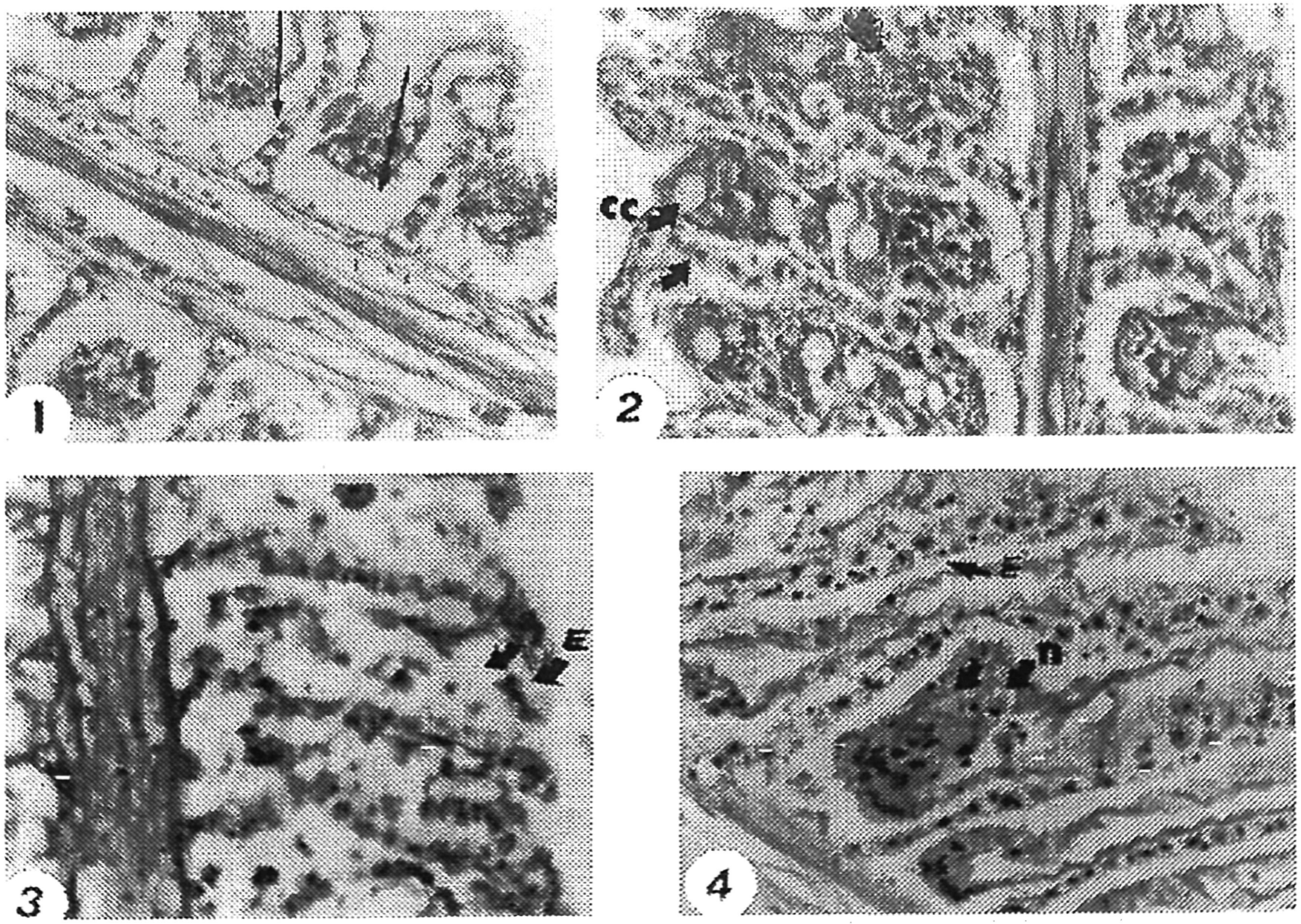
Qarun Lake:

Fig. (16): Glomerulonephrites (g). haemosidrosis (hs) and necrosis of proximal (pt) and distal (dt) convoluted tubules (Shakshouk station).

Fig. (17): Hydropic swelling. degeneration and haemosidrosis (hs) (Ayoub station).

Bardawil Lake:

Fig. (18): Degeneration. necrosis of the proximal (pt) and distal (dt) convoluted tubules with cystic appearance of glomerulei (g).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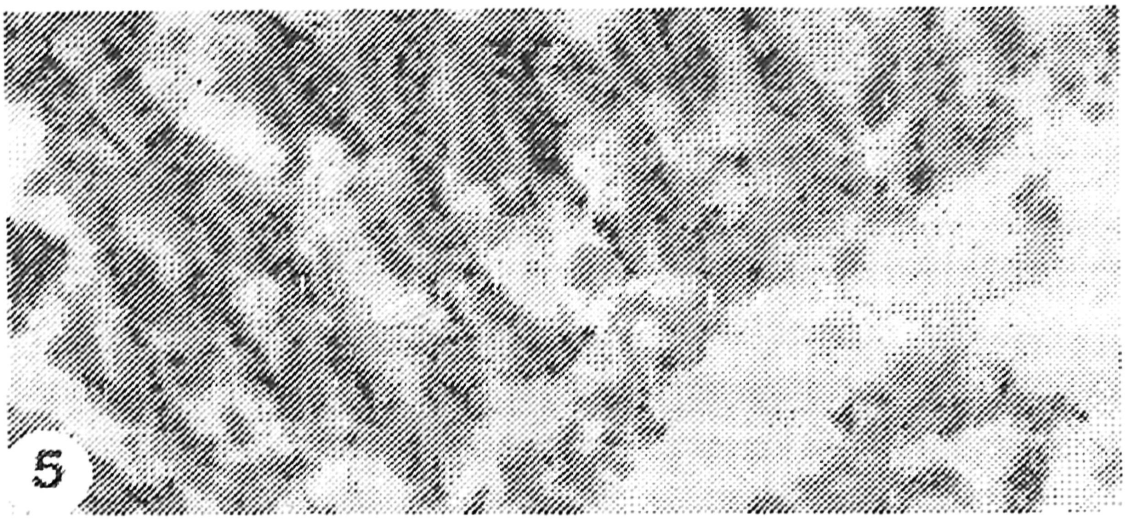
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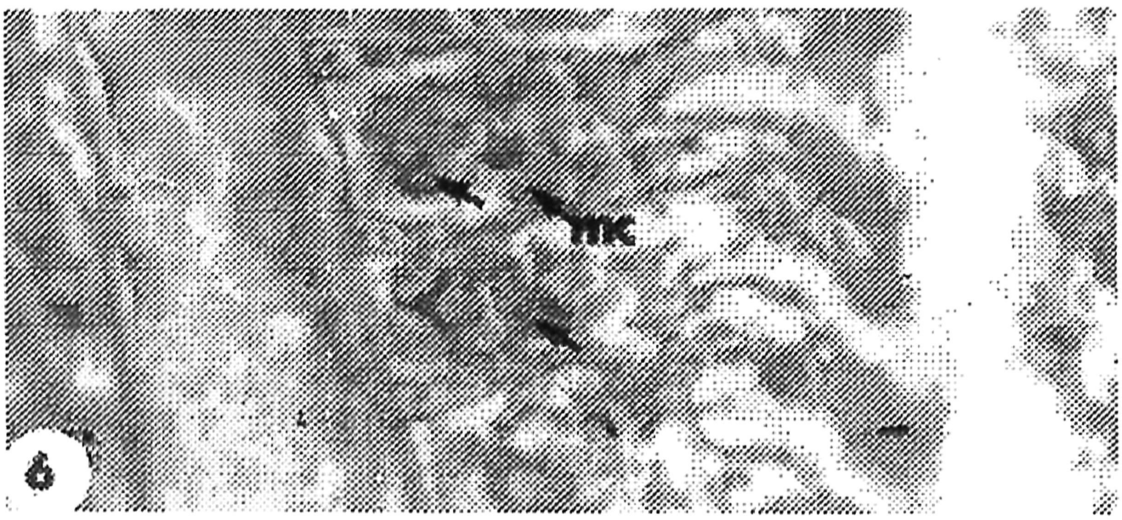
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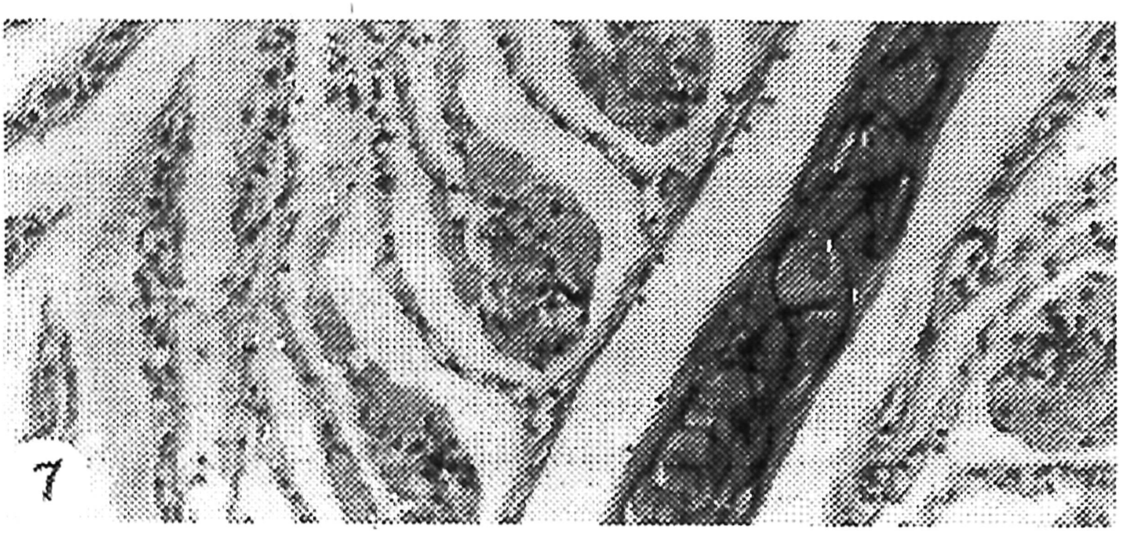
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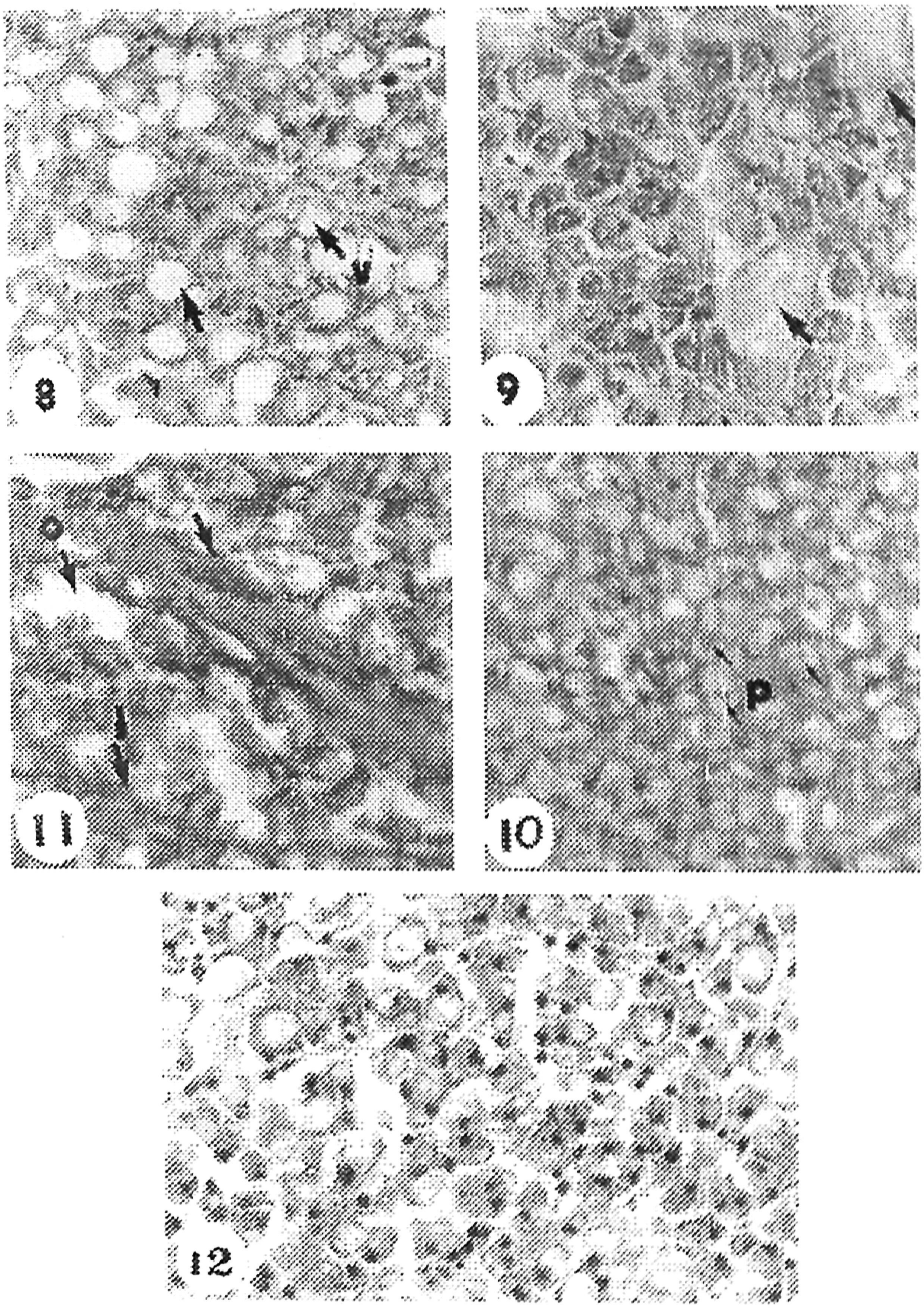
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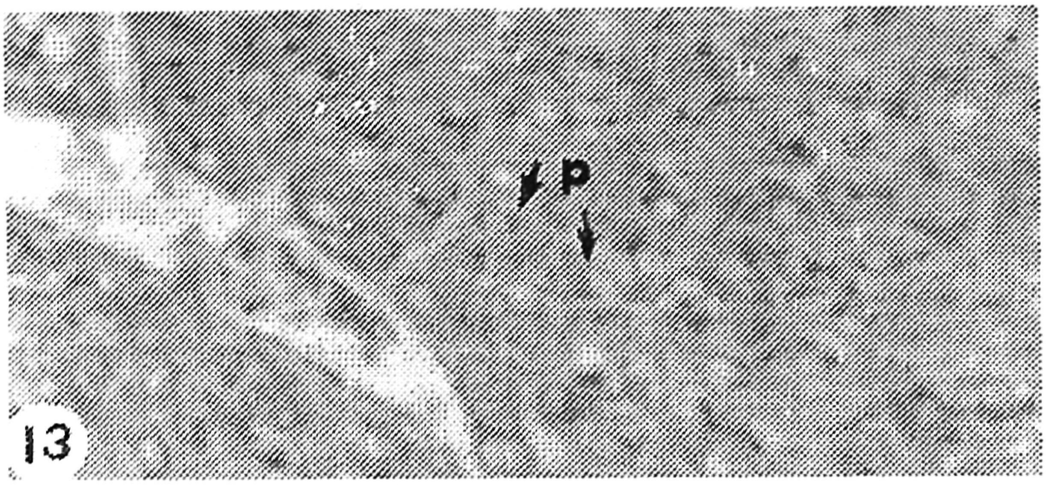
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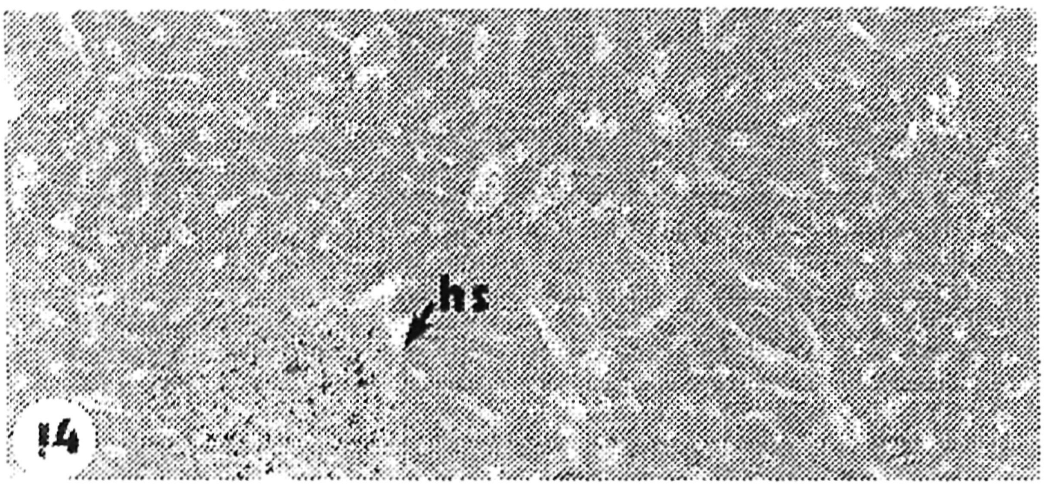
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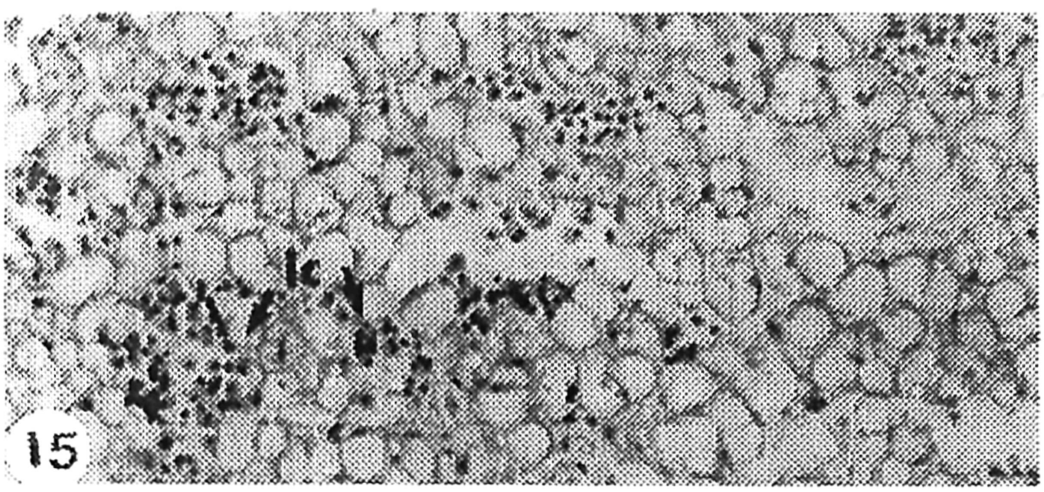
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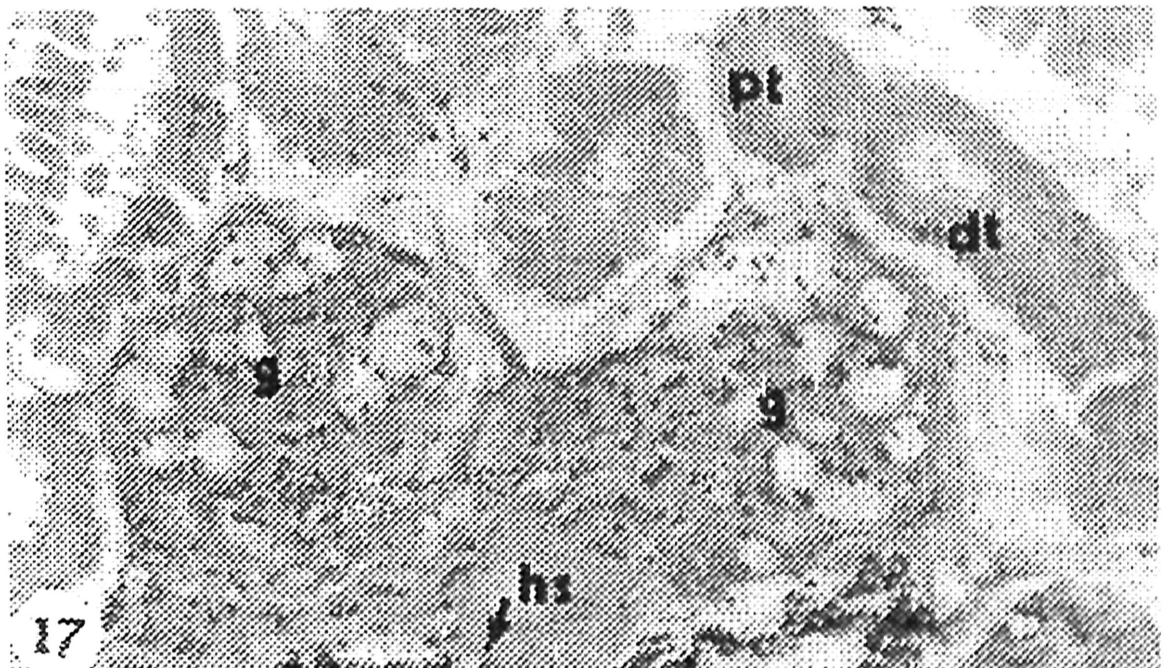
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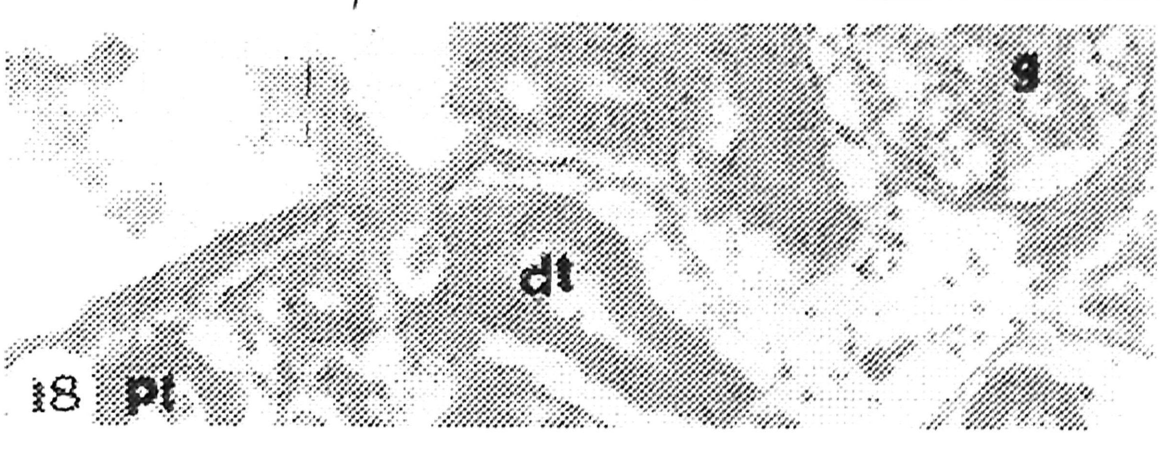
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