Egypt. J. Aquat. Biol. & Fish., Vol. 9, No. 1: 185-202 (2005) ISSN 1110-6131

IMMUNOHISTOPATHOLOGICAL EFFECTS OF FENTHION TOXICITY ON THE COMMON CARP (*CYPRINUS CARPIO*)

Hekmat M. Tantawy ; Mariam M. Sharaf ; Ismail M. Abd Elnabi and Hend M. Tag
Dept. of Zoology, Faculty of Science, Suez Canal University, Ismailia, Egypt.

Key words: *Cyprinus carpi*, pesticide: fenthion: histopathology: immunity.

ABSTRACT

Fenthion is an organophosphate pesticide, widely used all over the world as a broad-spectrum insecticide for the protection of numerous crops especially rice. Due to these agricultural activities Fenthion has repeatedly been detected in surface waters and soil. Thus, Fenthion is among the most toxic pesticides for aquatic life especially fish. This study had investigated the toxic effect of Fenthion on some immunological and histopathological parameters. Fish were exposed to concentrations of 0.00 (control), 0.83 ppm and 2.1 ppm for 96 hours. The histopathological alterations for lymphoid and non-lymphoid organs, as compared to the control group, included hemosiderosis and atrophy in spleen and pronephrous. In gills, hemorrhage, progressive degeneration and lymphocyte infiltration were reported: in liver and pancreas atrophy has been shown in addition to excessive zymogen granules secretion in pancreas. The kidney trunk showed glomerular and tubular atrophy and lymphocyte infiltration. For the alterations of plasma immunoglobulin level, Fenthion at all concentrations suppressed plasma IgM level ($P < 0.05$). So it can be concluded that Fenthion causes adverse effects on immunological and histopathological parameters of exposed fish.

INTRODUCTION

Pollution presents one of the most challenging unsolved problems which have deleterious effects on biological systems. These dangerous and undesirable effects are mainly due to the toxic substances. present in the industrial. domestic and agricultural wastes, especially pesticides.

In the last decades. organophosphate insecticides were produced and entered the environment in greater quantities than other pesticides. This is due to the general fact that these organophosphates are degraded more rapidly in environment (Abo Nour and Amer, 1995). They ultimately find their way into water bodies as consequence of rain and leaching from the soil and polluted water in which fish exist (Danasoury, 1997).

The dangerous effects of organphosphorous insecticides on fish present as accumulation of pesticides residues directly from water through their respiratory processes. and also from food. The effects may be acute, resulting in mass mortality or chronic involving changes in survival. growth and reproduction (Khallaf *et al.*, 1994).

Cyprinus carpio or common carp is a large minnow. That is considered a freshwater fish (Webb, 1981). It follows order Cypriniformes which comprises two families. eight genera and eight species according to Robins *et al.* (1991).

Cyprinus carpio is one of most important freshwater fish in the world since it is considered a prized food in Asia. Europe and Middle East (Eddy and Underhill, 1974). Banarescu and Coad (1991) reported that the carp is an important food throughout of the world. except for Australia and North America. where the fish is considered unpalatable. They also reported that the world catch rate of carp per year exceeds 200.000 tons.

The aim of the present work is to investigate the hazardous effects of the organophosphate insecticide Fenthion. which is widely used throughout the world as a wide spectrum insecticide for the protection of numerous crops (Kitamura. 2003). in the teleost fish *Cyprinius carpio*.

MATERIALS AND METHODS

Test animals and treatments :

The common carp (*Cyprinus carpio*) were purchased from the Central Laboratory for Aquaculture Research (CLAR) at Abbassa. Agricultural Research Center. Ministry of Agriculture. Fish were transported live to the laboratory in nylon bags under continuous aeration. Fish were reared in glass aquaria of 40 liters capacity. continuously aerated through stone diffusers connected to a mechanical air compressor. Fish were fed on commercial pellets with an average protein content of about 30%. Fish were fed twice a day at a rate of 3% of wet weight, while faeces and unused pellets were collected regularly. Fish density was in the range of 30 fish of each aquarium. Fish used in bioassay tests had an average weight of 12±3 g. and an average length of 7±3 cm.

Fish were maintained in a natural illuminating system under normal prevailing photoperiod conditions. Water used in aquaria was dechlorinated tap water: its temperature was maintained at around 25 C throughout. Meanwhile, the physicochemical properties of water were: pH 7.5±0.03, dissolved oxygen 5.95±0.26 mg L and alkalinity 3.48±0.29 meq L as Ca Co_3.

Tested compound:

Fenthion is an organophosphorus insecticide. The empirical formula is $C_{10}H_{15}O_3PS_2$. The purity of technical-grade Fenthion is generally > 95%. It smells like mercaptans and is a colourless- to-yellow liquid with a specific gravity of 1.25 and a fairly low vapour pressure, slightly soluble in water, and very soluble in various organic solvents.

Determination of Half Lethal Concentration (LC50) of Fenthion, and Route of Exposure:

Toxicity tests were performed using apparently healthy fish of approximately equal size and length. Fish were starved for performing the toxicity tests, and all through the exposure time to rule out differences in toxicity ascribed to variations in feeding habits and to prevent any loss of tested compound if adsorbed on the pellets surface, and to ensure that the only route of pesticide assimilation would be via gills. A series of concentrations of the tested pesticide was made, each concentration in a separate aquarium and replicated for at least three times.

The different concentrations of the pesticide were transferred to the glass jars, each filled with ten liters of dechlorinated tap water. Ten fish, which were previously acclimatized, were transferred to each jar and confined for an exposure period of 96 hours. On the other hand, control fish were maintained in similar aquaria that contain only water.

Dead fish were removed as soon as death was confirmed. At the end of the exposure period final mortality records were made in treated and control aquaria. Results were subjected to probit analysis method (Finney, 1971) to produce regression lines and determine the median lethal concentration (LC50) and the 95% confidence limits.

No fish died at the concentration below 5 ppm and no one survived at concentration higher than 8.9 ppm. According to this method. LC50 obtained for Fenthion was 8.3 ppm, and the two concentrations used were 0.83 ppm and 2.1 ppm (1/10 and 1/4 LC50, respectively)

Determination of *in vivo* effect of sub-lethal concentration of tested pesticide on parameters under investigation of common carp :

Determination of serum IgM:

The IgM level was determined by using enzyme immunoassay test kit.

Histological preparation:

Control and insecticide treated fish were anaesthetized and dissected immediately. Tissue samples of fish liver. kidney. gills. spleen. and thymus were excised and cut into small pieces. Tissues of different organs were fixed rapidly in 10% formalin for 24 hr. washed under running tap water for 24 hr.. dehydrated in ascending grades of ethyl alcohol, cleared in terpineol for two days. then washed in benzene for 10 minutes and embedded in three changes of pure paraffin wax. Serial transverse sections of all selected organs. 5 microns thick were cut and amounted on clean glass slides. stained in haematoxylin (Harris HX) and eosin. cleared in xylene and mounted in Canada balsam(Drury and Wallington. 1980).

After that sections of selected organs were carefully examined and photographs were taken as requested.

RESULTS AND DISCUSSION

External manifestation and anatomical signs of the exposed common carp due to exposure to Fenthion:

A neural paralytic syndrome was typical for fish poisoned with Fenthion. Strong restlessness started when fish come into contact with the poisoning bath. Fish excitation was reflected by an increased reaction to exogenous stimuli and by cramp movements of fins and mouth. Loss of movement coordination began. as well as loss of orientation in water. The fish turned on the flank and swam in half-circles. Reaction to excitation was manifested by sudden movements and fin tremor. Body surface darkening was noticeable in this phase of poisoning. mainly on the dorsal part. Weakening of jerks or areflexia. paralysis. arrhythmia and block of respiration movements began in the terminal phase of poisoning. The fish fell into agony and died in a short time.

Pathological and anatomical signs found in fish poisoned with Fenthion were not specific. Body surface was opaque with slightly increased amount of mucus and with excessive pigmentation mainly on the dorsal part. No changes on the eye. Gills had straight edges, normal color, and slightly increased amount of mucus. In the body cavity there was an evident injection of internal organ vessels, mainly hyperaemia of hepatopancreas.

Immunological Studies:

The immunology of fish species is less well characterized than in mammalian species, although the knowledge has increased rapidly in recent years. As in mammals, the immune system biomarkers in fish to have considerable potential for application in pollution biomonitoring (Wester *et al.*, 1990).

The immunological parameters used as biomarkers in the present study included morphology of leukocyte producing organs (spleen and pronephorus) and level of serum immunoglobulin M (IgM).

The effect of Fenthion administration on the level of serum IgM of *Cyprinus carpio* was presented in Table (1) and Figure (1). The results indicated that treating the fish with high and low concentrations (2.1 and 0.83 mg/l respectively), caused a highly significant decrease ($p<0.001$) in the level of serum IgM. The first group treated with high concentration, showed highly significant decrease (1.11, 0.91, 0.77, and 0.40 *vs* 1.43 control) at different time peroids (12, 24, 48 and 96 hours respectively), and the second group treated with a low concentration, showed also highly significant decrease (1.21, 0.97, 0.84 and 0.63 *vs* 1.43 control) after the same exposure periods.

The obtained data recorded in Table (1) indicated that there was a significant decrease in IgM level of common carp serum after exposure to low and high concentrations of the tested insecticide for 12, 24, 48 and 96 hours. These results are in agreement with those of O'Neill (1981) who mentioned that heavy metals caused a depression of humoral antibody titer in brown trout (*Salmo trutta*). Also, Barnett and rodgers (1994) reported that administration of 5 to 10 mg/kg malathion to rabbit for 5 to 6 weeks significantly decreased the serum antibody titers generated in response to *Salmonella typhi* vaccination.

Immunohistopathological studies:

In this study the normal histological structure of the examined organs (gills, pseudobranch, kidney trunk, pronephrous, spleen and liver) are illusterated in Figures 2, 5, 8, 11, 14, and 17 respectively.

The gills of fish are the main target organs for toxic action of chemical pollutants, as well as for detoxification process (Kumar and Pant, 1984). Swelling and lifting of the epithelial layer of the gill lamellae (Fig. 3) as a result of the effect of low concentration (0.83 mg/ l) of Fenthion for a period of 96 hours was recorded. Similarly a morphological study of the gills of channel catfish exposed to malathion, showed edema with epithelial separation from basement membranes, general necrosis, and epithelial desquamation (Areechon and Plumb, 1990). Also, Mallatt (1985) reviewed gill structural changes in fish exposed to chemicals, and concluded that most lesions were separation of the branchial epithelium from the basement membrane, and mucous hypersecretion, lamellar fusion, and hyperplasia of the gill epithelium.

Fusion of the gill lamellae (Fig. 4) was noticed with the increase of Fenthion concentration (2.1 mg/l).Fusion of secondary lamellae as recorded in the present investigation was also noticed in bluegill *Lepomis macrochirus* exposed to malathion, which clearly implies a reduction in the respiratory surface (Richmonds and Dutta, 1989). Nevertheless, fusion of the adjacent lamellae also occur after exposure to heavy metals, such as cadmium and copper (Randi *et al*., 1996 ; Arellano *et al*., 2000).

As for the pseudobranch, the impact of the two concentrations used in the current study showed a separation in the branchial filaments (Fig. 6) and degeneration of both the acidophilic cells and the connective tissue surrounding the filaments (Fig. 7).

Atrophied glomeruli were a marked lesion in the kidney of fishes treated with the low concentration of Fenthion (Fig. 9), with the epithelium degeneration of the renal tubules (Fig. 10). Gupta and Dalela (1987) reported histological changes in kidney of *Notopterus notopterus*, exhibiting degeneration and dissolution of epithelial cells of renal tubules, hypertrophy and necrosis following subtle exposure to phenolic compounds.such abnormalities of the kidney of medaka and guppies (*Poecilia reticulata*) exposed to organotin compounds developed degeneration of tubular epithelium and inflammatory glomerular lesions (Wester *et al*., 1990).

The head kidney (pronephrous) of fish treated with both concentrations elucidated infilteration of the lymphocytes and deformation of the haematopoietic tissues (Figs. 12 and 13). Moderate to marked cellular infilterations comprised mostly of mononuclear cells were discernible in the interstitial tissues, which might be explained as a defense mechanism in the fish to counteract toxic metabolites (Das and Mukherjee, 2000).

Hemosiderin granules were found in the spleen under the effect of low concentration of Fenthion (Fig.15). Furthermore ,proliferation of lymphocytes were shown in the spleen treated with higher concentration (Fig. 16). The amount of hemosidrin in the spleen was usually small, but this may be due to increase in the rat of destruction of erythrocytes after the exposure of pesticides (Hibiya, 1982).

In the liver of *Cyprinus carpio* the pancreatic tissue invades the liver along the branches of the portal vein, so it is called hepatopancreas. The liver of fishes treated with low concentration of Fenthion showed atrophied hepatocytes (Fig. 18). As for the high concentration effect (Fig. 19), zymogen granules (acidophilic substances) were presented in the glandular lumen of pancreas. A histopathology study in freshwater teleost *Channa punctatus* (bloch) due to the effect diazinon, (an organophosphate insecticide), showed liver hepatotoxic lesions of fatty infiltration, nuclear or general hypertrophy of hepatocytes and loss of hepatic glycogen (Anees, 1976). Hibiya (1982) reported that the atrophied pancreatic cells contain very few zymogn granules as an example of pathological changes. Such findings differ from what was found in this study as there was large amount of zymogen granules.

CONCLUSION

In conclusion, the current study focuses upon the immunology and histopathology as an important tool for detecting the effect of pesticide toxicity on fish.

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Fig. (3): A section of the gills of a fish treated with low concentration Fenthion showing epithelial layer lifting (→). (X400).

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Fig. (5): A section of the pseudobranch showing normal structure of the branchial filaments (BF) and the acidophilic cells (AC). (X400).

Fig. (6): A section of the pseudobranch of a fish treated with low concentration Fenthion showing the branchial filaments separation (→). (X400).

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Fig. (17): A section of the liver showing the normal structure of the hepatocytes and pancreatic cells (P). (X400).

Fig. (18): A section of the liver of a fish treated with low concentration Fenthion showing atrophied hepatocytes (→). (X400).

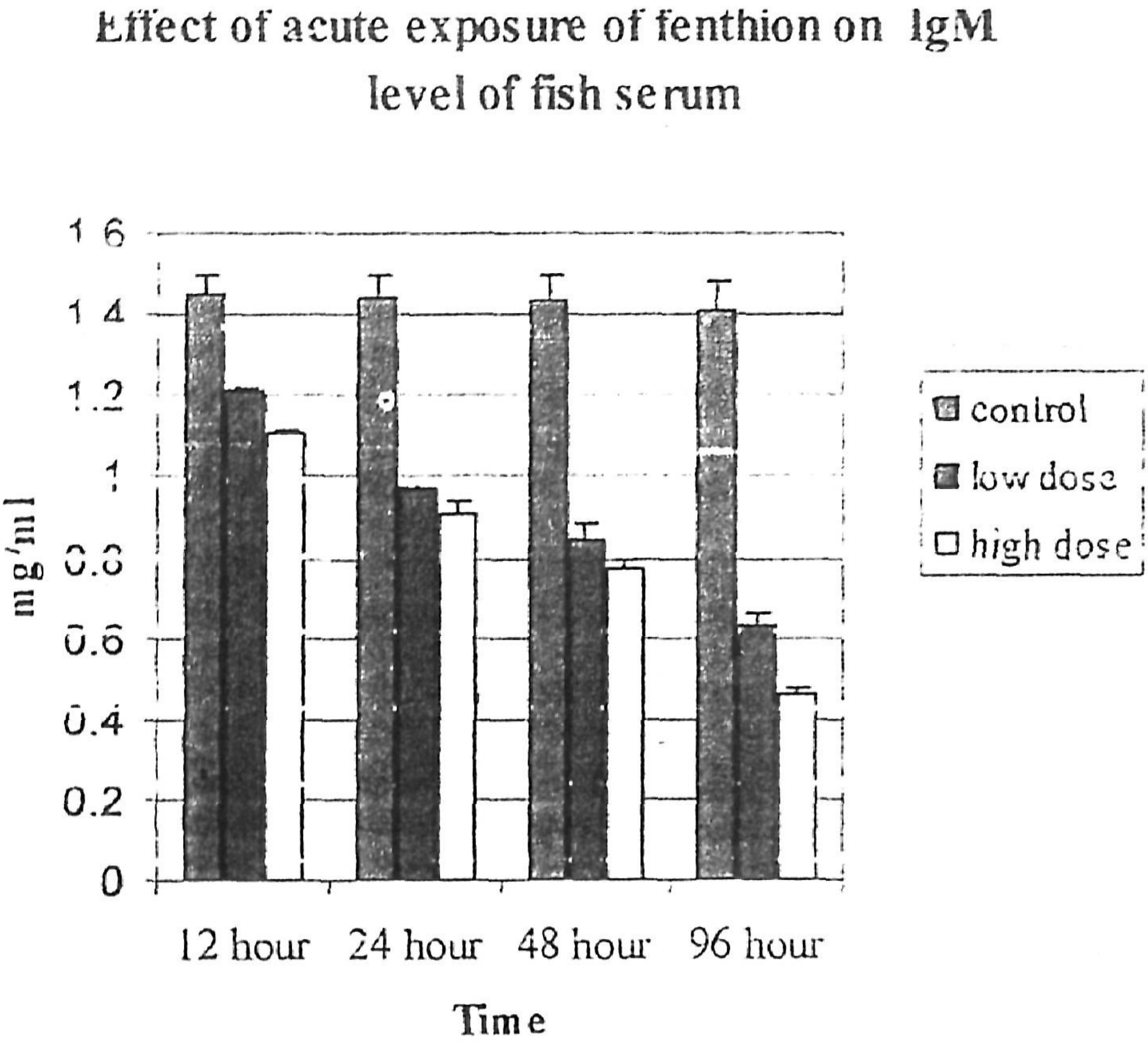
Fig. (19): A section of the liver of a fish treated with high concentration Fenthion showing zymogen granules (ZG) within the pancreatic cells. (X400).

Table (1): Changes in IgM level of fish serum after aquatic exposure of two concentrations of Fenthion (0.83 and 2.1 ppm).

Time hours	Control	Treated		% of change	
		0.83PPm (Low conc.)	2.1 PPm (High conc.)	Low conc.	High conc.
12 hr.	1.45± 0.05	1.21±.008**	1.11±.007**	-16.6	-23.4
24 hr.	1.44± 0.06	0.97±.02**	0.91±.03**	-32.6	-36.8
48 hr.	1.43± 0.07	0.84±.04**	0.77±.03**	-41.2	-46.2
96 hr.	1.41± 0.05	0.63±.03 **	0.46±.02**	-55.3	-67.4

Data represent the mean value ± S. E. from 6 fish / group

- Significant values by one way analysis of variance (ANOVA test) compared to control group (* $P<0.05$, ** $P < 0.01$)


Effect of acute exposure of fenthion on IgM
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Time
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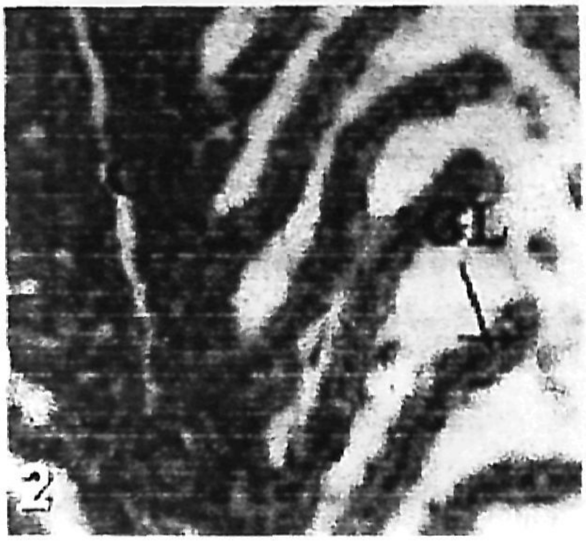
Fig. (1)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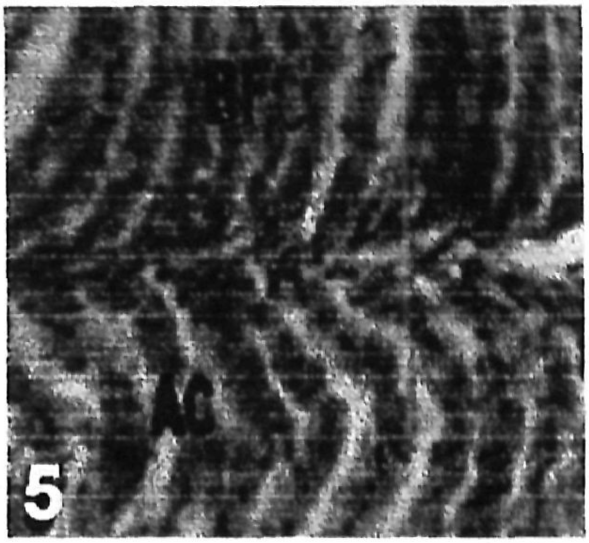
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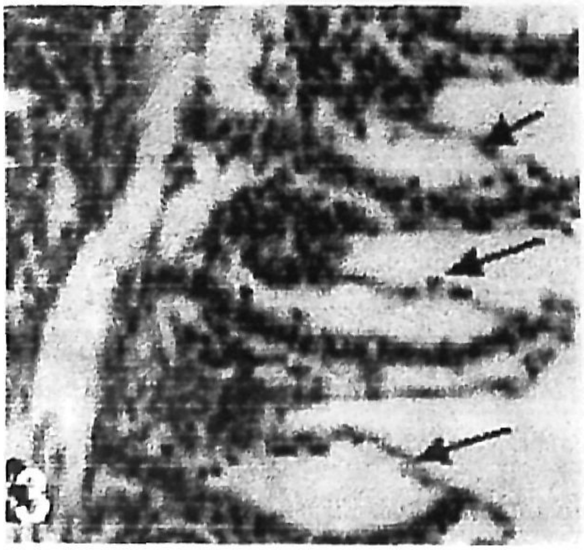
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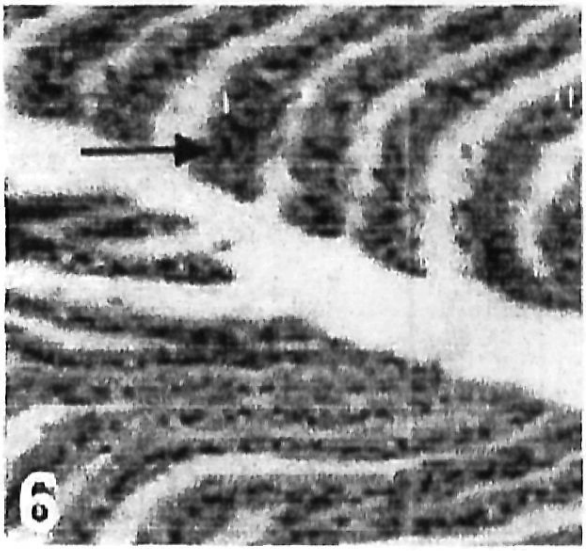
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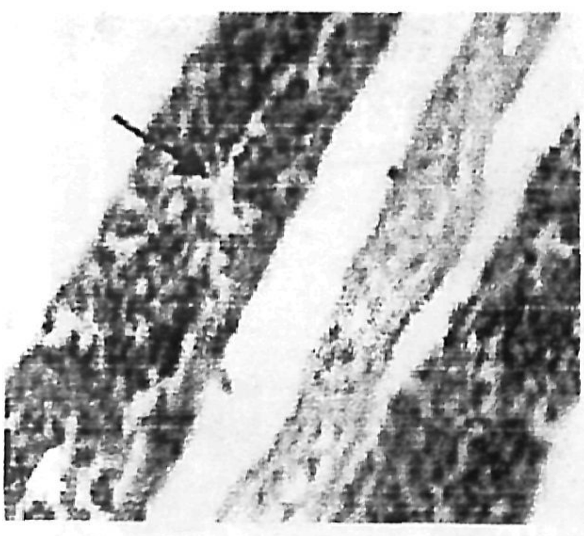

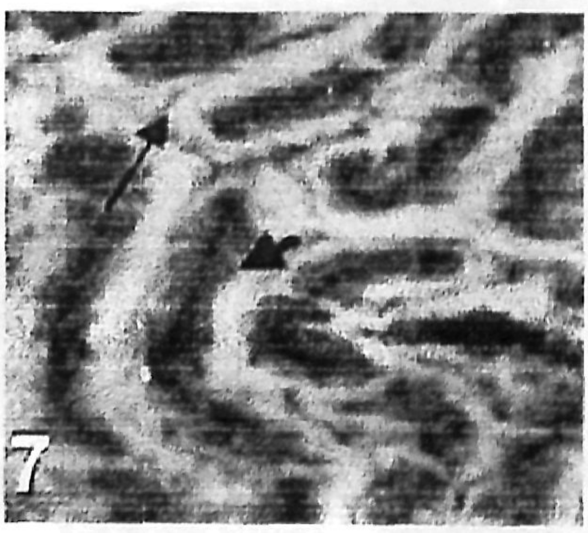
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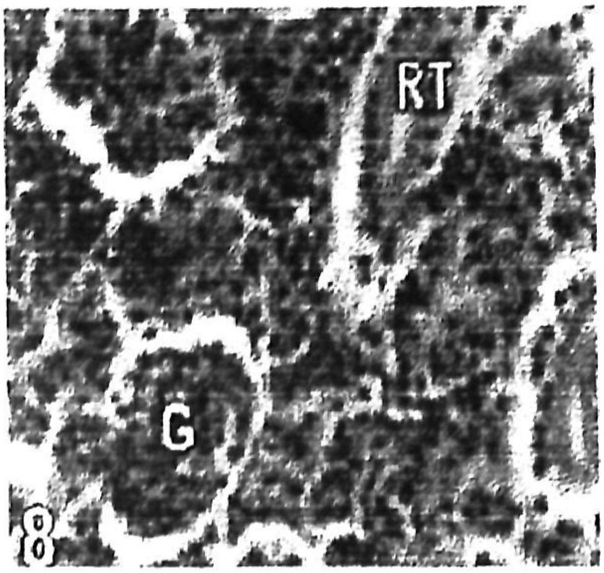
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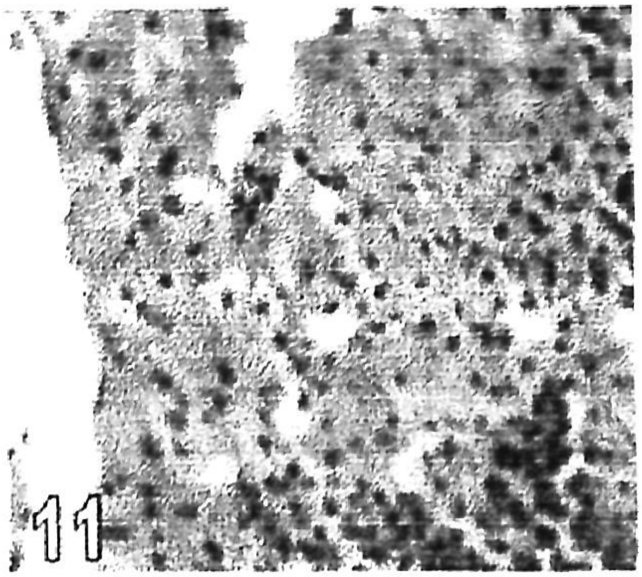
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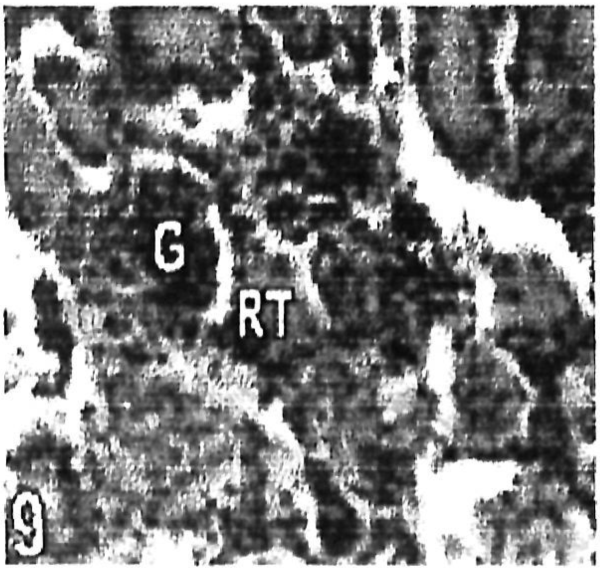
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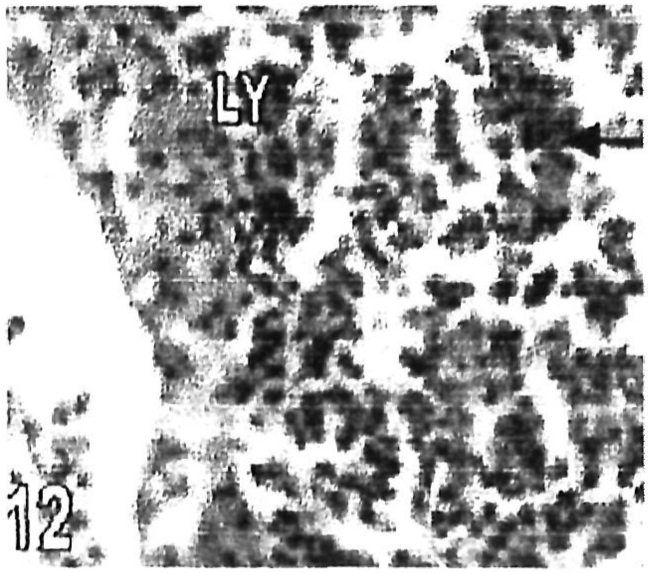
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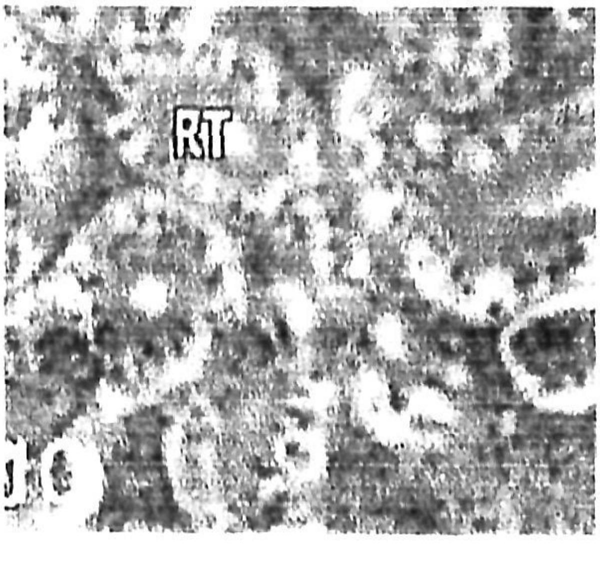
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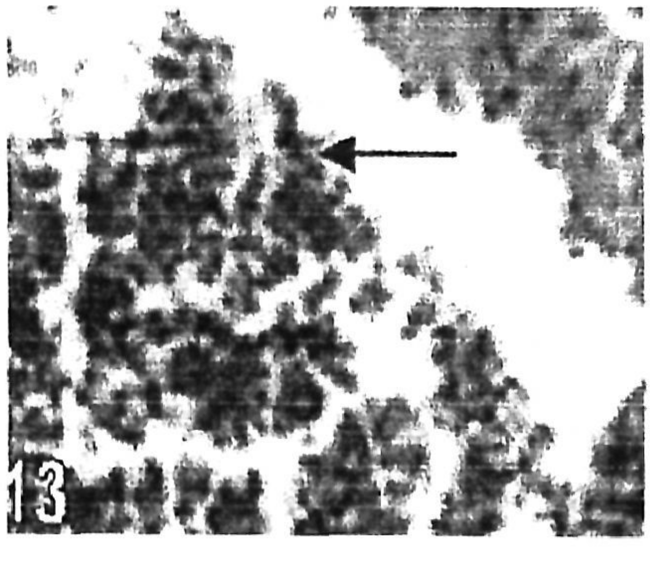
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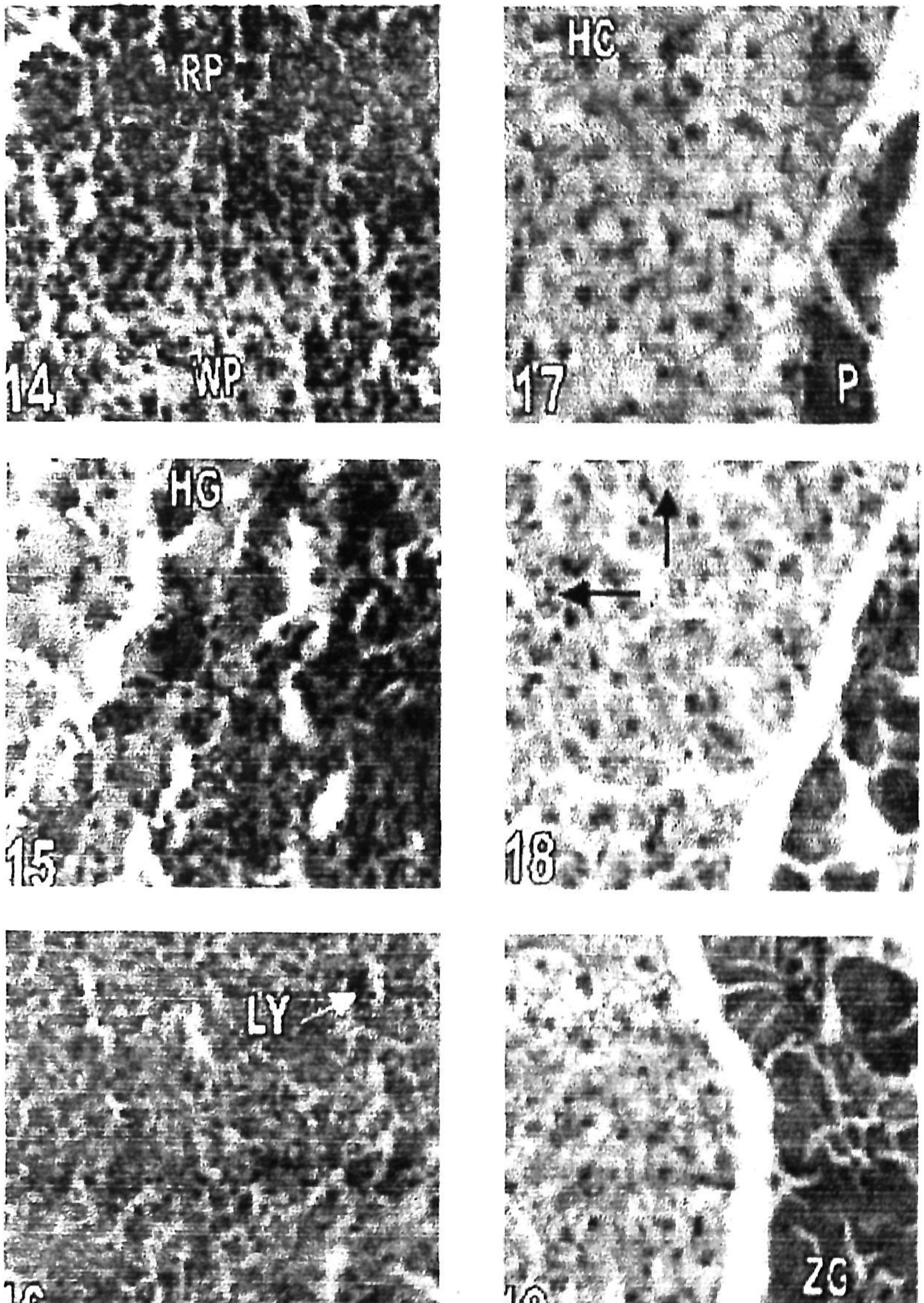
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