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RESEARCH ARTICLE

HISTOPATHOLOGICAL STUDIES ON CARP (CYPRINUS CARPIO) EXPOSED TO FENTHION

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Abstract

Indian carp (Cyprinus carpio) were exposed to Fenthion (0.387, 0.193, 0.096 mg/l) for the period of 60 days. Fishes were sacrificed at the end of the trial period to study light micrscopic changes associated with toxicity. Liver, kidney, gill, and intestine were examined for histopathological studies. Swelling of the hepatocytes with diffuse necrosis and marked swelling of blood vessels were observed in the liver tissue. Tubules of the kidney were distended, with tubular cells of posterior kidney exhibited marked necrotic changes. Gill tissue showed fusion of primary lamellae, congestion of blood vessels and hyperplasia of branchial plates. After the exposure of Fenthion the secondary lamellae of the gills were shortened, deformed, swollen, ruptured and hyperplasia was observed. Many intestinal villi were ruptured near the tips; cellular exudates were also observed. Enlarged mucous cells filled with secretary materials were observed. Proliferations of mucous epithelial cells with pycnotic nuclei were seen.

Introduction

Any organ can function normally only when its structure is normal but any structural damage to it is likely to affect the function of that organ. Virchow, (1958); Bell, (1968) & Brown et. al. (1968) suggested that there is a clear correlation between pathological condition of cell or tissue and it's affected functions. Thus, a study on histology provides a very important and useful data concerning changes in cellular or sub cellular structure of an organ much earlier than external notification. Histological criteria serve as a working approach for assessing toxicity in number of animals. Unfortunately, however, the effect of toxic substances on fish has to some extent been hampered because of the lack of proper histological literature on various fish organs. Such an experimental study helps in determining the extent of pollutant stress, well in advance to avoid any future disasters.

Lillie et. al. (1947), Durham et. al. (1963), Eller (1971) reported that nature and the extent of damage depend on the fish, the pesticide and its concentration. Schmid and Mann (1961) reported the damage to fish gills which resulted from exposure to sulphonate detergents. King (1962) described various histopathological changes in liver, intestine and kidney, particularly cell vacuolation in guppies and brown trout exposed to sublethal concentration of DDT. Mathur (1962 a & b) reported loss of parenchymatous cells of renal tubules and degeneration of the epithelium of Ophiocephalus sps exposed to DDT. Pathological lesions were noticed in liver, brain, spinal cord, kidney and stomach in fish (spots) after three weeks exposure to 0.075 /µg/l Toxaphene by Lowe (1964). Rainbow trout exposed to 5 ppb of Toxaphene for 11 days showed paranchymal cell necrosis and disruption of the cordal structure of liver tissue (Wood 1967). Eller (1971) observed that chronic exposure of Endrin to cut throat fish caused hyperplasia of islets of langerhans. He also noticed odema, haemorrahage, intercapillary congestion and hyperplasia in the gills after exposure to higher levels of Endrin. According to Boulekhache (1974), Lindane caused cell vacuolation in liver and muscle of trout fry. Bhattacharya et al. (1975) observed histopathological lesions in the hepatopancreas of Clarius batracus when exposed to Endrin. Gaikwad (1981) reported histopathological changes in the liver, kidney and gill of Thiodan exposed T. mossambica. Sastry and Sharma (1978) reported liver cord disarray, connective tissue damage, vacuolation in cytoplasm of hepatocytes, and degeneration of the nucleus in liver tissue

when Ophiocephalus punctatus was exposed to Diazinon. Ramalingham,& Reddy (1982) studied 24 hr and '96 hr sublethal concentrations effects of Lindane on Colisa falia and reported that after exposure for 95 hr. period showed extensive vacuolation, enlargement and pycnosis of nuclei. Crandell and Goodnight (1963) suggested that prolonged exposure of fish to low level of pollutant could lead to variety of internal damages. Arora et. al. (1971,72) reported that histopathological effect depended on concentration and duration of exposure. Virtanen (1986) reported shortened and deformed gill lamellae in Paecilla reticulatus when expsed to DDT. Soman (1987) studied histopathological changes in gills, liver and stomach of Colisa fasciata when exposed to Lebaycid. Gupta and Dalela (1987) noted histological changes in kidney of Notopterus notopterus exposed to sublethal levels of Phenol 2,4,-dinitrophenol and pentachlorophenol. Khillare& Wagh(1988) observed histopathological changes in gill after chronic exposure of Endosulfan, Malathion and Sevin (0.00038, 0.00I-390, 0.00421 mg/l) for 16 weeks in the fresh water fish Barbus stigma (Ham).

With a view to throw more light on the above facts in this chapter histopathological study on C. carpio exposed to sublethal concentrations of Fenthion was carried out on gill, liver, kidney and intestine.

Materials and Methods:

. Live specimen of cyprinus carpio measuring of approximately the same size (13 cm.) and 18-20 gm weight were collected from local fish market and were transferred into water containers 25 liters of chlorine free water for acclimatization after dipping them into low concentration of KMnO4 solution for few seconds in order to check microbial infection. Each group containing eight fishes was selected after successful acclimatization following the standard procedure. The selected fishes were exposed to three different sub lethal concentrations of Fenthion for the period of 60 days. A control set of fishes was also maintained simultaneously. At the end of experimental period four fish were sacrificed from each group and were subjected to histopathological studies.

The required tissues were taken from fish after killing it by decapitation, were fixed for 24 hours in Bouin‘s fluid and processed following standard procedure for routine microtechnique. The blocks were prepared in paraffin wax with melting point 54-56°C and sections were out to a thickness of 8 to 10 µ. Staining procedure was followed using Delafield's hematoxylin and Eosin.

Results and Discussion:

Gill – Control: Fig. 1a, 1b.

Below the operculum, are found four branchial arches which extend from either side of pharynx. Each branchial arch bears two hemibranchs consisting of two rows of tapered and flattened gill filaments (GF) which lie parallel to one another and perpendicular to the arch. Each filament is supported by an eccentrically placed cartilagenous gill ray (GR) acting as mechanical support. On the upper and lower surfaces of each filament are a series of flattened leaf like structure each called secondary lamella (SL) which form the respiratory surfaces. The epithelial wall (EW) of each secondary lamella is held apart and supported by the pillar cells (PC), leav- ing a blood space (BC) connecting the afferent (ABV) and efferent blood vessels (EBV). The filament consists of a layer of epithelial cells, basal laminae and a layer of connective tissue. Mucous goblet cells and chloride cells are also present.

Fenthion-chronic: Fig. 1c, 1d, 1e(1),1e(2).

On the exposure to Fenthion (0.387, 0.193, 0.096 mg/l) for the period of 60 days the changes observed were as follows :

i) Proliferation of primary lamellar cells.

ii) After the exposure of Fenthion the secondary lamellae of the gills were shortened, deformed, swollen, ruptured and hyperplasia was observed.

iii) The overall appearance of filaments was more cellular.

iv) In between the cells large number of blood cells were seen scattered in many places in the tissues.

v) Chloride cells were swollen.

Liver control; fig 2a

Normal liver shows following structures. It is irregular in shape and hepatic cells are arranged in cords. Each cord is separated from the adjacent one by blood space (Bs).The blood spaces are lined by connective tissue. The nuclei of the hepatic cells are round and centrally located and shows three to four nucleoli. Islets of langerhans are seen scattered in association with the hepatic blood vessels.

Fenthion chronic *(0.387, 0.193,0.096 mg/ml) Fig.2b,2c and 2d* .

1) The regular cordal arrangement of cells was not seen.
2) Hepatic cells were extensively damaged
3) These cells become vacuolated in peripheral region. The nuclei of these cells were pushed towards periphery (pycnotic).
4) In liver exposed to highest concentration, the cell boundaries were ruptured and disintegrated at certain places which lead to the leakage of cytoplasm and nuclear contents.
5) In between hepatic cell large number of blood cells was seen scattered in many places in the tissue.

Kidney-control : **Fig. 3a.**

The kidney consists of coiled uriniferous or renal tubules (T) (not arranged in any specific pattern) & malpighian body (M). Malpighian body consists of glomerulus(G) & Bowman's capsule(Bc). Each renal tubule can be differentiated into neck segment, proximal segment and distal segment.

Fenthion chronic (0.,387, O.193, 0.096 mg/l).Fig 3b, 3c, 3d, 3e, 3f.

i) Renal tubule cells were vacuolated and started degenerating.
ii) The glomeruli showed swellings and vacuolation.
iii) Uriniferous or renal tubules were swollen.
iv) Ruptured, vacuolated and disintegrated cells were more in number in higher concentration.
v) A visible increase in blood cells number was observed.

Intestine-control : Fig. 4a.

lt is a coiled, elongated structure showing following layers.

i) Serosa(S1);This is the outer most covering consisting of a single layer of epithelial cells.
ii) Subserosa (SZ) or muscularis layer consists of smooth muscle fibers arranged in definite pattern the outer being longitudinal and inner circular.
iii) Submucosa (S3); Consisting of connective tissue fibers blood vessels and nerve endings.
iv) Muscularis mucosa (M1); with two layers of muscles i.e. outer longitudinal and inner circular muscles.
v) Gastric mucosa (M2); Epithelial coat forming inner layer,formed of columnar prismatic cells with basically located nuclei. The columnar cells of mucosa seem to be modified to form globet cells which re secretory in function. The entire mucosa was seen folded into number of finger like processes.

Fenthion chronic; (0.387,0.193,0.096 mg/ml). Fig 4b,4c,4d.

1) Epithelial cells of villi show vacuolation.
2) Epithelial cells lesions were seen.
3) Many villi were ruptured near the tips; cellular exudates were also observed.
4) Enlarged mucous cells filled with secretary materials were observed.
5) Proliferation of mucous epithelial cells with pycnotic nuclei were seen.

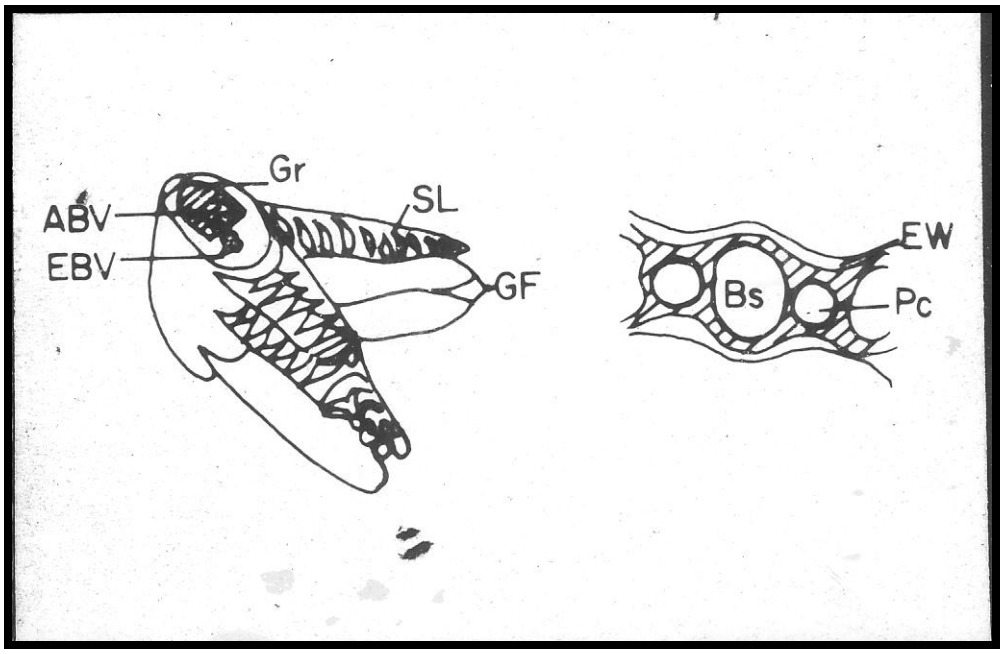

Fig1a Schematic diagram of filaments, lamellae and cross section of secondary lamellae of gill. GF. Gill filament, Gr. Gill ray, Sl.. Secondary lamellae, EW. Epithelial wall, Pc. Pillar cell, Bs. Blood space, ABV. Afferent blood vessel, EBV. Efferent blood vessel.

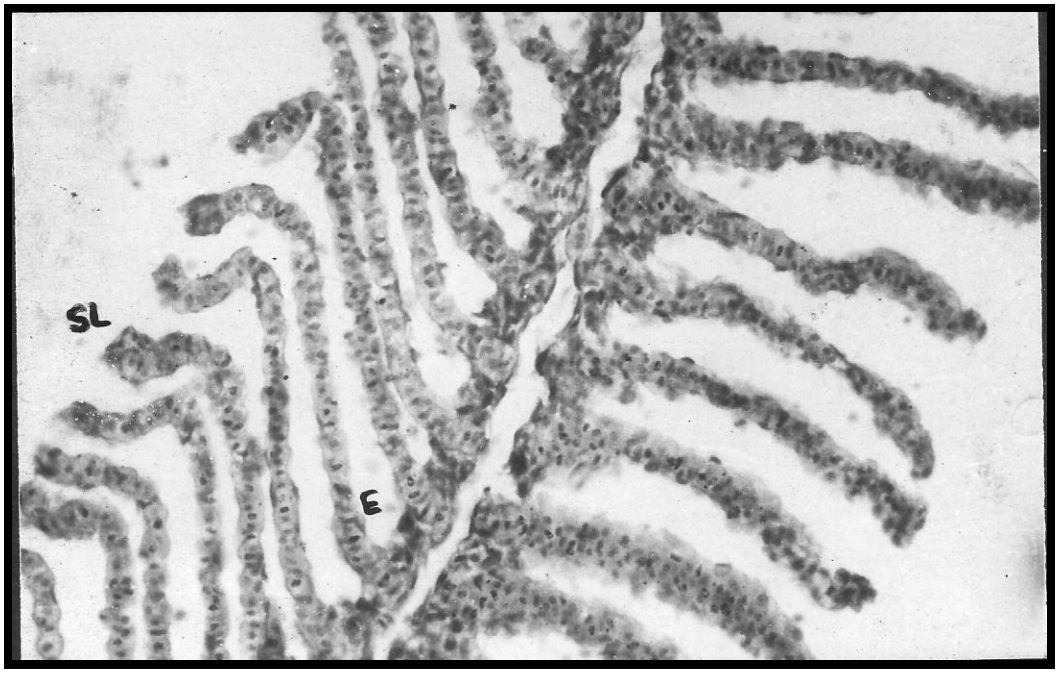

Fig.1b 40x Gills of control fish showing secondary lamellae (SL) and epithelial cell (E).

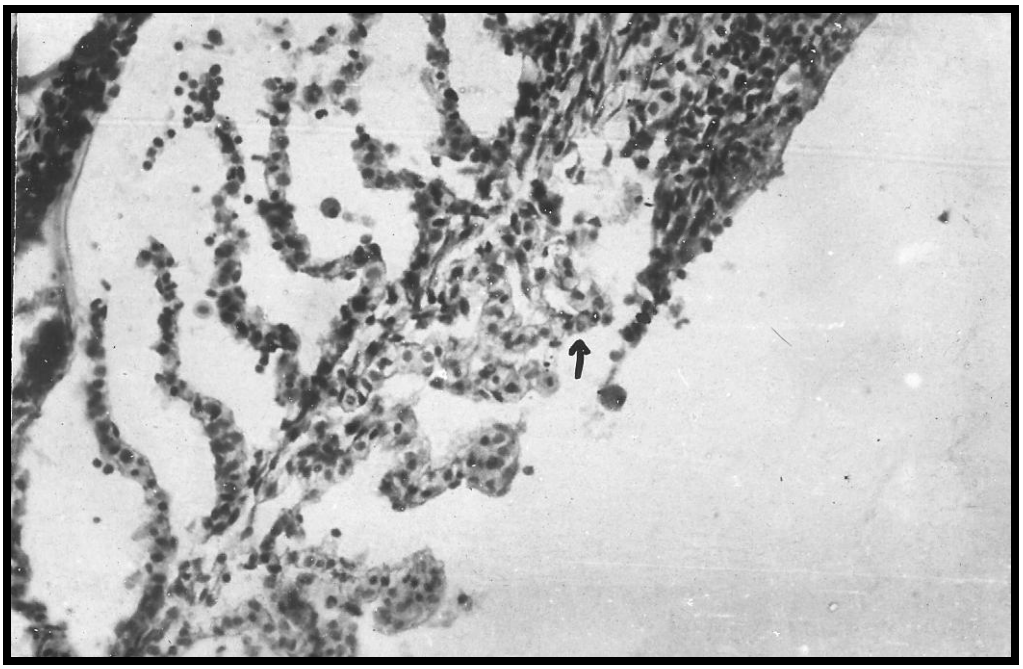

Fig1c 25x Gill of fish exposed to 0.38 mg/ml.Fenthion for 60 days exposure showing vacuolated, deformed and shortened secondary lamellae (arrow mark)

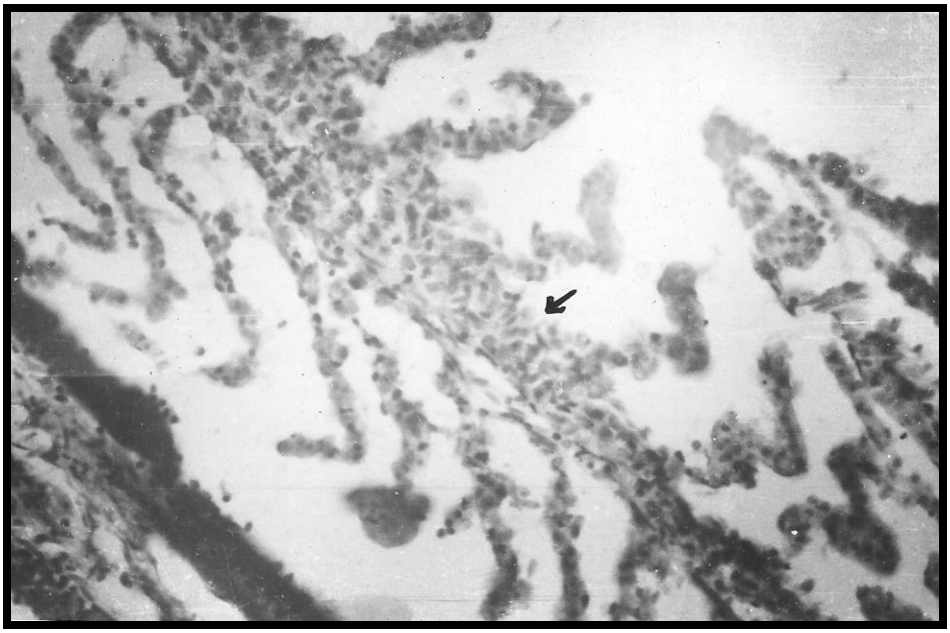

Fig 1d 25x Gills of fish exposed to0.193 mg/ml fenthion for 60 days showing ruptured secondary lamellae.

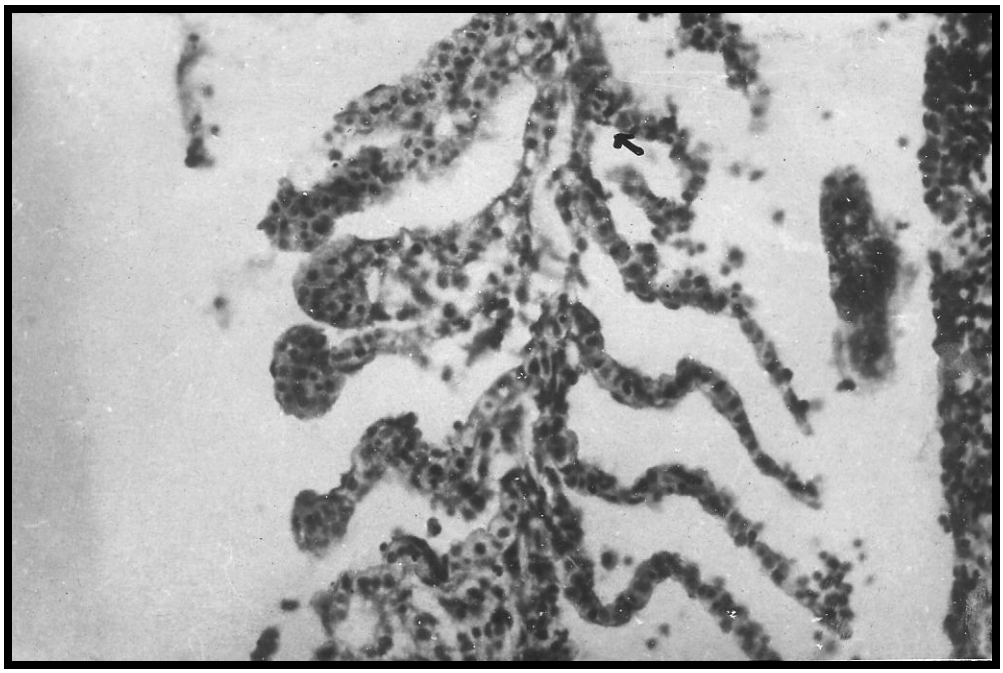

Fig 1e(1) 25x Gill of fish exposed to 0.96 mg/ml fenthion for 60 days showing disoriented epithelial cells and swollen chloride cells.

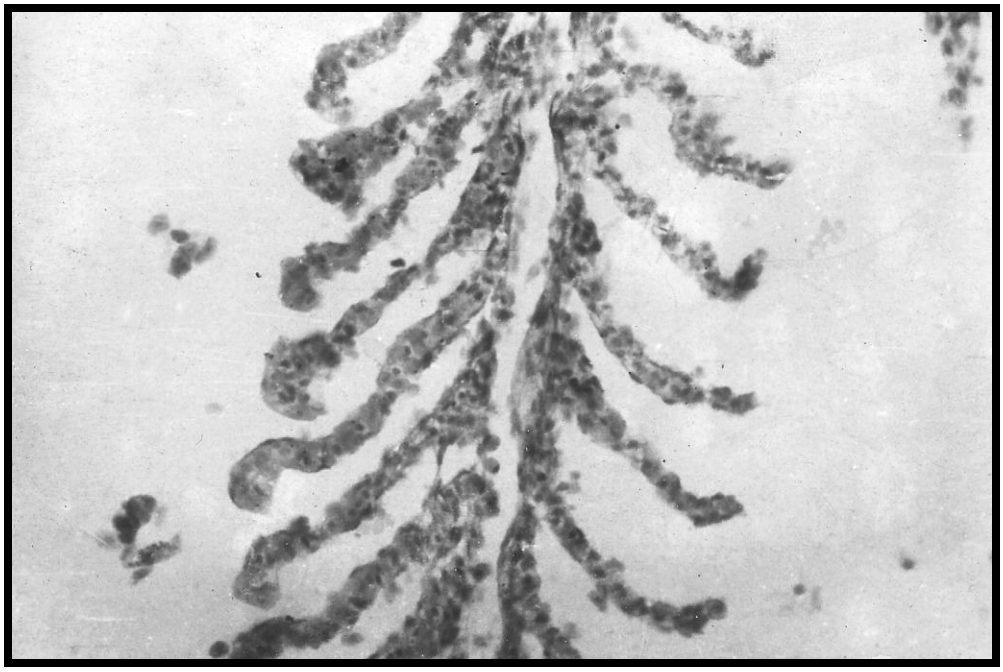

Fig 1e2 (40x) Gill of fish exposed to0.096 mg/ml fenthion for 60 days showing proliferation of primary lamellar cells and increase in number of haemopoietic tissue.

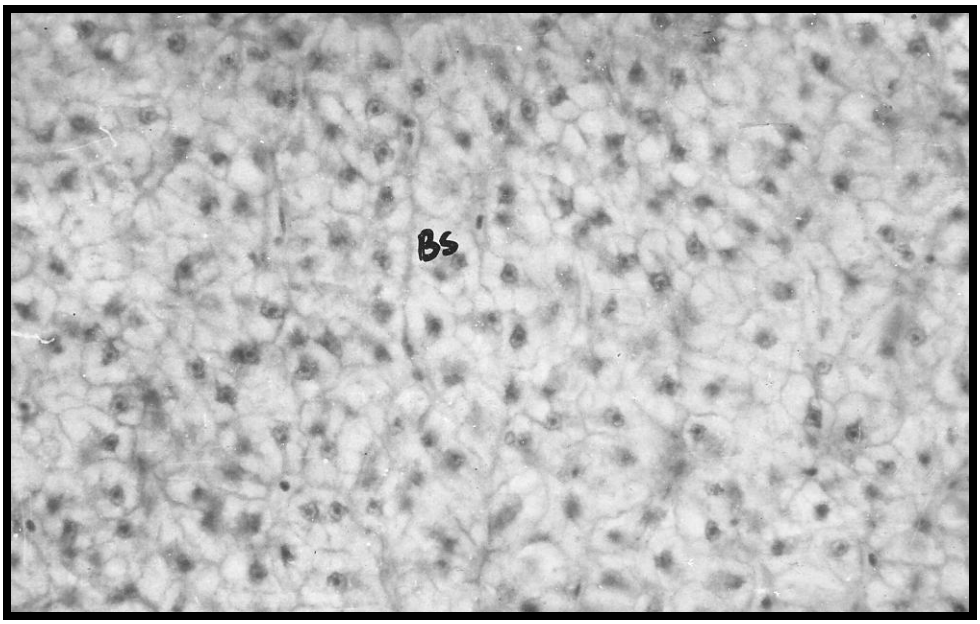

Fig 2a (40x) Liver of control fish showing cordal arrangement of hepatic cells and blood space (Bs)

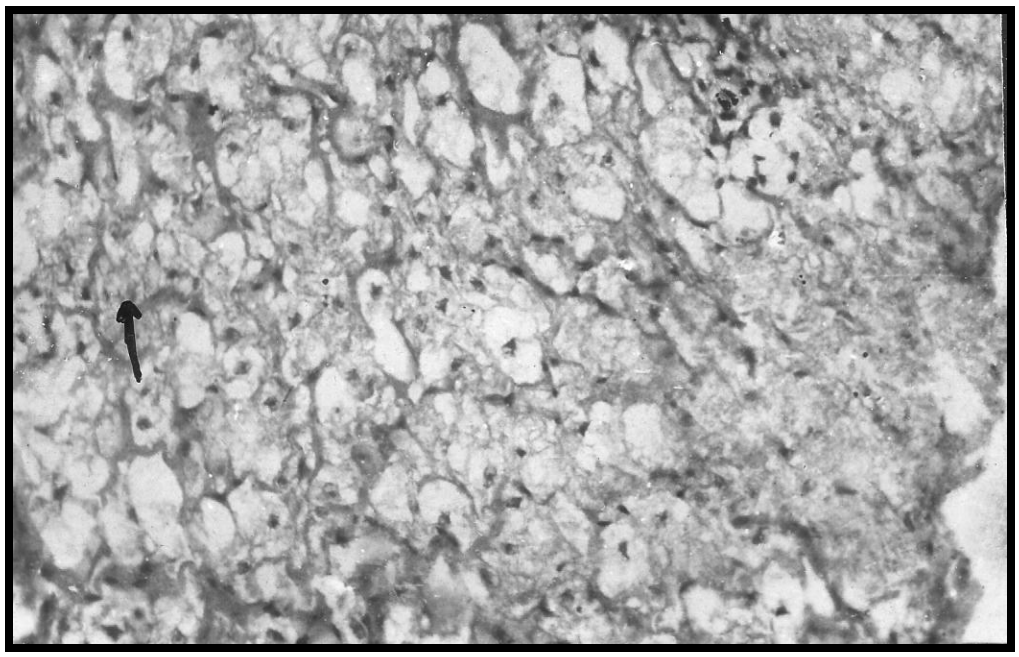

Fig.2b (40x) Liver of fish exposed to 0.38 mg/ml fenthion showing vacuolated, cloudy swollen, disintegrated and extremely ruptured hepatic cells.

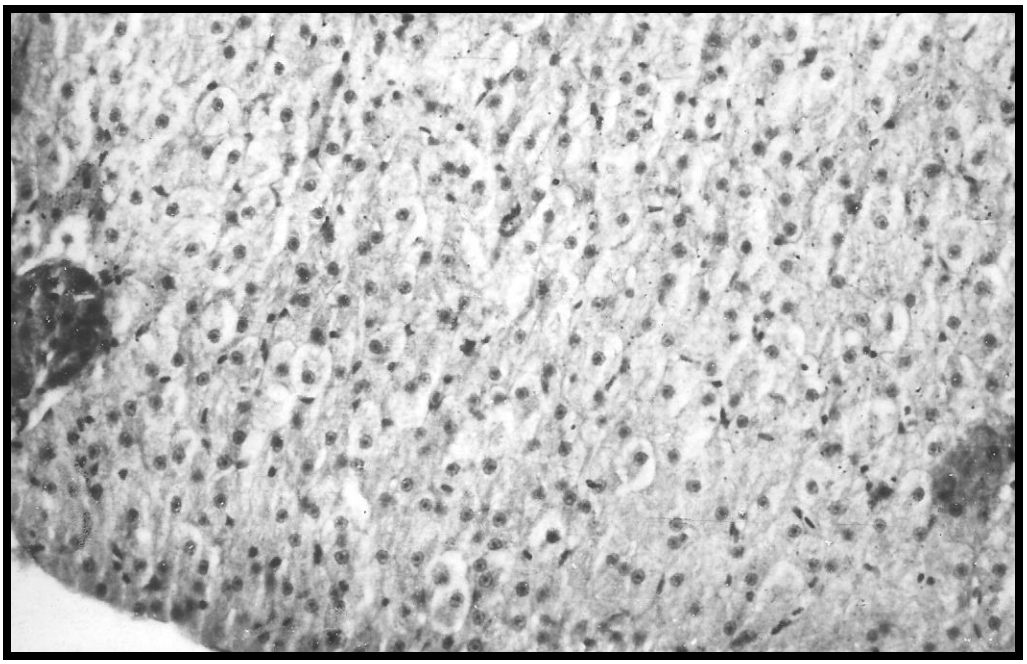

Fig 2c Liver of fish exposed to 0.096 mg/ml showing pycnosis and large number of necrotic regions.

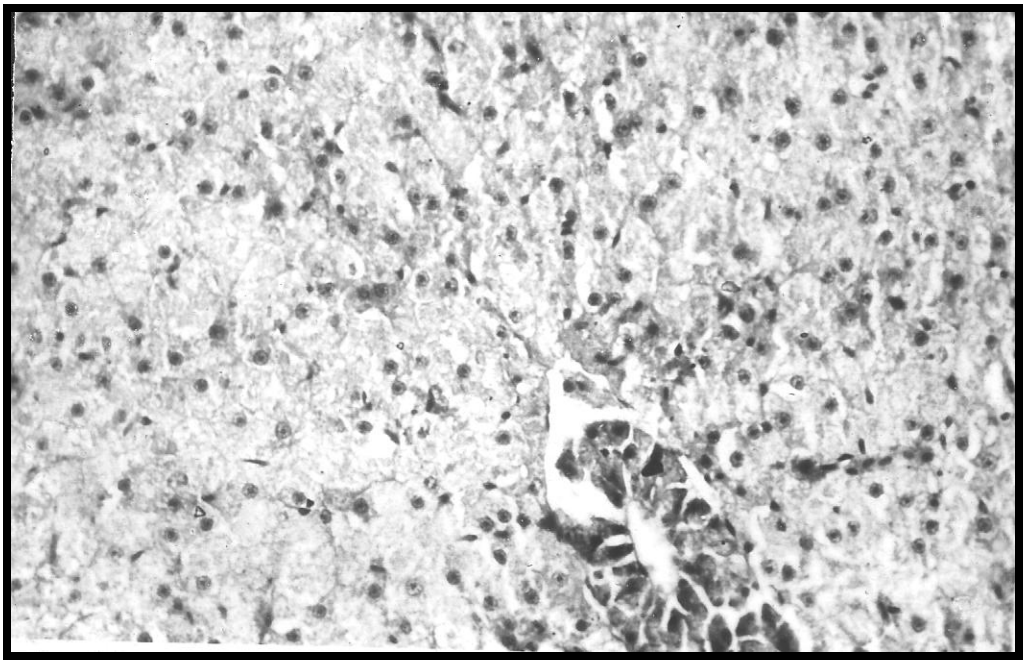

Fig 2d (40x) Liver of fish exposed to0.096 mg/ml showing large no of fatty degeneration and disturbed cordal arrangement of hepatocytes.

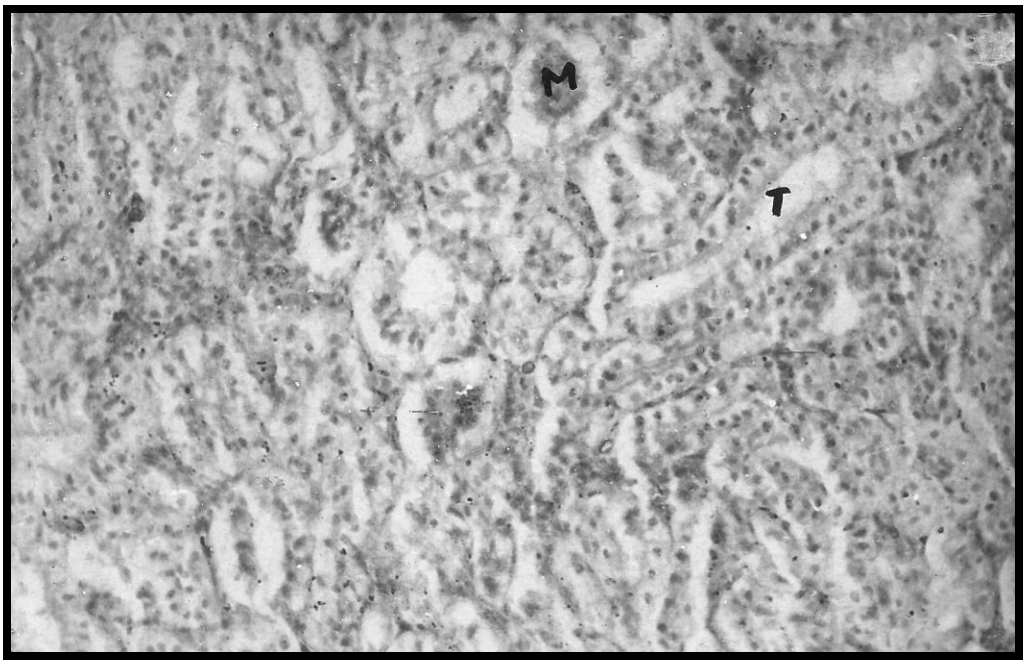

Fig 3a (40x) Kidney of control fish showing renal tubules(T) and malphigian bodies(M).

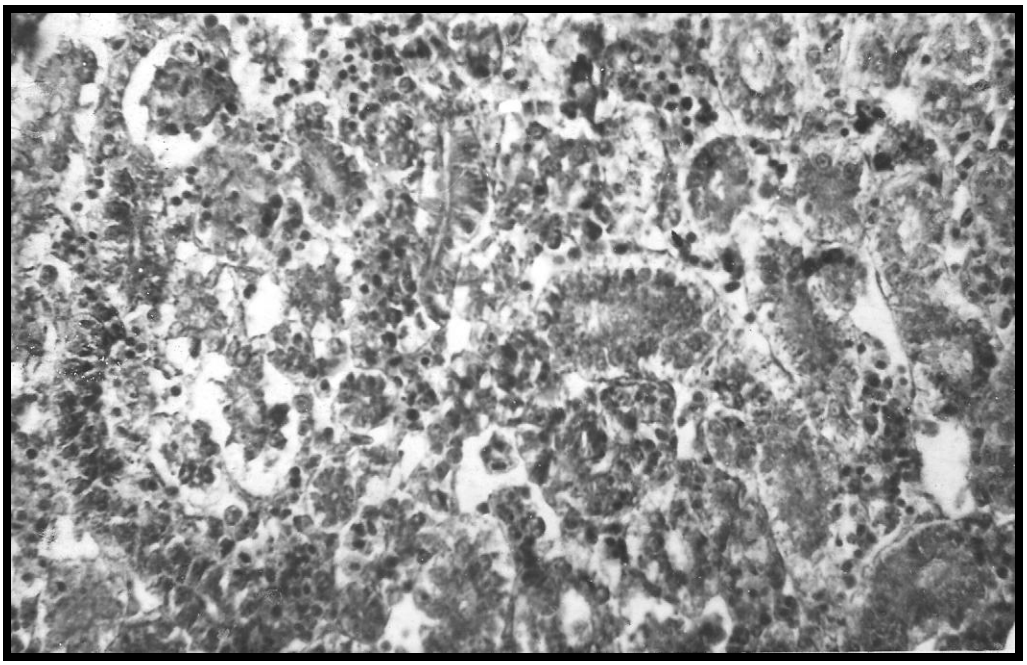

Fig 3b (40x)Kidney of fish exposed to0.38mg/ml fenthion showing swollen and damaged renall tubule and glomeruli with large number of necrotic regions.

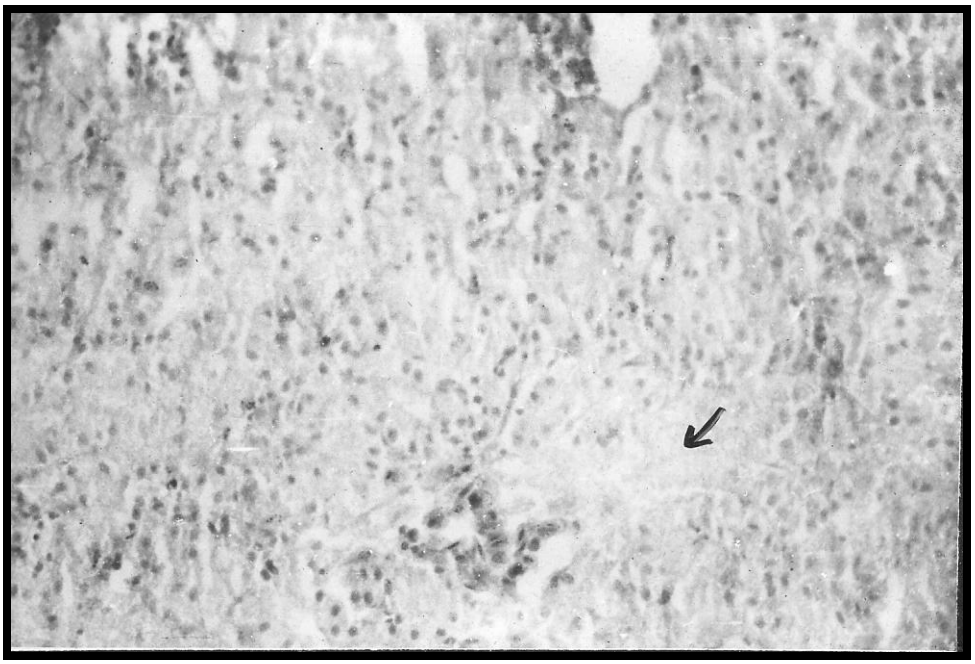

Fig 3c (25x)Kidney of fish exposed to0.193 mg/ml fenthion showing extreme degeneration of renal cells.

Fig 3d (10x) Kidney of fish exposed to 0.193mg/ml fenthion showing increase in haemopoietic tissue.

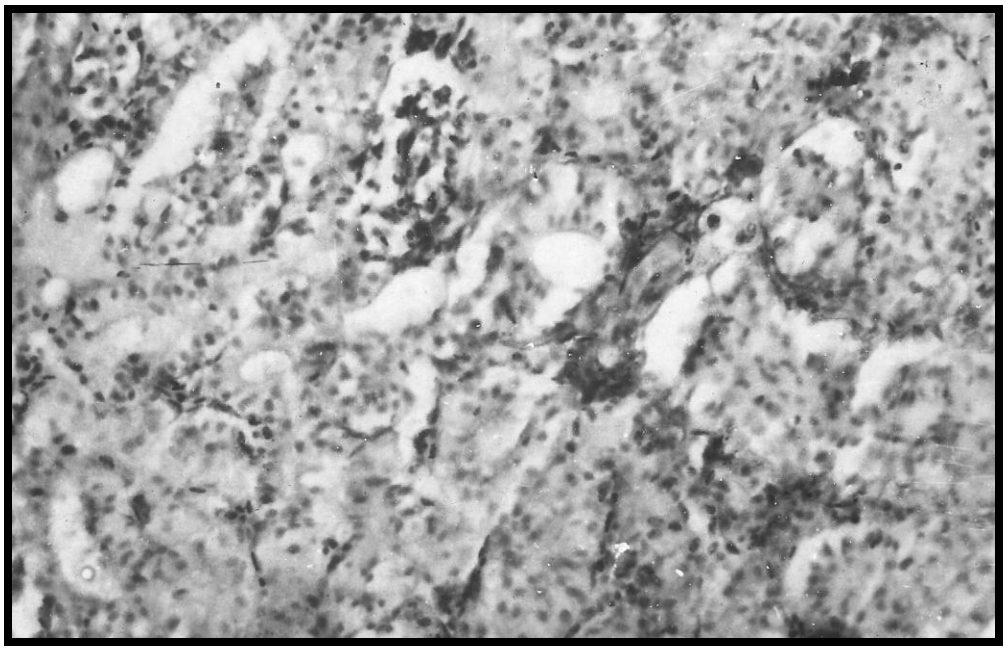

Fig 3e (60x)Kidney of fish exposed to 0.193mg/ml fenthion showing vaculated renal tubule.

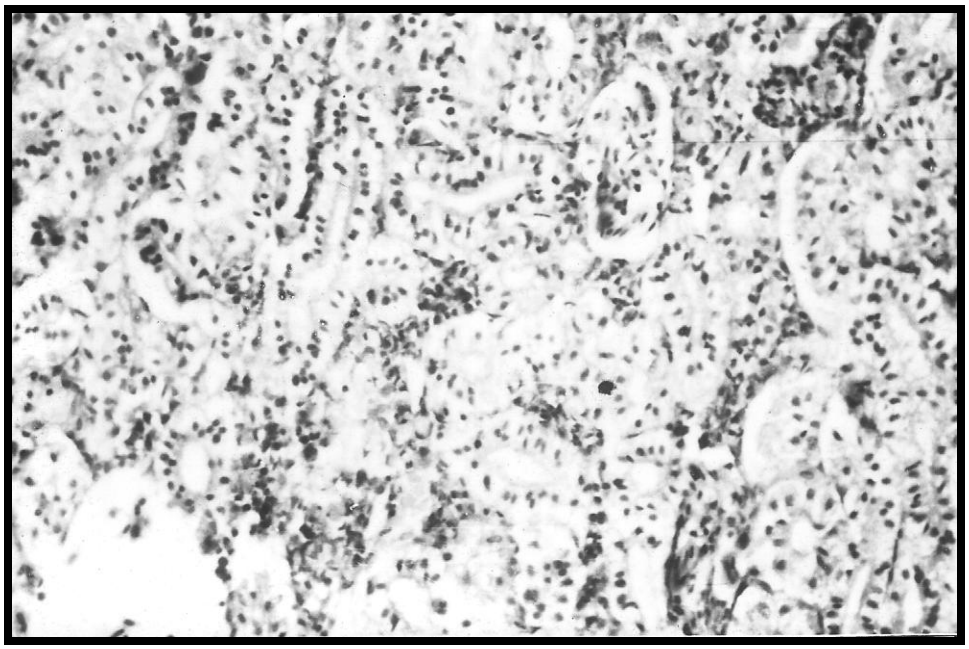

Fig 3f (40x)Kidney of fish exposed to 0.096mg/ml fenthion showing ruptured renal tubule and increase in number of blood cells.

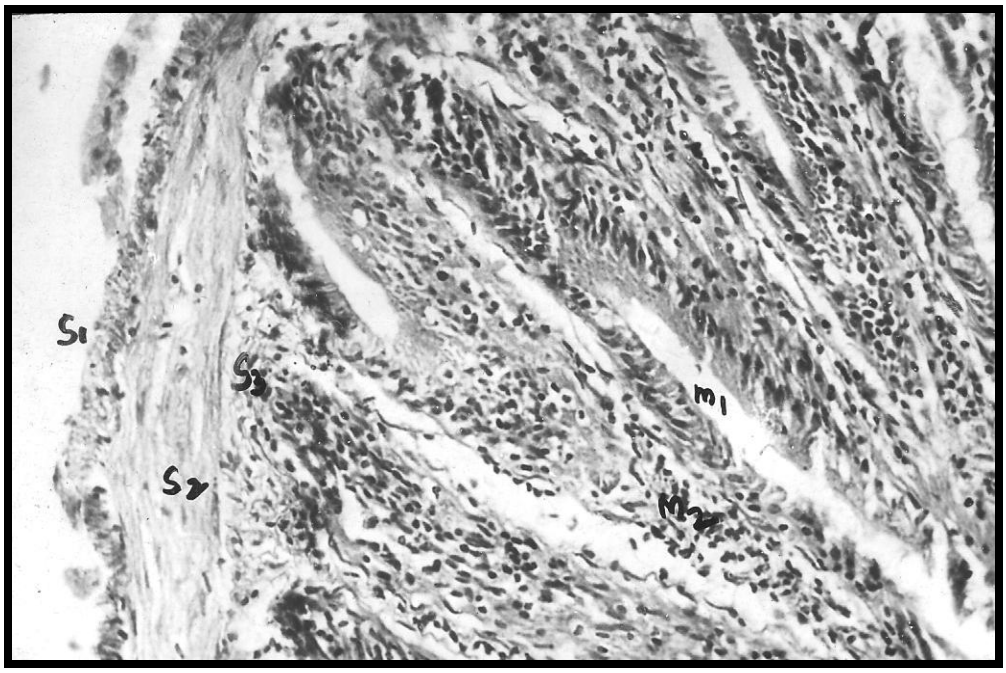

Fig 4a (10x) Intestine of control fish showing serosa (s_1), subserosa (s_2), submucosa (s_3), muscularis mucosa (m_1) and gastric mucosa (m_2).

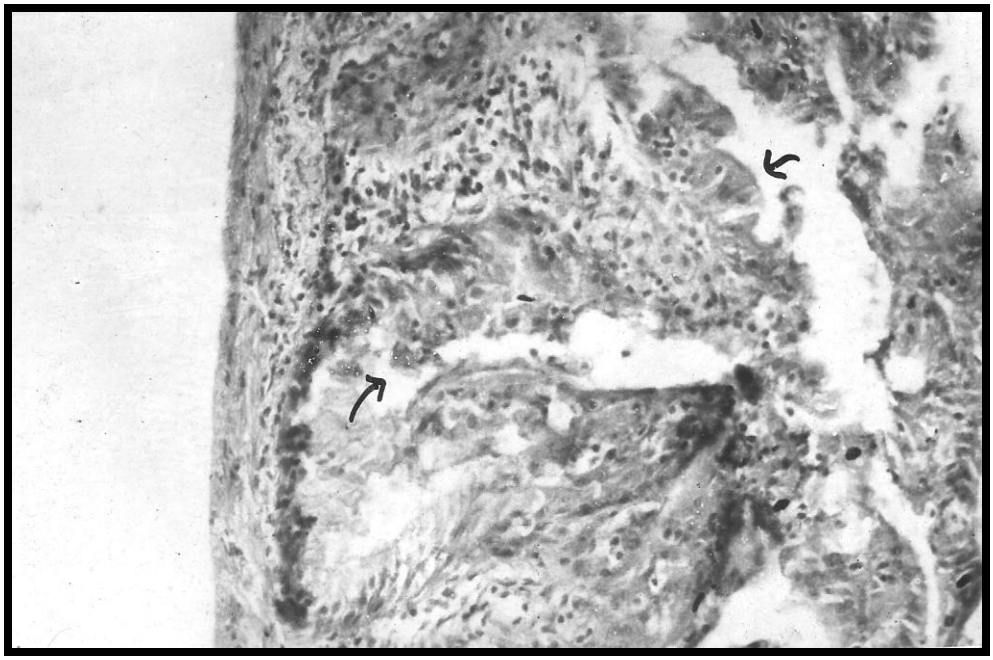

Fig 4b (25x) Intestine of of fish exposed to 0.38mg/l Fenthion showing epithelial cell lesion, shrunken and extremely damaged villi.

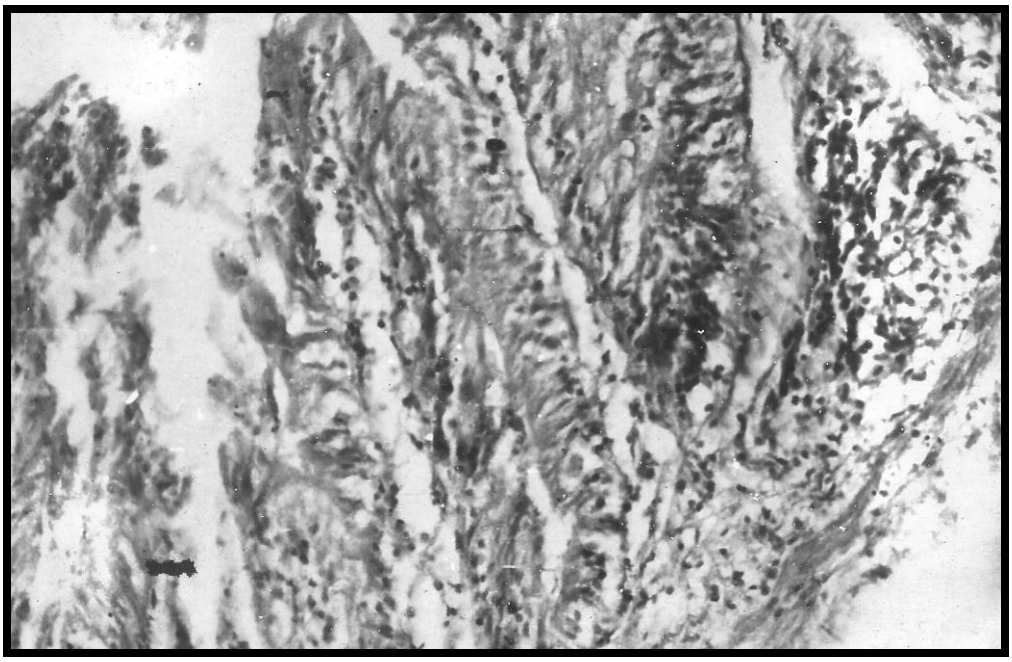

Fig4c (40x) Intestine of fish exposed to 0.193mg/l Fenthion showing proliferation of mucosal epithelial cells with pycnotic nuclei. Damaged serosa and villi are also seen.

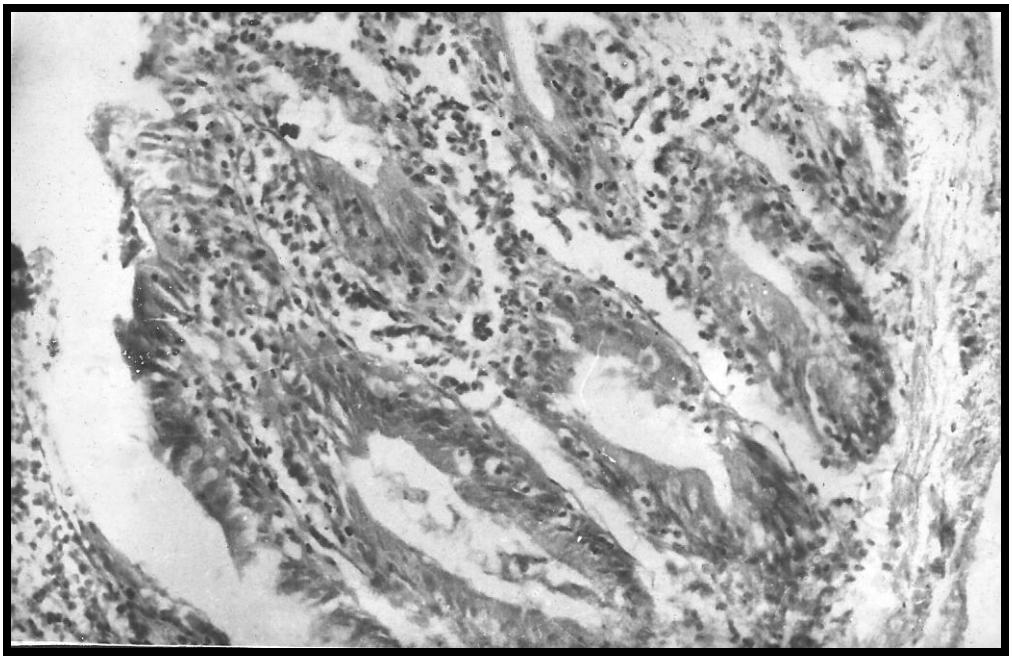

Fig 4d (40x) Intestine of fish exposed to 0.096mg/l Fenthion showing ruptured epithelial cells increase in number of goblet cells.

Discussion:

The extent of damage to the organs tested appeared to be dose dependent. The fish exposed to lowest concenteration do not appear to be severely affected when compared to fish exposed to the highest concenteration.

Gill being an important respiratory organ/ one can say that above changes might have been the cause for oxygen deficiency and further changes in enzyme activities . Gill seemed to be severely affected at the highest concentration (0.38 mg/l) of Fenthion. Similar observations were reported by Gaikwad (1981) and Amminikutty (1979) when T. mossambica and widow tetra were exposed to Thiodan 35 EC. Virtanan (1986) reported that secondary lamellae of gills were shortened and deformed on exposure to 1.2 and 3.0 ppm of DDT . in P. reticulatus. In the present study. it can be presumed that gill damage occurred in C.carpio exposed to Fenthion could also be due to the direct contact with the insecticide. This view is in support with Soman (1987) who related extent of damage to direct contact with insecticide. Liver, though not in direct contact with insecticide, the pale appearance of liver cytoplasm indicates tissue damage. Necrosis, loss in regular polygonal shape of hepatic cells, vacuolation and disintegration of typical cordal arangement of hepatocytes were observed at all concentrations. Similar observations were noted by Gaikwad (1981) in T. mossambica exposed to Thiodan. In the present study it can be stated that liver being an important detoxifying organ, damage caused to its cells could be due to the accumulation of Fenthion .The view also supports Soman (1987) who suggested accumulation to be the principal cause of damage to liver in C. fasciata exposed to Lebaycid. Liver being one of the important metabolic organs/ damage to liver may cause severe metabolic disorders. Kidney of the fish also appeared to have been affected by Fenthion treatment. A general increase in blood cells, swollen renal tubules and vacuolated glomeruli indicate the extent of damage to the tissue as a result of insecticide exposure. Amminikutty (1979), Joshi (1978), Gaikwad (1981) , Soman (1987) and Das(2000) observed similar changes in various fresh water fishes exposed to chronic concentrations of different pesticides. Kidney being the principal organ involved in general physiological activities of fish, this loss of structural form may prove fatal to the fish.

Intestine showed distinct changes after Fenthion treatment. Rupturing of epithelial cells with pycnotic nuclei was the most prominent and revealing feature of tissue damage. Similar observations were made by Gaikwad (1981) and Amminikutty(1979) in T. mossambica and'widow tetra exposed to Thiodan. Intestine being an important organ involved in physiological activities of fish, this loss of structural form may cause the death of animal.

Conclusion:

The histopathological studies reveal that in gill, liver, kidney and intestine tissues the destructive changes are directly related to both, the concentration of dose and the period of exposure. The gills are the target organ to be affected. They are in direct contact with the insecticide present in water thus serving as the major path of entry of toxicant in the body. Liver showed extensive damage due to added burden of insecticide detoxification. This may lead to the impairment of all major metabolic activities. Extensive damage to intestine, an important absorptive organ, especially the villi region giving loss in structural form also could/lead to impairment of all metabolic activities. Kidney being an important excretory and osmoregulatory organ, extensive cell destruction may lead to the altered biochemical and physiological responses ultimately leading to the death of `the animal.

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