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EFFECT OF CONTRA/INSECT 500/50 E.C. ON THE HISTOPATHOLOGY OF OREOCHROMIS SPILURUS FISH

BY

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ABSTRACT

The present study is one of the serial studies dealt with the toxicity problems in the water environment that caused by compound type of pesticides. The impacts of one of these pesticides were examined on fish, which is considered one of the most important sources of animal protein. The present study investigates the effect of the Conrta/Insect 500/50 E.C.toxicity (one of the compound pesticides) on pesticidal histopathology of the gill, muscle, heart, and gonads of Orechromis spilurus fish.

Healthy fish were exposed to the sub lethal concentrations 0.015(1), 0.035(2), and 0.070(3) mg/l, of the contra/insect pesticide. Fish specimens were taken from each concentrations after 24, 72, 120 and 168 hours of post exposure time. Tissues were prepared and histologically examined by standard methods. The results of this study showed necrosis, myolysis, lesion, and hemorrhage in most of the examined tissues as a result of the pesticide toxicity.

INTRODUCTION

Fish is an important source of animal protein in most of the world countries. In nature, fish can be exposed not to a single pesticide only, but to more than one, that present either in considerable amounts or in traces in the aquatic environment. Some of these pesticides are applied as a mixture of two or more in the agricultural and industrial fields and their residuals reach the aquatic environment through drainages and runoff. Chemical interaction, if there is any, between these pesticides in the mixture may either increase or decrease its overall effect (Murty, 1986).

Knowing that fish can store about 58-93% of these insecticides in its tissue. One of these pesticides combinations, which are used extensively in the agricultural field in Saudi Arabia, is commercially known as Contra/Insect 500/50 E.C. It is a broad-spectrum insecticide that controls a wide range of, sucking and chewing insect, and pests in vegetables, fruit trees, field crops and ornamental plants. Contra/ Insect contains 50% of its weight Clorpyrifos and 5% cypermethrin. While the clorypyrifos is from the organophosphorus chemical group, the cypermethrin is from the pyrethroid group. This insecticide coming directly through the water resources or through food chain can affect different species of fish such as Tilapia species.

Tilapia species is a popular fish, which are grown in both fresh water and saltwater fish farming in Saudi Arabia. One of these species is *Oreochromis spilurus*, which has been acclimated to the red sea water in Saudi Arabia. This fish is indigenous to Athi and Tana rivers in East Africa. It is an omnivorous grazer, and according to (Peacock, 1979) no limitation of its salinity tolerance have been registered; it can tolerate a temperature range of 8 to 43°c. To our knowledge there was no previous attempt to investigate the toxicity effect of the contra/insect insecticide on the *Oreochromis spilurus* . However, there are many efforts have been done in these area of research to study the effect of different pesticides and other pollutants on different species of fish tissues. The histopathological effects of different pollutants on thyroid, gill, liver, kidney, bones, blood, brain, and other organs have been studied by many investigators using various toxic chemicals (Seenivasan, 1998; Kumar& Ansari, 1984; Girija and Inbamani 1985).

The present study investigates the effect of the conrta/insect toxicity on pesticidal histopathology of the gill, muscle, heart, and gonads of *Oreochromis spilurus* fish.

MATERIALS AND METHODS

Healthy specimens of the *Oreochromis spilurus* fish of average length ranging from 12.1 to 14.2 cm, and average weight ranging from 28.3 to 47.9 g., were collected from the fish farm of the faculty of oceanography at Abhor, Jeddah. Fish were kept in a concrete tank of about 6000 liters of water capacity, under the shaded farm. The holding tank received a continuous supply of, the Red sea water. Mechanical air pumps were used to aerate the fish tank. Fish were held six weeks in the tank under experimental conditions for acclimation. During the period of acclimation the fish were fed a commercial fish food ad libitum twice daily. After the end of the acclimation period, which was judged by normal activity of the fish; feeding was stopped 24 hrs before and during the experiments.

Glass aquaria of 60 liters capacity were prepared for this study. The aquaria were supplied with 50 liters of filtered seawater. For aeration air pipes, and air stones connected to an aeration pump, were used.

The sub lethal concentrations were estimated based on results obtained for the LC50 and LC90 expenments on of the exposed fish (Elnemaki, 2001) during the first stage of the present work. The fish were exposed to three pesticide sub lethal concentrations, 0.015(1), 0.035(2), and 0.070(3) mg/l. for 7 days. The experiment was run in triplicate, and for each insecticide concentration one aquarium was used as a control where the fish were exposed to water free of insecticides.

Two fish were taken from each of sub lethal concentrations at 24,72,120,and 168 hours post exposure time. Fish was dissected and tissue such as, muscle, gills and gonads were fixed in neutral buffer formalin for the histological analysis. Histological analyses were done using standard methods of Couch (1975) using the light microscope. Following fixations tissues were dehydrated, cleared, embedded in paraffin wax, then transverse and longitudinal sectionined. Finally tissues were stained by Hematoxylin and Eosin (H&E), Perodic acid & Schiff reagent (PAS), and Masson Trichrome.

RESULTS

GILLS

The gills of non-exposed *Oreochromois spilurus* to the contra/insect pesticide had the normal architecture of the gill filament. However, telangectiasis in few gill filaments at the distal end was observed (Fig. 1). Fig (2) showed the normal appearance of the chonodrocytes supporting the gill arch.

Exposed *Oreochromis spilurus* to the pesticide concentration (1) at 24 hours exposure time, showed area of hemorrhage and necrosis of the chondrocytes that supporting the gill arch (Fig.3). Prominent lamellar necrosis was also observed (Figs.4; 5). Fig. (6) showed hypertrophy of the mucus cells of the gill filaments in fish exposed to concentration (2) at 24 h. exposure period. These cells were PAS positive and stained red in color (Fig.7). Moreover, slight lamellar fusion was evident in fish exposed to concentration (3) at 24 h. (Fig.8).

Exposed *Oreochromis spilurus* to concentration (1)· at 72 h post-exposure showed marked proliferation of mucus cells and were PAS positive (Figs 9;10). Marked dilatation and engorgement of the efferent blood vessels was also observed (Fig.11). Fig.(12) showed wide spread telangectiasis in fish exposed to concentration (2), and collected at 72 h post-exposure time compared to the non-exposed fish (Figs.12; 13). Moreover, hemorrhage beneath the gill arch, and hyperemia of the afferent blood vessels was also observed (Fig.14).

Oreochromis spilurus exposed to concentration (1), at 120 h showed wide spread area of hemorrhage beneath the gill arch (Fig.15). Severe lamellar necrosis was also evident (Fig.16). Moreover, moderate necrotic changes of the chondrocytes supporting the gill arch were noticed (Figs.17; 18). Figs (19; 20) showed severe necrosis of the epithelial lining the secondary lamellae and necrosis of the chondrocytes supporting the gill arch in fish exposed to concentration (2), at 120 h post-exposure time. Mallory Trichrome stain showed deeply stained necrotic chondrocytes and loss of matrix homogeneity (Fig.21).

By 168 h post-exposure *Oreochromis spilurus,* subjected to the concentration (1), of the pesticide showed hyperplasia of the lamellar epithelium of the gill filament and slight exhaustion of the mucus cells (Figs.22; 23). While fish exposed to con. (2), and collected at 168 h exposure time, showed severe necrosis of both lamellar and interlamellar epithelium (Figs 24; 25). Figs (26; 27) showed well-expressed lamellar fusion and severe hemorrhage in the gills of fish exposed to concentration (3), and collected at 168 hours.

MUSCLES

Non-exposed muscles of *Oreochromis spilurus* showed the normal appearance, arrangement and striation of muscle fibers. There was no evidence for myolysis or Zenker's necrosis Figs. (28; 29).

Exposed *Oreochromis spilurus* to concentration (1), at 24 h exposure time, showed focal area of myolysis compared to the non-exposed group (Fig.30). While fish exposed to concentration (2), at 24 h had well expressed myolysis leaving remnants of necrotic fibers (Fig.31).

Figs. (32; 33) showed focal hypodermal myolysis of the muscles of *Oreochromis spilurus* exposed to concentration (1) at 72 h exposure time. On the other hand, fish

exposed to concentration (2), at 72 h showed wide spread area of hypodermal myolysis , which extended deeply into the musculature (Figs. 34;35). *Oreochromis spilurus* exposed to concentration (3) at 120h showed well-expressed deep myolysis and marked eosinophilia of the sarcoplasm (Figs.36; 37). The skin covering the muscles showed focal area of spongiosis and hypertrophy of mucus cells (Fig.38).

At 168 hours, *Oreochromis. spilurus* exposed to concentration (3), showed marked eosinophilia of the muscle bundles compared to the non-exposed fish (Fig.39).

INTESTINE

Oreochromis spilurus fish exposed to concentration (3), at 120 h exposure time showed marked proliferation of the mucus cells allover the intestinal villi and stained **red by PAS** (Fig.40). There were no other changes detected in the lamina epithelial , submucosa or muscularis.

HEART

The bulbous arteriosus of fish exposed to concentration (2) and collected at 120 hours had massive area of hemorrhage and disintegration of few elastic fibers (Fig.41). While, the atrium showed multi focal areas of hemorrhage, it also showed slight disintegration of the muscle fibers (Fig.42).

OVARY

Corrigation of the perinuclear ova membranes was observed (Fig.43). vaccuolation of the vitellogenic ova theca layers was also noticed (Fig.44). These changes were detected in *Oreochromis spilurus* exposed to concentration (2) at 120 hours exposure time.

TESTES

Oreochromis spilurus exposed to concentration (1) at 72 h exposure time showed degenerative changes of some of the seminiferous tubules. These changes were expressed by damage of their membranes and necrosis of the spermatogenic cell layers (Fig.45).

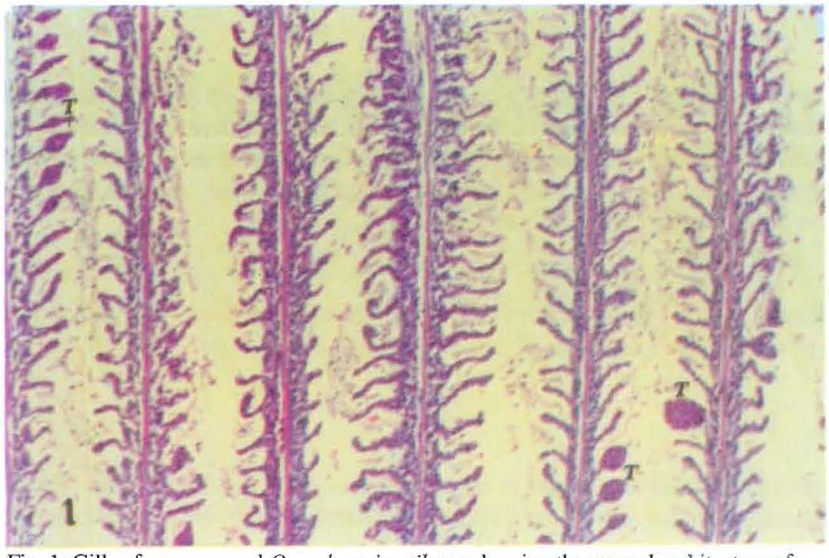


Fig. 1: Gills of non-exposed *Oreochromis spilurus* showing the normal architecture of gill tissue with few telangectaisis (T) at the distal end of the gill filaments. Haematoxylin and Eosin X 33.

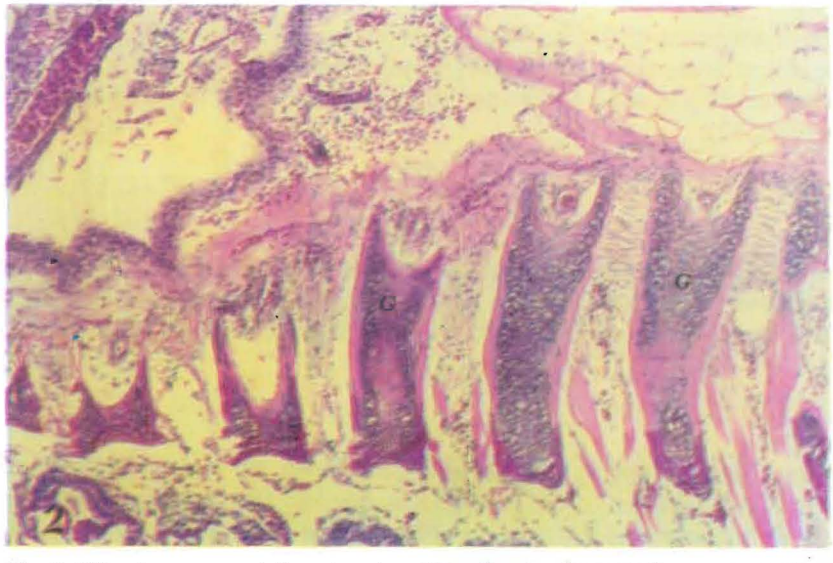


Fig. 2: Gills of non-exposed *Oreochromis spilurus* showing the normal appearance of the gill arch (G). Haematoxylin and Eosin X 33.

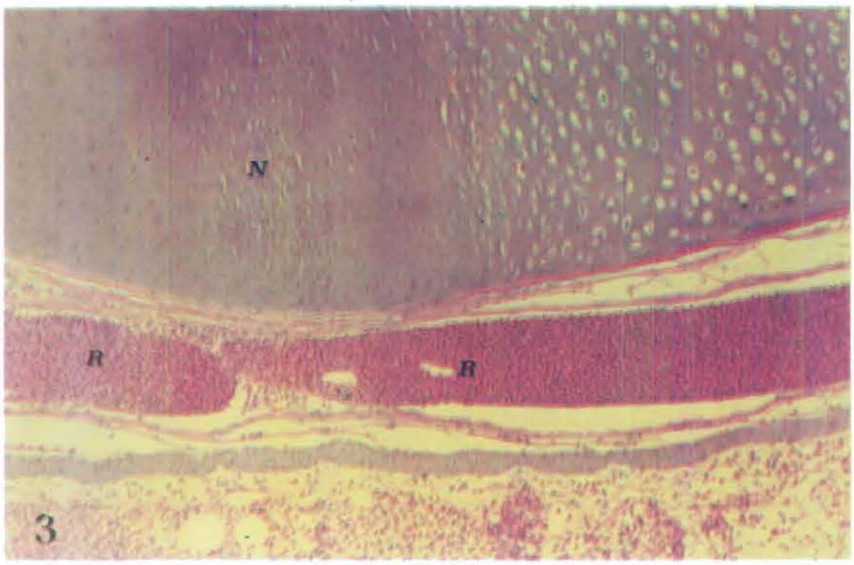


Fig. 3: Gills of exposed *Oreochromis spilurus* exposed to concentration (1) at 24 h. post-exposure showing area of extravasated red blood cells (R) and necrosis of the chondrocytes of the gill arch (N), Haematoxylin and Eosin X 33.

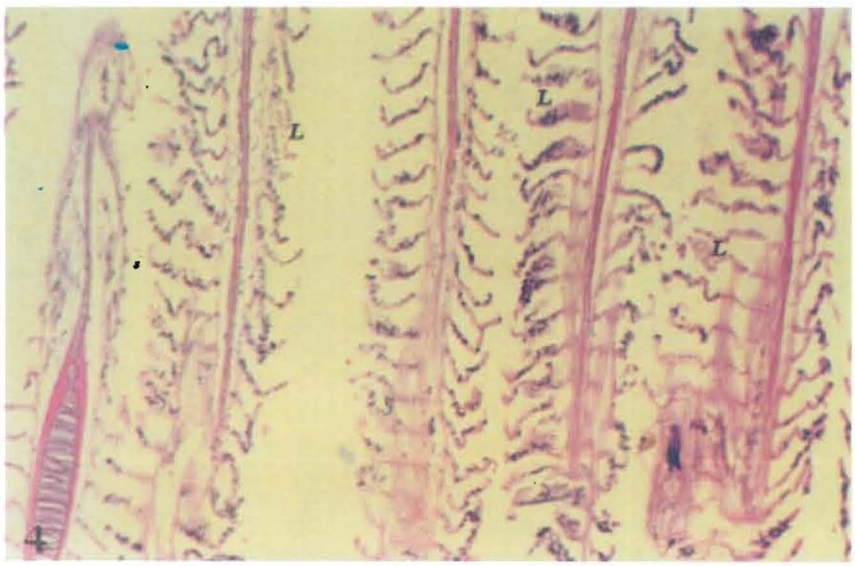


Fig. 4: Gills of *Oreochromis spilurus* exposed to concentration (2) at 24 h showing prominent lameller necrosis (L) result in irregular blue deposits representing the desquamated epithelium. Haematoxylin and Eosin X 33.

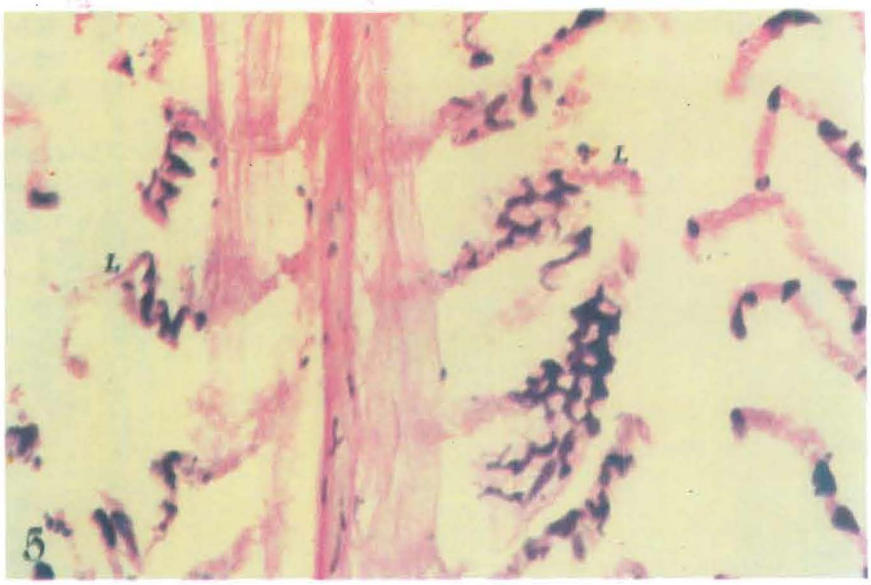


Fig. 5: Higher magnification of Fig. 4 showing the blue desquamated epithelium and necrosis of lameller epithelium (L). Haematoxylin and Eosin X 132.

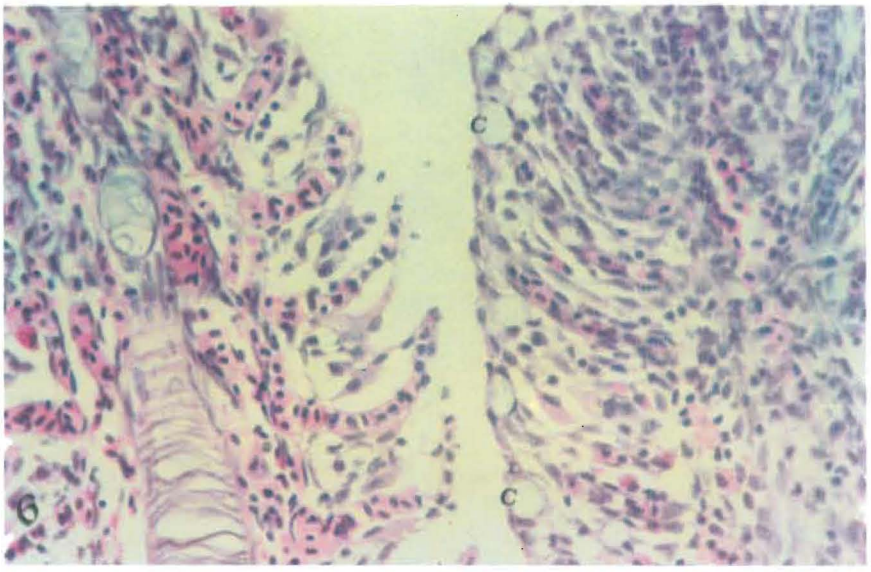


Fig. 6: Gills of *Oreochromis spilurus* exposed to concentration (2) at 24 h showing hypertrophy of mucus cells (C) of the gill. Haematoxylin and Eosin X 132.

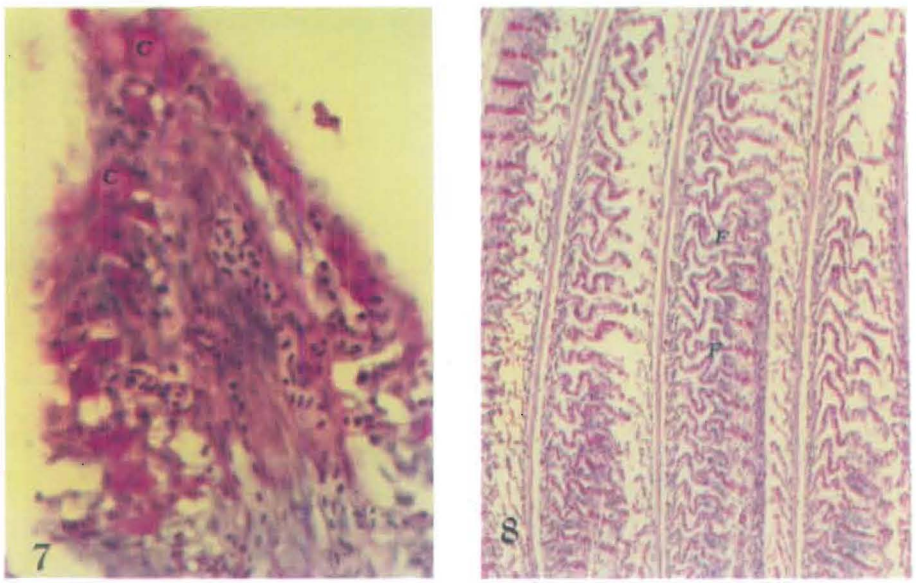


Fig. 7: Gills of *Oreochromis spilurus* exposed to concentration (2) at 24 h showing the typical PAS + ve reaction of the hypertrophied mucus cells (C). PAS stain reaction X 132.

Fig. 8: Gills of *Oreochromis spilurus* exposed to concentration (3) at 24 h showing lameller fusion (F). Haematoxylin and Eosin X 132.

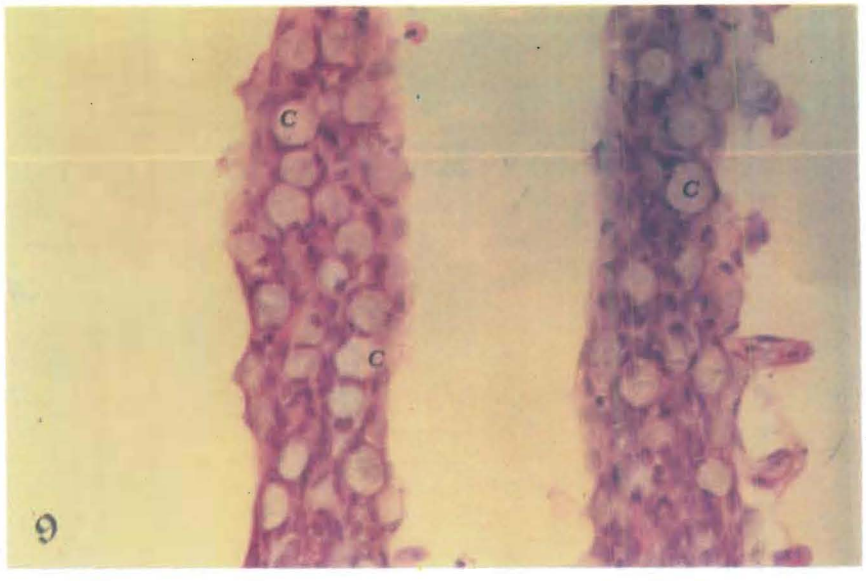
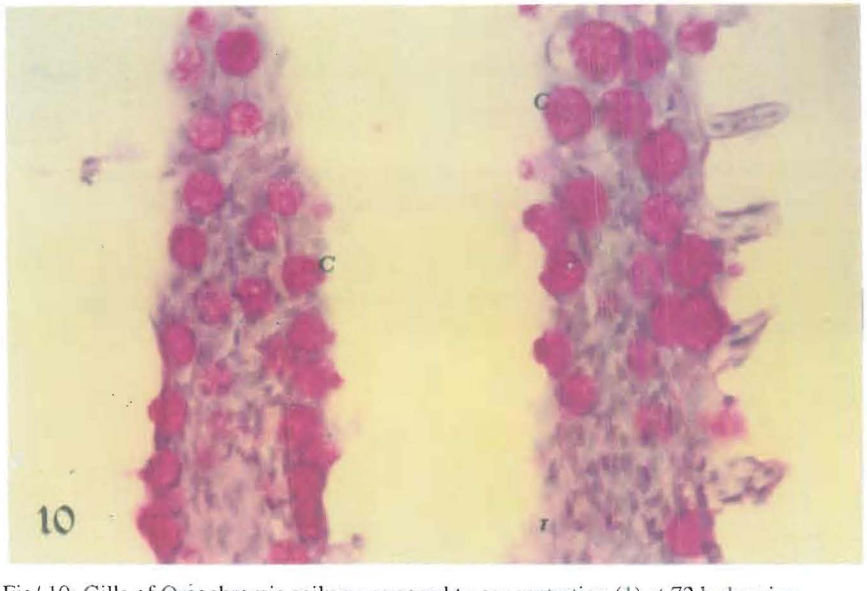


Fig. 9: Gills of *Oreochromis spilurus* exposed to concentration (1) at 72 h showing marked proliferation of mucus cells (C) of the gill. Haematoxylin and Eosin X 132.



Fig/ 10: Gills of Oreochromis spilurus exposed to concentration (1) at 72 h showing the staining affinity of proliferated mucus cells (C) with PAS + ve reaction. PAS stain reaction X 132.

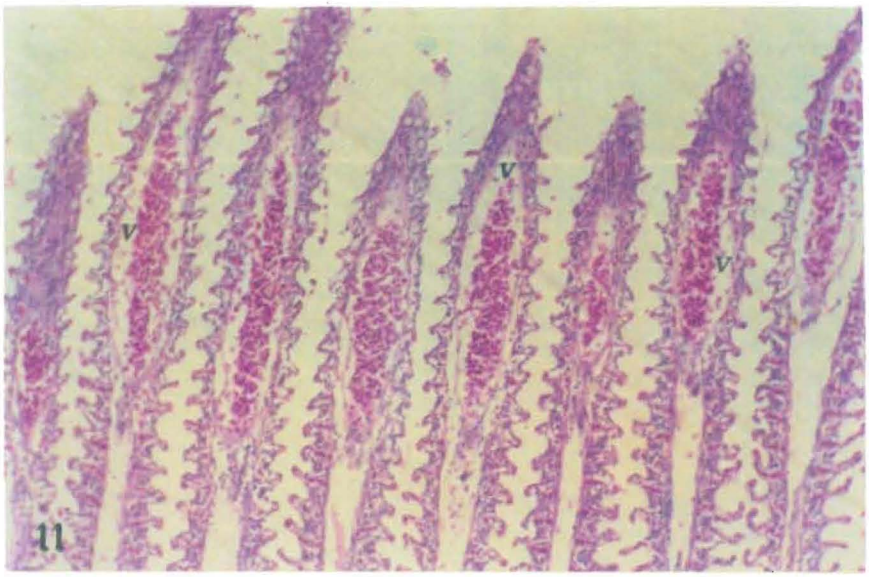


Fig. 11: Gills of *Oreochromis spilurus* exposed to concentration (1) at 72 h showing the dilatation and engorgment blood vessels (V). Haematoxylin and Eosin X 33.

Fig. 12: Gills of Oreochromis spilurus exposed to concentration (2) at 72 h showing pronounced telangectiasis (T). Haematoxylin and Eosin X 13.2.

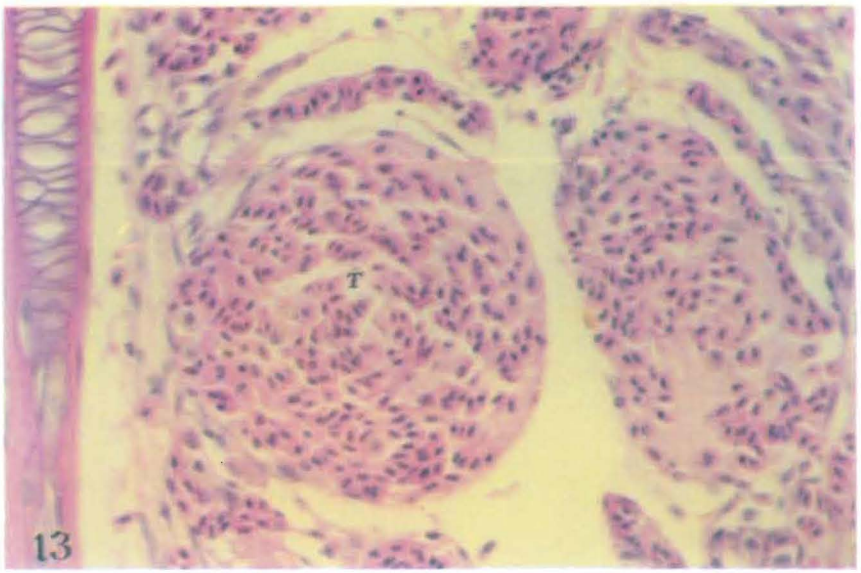


Fig. 16: Higher magnification of Fig.. 12. Haematoxylin and Eosin X 132.

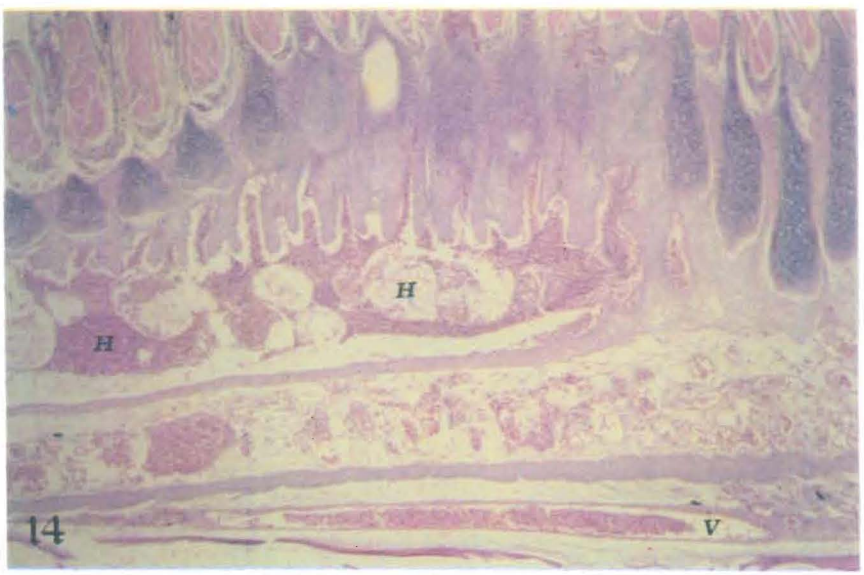


Fig. 14: Gills of Oreochromis spilurus exposed to concentration concentration (2) at 72 h showing the haemorrhage beneath the gill arch (H) and hyperemia of afferent vessels (V). Haematoxylin and Eosin X 13.2.

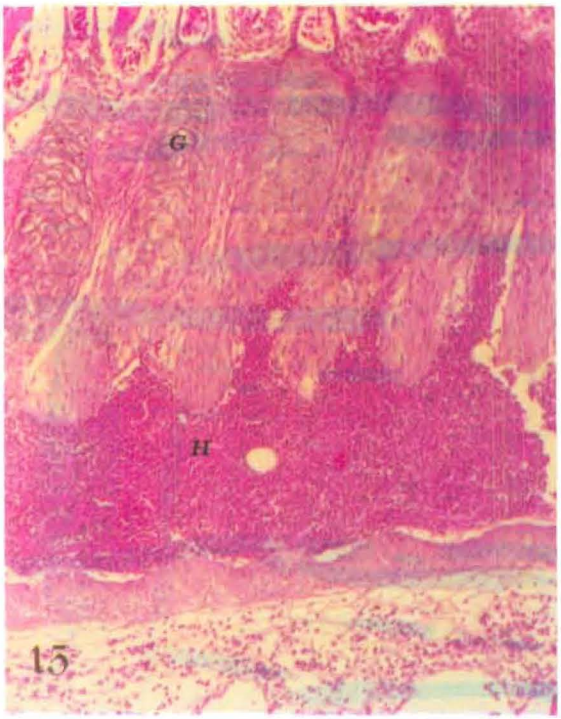


Fig. 15: Gills of *Oreochromis spilurus* exposed to concentration (2) at 120 h showing wid spead area of haemorrhage beneath the gill arch (H). Haematoxylin and Eosin X 33.

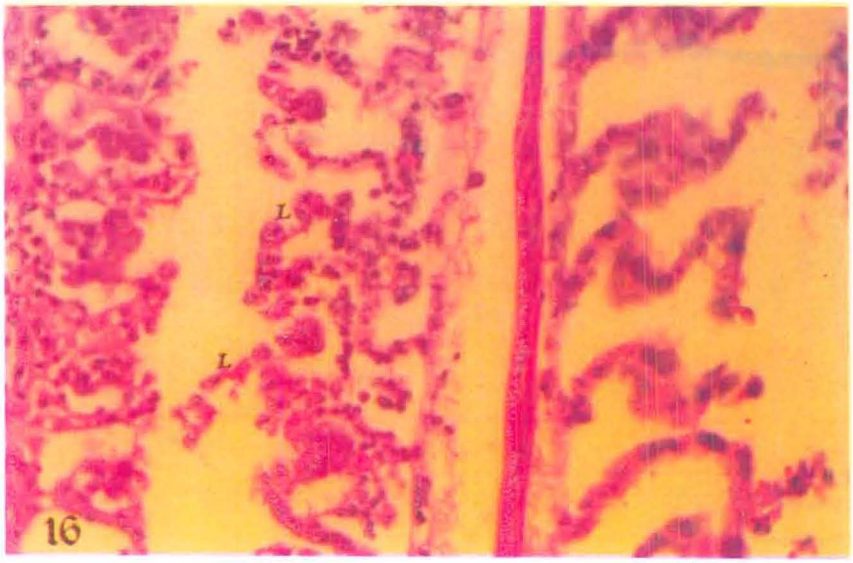


Fig. 16: Gills of *Oreochromis spilurus* exposed to concentration (2) at 120 h showing sever lameller necrosis (L) Haematoxylin and Eosin X132.

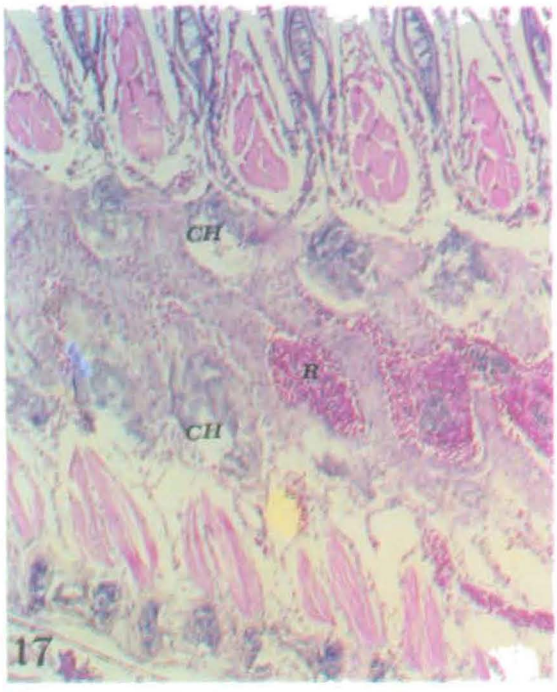


Fig. 17:Gills of *Oreochromis spilurus* exposed to concentration (1) at 120 h showing necrosis of chondrocytes (CH) supporting the gill arch and area of the haemorrhage (R). Haematoxylin and Eosin X 33.

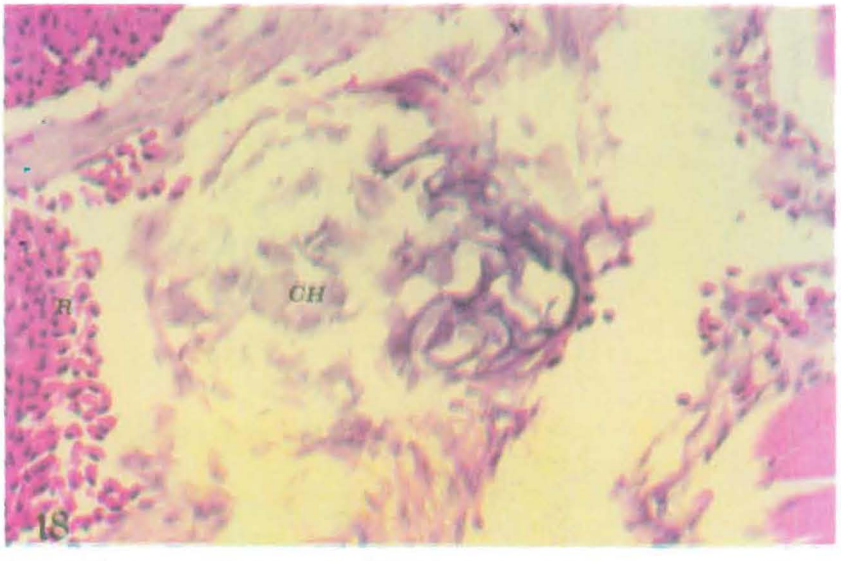


Fig. 18: Higher magnification of Fig.. 17 showing the necrotic chondrocytes (CH). Haematoxylin and Eosin X 132.

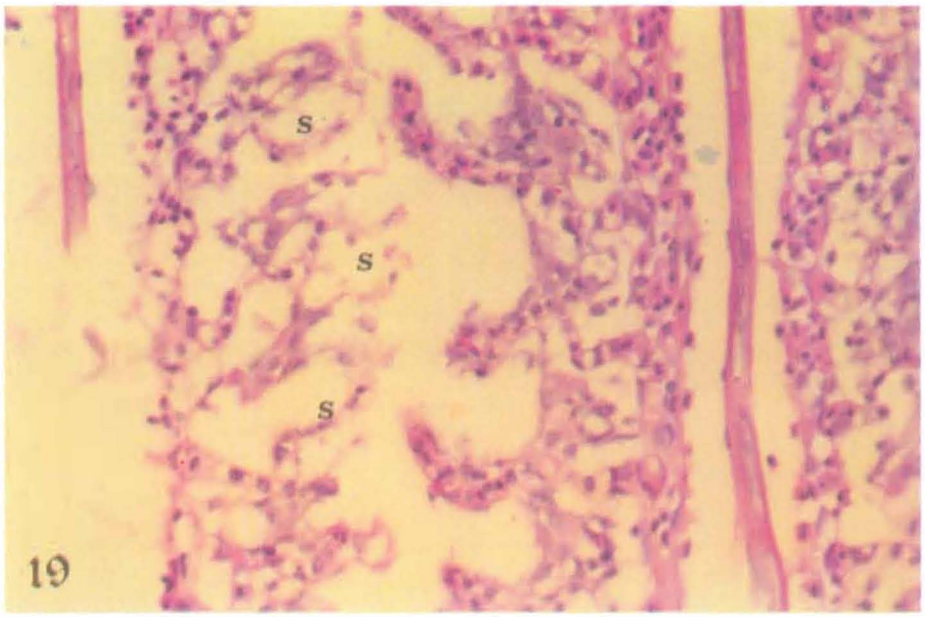


Fig. 19: Gills of *Oreochromis spilurus* exposed to concentration (2) at 120 h showing sever necrosis of secondary lamellae (S). Haematoxylin and Eosin X 132.

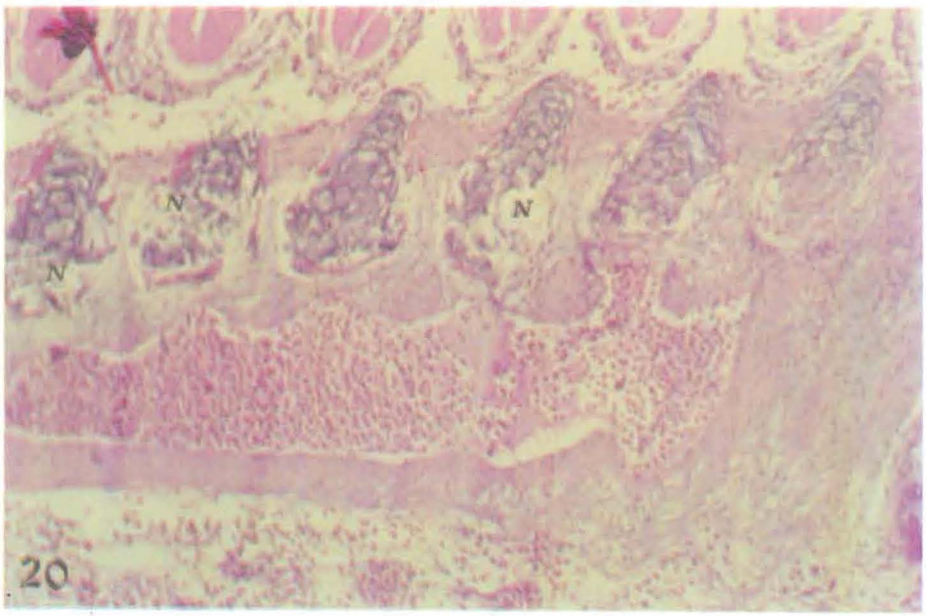


Fig. 20: Gills of *Oreochromis spilurus* exposed to concentration (2) at 120 h showing sever necrotic change of chondrocytes (N) of the gill arch. Haematoxylin and Eosin X 33.

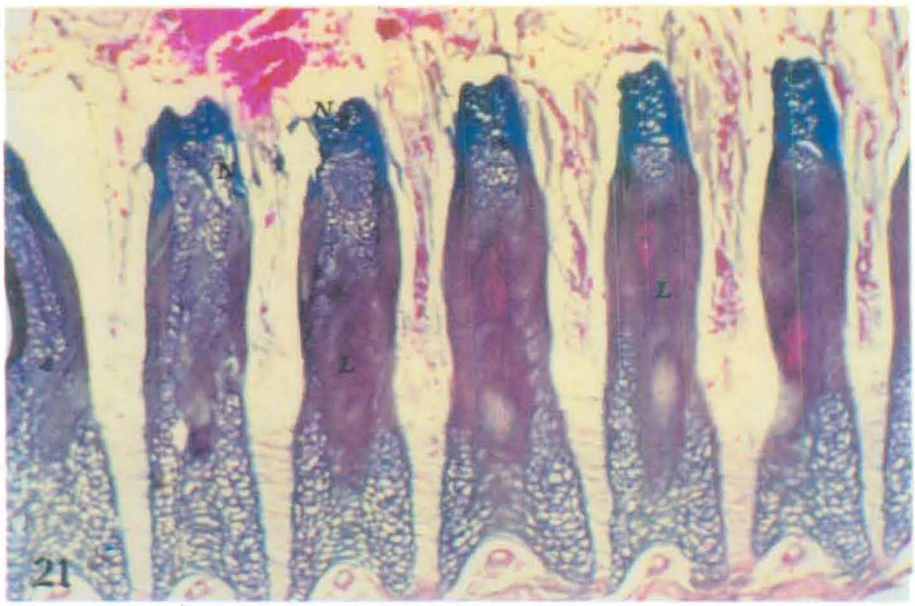


Fig. 21: : Gills of *Oreochromis spilurus* exposed to concentration (2) at 120 h showing loss of matrix homogenitiy (L) and necrotic chongrocytes (N) Mallory Trichrome stain X 33.

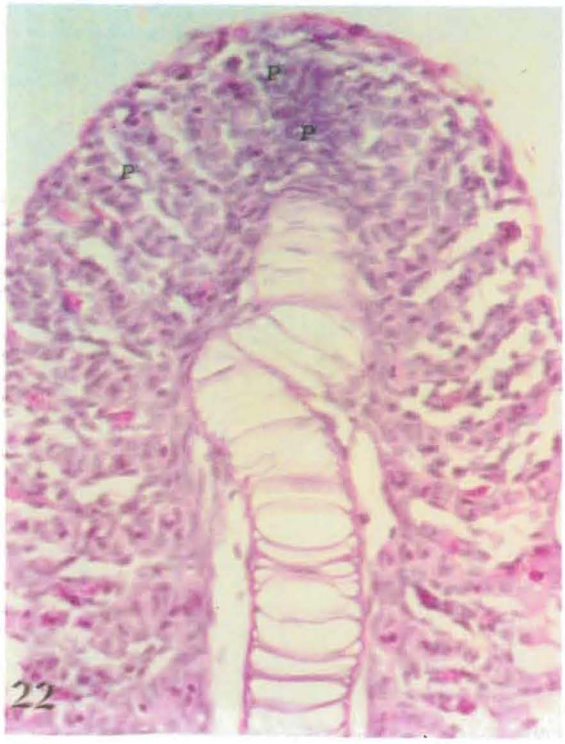


Fig. 22: Gills of Oreochromis spilurus exposed to concentration (1) at 168 h showing hyperplasia of the gill filament (P). Haematoxylin and Eosin X 133.

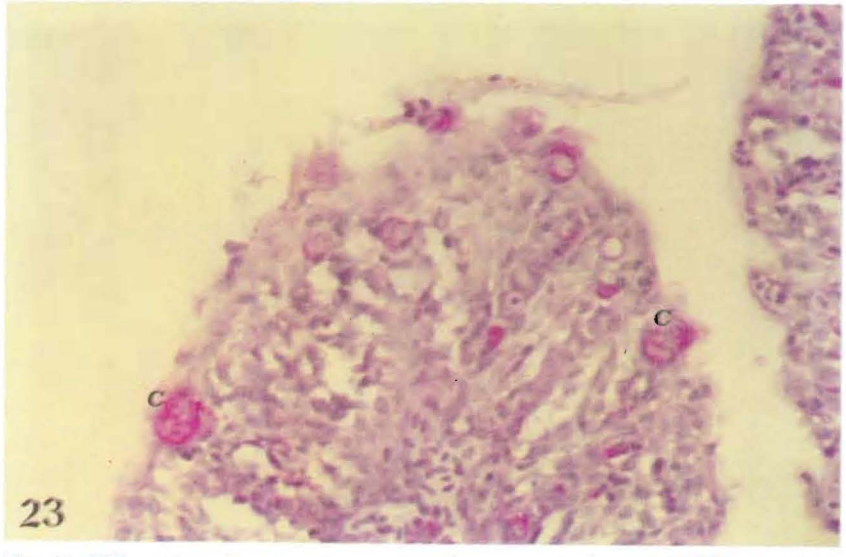


Fig. 23: Gills of *Oreochromis spilurus* exposed to concentration (1) at 168 h showing PAS + ve reaction of the mucus cells (C). PAS Stain reaction X 133.

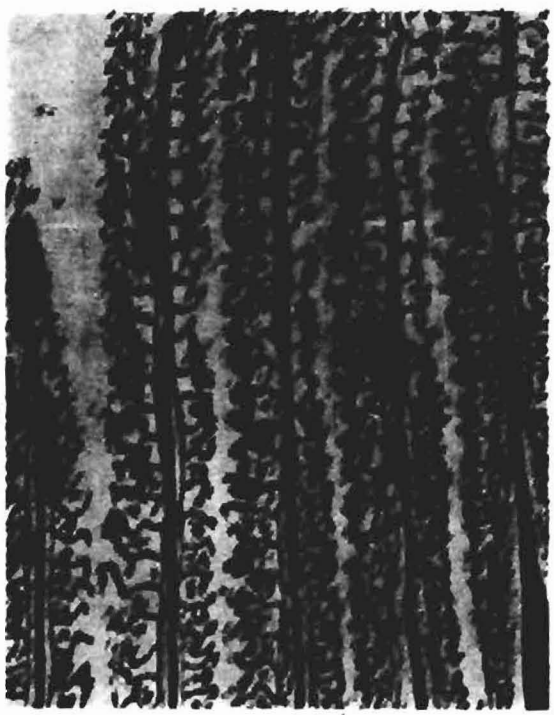


Fig. 24: Gills of *Oreochromis spilurus* exposed to concentration (2) at 168 h showing sever necrosis of the secondary lameller epithelium (S). Haematoxylin and Eosin X 33.

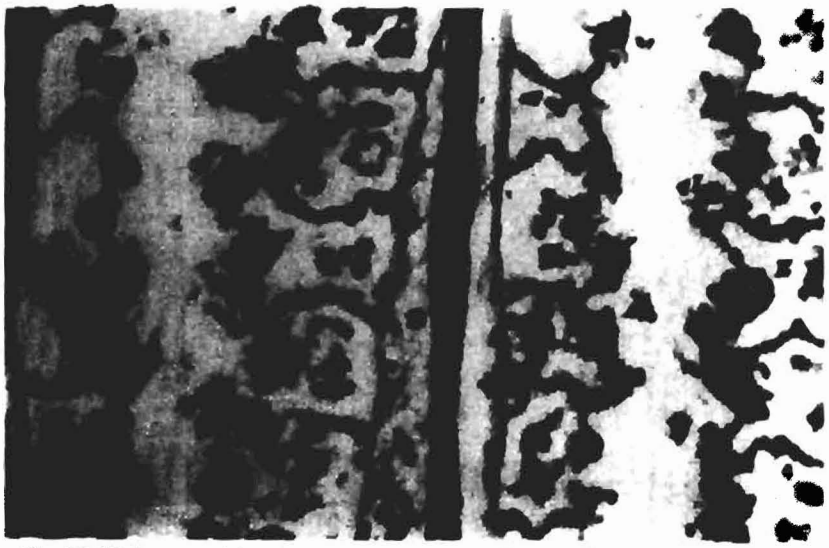


Fig. 25: Higher magnification of Fig.. 24 showing the necrotic change of both lameller (L) and interlameller epethlium (E). Haematoxylin and Eosin X 133.

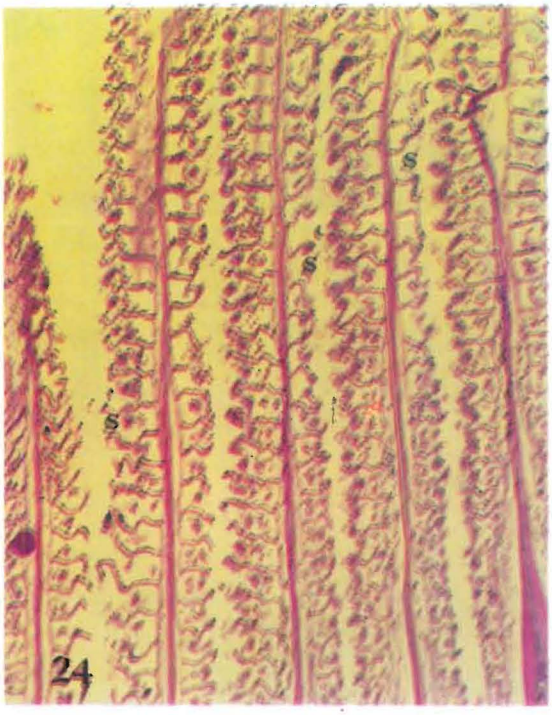


Fig. 24: Gills of *Oreochromis spilurus* exposed to concentration (2) at 168 h showing sever necrosis of the secondary lameller epithelium (S). Haematoxylin and Eosin X 33.

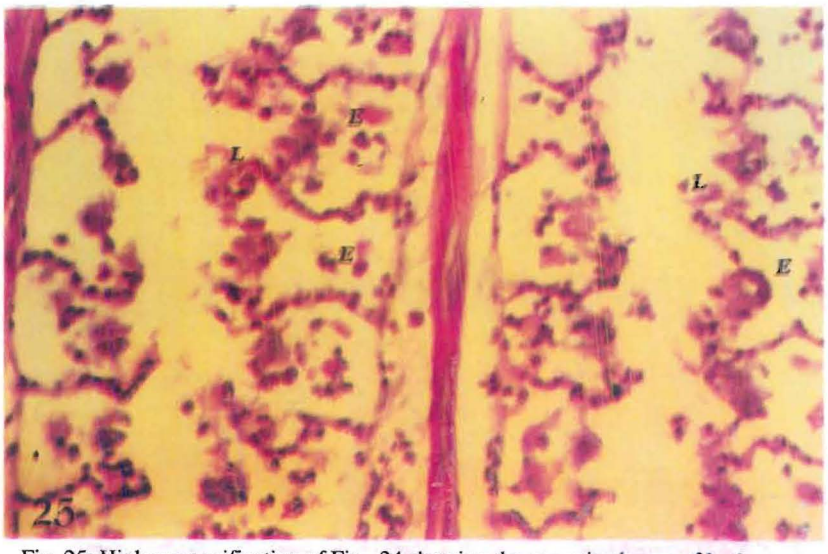


Fig. 25: Higher magnification of Fig.. 24 showing the necrotic change of both lameller (L) and interlameller epethlium (E). Haematoxylin and Eosin X 133.

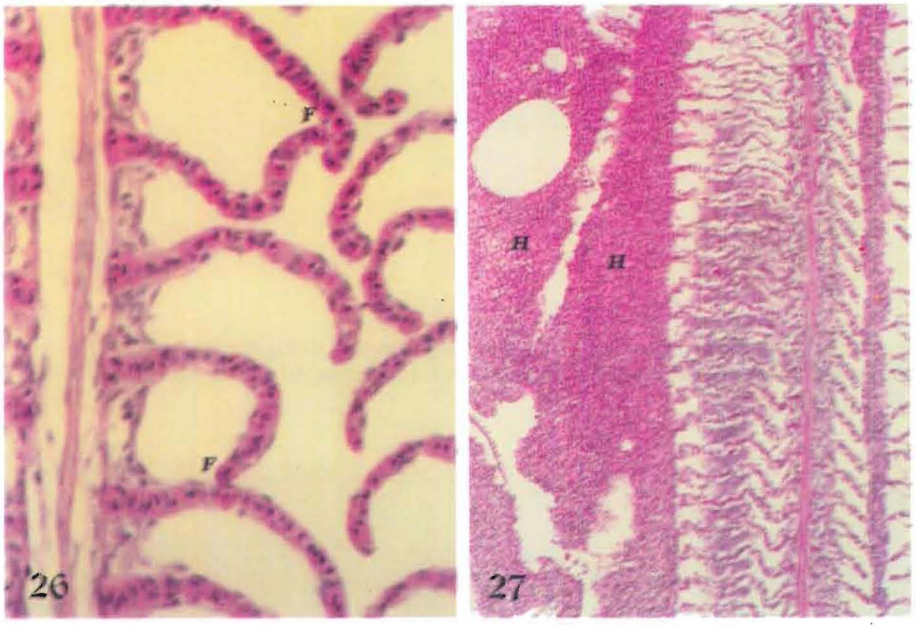


Fig. 26: Gills of *Oreochromis spilurus* exposed to concentration (2) at 168 h showing lameller fusion (F). Haematoxylin and Eosin X 132.

Fig. 27: Gills of *Oreochromis spilurus* exposed to concentration (3) at 168 h showing sever haemorrhage (H). Haematoxylin and Eosin X 33.

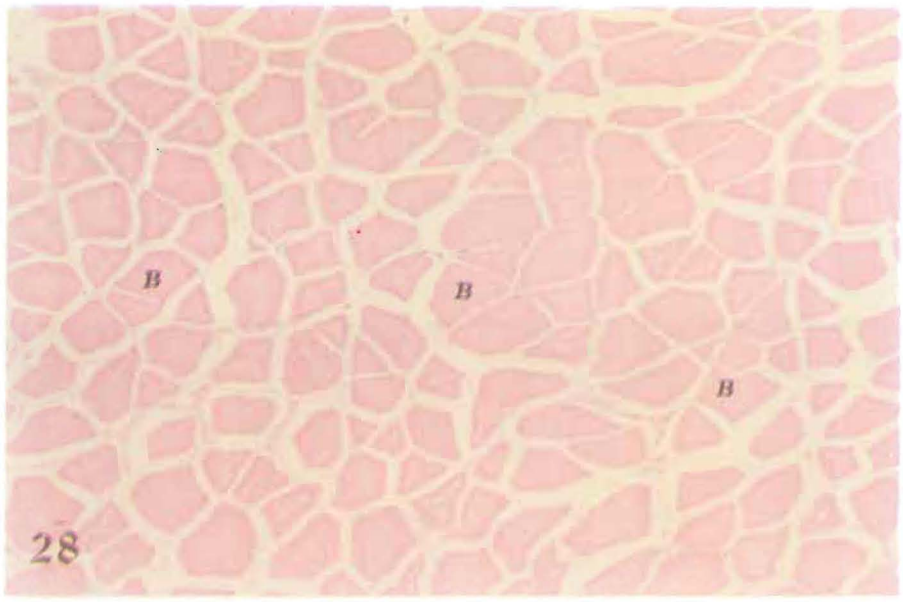


Fig. 28: Muscles of non-exposed Oreochromis spilurus showing the normal arrangement and appearance of muscle bundles. Haematoxylin and Eosin X 33.

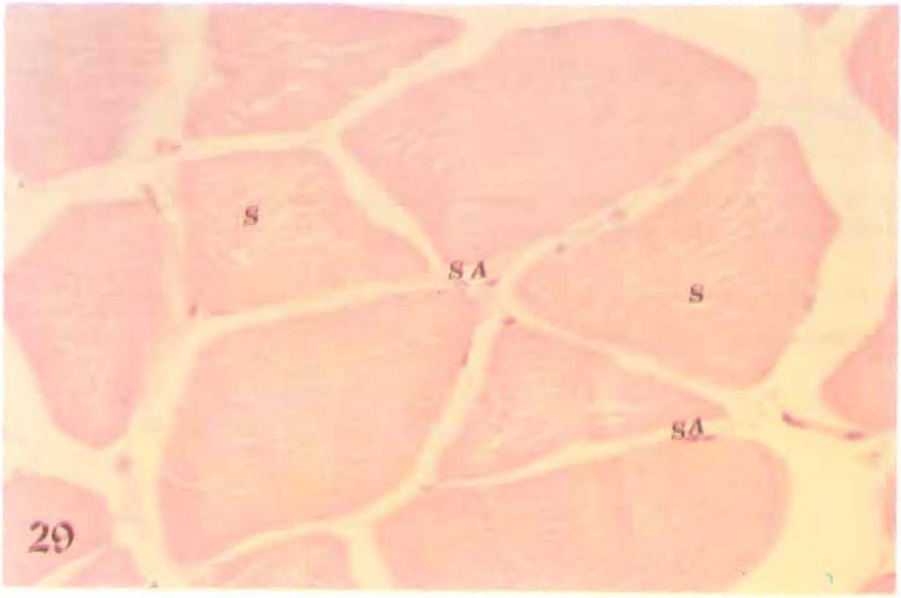


Fig.29: Higher magnification of Fig. 28 showing normal striation (S), with healthy nuclei (SA). Haematoxylin and Eosin X 132.

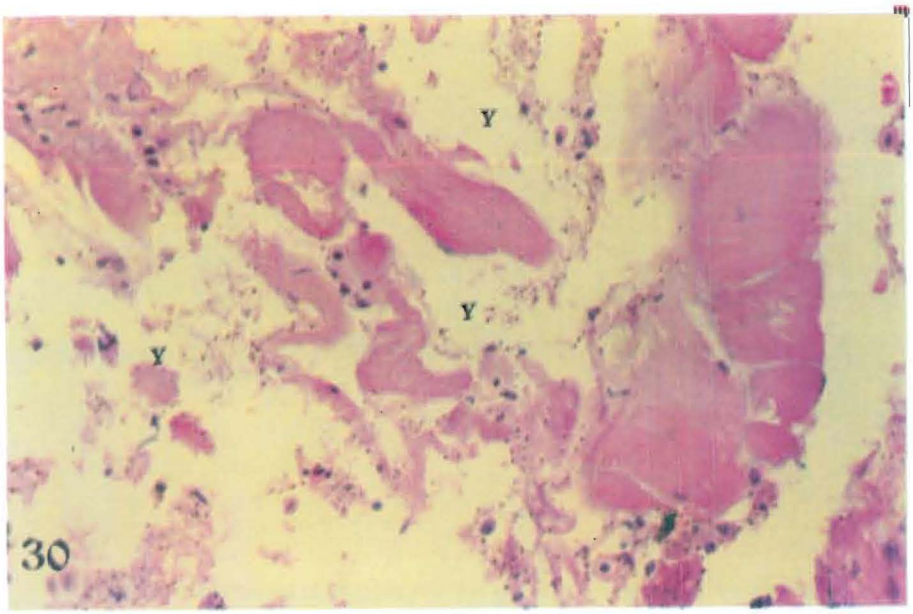


Fig.30: Muscles of exposed *Oreochromis spilurus* exposed to concentration (1) at 36h showing focal area of myolysis (Y). Haematoxylin and Eosin X 132.

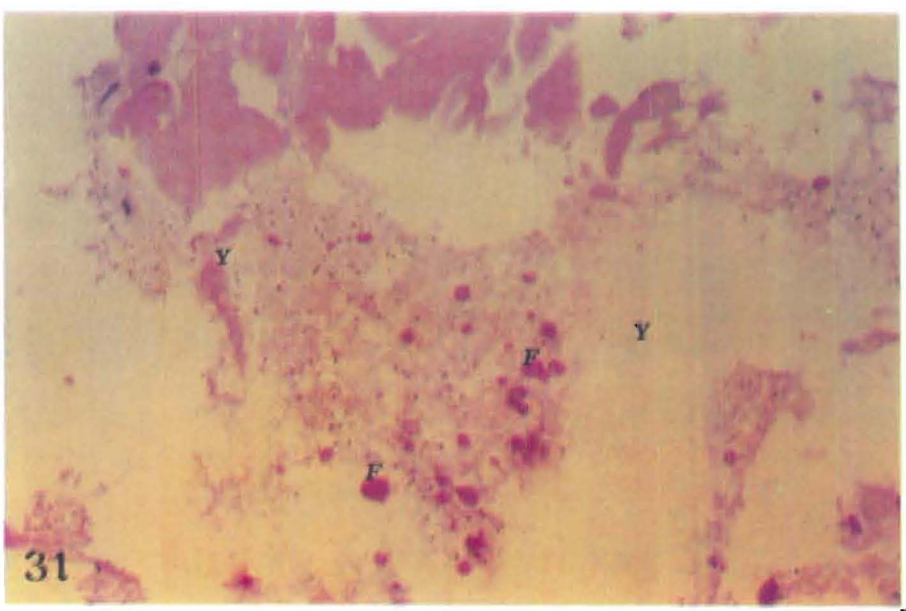


Fig.31: Muscles of exposed *Oreochromis spilurus* exposed to concentration (2) at 36h showing well expressed myolysis (Y) leaving ruminants of necrotic fibers (F). Haematoxylin and Eosin X 33.

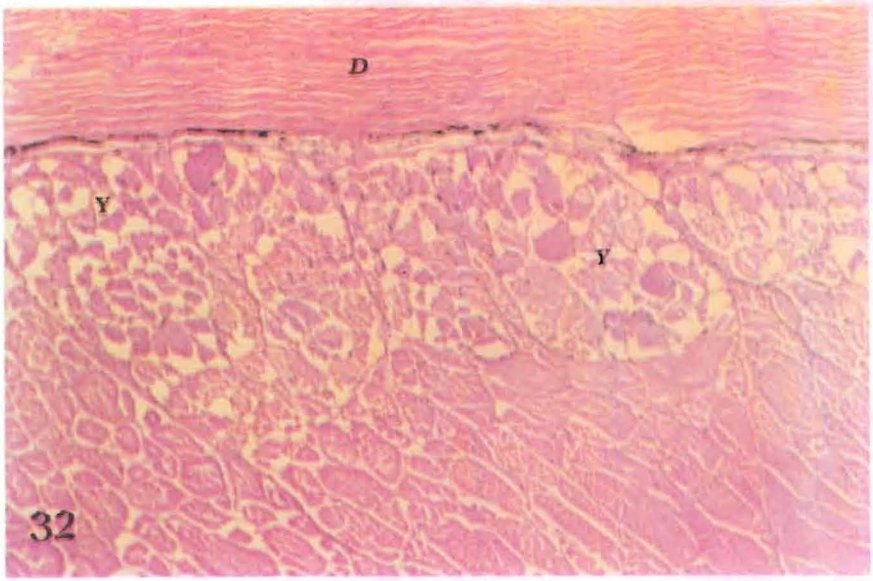


Fig. 32: Muscles of exposed Oreochromis spilurus exposed to concentration (1) at 72h showing focal area of myolysis (Y) beneath the hypodermis (D). Haematoxylin and Eosin X 33.

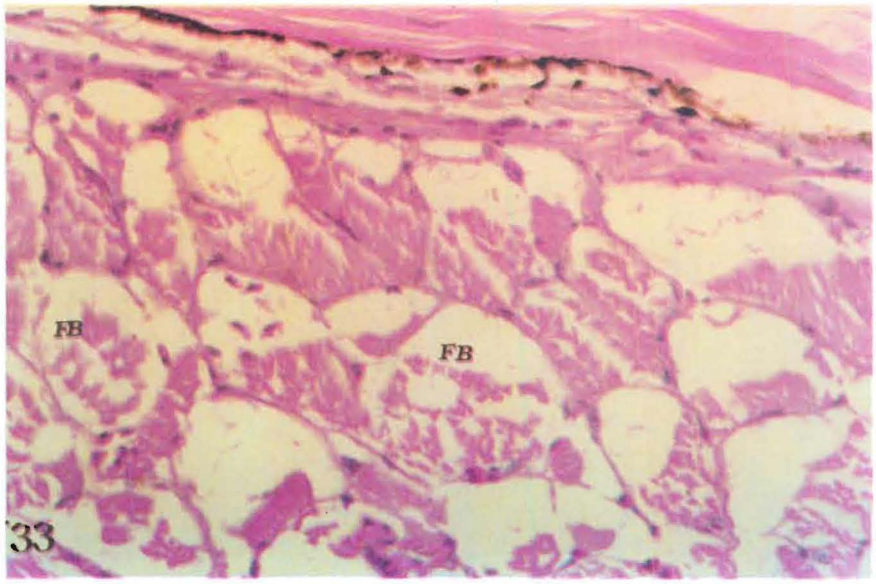


Fig. 33: Higher magnification of Fig. 32 showing fragmented individual muscle bundles (FB). Haematoxylin and Eosin X 132 .

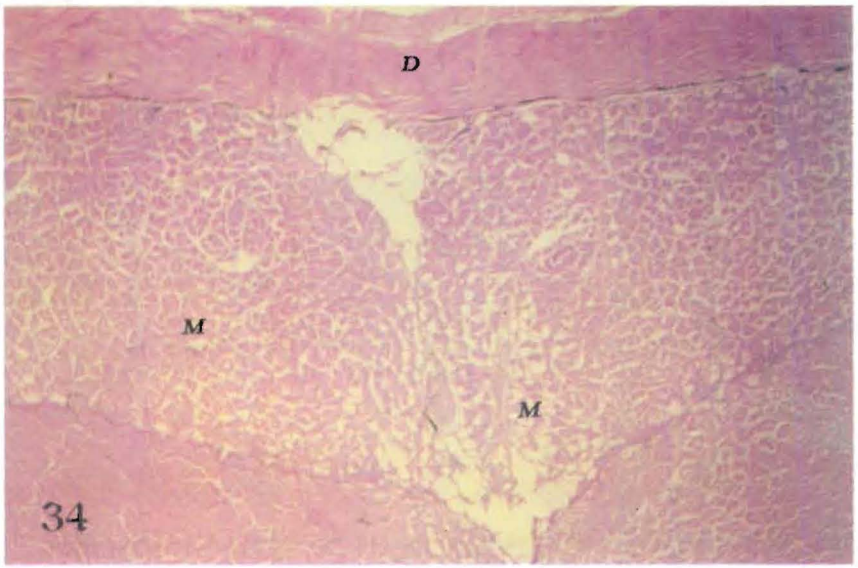


Fig. 34: Muscles of exposed *Oreochromis spilurus* exposed to concentration (2) at 72h showing widespread area of myolysis (M) beneath the hypodermis (D). Haematoxylin and Eosin X13.2.

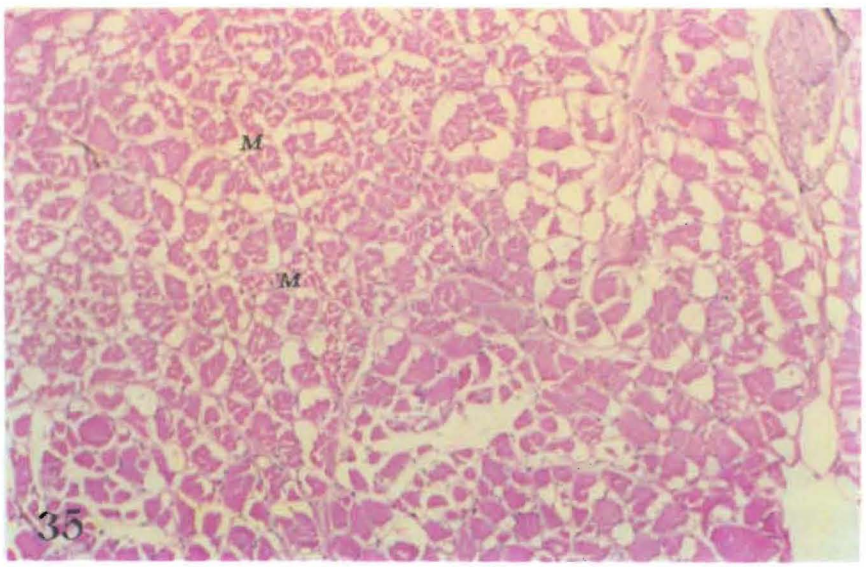


Fig. 35: Higher magnification of Fig. 34. Haematoxylin and Eosin X 33.

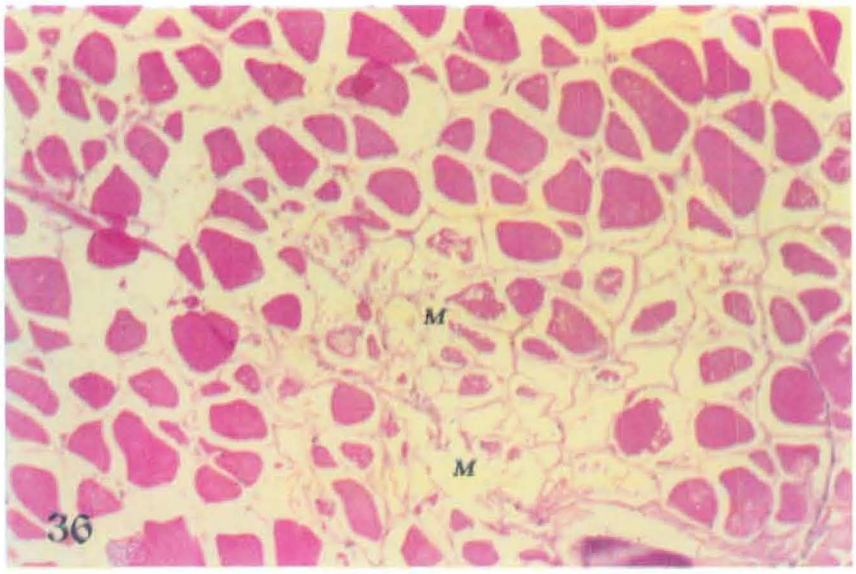


Fig. 36: Muscles of exposed *Oreochromis spilurus* exposed to concentration (3) at 120h showing deep myolysis (M). Haematoxylin and Eosin X 33.

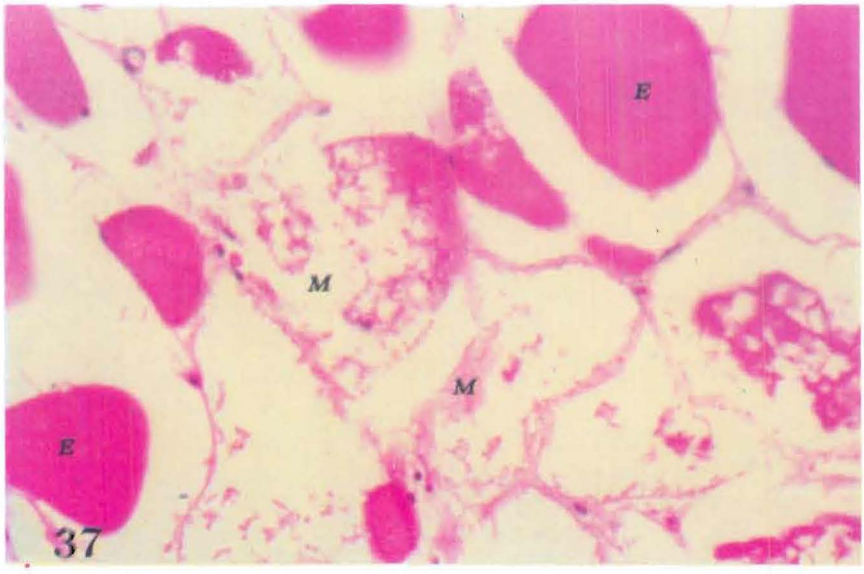


Fig. 37: Higher magnification of Fig. 36 showing the myolysis (M) and marked eosinophilia of the sarcoplasm (E). Haematoxylin and Eosin X 132.

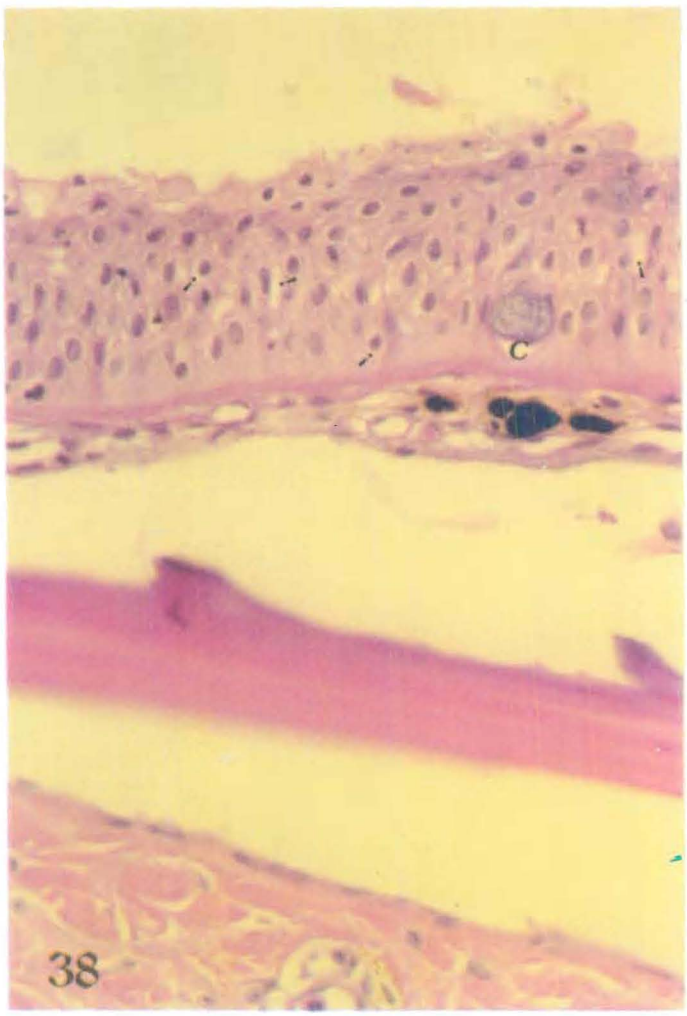


Fig.38: Skin covering the muscles of Oreochromis spilurus exposed to concentration (3) at 120h showing slight spongiosis (!) and hypertrophy of mucus cells (C). Haematoxylin and Eosin X 132.

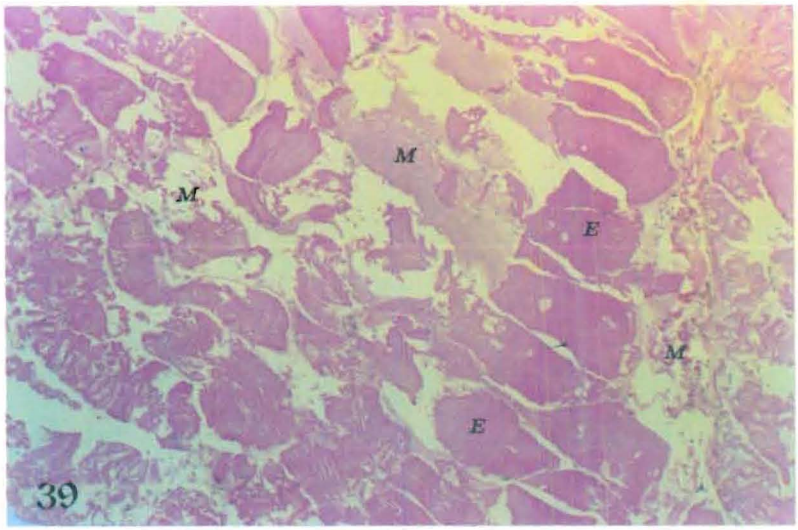


Fig. 39: Muscles of exposed *Oreochromis spilurus* exposed to concentration (3) at 168h showing well expressed myolysis (M) and marked eosinophilia of the muscle bundles (E). Haematoxylin and Eosin X 33.

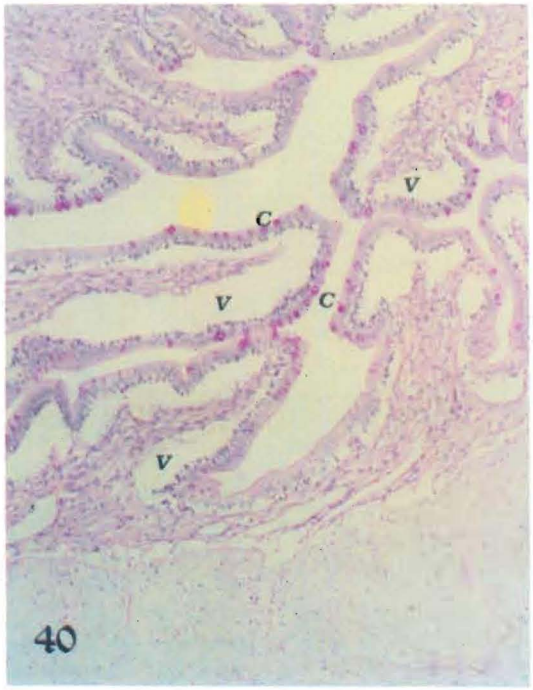


Fig. 40: Intestine of *Oreochromis spilurus* exposed to concentration (3) at 120h showing marked proliferation of mucus cells (C) in the intestinal villi (V). PAS Stain reaction X 33.

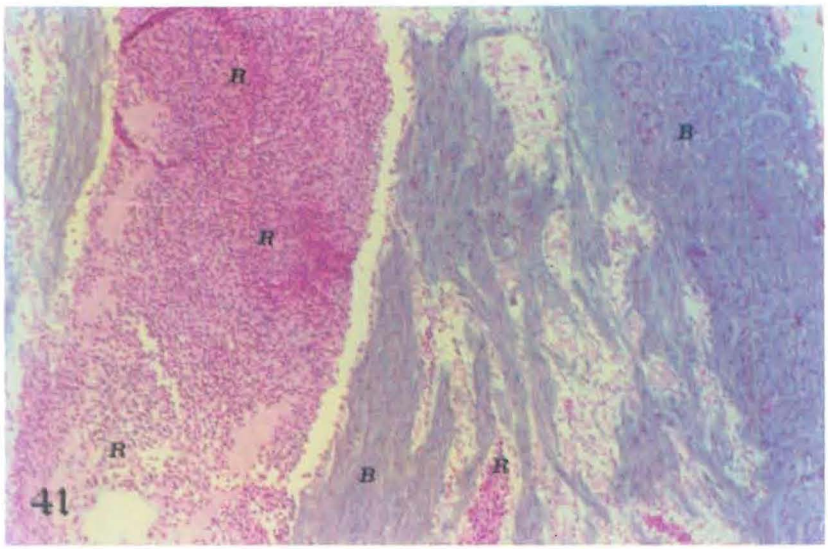


Fig. 41: Heart of *Oreochromis spilurus* exposed to concentration (2) at 120h showing massive haemorrhage (R) in the bulbus arteriosus (B). Haematoxylin and Eosin X 33.

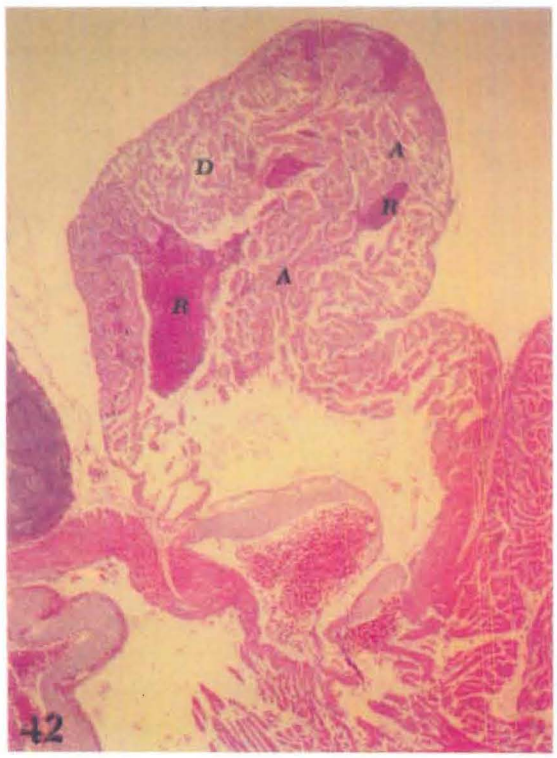


Fig.42: Heart of *Oreochromis spilurus* exposed to concentration (2) at 120h showing multifocal areas of haemorrhage (R) in the atrium (A) with slight disintegration of its muscle fibers (D). Haematoxylin and Eosin X 13.2.

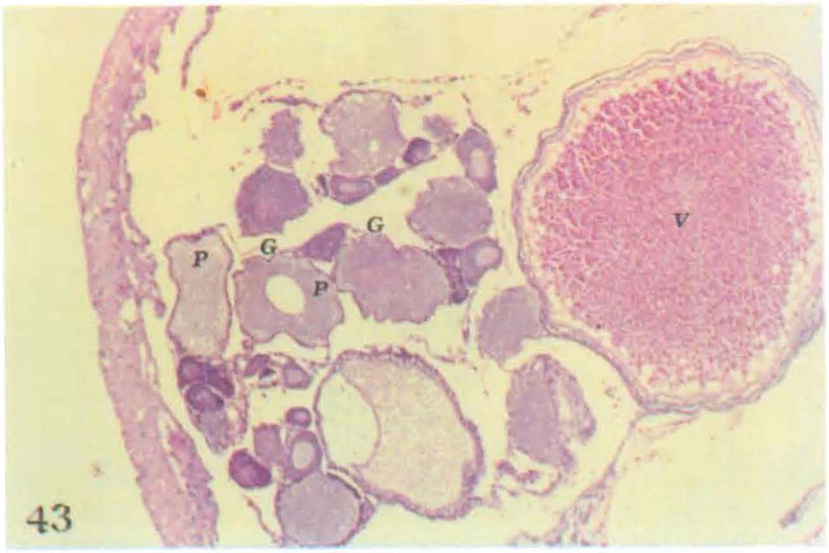


Fig.43: Ovary of *Oreochromis spilurus* exposed to concentration (2) at 120h showing degenerative changes of the perinuclear stage of the ova (P) expressed by corrigation of their memberanes (G).(V)= Vitellogenic ova. Haematoxylin and Eosin X 33.

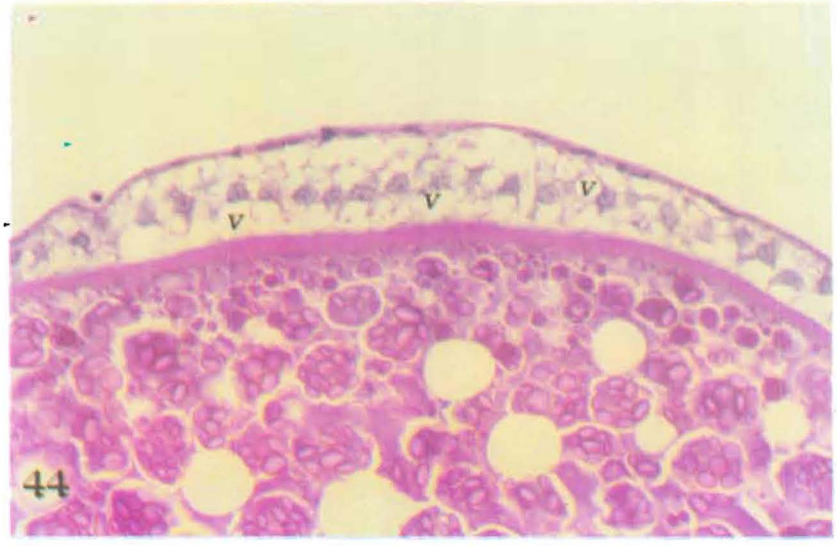


Fig.44: Ovary of *Oreochromis spilurus* exposed to concentration (2) at 120h showing vacuolation of the theca layer (V) of the vitellogenic ova. Haematoxylin and Eosin X 132.

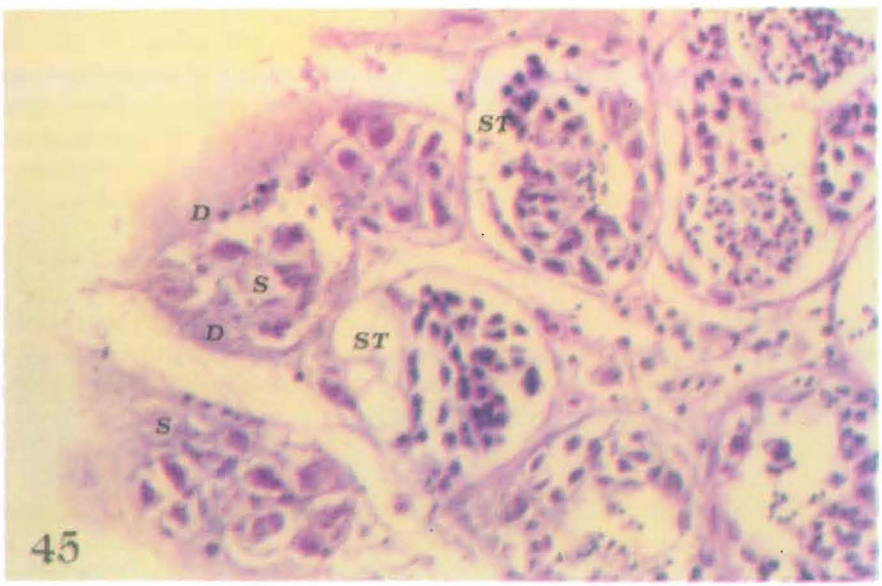


Fig.45: Testes of Oreochromis spilurus exposed to concentration (1) at 72h showing degenerative changes of the seminiferous tubules (ST) expressed by damage of their membranes (D) and slight necrosis of the spermastogenic cell layer (S). Haematoxylin and Eosin X 132.

DISCUSIONS

Branchial epithelium is comparatively vulnerable to environmental toxins because it is in direct contact with the medium in which toxins are delivered (Weindelaar and Vender, 1989). Gill lesions caused by toxins are predicted to have several physiological effects on the fish, which include gas exchange, ammonia excretion and ion regulation (Randall and Daxboeck, 1984; Heisler,1984 and Evans,1993).

Common lamellar changes include edema, fusion, epithelial necrosis, hyperplasia of mucus and chloride cells in response to diverse environmental toxins (Mitchell & Cech, 1983;Ewing *et al.*, 1994).The present study describes the gill lesions in *Oreochromis spilurus* exposed to the contra /insect pesticide. The hyperemia and hemorrhage observed in this study suggesting signs of acute toxicity. The hemorrhage in fish exposed to concentration (2) was more pronounced than those subjected to concentration (1). Moreover, the extent of hemorrhage was dependent on the duration of exposure. These results suggest that the acute toxicity of contra/insect in *Oreochromis* spilurus was dose and time dependent.

The lamellar and inter lamellar epithelial necrosis and necrosis of the chondrocytes observed in the present study suggest the direct toxic effect of contra/insect on gill tissue, which was also dose and time dependent. Several reports have indicated these changes in response to wide variety of toxins (Ewing et al., 1994).

Telangiectasis (or aneurysm) is a characteristic pathologic change of the gill, associated with physical or chemical trauma, after grading, with parasitic conditions, and in association with metabolic waste or chemical pollution (Roberts, 2001). These lesions start with rupture of the pillar cells, which join the dorsal surface of the secondary lamellae to the ventral. The result is dilatation of the lamellar capillary and pooling of the blood. If there are many telangiectatic lamellae, respiratory function may be impaired, especially at higher temperatures, when dissolved oxygen demand is high (Roberts, 2001). The present study describes many telangiectatic lamellae in fish exposed to concentrations (2,3). This result may indicate respiratory failure and explain the hemorrhage observed at higher doses. Roberts (2001) stated that extensive telangiectasis could result in fatal hemorrhage and takes considerably longer to resolve than hyperplastic lesions of the gill.

Lamellar hyperplasia observed in the present study in fish exposed to and collected at 168 h post-exposure may suggest a compensatory mechanism of the gills occurred. Lamellar hyperplasia is a more long-term response to lower levels of irritation (Roberts, 2001). The present study described lamellar hyperplasia at low concentration (1) by 168 h exposure time.

Mucus hyper secretion is a typical response of the pulmonary airways of mammals to many inhaled toxins (Gail & Lefant, 1983). The rate of mucus production in fish can be increased in response to infection or physical or chemical irritants (Roberts, 2001). Morewover, mucus is known to store IgM and also contain C -Reactive Protein (CRP), which help precipitation and colonization of microorganisms (Roberts, 2001). The mucus cell index, which is the number of of all mucus cells on the lamellae and dividing this value by the number of lamellae (Speare & Ferguson, 1989). Decrease number of mucus cells have been reported in juvenile rainbow trout in response to repeated exposure to chloramine-T and it is a stereotype response of fish to water-borne toxins (Mallatt,1985). Afifi et al. (2001) reported that the mucus cell index showed no difference between control and exposed fish to clove oil and suggested that clove oil had no irritant effect on the gills exposed to 3,6; 9 ppm. The present study showed hypertrophy of mucus cells in fish exposed to concentration (2) at 36 h, followed by proilferation by 72 h and slight exhaustion of its content by 168 h post-exposure. These observations may suggest a possible role of mucus in acute contra/insect toxicity in *Oreochromis spilurus*.

The present study describes the histopathological changes in the muscles associated with contra/insect toxicity in *Oreochromis spilurus*. Focal hypodermal myolysis was observed in fish exposed to low dose of contra/insect, which progressed to more pronounced myolysis and extended deeply in the musculature at higher doses and longer exposure. These results suggest that these toxic degenerative changes of the muscles observed in this study are dose and time dependent. Organophosphates interfere with the function of somatic motor neurons of the autonomic nervous system result in muscle contraction (Plumb, 1993).

One of the contra/insect components is from the organophosphates group. The heart hemorrhage observed in fish exposed to concentration (2) suggests also sign of acute toxicity. While, the degenerative changes observed in the ovary and testes of fish exposed to higher doses of contra/insect and for longer duration suggested direct toxic effect

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