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TISSUE DISORGANISATION IN A HYPERPLASTIC LIVER OF A CATFISH *MYSTUS GULIO* COLLECTED FROM THE HIGHLY POLLUTED HARBOUR WATERS OF VISAKHAPATNAM, INDIA

RAFIA SULTANA AND K. SRINIVASA RAO

Department of Zoology, Andhra University, Waltair 530 003

ABSTRACT

An unusually large sized long (26.0 cm) whiskered catfish *Mystus gulio* was collected from Visakhapatnam harbour having an abnormally bloated abdomen and emaciated tail. There were no external lesions, but the discoloured liver was hyperplastic. Microscopic examination of liver tissue revealed polymorphic liver cells with nuclear and nucleolar enlargements, sporadic binucleate and multinucleate cells and PAS and BPB positive intra cytoplasmic inclusions. Derangement of kidney tubules and appearance of melano macrophages were observed in kidney and gonads. High concentrations of heavy metals and hydrocarbons in the harbour waters seem to have affected the fish.

INTRODUCTION

THE MAGNITUDE of pollution in the harbour waters of Visakhapatnam is found to be very high (Panduranga Rao *et al.*, 1990). High concentrations of heavy metals such as Iron (10,000-11,000 µg/1), zinc (1000-1600 µg/1), lead (30-50 µg/1), copper (15-20 µg/1), cadmium (15-20 µg/1) and oil and grease (85-90 mg/1) are found in harbour waters when compared to normal levels in sea water (Bruland, 1983; mean values of 0.06 µg Fe/1, 0.39 µg Zn/1, 0.25 µg Cu/1, 0.08 µg Cd/1 and 0.002 µg Pb/1). Earlier studies on the physiological and pathological effects of cadmium (Calabress *et al.*, 1975) and lead (Holcomb *et al.*, 1976) were reported to have caused gill damage and spinal deformities respectively. Oil pollution is known to have caused papillomas (Russel and Kotin, 1957) and carcinomas (Halstead, 1972) in fishes. These studies were related to the effects of pollutants in isolation carried out under experimental conditions. Studies on synergistic effects of multiple toxicants on feral fish were few (Brown *et al.*, 1973; Dawe and Harshbarger, 1975; Baumann and Harshbarger, 1985). In the Visakhapatnam harbour there were earlier reports on mass mortality (Ganapati and Raman, 1976), eye opacity and fin erosion of fishes (Ram Bhaskar *et al.*, in press) and inferior condition of mullets (Ram Bhaskar *et al.*, 1989) as a result of combined effect of pollutants on feral fish. The present study is a continuation of the report on hepatic hyperplasia in a catfish *Mystus gulio* (Srinivasa Rao and Rafia Sultana, 1983) to give the details of the histological degeneration in the hyperplastic liver.

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Materials and Methods

The affected specimen of *Mystus gulio* (26.0 cm) is from a school of fish of the feral populations of Visakhapatnam Harbour, obtained by fishing with a castnet. It was the largest in the school of fish. The liver of the specimen was unusually large, while the ovary and kidney were discoloured. Histological preparations of the three organs were made.

All tissues were directly fixed in Bouin's fluid. Sections of 6 to 7 microns thick were cut and stained with Haematoxylin and Eosin (H & E), Heidenhan's azan (azan), Periodic acid and Schiff's (PAS) test and Bromophenol blue (BPB). Liver tissue was removed from the periphery and centre of the liver at four different regions for histological study. Sections of intestine were examined and found to be normal. Heart and spleen did not manifest any abnormality. In the survey of histopathology, gross appearance of lesions on the liver only were considered. Such lesions were found in two other specimens out of 350 specimens examined.

Results and Discussion

Microscopic examination of hyperplastic liver of *M. gulio* revealed a number of degenerative features. Liver architecture was completely altered. The liver section shows glandular pattern hyperplasy at the periphery and complete disruption of the glandular pattern towards the centre with basophilic cells in one area (Pl. I A). The peripheral glandular type of cells were pale staining and seem to possess multiple nuclei. The nuclear membranes were thick and nucleoli were prominent. The outer and inner margins of the glandular hyperplasy were bounded by thick fibrous layers with frequent red blood cells. In another area of liver (Pl. I B) polymorphic cells of different shapes and sizes were observed. Enlarged cells resembling tumour giant cells with prominent nucleoli can also be seen. At the border between hyperplasy and giant cells there were 2-3 layers of spindle shaped cells.

High frequency of cells with PAS and BPB positive intracellular cytoplasmic inclusions of varying shapes and sizes were present (Pl. I C). Mitotic figures were clear in some areas of liver tissue (Pl. I D). Melanomacrophage aggregates were present in some areas. But none of the liver cells exhibited cytoplasmic vacuolation.

There were no external lesions on the ovaries. The ovary was in the early stage of maturity and the ova were without the yolk. The cytoplasm, nucleus and the nucleolus of the intact ova were normal. Binucleate oocytes were observed in the middle of the macrophage aggregates (Pl. II A). In a few areas ova were found to be completely destroyed (Pl. II B).

The epithelial cells of the kidney tubules and renal corpuscles were found to be disintegrating (Pl. II C). Frequency of melanomacrophage aggregates was very high. The severity of degeneration is evident from the complete destruction of the renal epithelial cells of highly dilated tubules (Pl. II D).

Microscopic study of sections of liver, ovary and kidney of *M. gulio* with enlarged liver and discoloured ovary and kidney appears to be a case of hyperplastic necrosis of liver with derangement of kidney tubules and appearance of melano-macrophage aggregates

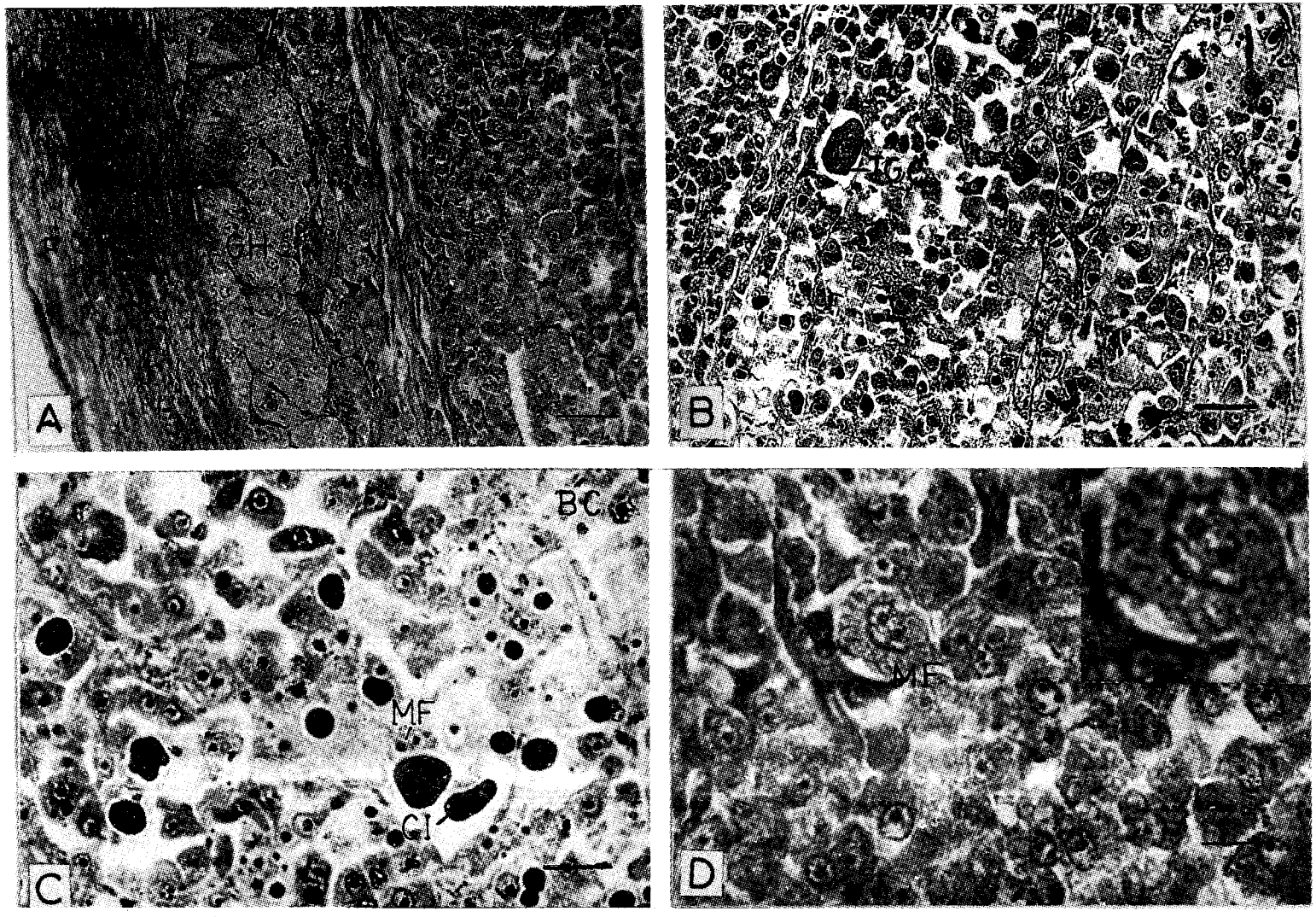

PLATE I. Photomicrographs of Hyperplastic liver of *Mystus gulio* : A. Disruption of liver architecture. Note the fibrosis (F) and glandular hyperplasy (GH) at the lower left corner. Scale bar = 50 μm Azan ×200; B. Polymorphic liver cells. Note the tumour giant cell (TGC) and spindle shaped cells (SSC). Scale bar = 30 μm Azan × 300; C. Liver cells with various sizes of PAS positive cytoplasmic inclusions (CI), binucleate cells (BC) and mitotic figures (MF). Scale bar = 40 μm PAS × 250; and D. Liver cells with nuclear and nucleolar enlargements and mitotic figures (MF). Scale bar = 56 μm Azan × 180; Inset : Note mitotic figure. Azan × 1000.

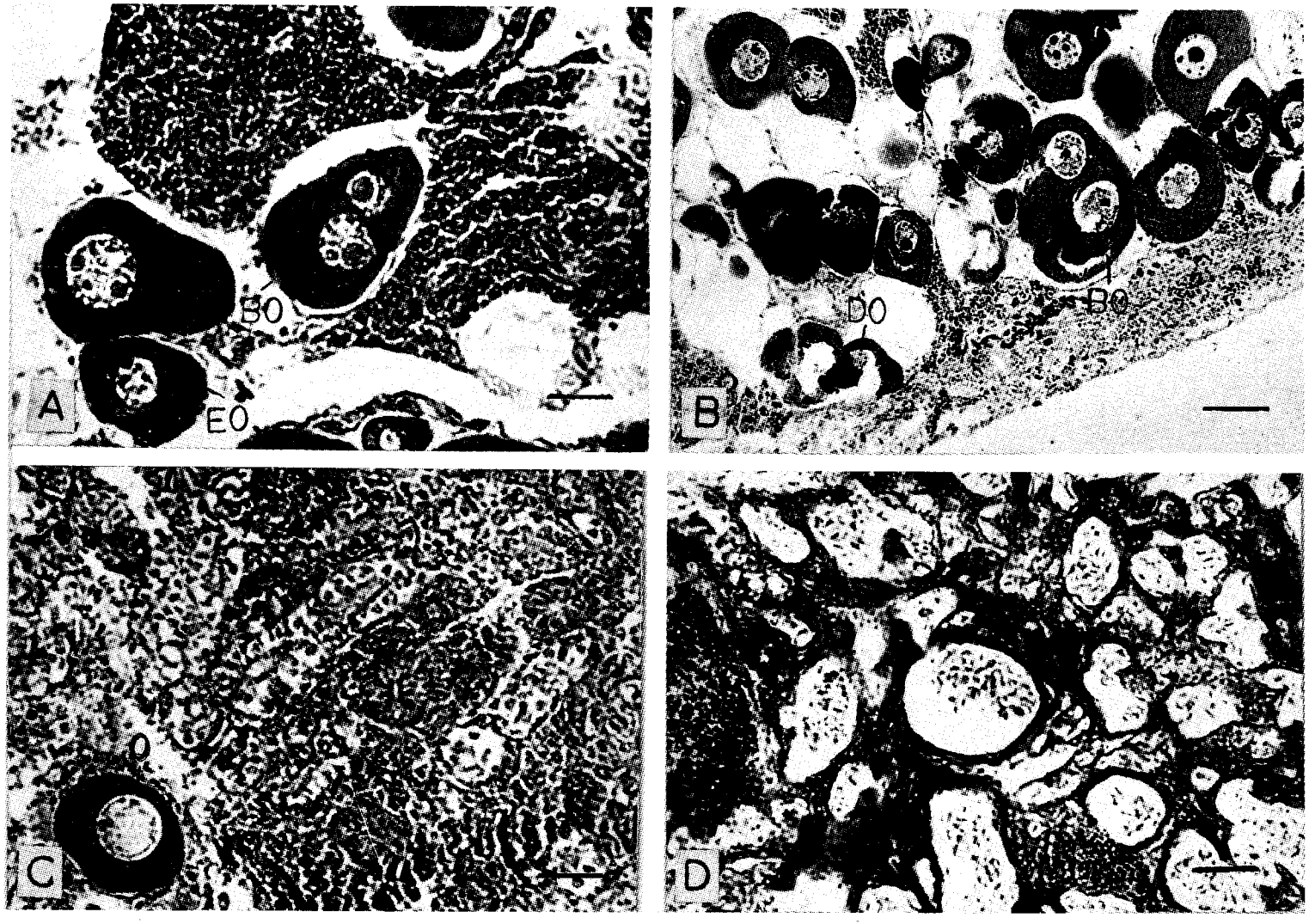

PLATE II. Photomicrographs of discoloured ovary and kidney of *M. gulio* : A. Binucleate oocyte (BO) in the middle of macrophage aggregate and early stage oocytes (EO). Scale bar = 50 μm H & E × 200; B. Some destroyed ova (DO) and a binucleate oocyte (BO). Scale bar = 55 μm H & E × 180; C. Kidney tubules with disintegrating epithelial cells. Note the oocyte (O) in the kidney. Scale bar = 50 μm Azan × 200, and D. Completely distructed renal epithelial cells of highly dilated tubules. Note the melanomacrophage aggregate at the left. Scale bar = 60 μm Azan × 170.

in kidney and gonad more than in the liver. The fish has been subjected to long term exposure to high concentrations of heavy metals and oil refinery products (Ganapati and Raman, 1973; Satyanarayana *et al.*, 1985) which may be individually or synergistically carcinogenic. Sunderman (1971) and Brown *et al.* (1973) reported that heavy metals associated with petroleum products in polluted watersheds can cause a high frequency of hyperplastic diseases that are not found in the same frequencies in less polluted areas.

The high frequency of liver neoplasia within feral populations of brown bullhead *Ictalurus nebulosus* in Black River Ohio (Baumann and Harshbarger, 1985) was attributed to polynuclear aromatic hydrocarbons which were found at an elevated level in liver tumours also. They have also stated that in the bullhead populations, hepatocellular neoplasms do not reach a stage where they are grossly observable and hence field studies may give an underestimation of the occurrence of neoplasia.

Some 350 specimens of *M. gulio* have been examined for gross manifestation of liver tumours. Small hepatic nodules were found only in two specimens (17 cm and 18.5 cm) (Rafia Sultana and Srinivasa Rao; in press) other than the one with hyperplasia currently reported. The livers of about 200 specimens (14 to 20 cm) were sectioned for microscopic examinations. None of the small size fish (other than the three mentioned above) showed lesions. Baumann and Harshbarger (1985) found the lesions in the bullhead populations to be of cholangiocellular origin in 3-4 years old fish only.

More detailed and careful studies of *M. gulio* are necessary to confirm whether or not the relative occurrence of lesions of liver is less prevalent in the younger age groups compared to older age groups. The present case study is that of an unusually large sized fish (26.0 cm) which is likely to be 6 years old according to the age-length relation of the species given by Pantulu (1961). The fish was observed to be actively moving in the school of fish from which it was caught (Srinivasa Rao and Rafia Sultana, 1983).

Hyperplasia of the liver in *M. gulio* did not show lesions as observed in the sole *Parophrys vetulus* (McCain *et al.*, 1978) exposed to experimentally oiled sediment. Some other fish exposed experimentally to crude oil (Solangi and Overstreet, 1982) and Aroclor 1254 (Couch, 1975) showed hepatocyte vacuolization commonly and their increase at higher concentrations was attributed to bioaccumulation and degradation mechanism in liver tissue. Such vacuolization of hepatocytes was not found in the liver of *M. gulio* of the present study and also in the estuarine fish *Trinectes maculatus* (Solangi and Overstreet, 1982). On the other hand, the cytoplasmic inclusions which were reported by Couch (1975) in the case of spot *Leiostomus xanthurus* exposed to Aroclor 1254 were found to be specific to that toxin. Cahn (1975) found in the case of menhaden, that the degree of vacuolization, cytoplasmic granulation and basophilia in the liver may vary not only between species, but also between individuals of the same species. Thus, histopathological changes may differ from toxin to toxin, species to species and between individuals of the same species. The response of *M. gulio* to the harbour pollutants appears to be confined to the appearance of cytoplasmic inclusions only in the hyperplastic liver. It appears to be more due to the influence of the high concentrations of heavy metals, rather than the fluctuating concentrations of oil and grease.

The degeneration of the renal tubules in some parts of the posterior kidney with only fibrous outlines remaining, indicate their disrupted architecture akin to the hepatorenal syndrome in turbot (Anderson *et al.*, 1976)

which was suspected to be due to contaminant heavy metal residues. Other features of the terminal stage of hepatorenal syndrome like 'fibrosis of the haemopoeitic tissue and extensive cast formation, parallel biliary proliferation and cirrhosis of the liver' (Roberts, 1978) are not observed in the present study. Liver necrosis appears to be the primary change and renal derangement appears to be an associated change as a result of exposure to high concentration of heavy metals.

Induced histopathological changes mostly resembled prehepatomatous lesion (Couch, 1975). The usual hepatoma induced in trout may be classified as minimal deviation tumour (Ashley, 1969) which is characterised by a mere increase in basophilic cells in which central and portal veins were absent (Sinnhuber *et al.*, 1977). The hyperplastic liver in *M. gulio* was devoid of blood vessels and portal canals in the centre of the liver as most of the liver is invaded by necrotic cells and seemingly without any regeneration taking place. But the fibrotic change, polymorphic, binucleate and multinucleate cells and tissue disorganisation of hyperplastic liver are indicative of prehepatomatous lesion formation.

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