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EFFECT OF CHROMIUM, CADMIUM AND MERCURY ON THE GILL HISTOLOGY OF *CLARIAS BATRACHUS* L.

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ABSTRACT

Effect of cadmium (Cd), chromium (Cr) and mercury (Hg) on the gill histology of *Clarias batrachus* L. was investigated following 28 days exposure to their sublethal concentrations under laboratory condition. Hyperplasia and hypertrophy of the lamellar epithelium, necrosis, partial lifting of epithelial layer, damage of gill ray, oedema of primary lamellae, damage of pillar cells and congestion of blood vessels were the main histopathological changes. In Cd treated fishes the interlamellar spaces were abolished but distended gill rays were characteristics of Cr treatment. Extensive cellular and tissue damages were prominent in Hg treated fishes.

Key Words: Heavy metal, Gill histology, Hyperplasia, Hypertrophy, Oedema.

INTRODUCTION

Heavy metals have been detected in abundance in many waterbodies of Bangladesh (Anonymous 1998, Quraishi and Akhter 2005). In the present work, effect of heavy metals Cd, Cr, and Hg on histology of the gill of *Clarias batrachus* L. was investegated following its exposure to sublethal concentrations for 28 days under laboratory condition.

MATERIALS AND METHODS

Fresh and live specimens of *Clarias batrachus* L. (average length 16 ± 3 cm and weight 70 ± 5 g) was bought from Chittagong city market and acclimatized for 7 days in 25 L aquarium in tap water. Fish species were given oligochaetes, prawn and small pieces of *Harpodon nehereus* as food once in a day and water was changed at 24h interval. Following it, test fish specimens (20 / aquarium) were exposed to sub-lethal concentration of each heavy metal (Cd– 9 ppm, Cr– 12 ppm and Hg – 0.3 ppm) separately (treatment) for 28 days along with a control without heavy metal and fed once a day with a change of solution after each feeding.

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Method of Humason (1961) was followed for histopathological study. At the end of the exposure period gills from the metal exposed as well as control fishes were isolated, cleaned with physiological saline solution, cut into pieces and fixed in freshly prepared Bouin's fixative. After 24 hours tissues were transferred to 70% alcohol for preservation. For histological slides preparation tissues were dehydrated in graded alcohol, cleared in benzene and embedded in paraffin. Thin sections (3-5μ) were cut by rotary microtome, double stained by eosin-haematoxylin, mounted with DPX for examination under microscope. Photomicrographs were taken by Ricoh 35 mm SLR camera.

RESULTS AND DISCUSSION

The gills are among the most delicate structures and owing to their external location and direct contact with surrounding water they are liable to more damage by any irritant materials, whether dissolved or suspended, in the water (Roberts 1989, Watson and Benson 1987). In the present work, some common degenerative changes in gill tissue were developed on exposure to all three heavy metals such as hyperplasia of the gill epithelia**,** degenerated epithelial cells with pyknotic nuclei, necrosis in epithelium, blood congestion, pillar cell degeneration, mucous exudation, separation of lamellar epithelium, etc. But there were also some metal dependent degenerative changes. Mallat (1985) and Heath (1995) also observed cell degeneration and even total damage of cells of gill epithelia by necrosis on exposure to pollutants.

In the present study extensive proliferation or hyperplasia on the gill tissue of *C. batrachus* was noticed under Cd treatment that led complete loss of interlamellar spaces between the adjacent secondary lamellae of the entire primary lamella and turned the epithelium a solid mass of cells (Plate 3). The cells of the entire respiratory epithelium were also hypertrophied (swollen) with pyknotic nuclei and clear cytoplasm. Necrotic spots were found at several places of the epithelium. The pillar cell system also showed hyperplasia, hypertrophy and necrosis (Plate 3). The primary lamellae at the base near its origin from the gill arch were degenerated and there were blood clots and mucous cell exudation at some places (Plate 4). Gill rays showed degeneration, necrosis and hyperemic blood vessels. Separation of the lamellar epithelium from the basement membrane was noted in some places of the primary lamellae. Hypertrophy and hyperplasia of the lamellar epithelial cells due to Cd exposure were reported in Zebra fish (Karlsson-Norrgren *et al*. 1985), in stickleback (Oronsaye 1989), and in the freshwater fish *Macropsobrycon uruguayanae* (Randi *et al*. 1996), in *Channa*

punctatus (Gupta and Rajbanshi 1988). Similar structural changes in the gill epithelium of different fish species due to other metal and non-metal toxicants have also been reported, e.g., gold fish (Nelson *et al*. 1999) and *Prochilodus scrofa* (Mazon *et al.* 2002) on exposure to Cu, and *Liza parsia* (Pandey *et al*. 1997) to Pb.

In course of present investigation the gill rays were extensively distended throughout their lengths (Plate 5) on exposure to Cr, and noted oedema at the tips of the primary lamellae (Plate 6). The oedematous separation of gill epithelium from the basement membrane could be due to the increased capillary permeability and/or lowered efficiency of the epithelial cells in maintaining normal water balance (Roberts 1989). At some places of the gill rays tumour like growth with blood clots were visible (Plate 7). The epithelial cells of the primary and secondary lamellae were hypertrophied and hyperplastic. The hypertrophied gill rays were so expanded that the interlamellar spaces increased greatly making the secondary lamellae distinct. The lamellar epithelium got separated in many places from the basal membrane (Plate 5). Blood cell congestion was found in many places of the respiratory epithelium and in the gill ray. Hyperplastic and hypertrophied tissues around the blood vessels were found in the gill arch at the base of the primary lamellae (Plate 8).

Mercury treated gills of *C. batrachus* showed extensive damage in the epithelium of primary and secondary lamellae. The epithelial and mucous cells were highly damaged, became vacuolated, leaving in some places only the pyknotic nuclei. Lamellar epithelium was necrotic at places and the pillar cells were also degenerated with pyknotic nuclei (plates 9, 10). The entire gill lamellae were atrophied. In the present investigation the gill rays were degenerated and shrunken, and blood sinuses in them were also degenerated. Damage of blood vessels and adjacent tissues at the base of the primary lamellae was evident (plate 9). Similar observations were recorded by Khangarot and Somany (1980) and Galat *et al*.(1985) while studying the effect of Hg exposure to *Puntius saphore* and trout respectively. Pandey *et al*. (1996) also observed hypertrophy at initial exposure to Hg at sublethal concentration (0.02 ppm) in *Liza parsia*, fusion of the secondary gill lamellae on day 8 and extensive cellular hyperplasia (complete filling of interlamellar spaces) after prolonged exposure (15 days). The above mentioned works are in conformity with the findings of the present study. However, Skidmore and Tovel (1972) and Roberts (1989) considered hyperplasia as an adaptive mechanism of the fishes for protection of the underlying tissues from irritant.

In the present observation mucous exudation was the general pathological symptom noted in the gills of Cd, Cr and Hg treated fishes. The mucous secretion along with proliferated primary lamellar epidermis could have form a respiratory exchange obstruction on its own (Roberts 1989). Damage of the pillar cell system, congestion of blood and necrosis in the lamellar epithelium were common in the cases of all three heavy metals which corroborated with that of others (Karlsson-Norrgren *et al.* 1985, Pandey 1994, Pandey *et al*. 1996). It may be inferred that this kind of lesion might affect the gill by reducing the supply of blood and might cause respiratory impairment.

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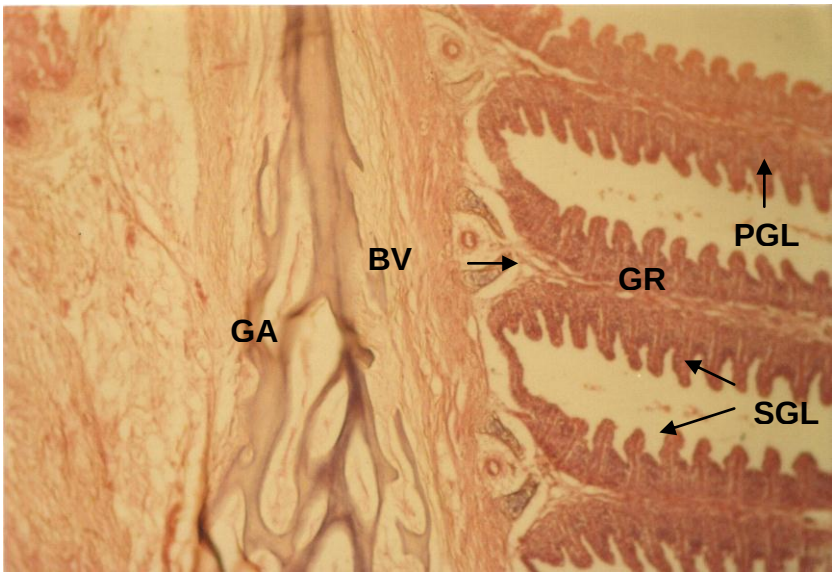

PLATE 1 PHOTOMICROGRAPH OF THE SECTION OF GILL OF CONTROL SPECIMEN OF *CLARIAS BATRACHUS* SHOWING GILL ARCH (GA), PRIMARY GILL LAMELLA (PGL), SECONDARY GILL LAMELLA (SGL), GILL RAY (GR) AND BLOOD VESSEL (BV). H&E 10×10.

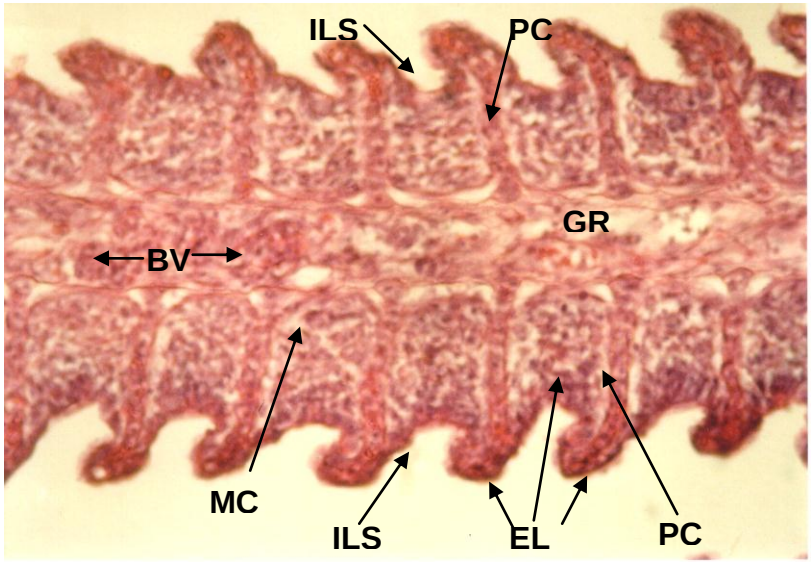

PLATE 2 PHOTOMICROGRAPH OF THE SECTION OF GILL OF CONTROL SPECIMEN OF *CLARIAS BATRACHUS* SHOWING EPITHELIAL LAYER (EL) OF PRIMARY AND SECONDARY GILL LAMELLAE, MUCOUS CELLS (MC), PILLAR CELLS (PC), AND BLOOD VESSELS (BV) WITHIN THE GILL RAY (GR), AND INTERLAMELLAR SPACES (ILS). H&E.10×40.

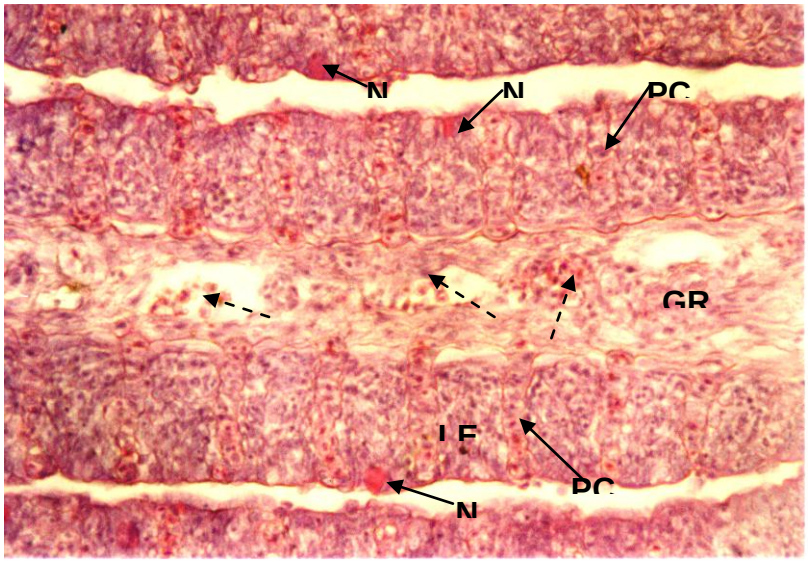

PLATE 3 PHOTOMICROGRAPH OF THE SECTION OF GILL OF CD-EXPOSED *CLARIAS BATRACHUS* SHOWING HYPERPLASTIC AND HYPERTROPHIED LAMELLAR EPITHELIA (LE) ABOLISHING INTERLAMELLAR SPACES, NECROSIS (N) IN LAMELLAR EPITHELIUM, NECROSED PILLAR CELLS (PC), AND CONGESTION OF BLOOD (BROKEN ARROW) IN GILL RAY (GR). H&E 10×40.

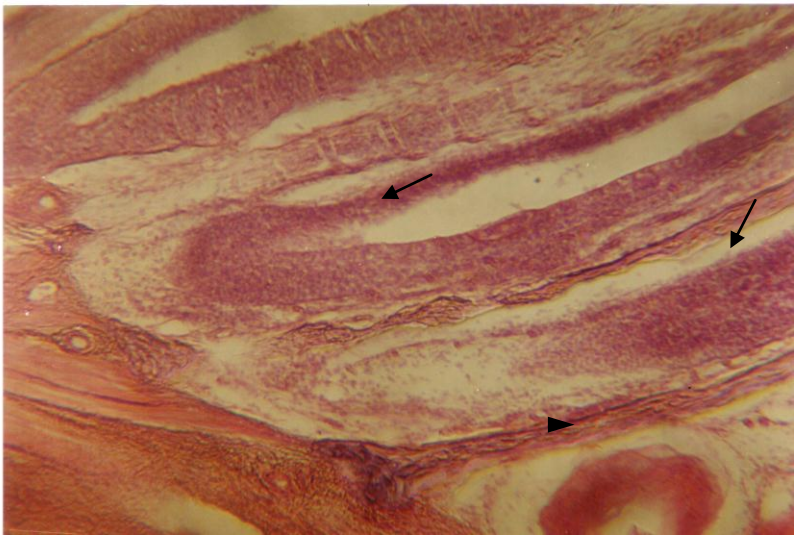

PLATE 4 PHOTOMICROGRAPH OF THE SECTION OF GILL OF CD-EXPOSED *CLARIAS BATRACHUS* SHOWING MUCOUS CELL EXUDATION AT THE BASE OF THE PRIMARY GILL LAMELLAE (ARROW), AND DILATION OF BLOOD VESSEL (ARROW HEAD). H&E 10×40.

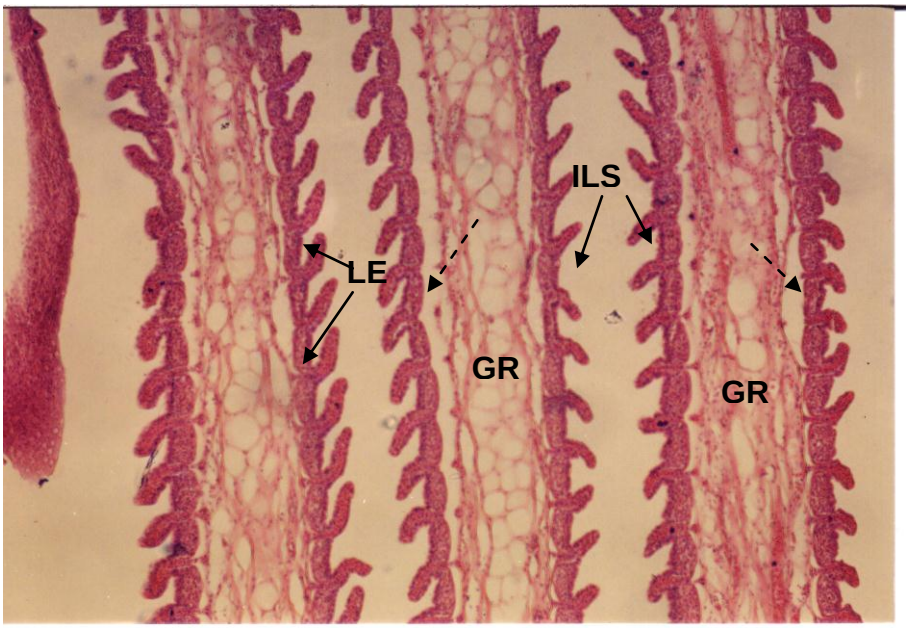

PLATE 5 PHOTOMICROGRAPH OF THE SECTION OF GILL OF CR-EXPOSED *CLARIAS BATRACHUS* SHOWING DISTENDED GILL RAY (GR), HYPERTROPHIED AND HYPERLASTIC EPITHELIA (LE), WIDE INTERLAMELLAR SPACES (ILS), AND SEPARATION (BROKEN ARROW) OF LAMELLAR EPITHELIUM (LE). H&E 10×10.

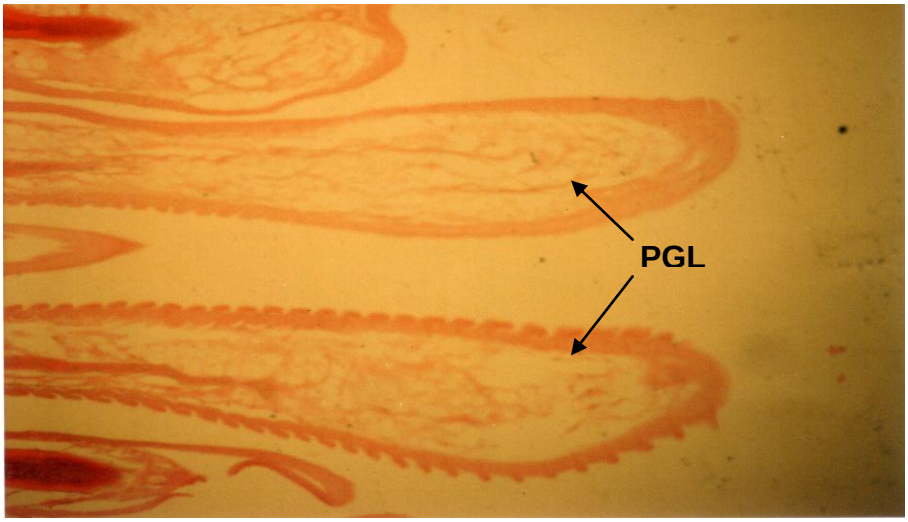

PLATE 6 PHOTOMICROGRAPH OF THE SECTION OF GILL OF CR-EXPOSED *CLARIAS BATRACHUS* SHOWING OEDEMA AT THE TIPS OF PRIMARY GILL LAMELLAE (PGL) H&E 10×10.

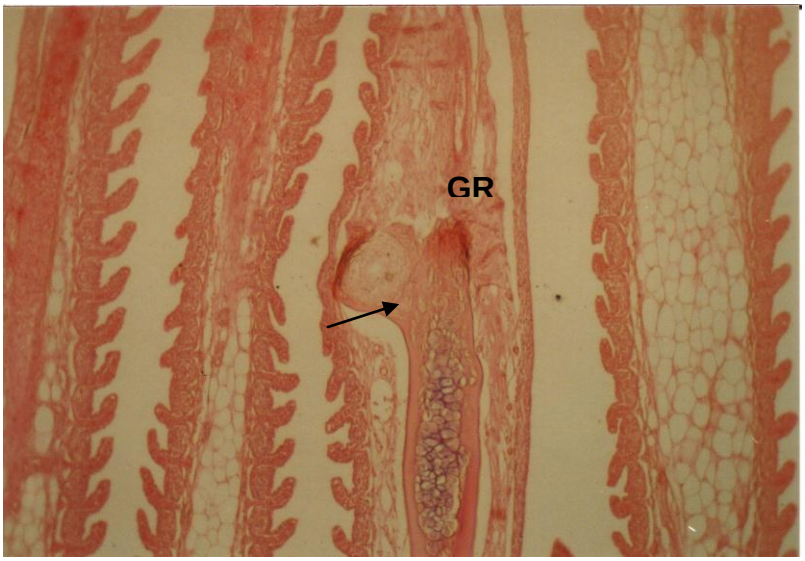

PLATE 7 PHOTOMICROGRAPH OF THE SECTION OF GILL OF CR-EXPOSED *CLARIAS BATRACHUS* SHOWING TUMOUR LIKE GROWTH (ARROW) IN THE GILL RAY (GR). H&E 10×10.

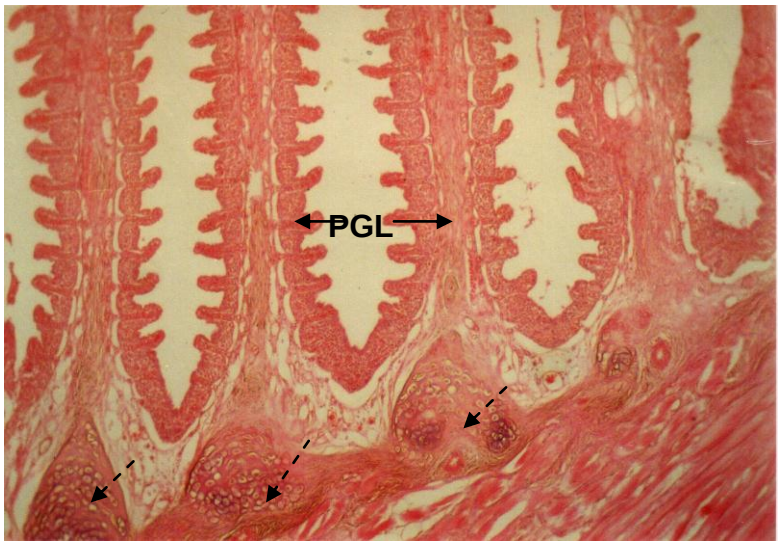

PLATE 8 PHOTOMICROGRAPH OF THE SECTION OF GILL OF CR-EXPOSED *CLARIAS BATRACHUS* SHOWING HYPERPLASIA AND HYPERTROPHY OF THE TISSUE AROUND THE BLOOD VESSELS IN THE GILL ARCH (BROKEN ARROW) AT THE BASE OF THE PRIMARY GILL LAMELLAE (PGL). H&E 10×10.

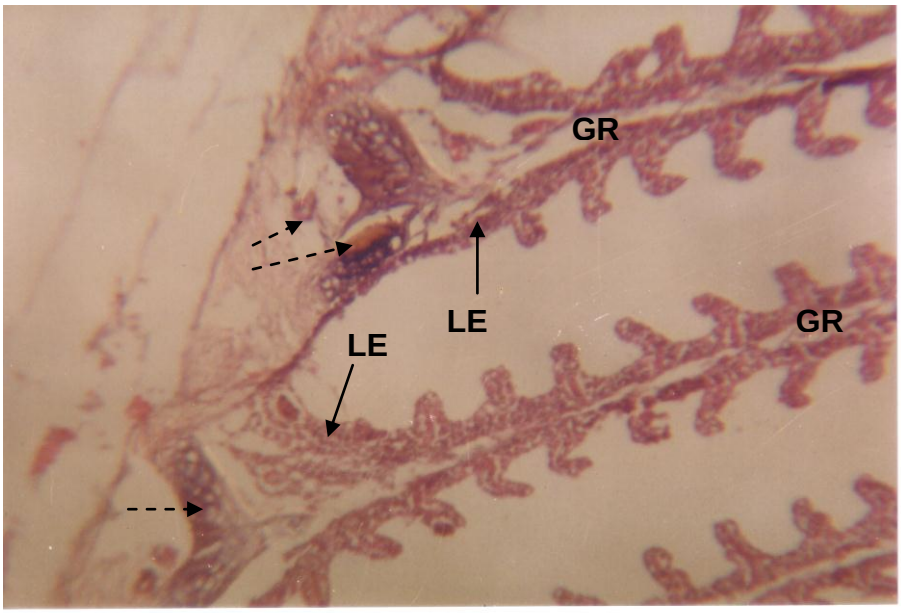

PLATE 9 PHOTOMICROGRAPH OF THE SECTION OF GILL OF HG-EXPOSED *CLARIAS BATRACHUS* SHOWING HIGHLY DAMAGED LAMELLAR EPITHELIA (LE) DEGENERATION AND SHRINKAGE OF GILL RAY (GR), AND DEGENERATION OF BLOOD VESSELS AND ADJACENT TISSUE IN THE GILL ARCH (BROKEN ARROW). H&E 10×10.

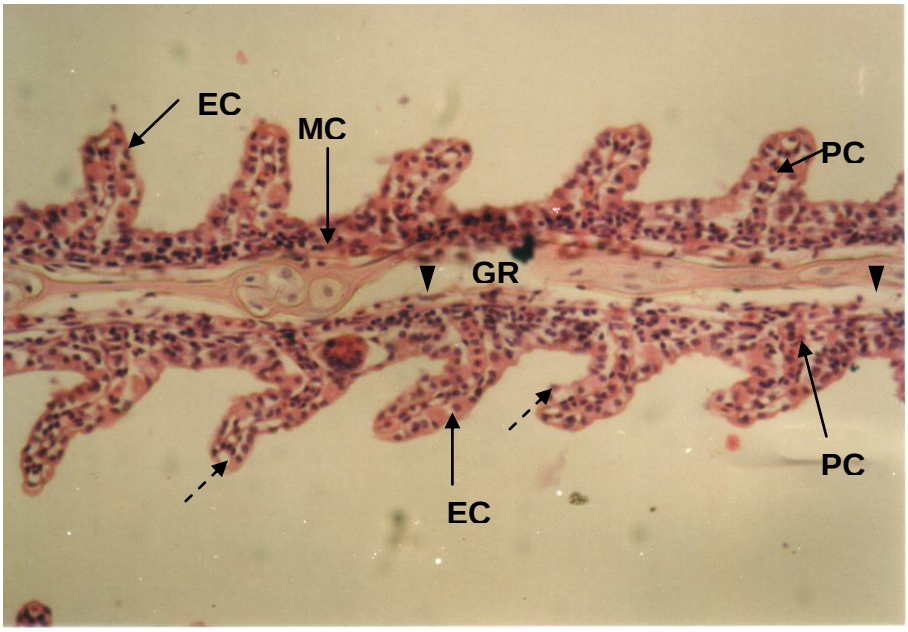

PLATE 10 PHOTOMICROGRAPH OF THE SECTION OF GILL OF HG-EXPOSED *CLARIAS BATRACHUS* SHOWING HIGHLY DEGENERATED EPITHELIAL CELLS (EC) AND MUCOUS CELLS (MC), VACUOLATION OF CELLS (BROKEN ARROW), PYKNOSIS OF NUCLEI, DEGENERATED AND SHRUNKEN GILL RAY (GR), DEGENERATED PILLAR CELLS (PC) WITH PYKNOTIC NUCLEI; AND SEPARATION OF NECROTIC LAMELLAR EPITHELIUM (ARROW HEAD).H&E 10×40.

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