Histopathological responses of the gill of gold fish, *Carassius auratus* to the tryramethylene dye malachite green

J.P.N. Singh †, Ram Yash Yadav and Ashutosh Kumar Singh

1Department of Zoology, R.H.S.P.G. College Singramau, Jaunpur-222175; 2Department of Zoology, S.M.M. Town P.G. College, Ballia

*e-mail: drjpsingh@gmail.com

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Abstract: Thickening of epithelium in gill filament and gill lamellae at different exposures of sub-lethal of the malachite green in *Carassius auratus* has been attributed to cell swelling in short term poisoning. Epithelial thickening is followed by fusion of secondary lamellae, lifting of epithelium from basement membrane of the gill filament and their secondary lamellae and finally dissociation of the epithelium. Fusion of gill lamellae in gills of *Carassius auratus* at sub-lethal concentration at 15d, 30d & 45d malachite green treatment in present study acquires significance. Fusion of gill lamellae is a natural defense mechanism to keep most of lamellar surface away from direct contact of toxic chemicals. However, such adaptations against the disturbed aquatic environment reduces the respiratory surface area and fishes fail to extract adequate oxygen for their metabolic activities and therefore asphyxiate. Also, sudden appearance of mitochondria rich chloride cells, in the gill filament region of *Carassius auratus*, is associated with the defense mechanism against toxicant. Possibly the appearance of chloride cells in the gill filament region at lethal exposure of 3h, 6h, 12h and 1d of the malachite green treatment is to protect the fish from the irritant present in the environment, either by providing energy or by facilitating the tissue to excrete nitrogenous or other toxic wastes by active ion extrusion method. Elongation of blood channel, their denucleation and constriction and finally disintegration in pillar cell system at late exposure hours in present investigation is significant. The normal microanatomy of lamellae with their blood channel, pillar cells and epithelial units get distorted and the blood is haemolysed. The haemolysis of lamellar blood causes hypoxemia and fishes leave their hiding places to float on water surface in search of oxygen. Also the sub-epithelial space between pillar cells and epithelial lining diminishes the effective osmoregulatory surface area in addition to the possibilities of normal gas exchange since the water flow through the above region is reduced. Laying down of a barrier layer of slime over the gill filament and gill lamellae by the sac-like mucous cells is perhaps the immediate response of the gill to the malachite green toxicity, in order to delay the penetration of toxic dye at least for a short period in the initial stage of exposure. Sudden evacuation of the contents of the mucocytes which results in their exhaustion and subsequent elimination causes a sudden decrease in the density of the mucous cells. Increase in protein contents in different cellular components of *Carassius auratus* under influence of malachite green treatment is quite significant. Increased protein synthesis against the toxicity of malachite green leads to the adaptation of organisms to a toxic environment and also induces tolerant stress.

Key words: Gill, *Carassius auratus*, malachite green

Introduction

Malachite green a tryramethylene dye extensively used as biocide in the aquaculture industry world wide, is readily absorbed, distributed and accumulated in different tissues and organs in fish and are feared to have adverse effect on fish, (Srivastava et al. 2004). Fish skin and gill are two main organs which come in direct contact to hazardous chemicals present in water bodies. Many pollutants are known to have detrimental effects on skin and gills. Any change in water quality is rapidly reflected in fish gill structure and function, since gills are continuously exposed to ambient water. Gills are the primary sites of gas exchange, acid-base regulation and ion transfer. That gills represent major sites for respiration, they are always in contact with water, which makes them important targets for water pollutants. Fish gills comprise more than half of the body surface, with an epithelial layer of only a few microns separating the interior of the fish from the external environment. As a result of this, a close association between water and blood occurs so that the gills are strongly affected by environmental contaminants. *Carassius auratus* is a surface feeder, larvivorous, predatory fresh water gold fish. It belongs to the family cyprinidae and order cypriniformes. In this communication we describe the structural organization of gill filament and gill lamellae of *Carassius auratus* and impact of Malachite green on different cellular component of gill filaments and gill lamellae using a battery of histochemical techniques.

Materials and Methods

Live specimens of Goldfish, *Carassius auratus* were purchased from Shivako fish aquarium Jaunpur (U.P.) during the month October, and were acclimatized with laboratory condition for 15 days before experiments were set. They were fed on every alternate days with dried Toyko food granules. Water was renewed after 24 hours leaving no faecal matter or uneaten food. Acclimated fish in batches of ten irrespective of their sex were subjected to 0.03 mg/L of sub lethal exposure. Similar conditions excepting the addition of malachite green were also maintained in control tanks. Feeding was continued throughout the tenure of experiment. Gill was excised from the fish rinsed in physiological saline and were fixed in 10% neutral formalin and aqueous Bouin’s fluid at 3 hour (h), 6h, 12h, 24 h, 2day (d), 3d, 4d of lethal (0.3310 mg/L) and 6h 12d, 1d, 2d, 3d, 4d, 5d, 6d, 7d, 8d, 9d, 10d, 15d, 30d, and 45d of sub-lethal (0.03310 mg/L) exposure of malachite green. Standard methods of dehydration, clearing and embedding were used. Paraffin sectors were cut of 6 µm and subjected to Ehrlich’s haematoxylin eosin (HE) to study general organisation of gills. Sections were subjected to various histochemical tests for carbohydrate, protein, following Lillie (1954), Gurr (1958), Bancroft and Stevens (1982) and Pearse (1985).

Results

Gill filament region: Gill filaments are also known as primary lamellae. In *Carassius auratus* gill filaments are fully developed. Gill filament includes cartilaginous axis cells, basement membrane, acidophil cells, gill filament epithelial cells and mucous cells.

Cartilagenous axis cells: The cartilaginous axis cells form the axis of gill filament region and platform for secondary lamellae. These cells were neglected by previous workers for study, but they show significant changes in present study.
Control: These cells are arranged in two rows lying adjacent to each other. These cells are rectangular containing an elongated nucleus. In HE and PAS their nuclei stain deep blue, the cytoplasm stain blue (Plate-Ia) in VHE their nuclei however, stain moderately black, the cytoplasm stains weakly pink and the cell peripheries stain moderately blue black. The nuclei stain strongly magenta, the cytoplasm stains moderately pink and the cell peripheries stain magenta with PAS, with and without prior diastase/saliva treatment. The nuclei stain moderately greenish blue, the cytoplasm stains moderately magenta with AB/PAS (Plate-Ib). These reactions suggest that the cartilaginous axis cells in general, contain a mixture of neutral and acidic glycoproteins. The cytoplasmic contents of these cells show weak reaction with mercuric bromophenol blue for general protein and acid Solochrome Cyanine R method for basic protein. With other histochemical techniques these cells do not show positive reaction for protein end groups.

Malachite green treatment: Cartilagenous axis cells respond to the malachite green treatment. The reaction in general, is reflected through distinct changes in their shape and size. At 3h, 6h & 12h exposures there no significant change in cartilaginous axis cells. At 1d, these cells are swollen and vacuolization occurs and shrinkage takes place in cytoplasmic content. At 2d, 3d, 4d & 5d exposures cells are degenerated and cytoplasmic content fills the intercellular spaces. At 6d, 7d, 8d, 9d & 10d exposures no significant changes are observed in cartilagenous axis cells. The cartilagenous axis cells in general show significant changes in the histochemical characterization as well as in the intensity of the reaction for protein and carbohydrate moieties. There is slight increase in the intensity of reaction for acid glycoprotein at 3h, 6h, 12h & 1d treatments as compared to moderate reaction in control. At 2d treatments cartilagenous axis cells display moderate reaction with AB at pH 2.5 and pH 1.00 and with AB/PAS sequences these cells show moderate greenish blue and magenta colour which indicate presence of acidic and neutral glycoprotein moieties. At 3d exposures the cartilagenous axis cells stain strong magenta with AB/PAS showing dominance of neutral glycoprotein moieties. At 4d, 5d & 6d exposures the cartilagenous axis cells stain strongly magenta with AB/PAS showing dominance of neutral glycoprotein moieties. At 7d treatments degenerative changes occur so these cells stain moderately magenta colour show the presence of neutral glycoprotein moieties. At 8d and 9d exposures cartilagenous axis cells stain strongly greenish blue with AB/PAS showing presence of acidic glycoprotein moieties. At 10d exposures these cells stain moderately magenta with AB/PAS showing presence of neutral glycoprotein moieties. No characteristic changes occurs in protein concentration of cartilagenous axis cells during treatments but only at 3d these cells show slightly increase in proteinaceous nature with different histochemical techniques.

Basement membrane: Basement membrane is thick non-cellular membrane enveloping the cartilagenous axis cells and it self being an attachment site of the pillar cell system.

Control: In HE and PS basement membrane stains weakly pink showing eosinophilic nature and stain black in VHE preparation. The basement membrane stains moderate pink with PAS with and without prior diastase restored deacetylation (Plate-XIIb). The basement membrane remains unstained with AB at pH 2.5 and pH 1.0, however, show weak magenta colour reaction with AB/PAS. These reactions suggest that basement membrane in general contains neutral glycoprotein (Plate-XIIa) and acid sulphated and non-sulphated glycoproteins are absent. The basement membrane show moderate reaction with mercury bromophenol blue method for general protein, weak reaction with acid solochrome cyanine method for basic protein and ninhydrin Schiff method for protein bound –NH2 groups. With the other histochemical technique basement membrane does not show positive reactions for protein end group.

Malachite green treatment: The basement membrane in general does not show significant changes in histological characterization as well as in the intensity of the reaction for carbohydrate and protein moieties at different duration of lethal and sub-lethal concentration of the malachite green treatment.

Gill filament epithelial cell-Control: In Carassius auratus gill filament epithelial cells are multilayered as compared to gill lamellar epithelial cells (Plate-Ic). These cells lie in close association with the basement membrane on gill filament. These cells are roughly polygonal having distinct nucleus (Plate-Ic). In HE and PS, their nuclei stain moderately blue, the cytoplasm stains weakly pink and the cell peripheries stain blue; however, in VHE their nuclei and the cell peripheries stain moderately black, the cytoplasm stains we Gill filament epithelial cells stain weakly magenta with PAS with and without prior diastase/saliva treatment blocked by prior acetylation and restored by deacetylation. These cells remain unstained with AB at pH 2.5 and pH 1.0, however, stain magenta with AB/PAS. These reactions suggest that gill filament epithelial cells, in general, contain neutral glycoprotein moieties. The cytoplasmic contents of these cells show weak reaction, where as, the nuclei and cell peripheries show moderate reaction with mercuric bromophenol blue method for general protein, acid solochrome cyanine method for basic protein and with ninhydrin Schiff method for protein bound –NH2 groups. With the other histochemical techniques, these cells do not show positive reactions for protein end groups.

Malachite green treatment - Lethal exposure: These cells respond quickly to the malachite green treatment. At 3h exposure due to hyperplasia, proliferation in gill filament epithelial cells is recorded which results in thickening of gill filament epithelium. A few chloride cells also appear at this treatment. At 6h treatment gill filament epithelium becomes more thick due to hyperplastic reaction. Increase in density and dimension of chloride cells is also recorded at this exposure (Plate-IId). At 12h & 1d treatments due to rapid hyperplastic reaction the interlamellar space between two adjacent lamellae is filled by gill lamellar epithelial cells. The chloride cells though small in number as compared to previous treatment but attain larger size. Due to filling of inter-lamellar space the lamellae appear fused. At 2d exposure vacuolization and flattening of gill filament epithelial cells is recorded indicating degenerative changes in these cells. No chloride cell is observed at this exposure. At 3d intoxication heavy degeneration is observed in gill filament epithelial cells characterized by heavy vacuolization, broken and dissolution of cellular membrane and pyknosis of nuclei. After 4d of exposure sloughing of gill filament and gill lamellar epithelial cells was markedly increased leaving only the rows of pillar cells (Plate Ie).

Sublethal exposure: At 3h, 6h and 12h exposures no apparent change is observed in gill filament epithelial cells. At 1d and 2d exposures hyperplastic reaction is observed in gill filament epithelial cells resulting thickening of gill filament epithelium. At 3d exposure gill filament epithelium
becomes more thick due to epithelial hyperplasia and cell hypertrophy. At 4d treatment thickening in gill filament epithelium is more marked as compared to previous treatment. Increase in intercellular spaces and vacuolization is also recorded at this exposure. At 5d & 6d exposures entire interlamellar spaces are filled due to proliferation of gill filament epithelial cells. Increase in intercellular spaces and vacuolization is observed at this treatment. At 7d exposure degenerative changes are at their peak characterized by broken cellular membrane, pyknosis of nuclei, heavy vacuolization and increase in intercellular spaces. At 8d treatment regenerative changes in epithelial cells appear. At 9d exposure entire interlamellar space is filled with hypertrophied gill filament epithelial cells with increased intercellular spaces. At 10d exposure vacuolization and increases in intercellular spaces is noticed in gill filament epithelial cells (Plate IIa). At 15d treatment degenerative changes are more marked with damaged cell membrane, vacuolization and increase in intercellular spaces. At 20d treatment cells membrane become dissoluted. And nuclei appear indistinct. Vacuolization and increase in intercellular spaces are well marked. At 30d and 45d exposures gill filament epithelium is extremely degenerated, quite thin and represented by only few damaged cells between two adjacent lamellae (Plate Iig). At 45d exposure clubbing in gill lamellae is observed at their tips (Plate Iih).

At lethal exposure gill filament epithelial cells show decline in acidic glycoprotein moieties at 3h treatment. At 6h exposure epithelial cells display purple colour reaction with AB/PAS indicating the presence of mixed glycoprotein moieties. At 12h treatment these cells stain greenish blue with AB at pH 2.5 and turquoise blue with AB/PAS suggesting the presence of acidic glycoprotein moieties. At 1d treatment there is shift in staining properties of these cells from acidic glycoprotein to neutral glycoprotein as they stain strongly magenta with AB/PAS. At 2d, 3d & 4d exposures these cells display reactions for neutral glycoprotein moieties. At sub-lethal exposures of 3h, 6h, 12h & 1d treatments epithelial cells show moderate reaction of purple colour with AB/PAS indicating presence of mixed glycoprotein moieties. At 2d, 3d, 4d, 5d & 6d exposures epithelial cells display magenta colour reaction with AB/PAS suggesting the presence of neutral glycoprotein moieties. At 7d, 8d, 9d and subsequent exposures intensity of reaction for acidic glycoprotein moieties increases. The cytoplasmic contents of these cells show weak reactions whereas the nuclei and cell peripheries show moderate reaction with mercury bromophenol blue (Hg BPB) method for general protein, acid solochrome cyanine R method for basic proteins and with Ninhydrin/ Schiff method for protein bound NH₂ groups. With the other histochemical techniques these cells do not show positive reaction for protein end groups. Gill filament epithelial cells display strong reaction for general protein and protein bound –NH₂ groups at different lethal and sub-lethal exposures of malachite green treatment.

**Mucous cells:** In control a very few mucous cells are observed at the surface of gill filament epithelium at inter-lamellar region. These mucous cells stain pink with PAS, greenish blue with AB at pH 1.0 and pH 2.5 and purplish with AB/PAS. These reactions suggest that mucous cells in control fish consist of mixture of neutral and acidic glycoprotein moieties. (Plate IIa). At the tip of gill filament mucous cell are abundantly recorded. **Malachite green treatment - Lethal exposure:** At 3h treatment no increase in density and dimension of mucous cell is observed. Mucous cells are sparsely distributed in the superficial layer of gill filament epithelium at inter lamellar region. These cells stain greenish blue with AB/PAS indicating presence of acidic glycoprotein moieties. At 6h exposure increase in density and dimension of mucous cell is recorded. Mucous cells are located in deeper layer and superficial region of the gill filament epithelium. Some flattened mucous cells are also recorded in lamellar epithelium. These mucous cells stain magenta with AB/PAS suggesting the presence of neutral glycoprotein moieties. At 12h exposure very small mucous cells with acidic glycoprotein moieties are observed. Mucous cells get exhausted due to release of their contents forming slimy layer on the surface of lamellar epithelium. At 1d exposure increase in density and dimension of mucous cells is recorded. These cells stain greenish blue with AB/PAS sequence suggesting the presence of acidic glycoprotein moieties. Mucous cells are discernible at the surface layer of gill filament and lamellae (Plate IIb). At 2d exposure there is decrease in density of mucous cells which stain for acidic glycoprotein moieties. At 3d treatment decline in mucous cell density and dimension is observed. These cells show staining for acidic glycoprotein moieties. These are located in the surface layer of gill filament epithelium. At 4d exposure mucous cells get exhausted due to release of their contents forming slimy coat on lamellar epithelium.

**Sublethal concentration:** No change in mucous cell density and dimension is observed at 3h, 6h and 12h treatments. At 1d exposure increase in density of mucous cell is recorded which stain greenish blue with AB at pH 1.0 and AB at pH 2.5 and AB/PAS suggesting the presence of acidic glycoprotein moieties (Plate IIc). At 2d intoxication mucous cells show increase in density staining for acidic glycoprotein moieties. At 3d exposure mucous cells stain for mixed glycoprotein moieties. At 4d and 5d treatments increase in density and dimension of mucous cell is recorded. These cells stain for acidic glycoprotein moieties (Plate IIe). At 9d and 7d exposures great decline in mucous cell density is noticed which demonstrate staining for acidic glycoprotein moieties. At 8d and 9d exposures again increase in mucous cell density and dimension is recorded. Some mucous cells stain purple and others greenish blue with AB/PAS suggesting presence of mixed population of mucous cells (Plate IIe). At 10d treatment abrupt increase in density of mucous cells is observed which display staining for acidic glycoprotein contents (Plate IIg). At 15d exposure very few mucous cells with smaller dimensions are observed at gill filament and gill lamellae due to liberation of their contents at the surface of gill filament and lamellae forming slimy coat of acidic glycoprotein moieties. At 30d and 45d exposures thick slimy coat of acidic glycoprotein moieties is observed at the surface of gill filament and lamellae. Only few evacuated mucous cells are discernible on the lamellar surface.

**Gill lamellae region:** Gill lamellae of *Carrasius auratus* are long leafy structures found alternately arranged on either side of the cartilaginous axis cells (Plate Ic). Each gill lamella consists of pillar cell system constituted of pillar cells and blood channels enclosed by well defined basement membrane and an outer covering of respiratory epithelial cells. In control mucous cell were absent from gill lamellae region however, they appear on gill lamellae after late exposure to malachite green.

**Pillar Cell System:** Pillar cell system consists of pillar cells alternately arranged along with blood channels enclosed by a thin basement membrane. In HE and PS blood channels appear eosinophilic with small dark blue nucleus however, pillar cells appear deep blue (Plate...
Ic). In VHE preparation pillar cells appear almost black and blood channels pink. Pillar cells stain moderately magenta where as the cytoplasm of blood channels stain purplish and the cell peripheries stain strongly magenta with PAS with and without diastase/saliva treatment blocked by prior acetylation and restored by deacetylation. Pillar cells and blood channels remain unstained with AB at pH 1.0 and pH 2.5 however, they show moderate reaction with AB/PAS. These reactions indicate that pillar cell system mainly consists of mainly neutral glycoproteins moieties. Pillar cells stain strongly bluish where as the cytoplasm of blood channels stains weakly; the cells peripheries and nuclei stain strongly with mercury bromo-phenol blue method for general proteins, acid solochrome cyanine method for basic proteins. With the other histochemical techniques, the pillar cell system do not show positive reactions for protein end groups.

**Malachite Green Treatment - Lethal Exposure:** At 3h exposure pillar cells and blood channels appear vertically compressed. At 6h treatment pillar cells and blood channels show swelling with well dilated nuclei. At 12h and 1d intoxication pillar cell system display constriction. At 2d and 3d treatment blood channels and pillar cells get elongated. Some of blood channels show denucleation. At 4d exposure pillar cells appear naked due to wear and tear and showing sloughing of lamellar epithelial cells (Plate Ie).

**Sub-lethal Exposure:** No change in pillar cell system is observed upto 2d exposure. At 3d exposure swelling in pillar cells and blood channels is observed. At 4d, 5d & 6d exposure flattening in pillar cell system is noticed. At 7d and 8d exposures pillar cells and blood channels display elongation. At 9d and 10d, exposure again swelling in pillar cell system is noticed. At 15d and 20d exposures elongation in pillar cell system is observed. At 30d exposure pillar cells and blood channels appear healthy. At 45d exposure linear arrangement of pillar cells and blood channels is disturbed and some blood channels appear disintegrated. The pillar cell system, in general, do not show significant changes in the histochemical characterization as well as in the intensity
of the reactions for carbohydrate and protein moieties at different durations for carbohydrate and protein moieties at different durations of lethal and sub-lethal exposure of the malachite green treatments.

**Gill Lamellae Epithelial Cells:** Gill lamellae epithelial cells cover the pillar cell system and together forms the respiratory surface area of gill lamellae. These are also called respiratory epithelial cells (Plate lc). These cells are roughly elliptical, arranged in a single layer so as to form the respiratory surface of gill lamellae. These cells are equipped with centrally placed flattened nucleus. These cells stain blue in HE, pink in PS and blue black in VHE (Plate Ic). These cells stain weakly magenta with PAS with and without prior diastase/saliva treatment blocked by prior acetylation and restored by deacetylation. These cells also remain unstained with AB at pH 2.5 and pH 1.0. These reactions suggest that gill lamellae epithelial cells, in general, contain neutral glycoprotein. The cytoplasmic contents of these cells show weak reaction, where as, the nuclei and cell peripheries show moderate reaction with Hg-BPB method for general protein, acid solochrome cyanine method for basic proteins and ninhydrin-Schiffs method for protein bound –NH₂ groups. With the other histochemical techniques, these cells do not show positive reactions for protein end groups.

**Lethal Exposure:** At 3h and 6h exposures proliferation in gill lamellar epithelial cell is recorded (Plate ld). At 12h and 1d exposures due to hyperplastic reaction proliferation occurs in gill lamellar epithelial cells and inter lamellar space between two adjacent lamellae is filled. At 2d exposure upliftment of gill lamellar epithelial cells from pillar cell system results the formation of sub-epithelial space. At 3d treatment the gill lamellar epithelial cells get broken at many places giving entry to toxicant borne water to come in direct to the pillar cell system. After 4d intoxication sloughing of gill lamellar epithelial cells was markedly increased leaving only the rows of pillar cells (Plate le).

**Sub-lethal Exposure:** There is gradual swelling or hypertrophy in gill lamellae epithelial cells from 1d to 15d exposures (Plate lf). At 20d exposure degeneration in gill lamellae epithelial cells is observed indicated by flattening of cells and pyknosis of nuclei. At 30d exposure gill lamellae epithelial cells are extremely degenerated characterized by broken cell membranes, vacuolization in cytoplasm and pyknosis of nuclei (Plate if). At 45d exposure epithelial cells at gill lamellae show hyperplasia resulting the formation of multilayered epithelium around pillar cell system and club shaped deformation at the apical end of lamellae (Plate Ig).
Gill tip region - Mucous cells:
Control: At the tip of gill filament most of the mucous cells on the surface stain for mixed glycoprotein moieties. While mucous cells in the deeper region stain for neutral glycoprotein moieties.

Malachite green treatment: At sublethal exposure from 1d to 7d mucous cells display reaction for acidic glycoprotein moieties. At 8d & 9d treatments these cells show reactions for neutral glycoprotein moieties. In onward treatments again acidic glycoprotein moieties is observed in mucous cells at gill tip region.

Discussion
Exposure to lethal and sublethal concentrations of malachite green had variety of effects on structural organization of gills. Commonly reported effects of toxicants on gills fall into two categories: Lesions and reactions. Lesions include necroses and rupture of respiratory epithelium, which are directed and deleterious effect (Tennmink et al., 1983). Lesions are dose dependent and developed by two mechanisms: autolysis induced by the cell’s own enzymes and rapid lysis caused by direct action of a toxicant (Mallat, 1985). Reactions that are also dose dependent includes epithelial lifting, fusion, hypertrophy, hyperplasia, mucous secretion, vascular stasis, mucous cell proliferation, chloride cell proliferation and leucocyte infiltration (Morgan and Tovel, 1973; Mallat, 1989; Dutta et al., 1996). These are defence responses of the fish.

Thickening of epithelium in gill filament and gill lamellae: Thickening of epithelium in gill filament and gill lamellae at different exposures of lethal and sub-lethal of the malachite green in Carassius auratus in present investigation is significant. Thickening of gill epithelium is also one of the earliest morphological changes that follow exposures to heavy metal salts, detergents and phenols (Baker, 1969; Skidmore, 1970). Epithelial thickening has been attributed to cell swelling in short term poisoning (Brown et al., 1968). Epithelial thickening is followed by fusion of secondary lamellae, lifting of epithelium from basement membrane of the gill filament and their secondary lamellae and finally dissociation of the epithelium (Skidmore, 1970). The most severe gill lesion is swelling or inflammation in many respiratory lamellae. This kind of damage could increase diffusion distance between water and blood thus being responsible for respiratory impairment. The inflammatory reactions perhaps resulted from direct contact with the malachite green. This has been previously reported with regard to detergents and other pollutants (Brown et al., 1968; Skidmore and Towell, 1972; Zaccone et al., 1985).

Fusion of gill lamellae: Fusion of gill lamellae in gills of Carassius auratus at sub-lethal concentration at 15d, 30d & 45d malachite green treatment in present study acquires significance. Fusion of gill lamellae is a natural defense mechanism to keep most of lamellar surface away from direct contact of toxic chemicals. However, such adaptations against the disturbed aquatic environment reduces the respiratory surface area and fishes fail to extract adequate oxygen for their metabolic activities and therefore asphyxiate. Lamellar fusion has also been reported by Ojha (1999) in Gara lamta due to lifting of the epithelium from the basement membrane of the gill filaments and their secondary lamellae and finally, dissociation of the epithelium due to biocidal plant sap. Munshi and Singh (1971) reported fusion of gill lamellae in some fish under the influence of several irritants. They were of the opinion that formation of inter lamellar bridges was due to reassociation or reaggregation of epithelial cells after dissociation. The mechanism by which cell recognizes and aggregates with one another at the cell surface is still unknown. However the term ‘mutual recognition’ in a cell population ‘surface coding’ and ‘preferential affinities’ have been used to explain such mechanism. Munshi and Singh (1992) have observed lamellar fusion in Channa punctatus exposed to low pH. Sirivastava, Singh and Srivastava (2008) also noticed fusion of gill lamellae in Colisa chuna under the influence of potassium dichromate. Singh and Yadav (2010) have also reported fusion of gill lamellae in Anabas testudineus under influence of the herbicide-2,4-Dichlorophenoxyacetic acid.

Proliferation of chloride cells: Sudden apperance of mitochondria rich chloride cells, in the gill filament region of Carassius auratus, is associated with the defence mechanism against toxicant. Possibly the appearance of chloride cells in the gill filament region at lethal exposure of 3h, 6h, 12h and 1d of the malachite green treatment is to protect the fish from the irritant present in the environment, either by providing energy or by facilitating the tissue to excrete nitrogenous or other toxic wastes by active ion extrusion method. The identity of cells responsible for ion transport was suggested by Keys and Willmer (1932) who observed the presence of certain spherical acidophobic granulated cell, the chloride secreting cells in the gill epithelium. Chloride cells are found in good numbers in the gills of many air breathing fishes (Munshi, 1964; Hungnes and Munshi, 1979). In most teleosts the so called chloride cells occur in two readily distinguishable forms, viz. active and inactive. The active chloride cells are rich in mitochondria that are typically small and rounded. Inactive type of chloride cells possess an electron transparent cytoplasm and a large basally located nucleus, both inactive and active type of chloride cells have been reported from the gills of Hoplerythrus a facultative air-breather of Amazon, Monopterus unofficialis, Clarias magur and Channa punctatus (Munshi, 1980). Chloride cells for active ion extrusion were claimed to be present in the gill epithelium of marine teleosts (Smith, 1930; Keys, 1931; Bevelander, 1935; Kroghi, 1937; Liu 1942; Copeland, 1948; Pettengill and Copeland, 1948; Getman, 1960; Morris, 1957; Colombo, 1961; Kessel and Beams, 1960-1962; Phipott, 1962; Phipott and Copeland 1963, 1965). In some fresh water teleosts, Munshi (1964) reported the presence of chloride cells and opined that variation in the hypertencity in fishes necessitate the development of the cells according to the need of the fish. By employing the technique of autoradiography using (H) thymidine, Conte and Lin (1967) and Mackinnon and Enesco (1980) have shown that cell migration rather than cells proliferation characterizes the cell renewal in the gill epithelium of freshwater teleosts. It seems logical that the development of chloride cells in relation to ionic composition of the media is controlled by unknown humeral factor (s) as suggested by Wedelraar-Bonga et.al. (1976) and Wendelaar-Bonga and Balm (1987).

Significant structural changes in the chloride cells occur when the fish is transferred from fresh water to sea water (Kamak et al., 1976; Dunnel and Laurent, 1948; Pettengill and Copeland 1948; Getman, 1950; Threadgold and Houston, 1964; Conte, 1969; Conte and Line 1967; Clivereau 1970; Dunel 1975; Doyle, 1977; Laurent and Dunel, 1980; Pism et al., 1980; Hossler, 1980; Zaccone, 1981). These authors have clearly shown that chloride cells increase in size, number and exhibit darkening of cytoplasm when the fish is transferred from the fresh water to sea water thus supporting the observation in present
investigation that when Carassius auratus is transferred from fresh water to test solution, favouring an increase in ion loss, chloride cells develop on the gill filament-epithelium. Increase in the number of chloride cells has been noted by Baker (1969) in some cases of heavy metal poisoning. Presumably the chloride cells increase in number and size to cope with the influx of malachite green. Mathiesen and Brafield (1973, 1977) observed increase in number of chloride cells in the gills of stickle-back exposed to zinc. It is assumed that chloride cells which appear in the gill arch epithelium arise from existing basal cells. Subsequent decline in chloride cell numbers suggest that, they are the cells from which they arise, were being poisoned by malachite green and their effectiveness in removing the malachite green can be lessened by long term exposure to high concentration. O’ha (1999) observed prominent chloride cells in the interlamellar epithelium with well developed mitochondria in the cytoplasm in the gills of Garra lamta under the influence of Terminalia park sap. Singh and Yadava (2010) have recorded appearance of chloride cells in the gill arch, gill filament and gill lamellae of Anabas testudineus exposed to herbicide 2,4-Dichlorophenoxy acetic acid.

**Distortion of pillar cell system:** Elongation of blood channel, their denucleation and constriction and finally disintegration in pillar cell system at late exposure hours in present investigation is significant. The normal microanatomy of lamellae with their blood channel, pillar cells and epithelial units get distorted and the blood is haemolysed. The haemolysis of lamellar blood causes hypoxemia and fishes leave their hiding places to float on water surface in search of oxygen. Also the influx of malachite green can be lessened by long term exposure to high concentration. Singh et al. (2010) have recorded appearance of chloride cells in the gill arch, gill filament and gill lamellae of Carassius auratus. Singh and Yadava (2010) have recorded appearance of chloride cells in the gill arch, gill filament and gill lamellae of Anabas testudineus exposed to herbicide 2,4-Dichlorophenoxy acetic acid.

**Mucous cell proliferation:** An important mucous cell response to the malachite green treatment, in the gills of Carassius auratus is also associated with the significant changes in the density and dimensions of the mucous cells at different durations of the treatment. It seems that enlargement and increase in number of the mucous cells in gill filament and gill lamellar region at 1d of lethal exposure and 3h, 6h, 12h, 2d, 4d, 5d, 6d, 8d and 10d of sub-lethal exposure malachite green is due to intensive rate of synthesis of secretory contents in the mucous cells rather than their discharge at the surface, resulting in the accumulation of excessive secretory contents with them. The decrease in the dimensions of the mucous cell at 4d of lethal exposure and 6d, 15d, 30d of sub-lethal exposure likewise, may be correlated with the periodic regeneration of mucous cells and the loss of the secretory contents compensated by their production in sufficient quantities. Further, it seems that the mucous cells at these stages have exhausted after intensive secretions on the surface and are at rest. It is suggested that the time interval between the apparent resting phase and the intensive secretory phase of the mucous cells represents the time necessitated for the revival of their capacity for the excessive synthesis of their secretory content. In the gills of Lepomis macrochirus exposed to diazoin, Dutta, et al. (1997) reported that a mucous film was found over the treated filament and lamellae, several droplets of oozing material were seen on the cell surface, giving the epithelial cells a papulate appearance and this may be a way of eliminating absorbed and metabolised toxicants. However, this type of reaction occurred more frequently on gill filaments than on the gill arch, possibly because filaments are more exposed than the arch and also are more vascularized.

Mucous hypersecretion after exposure to different toxicants has been reported frequently (Burton et al., 1972; Dalela et al., 1979; Crespo 1982; Richmonds and Dutta, 1989; Dutta et al., 1993). Mucous restricts the toxicant entry. A wider larger of functions have been attributed to fresh mucous including protection against environmental contaminants and Ultraviolet radiations (Mckin and Lein, 2001, Hakkinen et al., 2003). Laying down a barrier layer of slime over the gill filament and gill lamellae by the sac-like mucous cells is perhaps the immediate response of the gill to the malachite green toxicity, in order to delay the penetration of toxic dye at least for a short period in the initial stage of exposure. Sudden evacuation of the contents of the mucoytes which results in their exhaustion and subsequent elimination causes a sudden decrease in the density of the mucous cells of the gill arch region, gill filament, region and gill lamellae region. Gills and other accessory respiratory organs of fishes exposed to various ambient xenobiotics also exhibit increase in density of mucous cells (Mattey, 1984; Misra et al., 1987; Paul and Banerjee, 1995). However, Linderer and Thulin (1994) did not notice any increased density of mucous cells of the gills following exposure to pulp mill effluents. According to Banerjee and Paul (1993), the primary role of this enhanced mucogenesis is perhaps to protect the individual from the irritant present in the environment. The present investigation reveals that mucous cells of three different regions of gills at different stages of experiment show varying intensities of PAS and AB pH 2.5 positive reactions indicating elaboration of slime mostly containing acidic or mixture of neutral and acid glycoproteins. Singh et al. (2007) have observed thick slimy cost of sulphated acidic glycoprotein moieties on the surface of gill lamellae in Channa striatus exposed to chlorocone.

**Alterations in protein constituents in different cellular components of gills:** Increase in protein contents in different cellular components of Carassius auratus under influence of malachite green treatment is quite significant. Chandravarthy et al. (1987) also found an increase in the total protein concentration of muscle, liver, gills, kidney and brain of the climbing perch Anabas scandens following lead nitrate exposure. Elevation in the ratio of protein contents was also noticed after treatments with other heavy metals (e.g. mercury) (Radi and Matkovics, 1988 and Joshi and Bose, 2002). Increased protein synthesis against the toxicity of heavy metals leads to the adaptation of organisms to a toxic environment and also induces tolerant stress.

**References**


Singh et al.

Effect of tryarylmethane dye malachite green on gold fish


