

CADMIUM INDUCED CHANGES ON THE SECRETION OF BRANCHIAL MUCOUS CELLS OF PEPPERED LOACH, *LEPIDOCEPHALICHTHYS GUNTEA*

Anand Prakash Singh¹, Ashutosh Kumar Singh² and J. P. N. Singh³

^{1,2}Department of Zoology, S.M.M. Town P.G. College, Ballia - 277 001, India

³Department of Zoology, R.H.S.P.G. College, Singramau, Jaunpur - 222 175 India

(Accepted 9 September 2006)

ABSTRACT – Histopathological effects of sublethal dose of heavy metal, cadmium have been studied on the mucous in the gills of peppered loach, *Lepidocephalichthys guntea*. Laying down of slimy coat by the mucous cells of gills following exposure to cadmium chloride has been correlated to delay penetration of the heavy metal at least in initial stage of exposure. Elaboration of glycoproteins by the mucous cells has been associated to trap the heavy metal ions. Mucus secreted by the mucous cells has detoxifying action against ambient toxins.

Key Words : *Lepidocephalichthys guntea*, cadmium, branchial mucous cells.

INTRODUCTION

Toxic metals are added in aquatic system from industrial processes, domestic sewage discharge, street dust, land run off and fossil fuel burning. Traces of heavy metals such as Hg, Cd, Pb, As, Ni, Cr. etc. have been identified as deleterious to aquatic ecosystem in general and fishes in particular.

Modern industrialization using metals for manufacture has however led an increase in heavy metal concentration in the environment (Mathiesen and Barfield 1973; Holcomb, et. al. 1987; Khalaf *et al*, 1985; Heath, 1987) and increase in the concentration of any of these hazards also be altering the physiological function in cells of the animal body (Heath, 1987; Flos *et al*, 1987)

Cadmium is extremely toxic and signs of cadmium poisoning in fish include impaired gills function (Calabrese *et al*, 1975; Majewski and Giles, 1981) hematological changes (Lareson, 1975; Johannson, Sjobeck and Larsson, 1978) and disturbances in osmotic-ionic balance and carbohydrate metabolism (Larsson, 1975; MC carty and Houston 1976; Larsson *et al*, 1976; Larsson and Houx, 1982).

MATERIALS AND METHODS

The experimental *Lepidocephalichthys guntea* were collected from local ponds at Ballia, stored in large plastic tanks containing 10 litre of tap water for two months for acclimation in the laboratory condition. They were fed on every alternate day with minced goat liver.

Water was renewed after 24 hours leaving no faecal matter or uneaten food. Acclimated fish in batches of ten irrespective of their sex of 8±2 cm standard body length were subject to 38.87 mg/L of cadmium chloride (10%

of the 96 h LC₅₀ value 388.7 mg/L). Similar conditions excepting the addition of the cadmium salt were maintained in the control tanks. Feeding was continued throughout the tenure of experiment.

Small fragments of gills were excised from fish in experimental as well as control tanks after 3h, 6h and from 1d to 10 d of sublethal exposures and fixed in 10% neutral formalin and aqueous Bouin's fluid. Standard methods of dehydration, clearing and embedding were used. Paraffin sections were cut at 5mm and sections were stained with Schiff without oxidation for demonstration of free aldehydes (Pearse 1968). Periodic acid Schiff (PAS) method for polysaccharides (MC Manus, 1946) diastase treatment followed by toluidine blue to distinguish mucopolysaccharides (=glycoprotein) and Clamp, 1978) from glycogen (Lillie and Campbell 1947). Alcian blue (AB) at pH 2.5 method for sulphated mucopolysaccharides. Combined AB at pH 2.5 and AB at pH 1.0 (PAS technique (Mowry 1963; Mowry and Henson 1967; Johanner and Klesson, 1984) were employed. With these techniques mucopolysaccharides stain magenta, mucopolysaccharide stain blue or greenish blue, mixed (neutral and acid) mucopolysaccharides stain purple or violet.

RESULTS

A complete gill consists of a gill arch. Each arch consists of gill rakers towards inside and gill filament towards outside. Each gill filament of *Lepidocephalichthys guntea* possesses four pairs of gills. Each gill filament consists of secondary lamellae arranged on both sides alternately.

Gill Arch Region

Control :

The mucous cells are arranged in three rows. The mucous cells in the upper layers opening to the surface are flask shaped and stain strongly purplish with AB/PAS suggesting the presence of mixture of acidic and neutral glycoprotein moieties. The mucous cells just under lying flask shaped mucous cells are oval or rounded and show affinity for acidic glycoproteins as indicated by strong greenish blue reaction with AB/PAS. The mucous cells occupying lower layers are rounded or oval and stain strongly magenta with AB/PAS suggesting presence of neutral glycoprotein contents. (Plate Ia)

Heavy metal treatment

Sub-lethal exposure :

At 3h, all the mucous cells having flask shaped appearance with acidic glycoprotein contents are observed crowded in the surface layers (Plate Ib).

At 6h, treatment, there is decline in the mucous cells density. The mucous cells get elongated and stained for acidic glycoprotein.

At 12 h, decline in mucous cell density is observed. The mucous cells get more elongated. The intensity for glycoprotein contents is also reduced. The basal part of the mucous cells stain purplish while apical stain greenish blue with AB/PAS. These reactions suggest that apical part consists of acidic glycoprotein while basal part has mixed glycoprotein.

At 1 d, increase in mucous cells density is recorded. Most of the mucous cells are rounded, swollen and distributed throughout the gill epithelium. They stain for acidic glycoproteins.

At 3d, mucous cells with smaller size having neutral glycoprotein contents appear throughout the gill arch epithelium.

At 4d, roughly flask shaped smaller mucous cells are observed in surface layers. Some mucous cells stain for acidic glycoproteins while others for mixture of acidic and neutral glycoprotein (Plate Ic). The secretion of these cells form a slimy coat over the surface.

At 5d, there is increase in dimension of mucous cells which are flask shaped and arranged in surface layers having strong glycoprotein contents.

At 6d, most the mucous cells acquire rounded shape. Some stain for neutral glycoprotein contents while others for mixture of acidic and neutral glycoprotein.

At 7d, mucous cells become voluminous and mostly stain for acidic glycoproteins.

At 8d, decrease in the number of mucous is recorded. Some mucous cells stain for acidic glycoproteins while others for mixture of acidic and neutral glycoproteins.

At 9d, tremendous increases in mucous cells density is observed while stain for acidic glycoprotein.

At 10d, decline in mucous cells density is recorded. The cells also show decrease in the intensity for glycoprotein contents.

Gill filament and Gill lamellae

Control :

In normal mucous cells are interspersed in the superficial layer of epithelial cells to gill filament region (Plate). They are rounded, oval, flattened or irregular in outline. These cells stain greenish blue with AB at pH 1.0 and pH 2.5 and purplish with AB/PAS suggesting the presence of mixture of acidic and neutral glycoprotein (Plate-Id)

Heavy metal treatment

At 3h, exposure these cells show increase in dimension and stain of acidic glycoprotein (Plate).

At 6h, 12h and 1d, treatment decline in density and volume of these cells is observed and these cells stain for acidic glycoproteins.

From 2d to 5d treatment there is no apparent change in numbers and volume of mucous cells. These cells stain for neutral glycoprotein as indicated by magenta reaction with AB/PAS.

At 6d, increase in density and dimension of mucous cell is observed. The mucous cells in the surface layer stain magenta and the mucous cells in the deeper layer stain purple colour. These reactions suggest that surface layers mucous cells contain neutral glycoprotein moieties while deeper layer mucous cells have mixture of acidic and neutral glycoprotein contents (Plate-Ie).

At 7d, the mucous cells appear in surface layer and they stain greenish blue with AB/PAS suggest the presence of only acidic glycoprotein moieties. No distinct mucous cells appear on gill lamellae.

At 8d, 9d and 10 d a slimy coat of acidic glycoproteins is observed on surface layers of gill lamellae (Plate-Ie).

DISCUSSION

Increased density and dimension of mucous cells are adaptation noticed in the gills of *Lepidocephalichthys guntea* exposed to cadmium. Increased density and dimension of mucous cells are related to enhanced mucous secretion which is an in built defence mechanism of fishes against a disturbed aquatic environment.

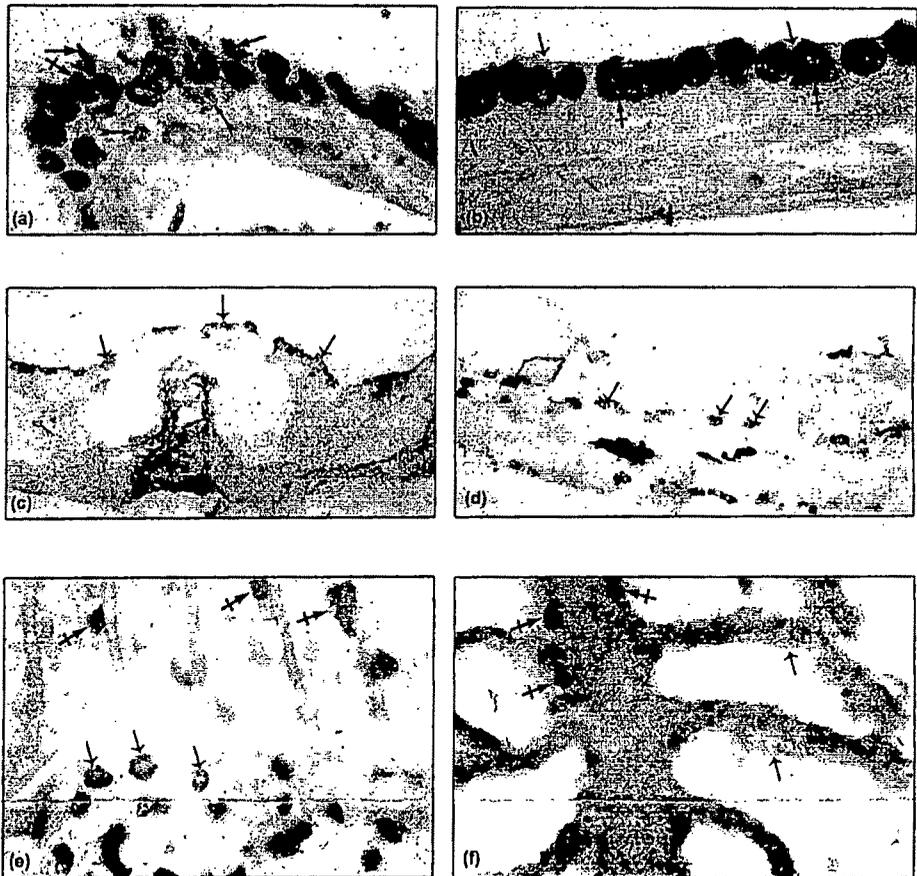


Plate I (a - f): Photomicrographs of the transverse section of gill arch, gill filament and gill lamellae region of *Lepidocephalichthys guntea* at control and at different durations of Cadmium chloride.

- (a): Showing flask shaped mucous cells in the surface layer staining purple in original (arrow), mucous cells in the outer middle layer staining greenish blue in original (barred arrow) and mucous cells in the inner middle layer staining magenta in original (winged arrow) [AB/PAS; Normal x1000].
- (b): Showing the crowding of flask shaped mucous cells in the surface (arrow). Note greenish blue reaction in original in these cells (barred arrow) [AB/PAS; 3h x1000].
- (c): Showing the formation of slimy coat (arrow) by the mucous cells in surface layer [AB; 4d x600].
- (d): Showing mucous cells staining greenish blue in original (arrow) in interlamellar region [AB; control x600].
- (e): Showing abundance of mucous cells staining purplish in original (arrow) in the gill filament epithelium and mucous cells, staining magenta in original (barred arrow) in gill lamellae region [AB/PAS; 6d x1000].
- (f): Showing slimy coat staining greenish blue in original (arrow) over the gill lamellae and rounded mucous cells staining purple colour in original (barred arrow) in the inter lamellar region [AB/PAS; 10d x1000].

cells of the gills following exposure to sub lethal concentration of $CdCl_2$ solution might delay the penetration to toxic heavy metal salt at least in the initial stages of exposure.

Several other ambient Xenobiotics also exert similar effect by activating the epithelial mucous cells of the gills and air sac (Matey, 1984; Misra *et al*, 1987; Wise *et al*, 1987; Paul and Banerjee, 1995, 1996a). Lindesjoo and Thulin (1994) on the other hand, did not notice any

increase in the density of mucous cells of the following exposure to pulp mill effluents. According to Baneerjee and Paul (1993) the primary role of enhance mucogenesis is perhaps to protect the individual from the irritant present in the environment.

The present investigation reveals that at most exposure period the mucous cells display elaborate slime containing mostly acidic or mixture of neutral and acidic glycoproteins. The ability of these glycoproteins to trap heavy metal ions is well documented (Coo

al, 1972; Varanasi and Markay, 1977; Lock and Van overbecke, 1981).

While studying the protective role of fish mucous against hexavalent chromium pollution, Arillo and Melodia 1990; suggested that some components of mucous, probably the protein bound sulphhydryl groups, may have a detoxifying function against the ambient toxins. These SH groups of mucous seems to bind with the toxicant and play a fundamental role in their reduction mechanism, specially for occasional and short term exposure.

The hygroscopic property of the mucous protect the delicate branchial units from desiccation when the fish is temporarily out of water. On certain occasions mucous with in micropockets on the lamellae epithelium also traps water molecules and helps respiration during the semi terrestrial state (Ojha and Hughes, 1988).

REFERENCES

- Arillo A and Melodia (1990) Protective effects of fishes mucous against chromium (VI) Pollution. *Chemosphere* 20, 373-402.
- Banerjee T K and Paul V I (1993) Estimation of acute toxicity of ammonium sulphate to the freshwater cat fish *Heteropneustes fossilis*. II. A histopathological analysis of the epidermis. *Biomed Environ. Sci.* 6, 45-48.
- Calabrese A, Thurbery F P, Dawson M A and Wenzloft D R (1975) Sublethal physiological stress induced by cadmium and mercury in winter flounder *Pseudopleuronectes americanus*. In: *Sublethal effect toxic chemicals on aquatic animal* (ed. Koeman III strick JTTWA) Elsevres Amsterdam.
- Coombs T L, Fletcher T C and White A (1972) Interaction of metal ions with mucous from the plaice *Pleuronectes platessa* (L). *Biochem J.* 128-129.
- Flos R, Riva M C and Balasch J (1983) Chromium and Potassium accumulation influenced by body weight in gold fish *Carassius auratus*. *Bull. Environ. Contam. Toxicol.* 30, 331-336.
- Heath A G (1987) *Water pollution and fish physiology*. C.R.C. Press Boca Ratan Florida, P. 2450.
- Holcobe G W, Benoit D A and Leonard E N (1979) Longterm effects of zinc exposure on brook trout *Salvelinus fontinalis*. *Trans. Am. Fish. Soc.* 108, 76-87.
- Johanner M L and Klessen C (1984) Alcian blue/PAS or PAS. Alcian blue? Remarks on a classical technic used in carbohydrate histochemistry. *Histochemistry* 80, 129-132.
- Johansson, Sjobeck M L and Larsson A (1978) The effect of cadmium or the hematology and on the activity of delta-aminolevulinic acid dehydrates (ALA-D) in blood and hematopoietic tissues of the flounder. *Pleuronectes flesus*. L. *Environm. Res.* 17, 191-204.
- Larsson A and Haux C (1982) Altered carbohydrate metabolism in fish exposed to sublethal levels of cadmium. *J. Environ. Biol.* 3, 71-81.
- Larsson A, Bengisson B E and Svanberg O (1976) Some hematological and biochemical effect of cadmium in fish. In: *Effects of pollutants on aquatic organisms combridge* (eds. Lock. Wood APM), University Press, London, pp35-47.
- Lilie R D and Greco J (1947) Malt diastase and ptyalin in place of saliva in the identification of glycogen. *Stain Tech.* 22, 67-70.
- Lindesjoo E Thulin (1994) The histopathology of skin and gills of fish in pulp mill effluent. *Dis. Aquat. org.* 18, 81-93.
- Lock RAC and Van Overbecke A P (1981) Effects of mercuric chloride and methylmercuric chloride on mucous secretion in rainbow trout *Salmo gairdneri* (Richardson). *Comp. Biochem. Physiol.* 69, 67-73.
- Matthiessen P and Brafield A E (1973) The effect of dissolved zinc on the gills of the streckleback *Gasterosteus aculeatus* (L). *J. Fish Biol.* 5, 607-613.
- Maey V E (1984) Comparative analysis of the gill epithelium. Ultrastructure in the perch *Perca pluvialis* from basins with different composition. *Tritologiya* 768-772.
- M C Manus J F A (1946) Histolical demonstration of mucin after Periodic acid. *Nature* 158, 202.
- Mc Carty L S and Houston A S (1976) Effects of exposure to sublethal levels of cadmium upon water electrolyte status in the gold fish *Carassius auratus*. *J. Fish Biol.* 8, 11-19.
- Mc Kone C E, Young R G, Bache C A and Lisk D J (1971) Rapid uptake of mercuric ion by gold fish. *Eviron. Sci. Technol.* 5, 1138-1139.
- Misra V, Chawla G, Kumar V, Lal H and Viswanthan P N (1987) Effect of linear alkylbenzene sulphonate in skin of fish finger *Cirrhinus mrigala*. Observation with (SEM). *Exotoxicol. Environ. safety* B:164-168.
- Mowry R W (1956) Alcian blue technique for the histochemical study of acidic carbohydrates. *J. Histochem. Cytochem. A:* 407.
- Mowry R W (1963) The special value of method that color both acidic and vicinal hydroxyl groups in the histochemical study of mucine with revised direction for the colloidal. Iron
- Ojha J and Hughe G M (1988) SEM study of the gills of a fresh water cat fish *Rita rita*. *Jpn. J. Ichthyol* 35, 56-67.
- Pearse A G (1968) *Histochemistry. Theoretical and applied*. Vol.2. Analytical technology. London Churchill, Living stone.
- Pual V I and Banerjee T K (1995) Acute toxicity of ammonium sulphate to the air breathing organ of the line fish *Heteropneustes fossilis* (Bloch). *Curr. Sci.* 68, 845-849.
- Paul V I and Banerjee T K (1996) Analysis of ammonium sulphate toxicity in the cat fish *Heteropneustes fossilis* using mucocyte indexing. *Pol. Arch Hydrobiol* (in press).
- Reid L and Damp J R (1978) The biochemical and histochemical nomenclature of mucous. *Br. Med. Bull.* 38, 5-8.
- Spicer S S and Henson J G (1967) Methods for localizing mucosubstances in epithelium and connective tissues. *Achievem Exp. Path.* 2, 78-112.
- Steedman H E (1950) Alcian blue 8. GS: A new stain for mucin. *Quart. J. Micros. Sci.* 91, 477-479.
- Varanasi V and Markey D (1977) Uptake and release of lead and cadmium in skin and mucus of coho salmon (oncorhynchus kisutch). *Comp. Biochem. Physiol.* 60C, 187-191.
- Wise M L, Stiebel C L and Grizzp J M (1987) Acute toxicity of nitrofurazone to channel catfish *Ictalurus punctatus* and gold fish *Carassius auratus*. *Bull. Environ. Contam. Toxicol.* 38, 42-46.

IMPACT OF MALACHITE GREEN ON THE CHLORIDE CELLS IN THE GILLS OF CLIMBING PERCH, *ANABAS TESTUDINEUS*

Ashutosh Kumar Singh, Anand Prakash Singh and J. P. N. Singh*

Department of Zoology, S. M. M. Town (P.G.) College, Ballia - 277 01, India

*Department of Zoology, R. H. S. (P. G.) College, Singramau, Jaunpur - 222 175, India

(Accepted 15 September 2006)

ABSTRACT - Gill of *Anabas testudineus* exhibited change in density and dimension of chloride cells at different duration of malachite green treatment. Appearance of mitochondria rich chloride cells at 4 day of malachite green treatment and increase in density and dimension at subsequent treatments has been associated to protect the fish from the dye present in the environment either by providing energy or by facilitating the tissue to excrete toxic wastes by active ion extrusion.

Key Words : Malachite green, *Anabas testudineus*, Chloride cells.

INTRODUCTION

The main feature of the gill epithelium is presence of chloride secreting cells. Key and Willmer (1932) observed chloride secreting cells in the gills of eel fishes. Copeland (1948) made cytological study of chloride cells in the gills of *Fandulus neteroclitus*. The first electron microscopic studies were done by Kessel and Beams (1962), Doyle (1960) and Philpott (1962). Since then numerous papers have been devoted to the fascinating subject. Chloride cells have been observed in good number in the gills of many air breathing fishes (Munshi, 1964; Hughes and Munshi, 1973, 1979). Effect of different pollutant to the chloride cells is sporadic and scanty. Baker (1969) observed effect of copper poisoning on the chloride cells in the gills of *Pseudopleuronectes americanus*. Crespo (1982) observed effect of zinc sulphate on chloride cells in the gills of dog fish *Scyliorhinus canicula*. Oronsaye &

Brafield (1984) studied effect of cadmium on the chloride cells of the gills of the stickleback *Gasterosteus aculeatus*.

The present study aims to determine impact of malachite green a triaryl methane dye, widely used as biocide in aquaculture on chloride cells in the gills of climbing perch *Anabas testudineus*.

MATERIALS AND METHODS

Live specimens of *Anabas testudineus* (length 9.0 ± 10 cm, weight 18.0 ± 5.0 g) were collected from Suraha tal of Ballia. Healthy fishes were acclimatized to laboratory condition for 15 days before experiments were started. The feeding was stopped 24h before the experiment begin and the fishes were not fed throughout the experiments. Fishes were treated with test solution of malachite green. The 96 hrs LC_{50} was calculated by Spearman-Kärber method (Byron and Brown, 1970) and was found to be 1.45 mg/L. Fishes were exposed to 1.45 mg/L of the dye and the gills were selected



for this study.

Fishes kept under control and experimental conditions were cold anesthetized following Mittal and Whitear (1978) at ½ h, 6h, 12h and from 1d to 10d intervals. The gill pieces were excised, rinsed in physiological saline and were fixed in aqueous Bouin's fluid and 10% neutral formalin. Paraffin section were cut at 5µm and were stained with Ehrlich's haematoxylin/Eosin (H/E) to study general organization of gill and stained with Fleming's (Gateby and Beams, 1950) and Heidenhain's iron haematoxyline methods (Claydon, 1953) to localize chloride cells.

OBSERVATION

Control

The chloride cells could not be located in the gills of untreated *Anabas testudineus* (Plate-Ia).

Malachite green treatments

From ½h to 3 day treatments chloride cells were not observed in gill epithelium.

These cells were observed at 4d treatment in interlamellar region where they were irregularly spaced or form clusters of cells in intercellular epithelium (Plate-Ib).

At 5d, exposure significant increase in the number of chloride cells were observed which spread in the epithelium surrounding gill lamellae (i.e. secondary epithelium).

At 6d and 7 d exposure increase in dimension of these cells was noticed (Plate-Ic). At 8d and 9d treatment decline in chloride cells density was observed which disappear all together at 10d treatments.

Chloride cells are almost spherical with slightly eosinophilic cytoplasm which appear to be homogenous or finely granular in H/E.

The nucleus in basophilic and occupies central position in the cells (Plate I b, c).

The cytoplasmic contents of these cells are packed with numerous spherical mitochondria which are stained black in Flemming's (without acetic acid) method and Heidenhain's iron haematoxylin methods.

DISCUSSION

Appearance of mitochondria rich chloride cells in the gill epithelium of *Anabas testudineus* is associated with the defence mechanism against the toxicant. Possibly the appearance of chloride cells in the gills epithelium at 4d, 5d, 6d, and 7d, 8d, 9d of the dye 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 role of chloride cell in excretion and neutralization of toxicants has also been studied (Karnaky, 1980, Crespo *et al.*, 1981, Oronesay and Brafield, 1984). The chloride cells proliferation may occur with concomitant excretion of the toxicants (Dutta *et al.*, 1996). According to Dutta *et al.* (1996), following 48 h of exposure of *H. fossilis* to melathion the chloride cells become swollen and traversed to whole lameller epithelia and were in direct contact with the lymphoid space and exterior to the epithelium. They proposed that the chloride cells play a role in the excretion of circulating secondary metabolites generated from the pesticide and some accumulated ions.

Ojha (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* bark sap and suggested that the mitochondria in chloride cells is to provide

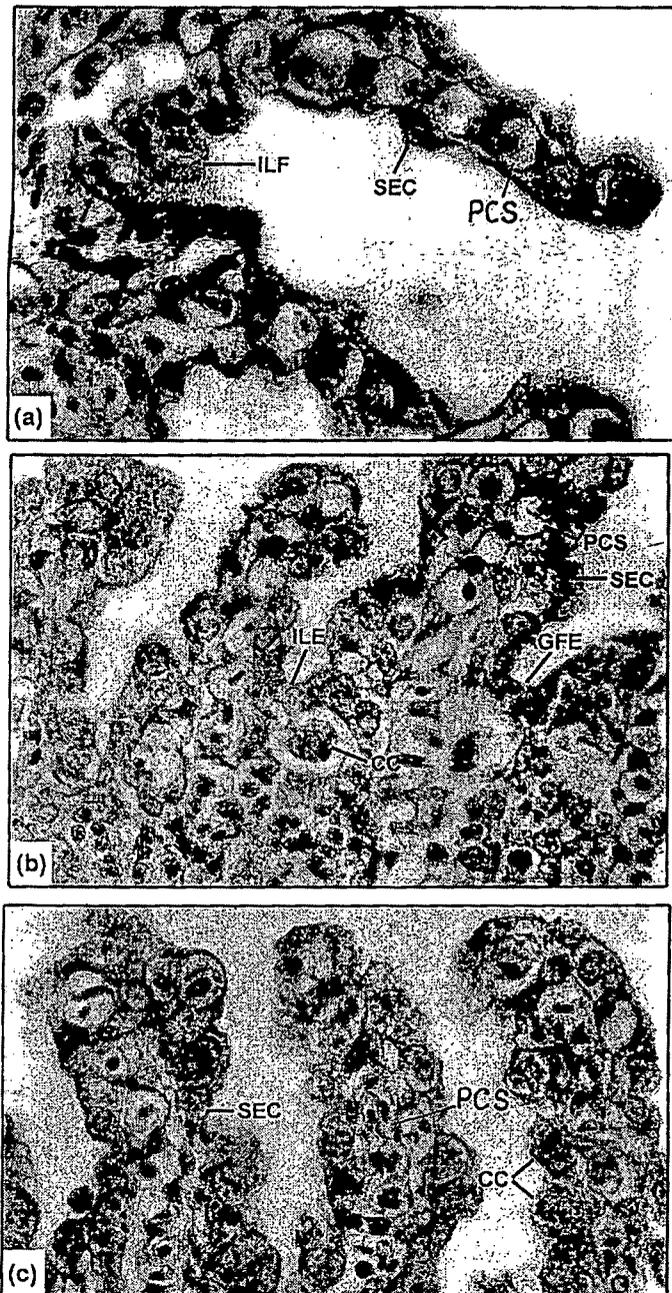


Plate I (a-c) : Photomicrographs of the transverse section of gill filament and gill lamellar region of *Anabas testudineus* showing distribution of chloride cells at control and at 4d and 6d of malachite green treatment.

(a) : Showing pillar cell system (PCS) in lamellae surrounded of secondary epithelial cells (SEC). Note absence of chloride cells in interlamellar epithelium (ILE)[Control, HE x 1000].

(b) : Showing presence of chloride cells (CC) in interlamellar epithelium [4d, HE x 1000].

(c) : Showing abundance of chloride cells in secondary epithelial cells [6d, HE x 1000].

energy for ionic regulation in disturbed aquatic environment.

Chloride cells for active ion extrusion were claimed to be present in the gill epithelium of marine teleosts (Vickers, 1961; Philpott and Copeland, 1963, 1965). In some fresh water teleosts, Munshi (1964) reported the presence of chloride cells and opined that variation in the hypertonicity in fishes necessitate that development of the cells according to the need of the fish. By employing the technique of radiography using (H) thymidine, Conte and Lin (1967) and Mackinnon and Enesco (1980) have shown that cell migration rather than cell proliferation characterizes the cell renewal in the gill epithelium of fresh water teleosts. It seems logical that the development of chloride cells in relation to ionic composition of the media is controlled by unknown humoral factor (S) as suggested by Wendelaar-Bonga *et al* (1976).

Significant structural changes in the chloride cells occur when the fish is transferred from fresh water to sea water (Karnaky *et al*, 1976; Dunel and Laurent, 1973; Dunel, 1975) and vice versa (Dunel, 1975; Doyle, 1977; Laurent and Dunel, 1980; Pizam *et al*, 1980; Hossler, 1980; Zaccone, 1981). These authors have clearly show that chloride cells increases in size number and exhibit darkening of cytoplasm when the fish is transferred from the fresh water to sea water. Thus when *Anabas testudineus* is transferred from fresh water to test solution favouring an increased in ion loss chloride cells develop in the gill epithelium and there is significant increase in the density and dimension of chloride cells.

Presumably the chloride cells increase in number and size to cope with the influx of

malachite green. Matthiessen and Brafield (1973, 1977) observed increase in number of chloride cells in gill of stickle back exposed to zinc.

REFERENCES

- Baker J T P (1969) Histological and electron microscopical observation on copper poisoning in the winter flounder (*Pseudopleuronectes americanus*). *J. Fish Res. Bd. Can.* **26**, 2785-2793.
- Byron W and Brown J R (1970) Quantal-response assay. In: *Statistics in Endocrinology* (eds: Mc Arthur and J W Colton. T) MIT Press Cambridge, MAI London.
- Clayden E C (1953) *Practical section cutting and staining*. J. A. Churchill Ltd. London.
- Conte F P and Lin D (1967) Kinetics of cellular morphogenesis in gill epithelium during sea water adaptation on *Oncorhynchus* (Klalb) *Comp. Biochem. Physiol.* **23**, 945-957.
- Copeland D E (1948) The cytological basis of chloride transfer in the gills of *Fundulus heteroclitus*. *J. Morph.* **82**, 201-227.
- Crespo S (1982) Surface morphology of dog fish (*Scyliorhinus canicula*) gill epithelium and surface morphological changes following treatment with zinc sulphate : a (SEM) study. *Mar. Biol.* **67**, 159-166.
- Crespo S, Soriano E, Sampera C and Balasch J (1981) Zinc and copper distribution excretory organs of the dog fish *Scyliorhinus canicula* and chloride cells response following treatment with zinc sulphate. *Mar. Biol.* **67**, 117-123.
- Doyle W L (1960) The principal cells of the salt glands of marine birds. *Exp. Cell. Res.* **21**, 386-393.
- Doyle W L (1977) Cytological changes in the chloride cells following altered ionic media. *J. Exp. Zool.* **199**, 427-434.
- Dunel S E (1975) Contribution a l'etude structural et ultrastructural de la pseudobranchie et de son innervation chez les Teleosteens. *Ph.D. Thesis*, University of Strasbourg.
- Dutta H M, Munshi J S D, Roy P K, Singh N K, Adhikari S and Killius J (1996) Ultrastructural changes in the respiratory lamellae of the cat fish, *Heteropneustes fossilis*, after sub-lethal

- exposure to malathion. *Environ Pollut.* 9, 329-341.
- Gatenby J B and Beams H W (1950) The microtometist's vade-mecum (Bolles Lee). A hand book of the methods of animal and plant microscopic technique. J. and A. Churchill Ltd. London.
- Hossler F E (1980) The gill arch of mullet, *Mugil uphalus* III Rate of response to the salinity changes. *Am. J. Physiol.* 238, 160-164.
- Hughes G M and Munshi J S D (1973) Fine structure of respiratory organs of the climbing Perch *Anabas testudineus*, Pisces: Anabantidae. *J. Zool. London* 170, 201-225.
- Hughes G H and Munshi J S D (1979) Fine structure of the gills of some Indian air-breathing fishes. *J. Morphol.* 160, 169-194.
- Karnaky K J, Ernst S A and Philpott C W (1976) Teleost chloride cell I. Response of pupfish, cyprinodont variegates gill Na, K-ATPase and chloride cell fine structure to various high salinity environment. *J. Cell Biol.* 70, 144-156.
- Kessel R and Beam H (1960) An electron microscopic study of the mitochondria rich "chloride cells" from the gill filaments of fresh water and sea water adapted. *Fundulus heteroclitus*. *Biol. Bull.* 119, 322.
- Kessel R and Beam H (1962) Electron microscopic studies on the gill filaments of *Fundulus heteroclitus* from the sea water and fresh water with special references to the ultrastructure organization of the "chloride-cell". *J. Ultrastruct. Res.* 4, 77-87.
- Keys A and Willmer E N (1932) "Chloride secreting cells" in the gills of fishes with special reference to the common eel. *J. Physiol.* London 76, 568-578.
- Laurent P and Dunel S (1980) Morphology of gill epithelia in fish. *Am. J. Physiol.* 238, 147-159.
- Mackinnon M and Enesco H (1980) Cell renewal in the gills of the fish *Barbus conchoniensis*. *Can. J. Zool.* 5, 650-653.
- Matthiessen P and Brafield A E (1973) The effect of dissolved zinc on the gills of stickleback *Gasterosteus aculeatus* (L.). *J. Fish Biol.* 5, 607-613.
- Matthiessen P and Brafield A E (1977) Uptake and loss of dissolved zinc by the stickle-back *Gasterosteus aculeatus* L. *J. Fish Biol.* 10, 399-410.
- Mittal A K and Whitear M (1978) A note on cold anaesthesia of poikilotherms. *J. Fish Biol.* 13, 519-520.
- Munshi J S D (1964) "Chloride cells" in the gill of fresh water-teleosts. *Quart. J. Microsc. Sci.* 105, 78-89.
- Ojha J (1999) Fish gills: Potential indicators of ecodegradation of aquatic environment. In. *Water/Air Transition in Biology* (Mittal, A.K., Eddy, F.B. and Munshi, J.S.D. (eds) Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi.
- Oronsaye J A O and Brafield A E (1984) The effect of dissolved cadmium on the chloride cells of the gill of the stickleback, *Gasterosteus aculeatus* L. *J. Fish Biol.* 25, 253-258.
- Philpott C W (1962) The comparative morphology of the chloride secreting cells of three species of *Fundulus* as revealed by electron microscope. *Anat. Res.* 142, 267-268.
- Philpott C W and Copeland D E (1963) Fine structure of chloride cells from three species of *Fundulus*. *J. Cell Biol.* 18, 389-404.
- Philpott C W and Copeland D E (1965) Halide localization in the teleost chloride cells and its identification by selected area electron differentiation. *Protoplasma* 60, 7-23.
- Vickers T (1961) A study of the so called "chloride cells" of the gills of teleosts. *Quart. J. Microsc. Sci.* 104, 507-518.
- Wendelaar-Bonga S E, Greven J A A and Veenhuis M (1976) The relationship between the ionic composition of the environment and the secretory activity of the endocrine cells types of stannous corpuscles in the teleost *Gasterosteus aculeatus*. *Cell Tissue Res.* 175, 297-312.
- Zaccone G (1981) Effect of osmotic stress on the chloride and mucus cells in the gill epithelium of the fresh water teleost *Barbus filamentosus* (Cypriniformes, Pisces). A structural and histochemical study. *Acta Histo. Chem.* 68, 147-159.