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Histopathological analysis of sublethal toxicity of zinc chloride to the respiratory organs of the airbreathing catfish *Heteropneustes fossilis* (Bloch)

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Sublethal toxicity of zinc chloride ($ZnCl_2$; 7.5 ppm: 10% of 96 h LC_{50} value) on the aerial (accessory respiratory organs or air sacs) and branchial (gills) respiratory organs of ***Heteropneustes fossilis*** *has been analysed histopathologically. Zinc chloride exposure causes certain common but severe damage to both tissues. The prominent changes in the accessory respiratory organs include periodic deformation of lamellar elements, haemorrhages due to necrosis and sloughing off of the respiratory epithelium, and hyperplasia accompanied by fusion of secondary lamellae. Subsequently, regeneration of the lamellar system from the focal inflammatory tissues takes place. Deposition of glycogen in the muscular layer indicates disturbed aerial respiration. The alterations in the gills include periodic lifting off of the respiratory epithelium, extensive intercellular vacuolization and occasional fusion of secondary lamellae, resulting in increased thickness of primary and secondary lamellae. Fusion of secondary lamellae reduces the surface area for gaseous exchange. The increased thickness of the respiratory epithelium due to uncontrolled hyperplasia of the epithelial cells, also increases the diffusion distance between the ambient and vascular components. Vasodilation in the secondary lamellae of the gills and periodic fluctuations in the mucous cell density are also observed at various stages of $ZnCl_2$ exposure.*

Key terms: *accessory respiratory organ, gills,* ***Heteropneustes fossilis,*** *histopathology, zinc chloride toxicity*

INTRODUCTION

Despite being an essential trace element (Harper *et al*, 1977; Holcombe *et al*, 1979; Alabaster & Lloyd, 1982), Zn in larger quantities adversely affects the fish populations (Lloyd, 1960; Skidmore, 1964; Benoit & Holcombe, 1978; Chapman & Stevens, 1978; Hughes & Flos, 1978; Banerjee & Mukherjee, 1994; Gupta & Chakraborti, 1993; Saxena *et al*, 1993). It finds its way into the aquatic ecosystem from a number of sources such as rubber, paint, ceramic, cosmetic, fertilizer and textile industries (Luckey & Venugopal, 1977). Extensive urbanisation and industrialization have further increased the heavy metal concentration in the environment.

Even though skin and gills are the main targets of direct contact stress, little is known about the interaction of Zn with the gills of the air-breathing fishes. Similarly, no much data on the toxicity of $ZnCl_2$ on the accessory respiratory organs (branchial diverticulum) of air-breathing fish(es) are available. The air-breathing fishes of the

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Indian sub-continent, that inhabit the swamps and delerict ponds (having very poor O_2 condition), have developed bimodal respiratory mechanism for the exploitation of water (via gills) as well as air (via accessory respiratory organs). Hence, this paper evaluates the toxicity of a sublethal concentration of $ZnCl_2$ solution on the gills and accessory respiratory organ of the air-breathing catfish *Heteropneustes fossilis*, which can also withstand the extreme conditions of drought when the ponds and various water bodies get dried up during the long period of summer. The accessory respiratory organ of *H. fossilis* comprises a pair of sacs like backward extensions of the suprabranchial chamber embedded deeply in the body myotomes one on each side of the body (Munshi, 1962, 1980, 1993). Although being a modified gill structure, this organ in *H. fossilis* does not come under the direct contact of the external media. Thus, a comparison of the toxic impact of the Zn salt on the gills and the accessory respiratory organ has also been made. This would allow in the future to use the accessory respiratory organ, in addition to the gills, as potential bioindicator of contaminated waters.

MATERIALS AND METHODS

Fish and their maintenance

Healthy specimens of *H. fossilis* of 18-20 cm length and 35-40 g body weight, belonging to a single population, were collected at Varanasi. Fish were maintained in large plastic aquaria for 30 days (d) for acclimation. They were fed daily with minced goat liver and the water was renewed after every 24 h, leaving no fecal matter, unconsumed food nor dead fish, if any.

Estimation of LC_{50}

Prior to the experiments, the 96 h median lethal concentration (96 h LC_{50}) of zinc chloride (E Merck India Ltd, Bombay; 99% pure) was estimated following trimmed Spearman-Karber method (Hamilton *et al*, 1977) and was found to be 75 ppm.

Experimental protocol

Five groups of 10 fish each were exposed to $ZnCl_2$ (7.5 ppm, 10% of 96 h LC_{50} value) for the estimation of sublethal toxicity. Each group was exposed separately to 50 litres of $ZnCl_2$ solution, each prepared in tap water, having dissolved oxygen 6 mg/l, pH 7.5, water hardness 23.2 mg/l and water temperature 24±2°C. Parallel groups of 10 fish each were kept in separate aquaria containing 50 litres of plain tap water (without the addition of $ZnCl_2$) to serve as controls. Feeding was allowed in the experimental as well as control aquaria for a period of 3 h every day, just before the renewal of the media with fresh ones, during the whole period of experiment.

Histopathological analysis

After 5, 10, 20, 30 and 45 days exposure, 5 fish each from the respective experimental as well as control groups were sacrificed. The second gill and the anterior portions of the air sac from both sides of the fish were fixed in 10% neutral formalin, aqueous Bouin's fluid and Helly's fluid. Tissue blocks were dehydrated in graded ethanol, cleared in xylene and softened in cedar wood oil before paraffin embedding. Six mm sections were stained in Ehrlich's haematoxylin/eosin (H/E) for routine histopathological analysis. Glycoproteins were histochemically detected by periodic acid Schiff (PAS) (Pearse, 1985)), Alcian blue pH 2.5 (AB 2.5) and AB 2.5/PAS (Pearse, 1985) methods. Sulphated mucopolysaccharides and water stable mucoproteins were localized by Alcian blue pH 1.0 (AB 1) and Bismarck brown (BB) techniques respectively (Pearse, 1985). Presence of glycogen was confirmed by prior treatment of the sections with salivary amylase before staining with PAS technique.

Statistical analysis

The densities (numbers) of mucous cells of the gills and air sacs were calculated from whole mount preparations, following Rajan

and Banerjee (1993). Standard statistical procedures based on random sampling of five different sites on each tissue block from the five experimental as well as control fish of each sacrificing intervals were taken into consideration. One way analysis of variance (ANOVA) followed by Duncan's multiple range test was performed to determine whether mucous cell density was significantly affected by exposure periods.

RESULTS

Control accessory respiratory organ (air sac)

The inner surface of the thin walled air sac is lined by a stratified epithelium (Figs 1A, B) next to which serially arranged are basement membrane, connective tissue layer, a thin membrane and a muscular coat (cucullaris muscle). The inner lining of the air sac may be differentiated into vascular areas bearing large and small respiratory islets (primary lamellae) and non-vascular areas. An islet is formed by double rows of modified secondary lamellae that project into the lumen of the air sac. In transverse section, these lamellae appear as vertical columns of alternately arranged pillar cells and blood channels. The PAS positive basement membrane also runs along with the secondary lamellae. The distal ends of the secondary lamellae in transverse sections often appear to project into the lumen as finger-like projections. The single layered respiratory epithelium of the secondary lamellae acts as a barrier layer between the respiratory blood and the external environment. The non-vascular area of the epithelium is made up of 7 to 8 layers of epithelial cells and several mucous cells. Patches of thin layer of slime cover the epithelial surface. A summary of the histochemical properties of the different cellular elements of the inner epithelial lining of control as well as experimental air sacs is given in Table I.

Experimental accessory respiratory organ

Five days after transferring the fish into the $ZnCl_2$ solution, the density of the mucous cells in the inner epithelial lining of the air sac is decreased. Simultaneously, detachment and lifting of the respiratory epithelium from the underlying pillar cell system is also noticed. Meanwhile, distal ends of many of the secondary lamellae are projected out prominently into the lumen. Large number of eosinophilic granular cells appearing identical to those reported in the gills are also noticed after 5 d of exposure (Fig 1C). Fusion and clubbing of secondary lamellae are also commonly observed. Highly vascular focal inflammatory tissues are frequently observed after 10 d of exposure (Fig 1D). Subsequently the fusion of secondary lamellae becomes more pronounced and after 20 d, several secondary lamellae fuse together to form a solid mass of cells (Fig 1E). Large scale necrosis of the epithelium also takes place at this stage resulting in haemorrhage into the lumen. Although telangiectasis of the secondary lamellae starts after 10 d, collapsing of the pillar cell system in many of the secondary lamellae is also noticed after 30 d and 45 d of exposure. The areas thus vacated by the dismantled secondary lamellae get haphazardly filled with a dense population of undifferentiated cell types which, excepting the blood cells, remain connected with each other by long filamentous intercellular cytoplasmic bridges. After 30 d, although the secondary lamellae regenerate, the respiratory epithelium shows detachment and lifting from the underlying pillar cell-blood capillary system (Fig 1F). However, haemorrhage decreases greatly from this and onward stages of exposure. Hyperplasia of the epithelial cells, resulting in the thickening of the distal tips of the secondary lamellae and filling of the paces between two secondary lamellae, is also prominently observed after 45 d of exposure (Fig 1G). The amount of blood in the secondary lamellae also decreases at this stage of exposure. The density of mucous cells decreases progressively throughout the experiment (Fig 1H). The staining behaviour of the mucous cells and the surface layer epithelial cells also show marked variation at different stages of exposure (Table I).

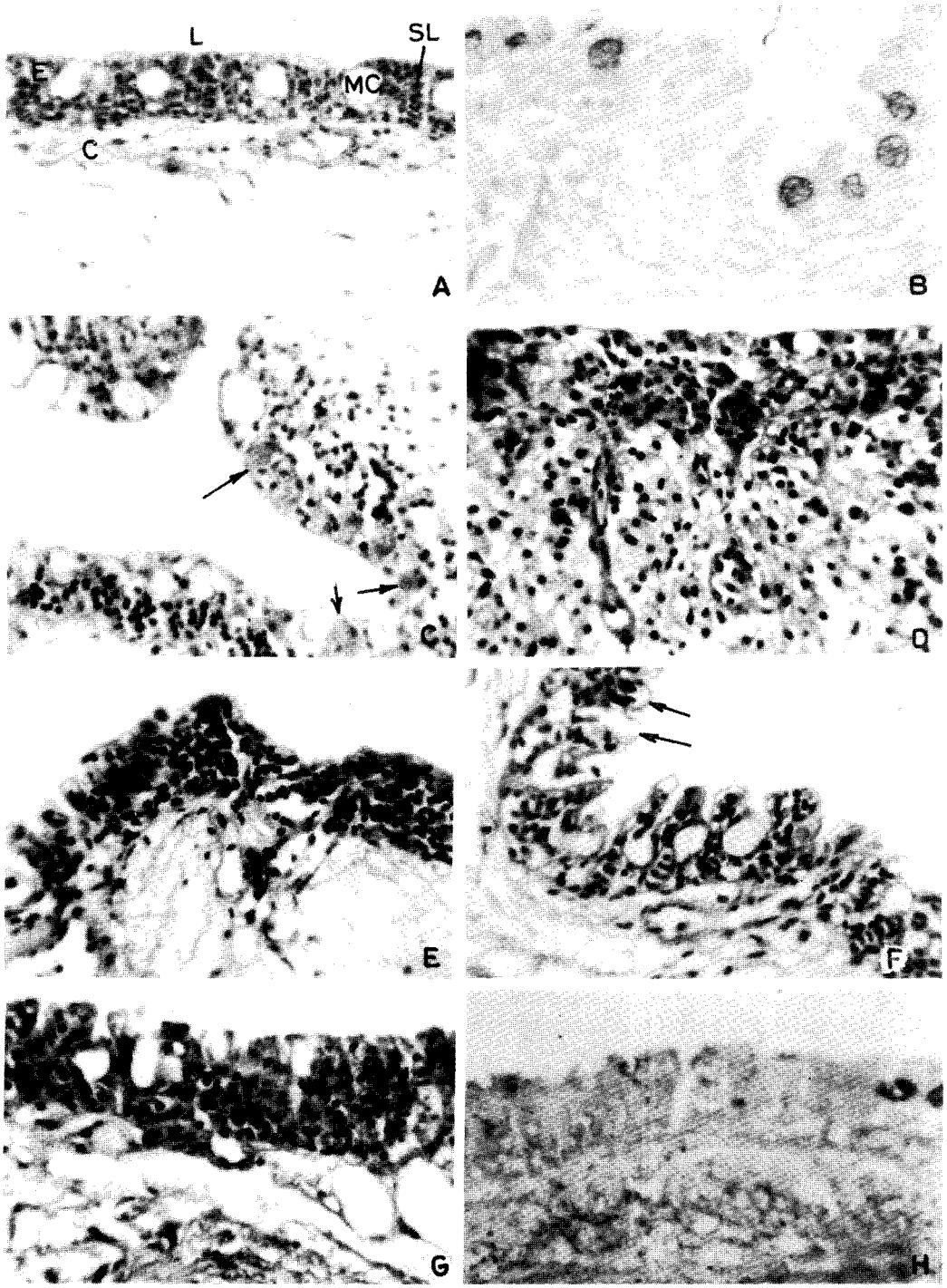

Fig 1. Transverse sections of specimens of accessory respiratory organ (ARO). **A.** Control fish, showing structural organization of inner epithelial lining. C, connective tissue; E, epithelium; L, lumen; MC, mucous cell; SL, secondary lamella. (H/E; x 475). **B.** Part of ARO in control fish, showing normal distribution of carbohydrates mostly in its mucous cells, generally present between secondary lamellae. (AB 2.5/PAS; x 475). **C.** Large number of eosinophilic granular cells (arrows) in epithelial lining after 5 d exposure. (H/E; x 475). **D.** A focus of undifferentiated cells (highly vascular inflammatory tissue) after 10 d exposure. (H/E; x 475). **E.** Collapsing of secondary lamellae after 20 d exposure, giving the epithelium a solid mass-like appearance. (H/E; x 475). **F.** Lifting of respiratory epithelium (arrows) of newly regenerated secondary lamellae after 30 d exposure. (H/E; x 475). **G.** Hyperplasia of interlamellar epithelium after 45 d exposure. (H/E; x 475). H. Section showing distribution of carbohydrate moieties especially in mucous cells after 45 d exposure. (AB 2.5/PAS; x 475).

Table I

Summary of histochemical alterations in carbohydrate contents of respiratory epithelium of accessory respiratory organ of *Heteropneustes fossilis* at various intervals of exposure to ZnCl2.

Technique	Cells	Control	5 d	10 d	20 d	30 d	45 d
PAS	Epithelial	0 ~ ±	0 ~ ±	0	1	1	1
	Mucous: periphery	3 ~ 4	3. ~ 4	3 ~ 4	3 ~ 4	3 ~ 4	3 ~ 4
	contents	2 ~ 3	2 ~ 3	1 ~ 2	2 ~ 3	2 ~ 3	1 ~ 2
AB 1.0	Negative reaction throughout epithelium in all stages						
AB 2.5	Epithelial	± ~ 1	0 ~ ±	± ~ 1	± ~ 1	± ~ 1	±
	Mucous: periphery	4	2 ~ 3	1 ~ 2	1 ~ 2	1 ~ 2	2
	contents	2 ~ 3	3	4	2 ~ 3	1 ~ 2	± ~ 1
AB/PAS	Epithelial	± ~ 1G	0 ~ ±G	0 ~ ±G	± ~ 1Gp	± ~ 1Gp	± ~ 1Gp
	Mucous: periphery	4Gp	2 ~ 3Gp	2 ~ 3Gp	1 ~ 2Gp	1 ~ 2Gp	3 ~ 4B
	contents	2 ~ 3Gp	3Gp	3Gp	2 ~ 3Gp	1 ~ 2Gp	2 ~ 3B
PAS/SD	Negative reaction throughout epithelium in all stages						
BB	Epithelial	0	0	0	0	0	0
	Mucous: periphery	2 ~ 3	1 ~ 2	1 ~ 2	1	1 ~ 2	4
	contents	2 ~ 3	1 ~ 2	1 ~ 2	1	1 ~ 2	2 ~ 3

Techniques: PAS = periodic acid Schiff, for neutral glycoproteins (1,2 glycols); **AB 1.0** = Alcian blue at pH 1.0, for sulphated mucopolysaccharides; **AB 2.5** = Alcian blue at pH 2.5, for acidic glycoproteins; **AB/PAS**, for neutral & acidic glycoproteins; **PAS/SD** = PAS/saliva digestion, for glycogen; **BB** = Bismarck brown, for water stable mucoproteins.
Staining: B = blackish green; **G** = greenish blue; **Gp** = greenish blue with pinkish tinge.
Reaction: 0 = negative; ± = faint/doubtful; **1** = weak; **2** = moderate; **3** = strong; **4** = very strong; ~ = *to*.

Control gill

H. fossilis possesses four pairs of gills, each bearing two rows of primary gill lamellae (gill filaments) which in turn bear a series of secondary lamellae (respiratory lamellae) (Figs 2A, B) alternately arranged on both sides. The secondary lamellae consists of single layered epithelium which rests on a basement membrane covering the serially arranged row of pillar cells and blood channels. These pillar cells along with the alternately arranged blood channels constitute the vascular components of the secondary lamellae (Fig 2A). The mucous cells are mostly observed in the region of primary lamellar epithelium between two secondary lamellae (Fig 2A) and at the distal ends of the primary lamellae. The histochemical properties of the cellular components of the control as well as experimental gills have been listed in Table II.

Experimental gill

On transferring the fish into the $ZnCl_2$ solution, no marked histopathological alteration is noticed in the gills of *H. fossilis* up to 10 d of exposure. However, the length of the secondary lamellae appears to increase slightly with marked increase in the amount of blood material in the vascular capillaries between pillar cells. Although the density of mucous cells in the epithelial lining between adjacent secondary lamellae and at the tip of the primary lamella increases markedly after 5 d, it decreases substantially after 10 d, but still maintaining above control level (Fig 3). The respiratory epithelium of the secondary lamellae also stains positively for carbohydrates, giving moderate AB 2.5, negative PAS and AB 1.0 reactions (Table II). After 20 d, hyperplasia of the respiratory epithelium along with that of the inter-lamellar epithelial linings of the primary lamellae is noticed (Fig 2C).

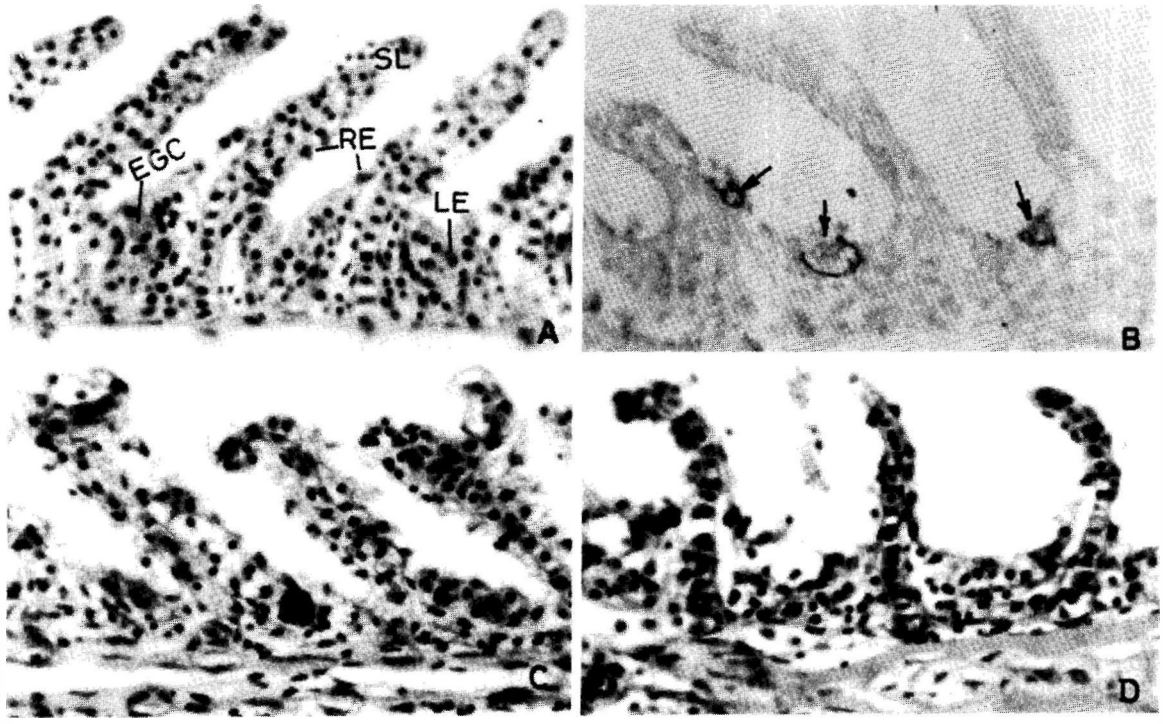

Fig 2. Transverse sections of specimens of gills. **A.** Second gill of control fish showing its structural organization. RE, respiratory epithelium; SL, secondary lamella; EGC, eosinophilic granular cells. (H/E; x 475). **B.** Second gill of control fish showing the distribution of carbohydrate moieties especially in mucous cells (arrows). (AB 2.5/PAS; x 475). **C.** Hyperplasia of epithelial linings of primary and secondary lamellae after 20 d exposure, with prominent vacuolization and necrosis in hyperplastic epithelial lining of secondary lamellae. (H/E; x 475). **D.** Hyperplasia of epithelial lining of primary lamella, causing decreased heights of secondary lamellae after 45 d exposure; note decreased thickness of epithelial lining of secondary lamellae. (H/E; x 475).

Table II

Summary of histochemical alterations in carbohydrate contents of gill epithelia of *Heteropneustes fossilis* at various intervals of exposure to $ZnCl_2$.

Technique	Cells	Control	5 d	10 d	20 d	30 d	45 d
PAS	Epithelial: primary lamellae	±	0 ~ ±	0	0	0	±
	secondary lamellae	±	0 ~ ±	0	0	0	±
	Mucous: periphery	1 ~ 2	2 ~ 3	1 ~ 2	2	2	1 ~ 2
	contents	1 ~ 2	1 ~ 2	1 ~ 2	± ~ 1	1	1
AB 1.0	Negative reaction throughout epithelium in all stages						
AB 2.5	Epithelial: primary lamellae	1	±	1	± ~ 1	± ~ 1	± ~ 1
	secondary lamellae	1	± ~ 1	1	± ~ 1	± ~ 1	± ~ 1
	Mucous: periphery	1 ~ 2	2 ~ 3	2	2	2	2
	contents	1 ~ 2	1 ~ 2	2	2	2	2
AB/PAS	Epithelial: primary lamellae	1Gp	±Gp	1Gp	± ~ 1Gp	± ~ 1Gp	± ~ 1Gp
	secondary lamellae	1Gp	± ~ 1Gp	1Gp	± ~ 1Gp	± ~ 1Gp	± ~ 1Gp
	Mucous: periphery	1 ~ 2Gp	2 ~ 3Gp	2Gp	2Gp	2Gp	2Gp
	contents	1 ~ 2Gp	1 ~ 2Gp	2Gp	1 ~ 2Gp	1 ~ 2Gp	2Gp
PAS/SD	Negative reaction throughout epithelium in all stages						
BB	Epithelial	0	0	0	0	0	0
	Mucous: periphery	1	1 ~ 2	2	1 ~ 2	2 ~ 3	1 ~ 2
	contents	2	1 ~ 2	2	1	2 ~ 3	1

Techniques: PAS = periodic acid Schiff, for neutral glycoproteins (1,2 glycols); **AB 1.0** = Alcian blue at pH 1.0, for sulphated mucopolysaccharides; **AB 2.5** = Alcian blue at pH 2.5, for acidic glycoproteins; **AB/PAS**, for neutral & acidic glycoproteins; **PAS/SD** = PAS/saliva digestion, for glycogen; **BB** = Bismarck brown, for water stable mucoproteins.
Staining: **Gp** = greenish blue with pinkish tinge.
Reaction: **0** = negative; ± = faint/doubtful; **1** = weak; **2** = moderate; **3** = strong; **4** = very strong; ~ = to.

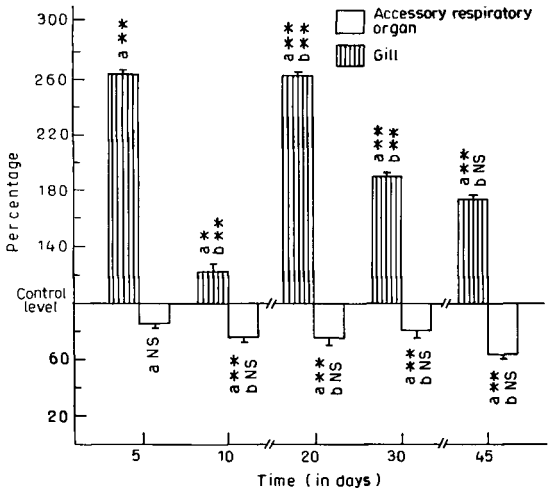

Fig 3. Periodic fluctuations in percentages of density of mucous cells in gills and accessory respiratory organs at different stages of sublethal intoxication by $ZnCl_2$. Means SEMs. Data analysed through Duncan's multiple range tests. a, difference between experimental and control groups; b, differences between each experimental group and the preceding experimental group; *, $P < 0.05$; **, $P < 0.01$; NS, non significant.

Telangiectasis of secondary lamellae becomes prominent at this stage and the density of mucous cells again increases greatly (Fig 3) along with further increase in the intensity of respiratory epithelium. After 30 d, the hyperplasia of the epithelial linings of the primary lamellae as well as secondary lamellae becomes more prominent, also exhibiting great vacuolization. Even though the density of the mucous cells decreases from that of the previous stage, it remains substantially above the control level (Fig 3). The haphazardly arranged epithelial cells remain connected with each other through prominent intercellular cytoplasmic bridges. After 45 d, although the respiratory epithelium on secondary lamellae becomes less hyperplastic, the primary lamellae still show prominent hyperplasia (Fig 2D) causing increased thickness of its epithelial lining especially at the inter- lamellar region. This results in decreased length of the secondary lamellae causing significant decrease in the surface area available for gaseous exchange (Fig 2D). The density of mucous cells, although increasing in the primary lamellae, is not found in the secondary lamellae, thus maintaining the density of mucous cells more or less at the level of the previous stage (30 d) (Fig 3).

DISCUSSION

Laying down of a barrier layer of slime by the goblet mucous cells of the gills following exposure to sublethal concentration of $ZnCl_2$ solution might delay the penetration of toxic heavy metal salt at least in the initial stages of exposure. The air sac of *H. fossilis*, which are modified gill structures, also show similar mucogenic activity following $ZnCl_2$ exposure (Fig 3). The increased mucogenic activity of the epithelial linings of many other tissue systems following Zn intoxication is well known (Banerjee & Sinha, 1993; Roy *et al*, 1993; Saxena *et al*, 1993). 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 & Banerjee, 1995, 1996a). Lindesjoo and Thulin (1994), on the other hand, did not notice any increase in the 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 the same or different mucous cells of the gills and air sacs at different stages of experimentation show varying intensities of PAS and/or AB 2.5 positive reactions indicating secretion and elaboration of slime containing mostly acidic or a mixture of neutral and acidic glycoproteins (Tables I, II). Electron microscopic observations of the mucous cells of *Cyprinus carpio*, by Iger *et al* (1994), revealed also the presence of mucosomes of different electron density in the same mucous cells. According to Zaccone *et al* (1989), stressed fish contain a mixture of neutral and acidic complex carbohydrates, the latter including *O*-acetylated sialic acids.

The ability of these glycoproteins to trap heavy metal ions is well documented (McKone *et al*, 1971; Coombs *et al*, 1972; Varanasi & Markey, 1977; Lock & van Overbeeke, 1981). Further, according to many workers, the mucous coating acts as mercury binding resin due to the capability of mercury to form a covalent bond with SH-group of proteins, S-containing amino acids

and a wide range of biological molecules present in the mucus (McKone *et al*, 1971; Olson & Fromm 1973; Friberg *et al*, 1974; Friberg & Vostal, 1976; Webb, 1979). The tendency to form covalent bonds is also observed with Zn and the property decreases in the order Zn > Cd > Hg amongst the subgroup II B metals (Venugopal & Luckey, 1978). All these three exhibit high affinity for thiol groups also in the order Hg > Cd > Zn (Venugopal & Luckey, 1978). Hence it can be expected that Zn might also be getting bound with the proteinaceous moiety of the mucus which ultimately gets substantially dissolved and lost into the medium. This might perhaps be one of the important ways for elimination of a part of the Zn from the surface of the gills, thereby reducing their concentration and uptake. While studying mercury intoxication, Part and Lock (1983) have indicated that due to the binding of the heavy metal salt with mucus, the diffusion rate of the heavy metal into the solution of mucus slows down significantly, thereby minimizing the uptake of the heavy metal.

While studying the protective role of fish mucus against hexavalent chromium pollution, Arillo and Melodia (1990) suggested that some components of mucus, probably the protein bound sulphydryl groups, may have a detoxifying function against the ambient toxins. These SH groups of mucus seem to bind with the toxicant and play a fundamental role in their reduction mechanism, especially for occasional and short term exposure. Apart from the mucous cells, the epithelial cells of the respiratory epithelium of the secondary lamellae of the gills also show periodic increased secretory activity at many stages of exposure. The mucoid secretion of epithelial cells of the gills might also be eliminating some amount of the Zn after binding with the heavy metal.

The increased mucogenic activity of the epithelial linings of the gills and air sac following the exposure (Fig 3) to $ZnCl_2$ might perhaps be due to the involvement of this important trace element in DNA and protein synthesis (Venugopal & Luckey, 1978). The additional amount of mucus provides an un-interrupted protective coating, as the slime is known to have detoxifying action against ambient toxicants (Arillo & Melodia, 1990), including heavy metals. According to Eddy and Fraser (1982), any loss of mucus from gill epithelia may result in decreased ionic and osmoregulatory ability.

Due to continuation of exposure, the mucoid barrier becomes short lived and collapses. Consequently, the cellular constituents of the gills are also subjected to the toxicity of the Zn salt, which leads to the detachment of the respiratory epithelia from their basement membrane, leading to the formation of non-tissue spaces and increased width of the secondary lamellae. Hyperplasia of the epithelia of primary lamellae as well as secondary lamellae also takes place (Fig 2C). Although all these lead to an increased xenobiotic diffusion barrier, they also result in increased respiratory blood-water distance and decreased surface area on the secondary lamellae available for gaseous exchange. Increased width of primary lamellae due to hyperplasia following exposure also causes decreased height or length of the secondary lamellae resulting in further decrease in respiratory surface area.

It is interesting to note that some of the secondary lamellae of the air sac also show fusion and clubbing. Rajan and Banerjee (1993) and Paul and Banerjee (1996b) also noticed fusion of secondary lamellae of the air sac following exposure to sublethal concentration of ammonium sulphate solution. Xenobiotics are also known to induce fusion of secondary lamellae of gills (Leino *et al*, 1987). In the later stages of exposure, the secondary lamellae differentiate themselves from the fused mass of tissue on the primary lamellae and the gills acquire more or less control like morphology. Vasodilation accompanied by increased amount of blood material in the vascular channels of the gills and air sac causes impaired aquatic as well as aerial respiration. The number of eosinophilic granular cells also increases.

The air sacs which do not come under the direct contact stress of the heavy metal salt, also exhibit periodic histopathological alterations. These alterations include detachment of the respiratory epithelia of

the secondary lamellae, severe necrosis of the epithelial lining causing haemorrhage from the secondary lamellae into the lumen of the air sac, collapsing and disintegration of the secondary lamellar system, loosening of cell junctions due to enormous increase in intercellular vacuolization. Side by side regeneration of the inner lining of the air sac takes place, resulting in the hyperplasia of the epithelial layer, as shown by the appearance of focal inflammatory tissues. Regeneration followed by differentiation of the lamellar system also takes place from these foci. The eosinophilic granular cells, which are commonly observed in the epithelial linings of the gills, also appear in the inner lining of the air sac (absent in control one) at several stages of exposure.

Fusion of secondary lamellae also causes reduced surface area for exchange of gases. Due to lifting of the respiratory epithelia, large non-tissue spaces appear in the gills of the $ZnCl_2$ exposed fish. Karlsson-Norrgren *et al* (1985) also observed similar non- tissue spaces in the gills and a consequently increased diffusion distance. They found that the non-tissue space formed within the respiratory epithelium was filled with a fluid consisting of myelin bodies and cellular debris. The non-tissue spaces along with hypertrophied epithelial lining could result in an inadequate gas exchange and consequently a reduced diffusion capacity, although they also create additional barrier for penetration of the ambient xenobiotics.

While studying the toxicity of chronic acid stress (pH 5.6) on the pearldace *Semotilus margarita,* Leino and McCormick (1984) also suggested that the histopathological changes noticed in the gills of exposed fish might signify or contribute to problems with respiration and ionic or acid base balance. The periodic decrease in density of epithelial cells may be due to periodic apoptosis of these cells. A new balance between cell proliferation and cell loss following acid water exposure due to mitotic and apoptotic activities has also been observed by Balm *et al* (1995). Apart from apoptosis $ZnCl_2$ exposure also stimulates shedding "of still living" cells due to the wear and tear of epithelial cells.

While reviewing the gill morphology affected by chemical and physical irritants/ stresses, Mallatt (1985) concluded that irritants induced alterations in gill histology are largely non specific and similar types of lesions occur for a wide range of irritants and no unique class of lesions has been reported for a particular stress. The present study is in agreement with this observation. Even though the air sac of *H. fossilis* does not come under the direct contact with the aquatic environment, the toxic manifestations of the Zn salt on the air sac have many similarities with those observed in the gills. This may be due perhaps to the common origin of both respiratory structures. The appearance of a large amount of glycogen in the muscular layer of air sac following exposure to $ZnCl_2$ solution may also disturb the physiology of air-breathing organs.

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