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Toxic effects of sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) on the skin epidermis of air-breathing catfish *Clarias batrachus* (L.)

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ABSTRACT

The toxicopathological effects have been investigated of a sublethal concentration (1 ppm) of sodium arsenate on the epidermis of the skin of air-breathing catfish *Clarias batrachus* L. The skin that acts as an accessory respiratory organ in this fish, faces direct contact stress of the toxicants and exhibits extensive damage, including massive wear and tear, sloughing of the epithelial cells (ECs) along with degeneration of the club cells (CCs) whose contents get squeezed out onto the body surface. This causes altered histomorphology of the epidermis. The mucous cells (MCs) show great hyperplasia and hypertrophy at most exposure periods. The staining properties of MCs also showed periodic alterations exhibiting more affinity for sulphate moieties. A thick layer of slime very often protects the surface of the skin. The epidermis also exhibits periodic but independent fluctuations in its protein, RNA and DNA contents. This is due to periodic synthesis, accumulation and sloughing of the slime, along with degeneration followed by regeneration of its different cellular elements, especially in the earlier stages of the treatment.

Key words: accessory respiratory organ, *Clarias batrachus,* histopathology, skin, sodium arsenate

Introduction

Arsenic, an important environmental contaminant, arises not only from anthropogenic activities but also from rocks, possibly due to geothermal activities and leaching. The arsenates (e.g. $Na_2HAsO_4 \cdot 7H_2O$), being thermodynamically more stable overwhelm the arsenites in the surface water and well-oxygenated freshwater systems (IRGOLIC, 1982; CUI and LIU, 1988). The toxic effects of arsenic on the human and other mammalian subjects have been a matter of great concern, hence thoroughly investigated by different

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investigators (EGUCHI et al. 1997; OHNISHI et al. 1997; HU et al. 1998; ANONYM., 1999; BISWAS et al. 2000; LIU et al. 2000; WAALKES et al. 2000; ANONYM., 2002). However its toxic effect on aquatic animals, especially on fish that serve as reliable indicators of arsenic toxicity has not much been studied. A few studies have advocated that the sub-lethal toxicity of arsenic involves stress mediated pathways (BEARS et al., 2006). The skin (along with gills) constitutes the boundary tissue of the fish and, being continuously hydrated, unkeratinized, and covered by a layer of slimy coating, is more vulnerable to water-borne toxicants. The skin of *Clarias batrachus* (L.) which inhabits hypoxic waters, also acts as an accessory respiratory organ (BANERJEE and MITTAL, 1976) and supplements any deficiency in oxygen uptake through conventional respiratory organs (gills) (GÜNTHER, 1880). Hence in this paper an effort has been made to explore the toxicity of an arsenic salt on the epidermis of the skin of important edible catfish *C. batrachus* (L.). This would also help to reinforce the importance of the skin as a reliable bio-indicator.

Materials and methods

Live specimens of *Clarias batrachus* (L.) (15 ± 1 cm in length and 45 ± 5 gm body mass) from a single population were acclimated in the laboratory in 25 litre plastic tubs containing tap water (having dissolved O_2 6.3 mg/L, pH 7.2, water hardness 23.2 mg/L and room temperature 28 ± 3 ºC) for 30 days. Regular feeding followed by renewal of water was done at every 24 h interval. Ten groups of ten fish each were exposed separately to 10 L of sublethal concentration (1 ppm) of sodium arsenate (s.d. fine-chem. Ltd. Mumbai, Min. assay 99.0-102.0%) prepared in ten litres of tap water. Parallel control fish were exposed to similar plain tap (10 L.) water only, under identical laboratory conditions. Three fish each from the experimental as well as the control aquaria were sacrificed after different exposure periods and the skin pieces (5×5 mm), just below the anterior end of the dorsal fin, were fixed in 10% neutral formalin (LILLIE and FULLMER, 1976), aqueous Bouin's fluid (BOUIN, 1897), 70% alcohol and Helly's fluid (PEARSE, 1985) for histopathological analyses. Lugol's iodine was used to remove mercury from Helly's fluid fixed tissue (PEARSE, 1985). Paraffin sections (6 µm) were stained with Ehrlich's haematoxylin and eosin (H/E) (EHRLICH, 1886) for routine histopathology, periodic acid-Schiff (PAS) (MCMANUS, 1948) for neutral glycoproteins, alcian blue (AB) pH 1.0 (LEV and SPICER 1964) and aldehyde fuchsin (AF) (PEARSE, 1985) for sulphated glycoproteins, alcian blue (AB) pH 2.5 (MOWRY, 1956) for acidic glycoproteins, AB 2.5/PAS (MOWRY, 1956) for differentiation of acidic and neutral glycoproteins and bismark brown (BB) (GURR, 1958) for water stable mucopolysaccharides. The density and area of mucocytes were measured using the software motic images 2000, version 1.3.

Skin fragments were also subjected to biochemical estimation for proteins (LOWRY et al., 1951) and nucleic acids (both RNA and DNA) (SCHNEIDER, 1945). One-way analysis

of variance (ANOVA) followed by Dunnett t-test was performed using the software SPSS, version 10. Since the differences between the measurements taken from various control groups at different time intervals of exposure were not significant, the averages of all the control groups were taken into consideration. Similarly, 0 h exposure data also appeared identical to those of the control fish; hence these data are not described separately.

Results

Histopathological observations. Control fish. The epidermis, the outer stratum of the skin of *C. batrachus* (L.) is a typical stratified epithelium (Fig. 3a). It is divided into an outermost layer (OML), a middle layer (ML) and a basal layer (BL). It is mainly composed of epithelial cells (ECs), mucous cells (MCs) and club cells (CCs) (Fig. 3a). The ECs of OML stained variously for carbohydrates (Figs. 4a and 4b) (Table 1). The slime on the surface also stained moderately to strongly with AB 1.0. The MCs of OML stained moderately to strongly with PAS (Fig. 4c), negatively with AB 2.5, moderately to AB 1.0, moderately to strongly with AF, moderately with BB and showed magenta with a bluish tinge with the AB 2.5/ PAS technique. The ML of the epidermis is mainly composed of large sized binucleated CCs (Fig. 3a). These cells are somewhat oval or elongated in shape and are present vertically in 1 to 2 layers. The slightly eosinophilic contents of CCs showed some degree of shrinkage and exhibited almost negative PAS and AB 2.5 reactions (Figs. 4a, 4b and 4c).

Experimental fish. Exposure to sodium arsenate (1 ppm), caused mild to moderate degree of wear and tear of the OML within 3h of exposure (Fig. 3b). This was followed by sloughing of ECs from the surface. Simultaneously the contents of the CCs at the surface of the epidermis was squeezed out, leaving empty spaces behind. Many of the CCs at this stage showed extensive vacuolization, especially around their nuclei. During the initial stages of exposure, the density and dimension of the MCs decreased (Fig. 1) with altered staining intensity for certain carbohydrates (Table 1).The peripheries of the MCs invariably remained strongly stained with BB. With AB 2.5/PAS, most of these MCs stained magenta. The ECs at the OML also showed altered carbohydrate staining (Table 1). While the slimy coatings on the epidermis remained unstained with PAS, AB 2.5, BB, and AB2.5/PAS techniques, they stained strongly with AB 1.0 and AF. Subsequently the staining properties of the MCs, along with their slimy secretion, showed periodic fluctuations at different stages of exposure (Table 1).

After 6 h the CCs showed extensive hyperplasia (Fig. 3c) and remained compactly and parallely arranged in four layers, occupying most of the space of the epidermis. They stained almost negatively for carbohydrates throughout the period of exposure. Sloughing of ECs from the surface and the squeezing out of the contents of the CCs were not noticed. The density and dimension of the CCs continued to increase even after 6h. New MCs

regenerated frequently on the inner layer. The staining property of MCs with AB 1.0, PAS, and AB2.5/ PAS techniques remained almost unaltered (Table 1).

After 12 hours the CCs became round, irregularly arranged with decreased vacuolization. The content of CCs showed condensation and shrinkage. The MCs showed further hyperplasia when they formed a linear layer on the OML. After 24h the density of CCs decreased significantly and their spaces were rapidly filled by newly formed ECs causing decreased thickness of the epidermis. The condensed contents of these CCs often contained glaring vacuole like structures. The density and dimension of the MCs at the surface increased substantially (Figs. 1 and 4d). They also showed increased secretion activity and often poured a thick layer of slime on the surface.

After 3 d the density of CCs increased, even though the number of layers of CCs were less than those in the initial stages of exposure. No squeezing out of the contents of the CCs was noticed. In the basal layer new CCs developed. The density and dimension of MCs decreased significantly, even though these cells stained strongly with various carbohydrate techniques (Table 1). Due to positive carbohydrate staining given by the ECs at the OML, this layer appeared as a separate stratum from the rest of the epidermis.

Due to progressive degeneration of the CCs, their density decreased after 7 d (Fig. 3d). The space left by the CCs was occupied by closely approximated ECs. The remaining CCs continued to show degenerative changes (Fig. 3d). The fine glaring vacuole-like structures continued to persist in the CCs. The rest of the area of the CCs remained filled with fuzzy substances (Fig. 3d). The density and dimensions of the MCs increased, some of them attaining very large size.

Degeneration of the CCs continued after 14 d when only a few of them were left (mostly in the basal layer). The entire space of the outer and middle layers remained densely occupied by small sized MCs (Fig. 4e), which contained moderately eosinophilic granulated secretory substances. The condensation of contents of the CCs continued.

Due to the massive wear and tear of the epidermis, a thick layer of degenerating sloughed cells covered the surface of the epidermis after 21 d (Figs. 3e and 4f) causing a further decrease in the number and size of the CCs in certain places. The surface of the epidermis remained covered with a thick layer of slime, which took on a magenta colour with AB2.5/PAS (Table 1).

Extensive alteration in the morphology of the epidermis was noticed after 30 d. The density of CCs decreased and remained located mostly in the inner layers. Often large empty spaces were noticed in the ML and BL in H/E preparation. The appearance of fine black granular deposits, especially around the nuclei of the CCs, was noticed. They were embedded within the condensed contents of the CCs and subsequently increased in density (Figs. 3f and 3g). Strongly PAS positive MCs of varying dimensions engorged

the OML of the epidermis. Some of the MCs also extended deep into the ML. Due to the great hyperplasia of MCs, the ECs could not be differentiated from goblet cells with PAS and AB 2.5/PAS preparations (Table 1).

No further alteration was noticed in the histomorphology of the epidermis after 45d. The number of the CCs continued to decrease greatly. The density of PAS positive MCs decreased slightly (Fig. 4g).

A partial regeneration of the epidermis was noticed after 60 d (Figs. 3g and 3h). The density of PAS positive MCs remained less. They however secreted a thick layer of (strongly PAS positive) slime on the surface. Very often a large number of MCs poured their contents into common pit like depressions on the epidermal surface (Fig. 4h).

Damage in the epidermis became more pronounced after 90d. At certain places most of the cells in the lower layer of the epidermis lost their integration and the cell boundaries of the neighboring cells were not visible. Instead an eosinophilic hazy material was observed, in which a large number of nuclei were embedded. Even the boundaries of certain CCs at basal layer were not visible. The density of the MCs decreased substantially with the decrease in the slime layer on the surface.

Biochemical observations. Nucleic Acids.

(i) RNA: A survey of the Fig. 2 showed that the RNA contents of the skin started decreasing from 24 h of treatment onward, except after 7 d when it increased marginally. The decrease in the RNA became more prominent from 21 d of exposure. This might indicate that arsenic greatly disturbs protein synthesis. Another possible reason for the decrease in protein synthesis was the sloughing of RNA along with the slime.

(ii) DNA: The DNA contents of the epidermis also remained subnormal throughout the exposure period, except after 3 d where it increased substantially. This might perhaps be due to the hyperplasia of ECs observed at this stage (3 d) of exposure. Decreased DNA contents at the later stages coincided with the degenerative cells noticed in histopathological preparations.

Proteins. Following exposure, the protein contents also showed a decrease in concentration remaining subnormal throughout the period of exposure. However their concentration fluctuated at different stages. The decrease in the protein contents may primarily be due to sloughing of the damaged ECs and CCs from the surface of the epidermis. Sloughing of slime (containing glycoproteins mainly) from the surface may perhaps be the other reason.

Table 1. Summary of histochemical reactions given by the carbohydrates moieties in the epidermis of the skin of *C. batrachus* (L.) at different periods of exposure of sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$).

Histochemical techniques	Cells /Slime	Fluctuations in intensity of histochemical reactions after various intervals of exposure												
		Ctrl/0h	3h	6h	12h	24h	3d	7d	14d	21d	30d	45d	60d	90d
PAS for neutral Gp	ECs	1^+	$\pm \sim 1^+$	1^+	$1 \sim 2^+$	2^+	$1 \sim 2^+$	1^+	$0 \sim 1^+$	1^+	$\pm$	$\pm \sim 1^+$	$\pm$	$\pm$
	MCs	$2 \sim 3^+$	$1 \sim 3^+$	$1 \sim 3^+$	3^+	3^+	3^+	$1 \sim 3^+$	3^+	3^+	3^+	2^+	2^+	$2 \sim 3^+$
	Slime	$1 \sim 2^+$	0	$2 \sim 3^+$	$1 \sim 2^+$	3^+	3^+	3^+	$2 \sim 3^+$	3^+	3^+	3^+	3^+	$\pm \sim 1^+$
AB 1.0 for sulphated Gp	ECs	$2 \sim 3^+$	$1 \sim 2^+$	$1 \sim 2^+$	$2 \sim 3^+$	2^+	$2 \sim 3^+$	$\pm \sim 1^+$	0	0	1^+	$\pm \sim 1^+$	$1 \sim 2^+$	$1 \sim 2^+$
	MCs	2^+	4^+	4^+	4^+	0	$2 \sim 3^+$	$1 \sim 2^+$	$2 \sim 3^+$	$1 \sim 2^+$	1^+	3^+	3^+	0
	Slime	$2 \sim 3^+$	3^+	3^+	$1 \sim 2^+$	$1 \sim 2^+$	$2 \sim 3^+$	$1 \sim 2^+$	2^+	$1 \sim 2^+$	$1 \sim 2^+$	3^+	4^+	$\pm \sim 1^+$
AB 2.5 for acidic Gp	ECs	$1 \sim 2^+$	$2 \sim 3^+$	$2 \sim 3^+$	2^+	$2 \sim 3^+$	3^+	$1 \sim 2^+$	1^+	1^+	1^+	$\pm \sim 1^+$	$\pm$	$\pm$
	MCs	0	0	$1 \sim 2^+$	0	0	3^+	$2 \sim 3^+$	$2 \sim 3^+$	$2 \sim 3^+$	$1 \sim 2^+$	3^+	2^+	$2 \sim 3^+$
	Slime	$2 \sim 3^+$	0	$2 \sim 3^+$	3^+	2^+	3^+	$1 \sim 2^+$	$1 \sim 2^+$	$1 \sim 2^+$	1^+	3^+	0	$1 \sim 2^+$
AB 2.5/PAS for differentiating acidic and neutral Gp	ECs	$\pm$	b^{2+}	$2 \sim 3^+$	b^{3+}	1^+	b^{2+}	b	p^{3+}	1^+	$1 \sim 2^+$	m^+	b^{1+}	b^{1+}
	MCs	mb	m	m	m	m^{3+}	m^{3+}	m	mb	m^{3+}	m	4+	$m^{1+}b$	mb
	Slime	m	0	b-v	v	v^{1+}	v^{1+}	m^{3+}	m^{3+}	m	m	$2 \sim 3^+$	m^{3+}	$\pm$
AF for sulphated Gp	ECs	$\pm$	$1 \sim 2^+$	1^+	1^+	$\pm \sim 1^+$	3^+	1^+	$1 \sim 2^+$	$1 \sim 2^+$	$1 \sim 2^+$	2^+	$\pm$	$\pm$
	MCs	$2 \sim 3^+$	$2 \sim 3^+$	$3 \sim 4^+$	$2 \sim 3^+$	$\pm \sim 1^+$	3^+	$2 \sim 3^+$	3^+	$2 \sim 3^+$	4^+	3^+	3^+	2^+
	Slime	$1 \sim 2^+$	3^+	4^+	$1 \sim 2^+$	$1 \sim 2^+$	$2 \sim 3^+$	1^+	3^+	3^+	3^+	3^+	0	$2 \sim 3^+$
BB for water stable Gp	ECs	1^+	$\pm \sim 1^+$	1^+	3^+	$1 \sim 2^+$	$2 \sim 3^+$	$1 \sim 2^+$	0	1^+	$1 \sim 2^+$	$\pm \sim 1^+$	$\pm$	$\pm$
	MCs	2^+	0	1^+	3^+	0	$2 \sim 3^+$	$1 \sim 3^+$	$2 \sim 3^+$	$0 \sim 1^+$	$1 \sim 2^+$	$1 \sim 2^+$	2^+	0
	Slime	$2 \sim 3^+$	0	$2 \sim 3^+$	$1 \sim 2^+$	3^+	1^+	$\pm \sim 1^+$	1^+	0	0	0	0	$\pm$

Symbols and abbreviations: AB 2.5: alcian blue at pH 2.5, AB 1.0: alcian blue at pH 1.0, AF: aldehyde fuchsin, BB: bismark brown, b: blue (blue showing acidic glycoproteins), b~v: blue to violet d: days, ECs: epithelial cells, Gp: glycoproteins, h: hour (s), m: magenta (magenta showing neutral glycoproteins), mb: magenta with bluish tinge, MCs: mucous cells, p: pink, PAS: Periodic acid Schiff, v: violet (violet showing mixture of acidic and neutral glycoproteins), 0: negative reaction, ±: faint reaction, 1^+: weak, 2^+: moderate, 3^+: strong, 4^+: very strong reaction.

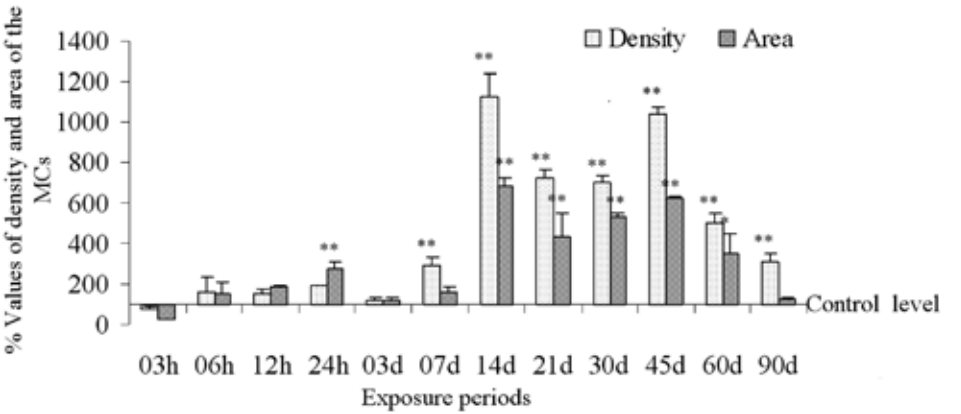


Fig. 1. Fluctuations in density and area of the mucous cells in the skin epidermis of Clarias batrachus (L.) after different periods of exposure of sodium arsenate. Values are expressed as mean ± SEM. * = P <0.05; ** = P<0.01. (Control value is taken as 100%).

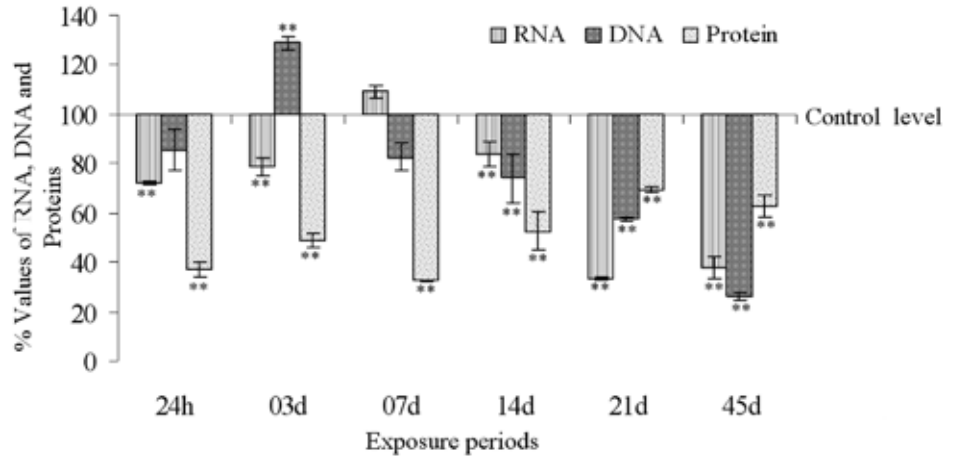
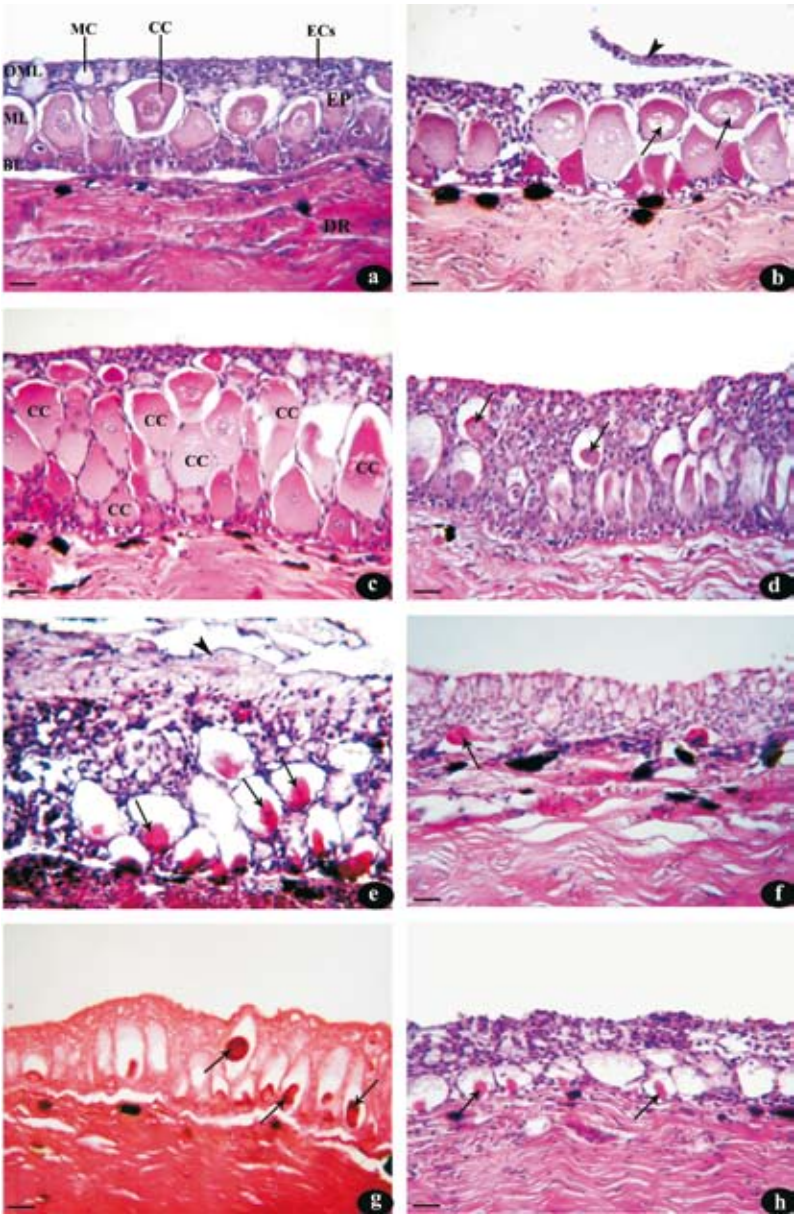


Fig. 2. Fluctuations in RNA, DNA and proteins in the skin of Clarias batrachus (L.) after different periods of exposure of sodium arsenate. Values are expressed as mean ± SEM. * = P<0.05; ** = P<0.01. (Control value is taken as 100%)


MC
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ECs
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Fig. 3a. Vertical section of the skin of untreated control fish *Clarias batrachus* (L.) showing its structural organization. H/E (scale bar = 20 µm)

Fig. 3b-3h. Vertical sections of the skin of *C. batrachus* (L.) showing various histomorphological alterations at different periods of exposure to sodium arsenate.

Fig. 3b. Wear and tear and sloughing (arrow head) of the epidermal cells at the OML after 03h. Note perinuclear vacuoles (arrows) in the CCs. H/E (scale bar = 20 µm)

Fig. 3c. Hyperplasia of CCs after 06h of exposure. H/E (scale bar = 20 µm)

Fig. 3d. Decreased density of the CCs in the OML after 07d. Note the shrinkage (arrows) of the contents of the CCs causing extensive peripheral vacuolization. H/E (scale bar = 20 µm)

Fig. 3e. Massive wear and tear of the epidermis after 21d. Note the degenerating CCs (arrows) and laying down of a thick layer of slime (arrow head) on the epidermal surface. H/E (scale bar = 20 µm)

Fig. 3f. Accumulation of dark granules (arrow) in the condensed contents of the CCs after 45d. H/E (scale bar = 20 µm)

Fig. 3g. Increased density of the granules (arrows) in the CCs following continuation (60d) of exposure. E (scale bar = 20 µm)

Fig. 3h. Greatly degenerated epidermis after 60d. Note the highly vacuolated damaged CCs (arrows) at the BL. H/E (scale bar = 20 µm)

Symbols and Abbreviations: BL: basal layer, CCs: club cells, d: day (s), DR: dermis, E: eosin, ECs: epithelial cells, EP: epidermis, h: hour, H/E: haematoxylin/eosin, MC: mucous cell, ML: middle layer, OML: outermost layer.

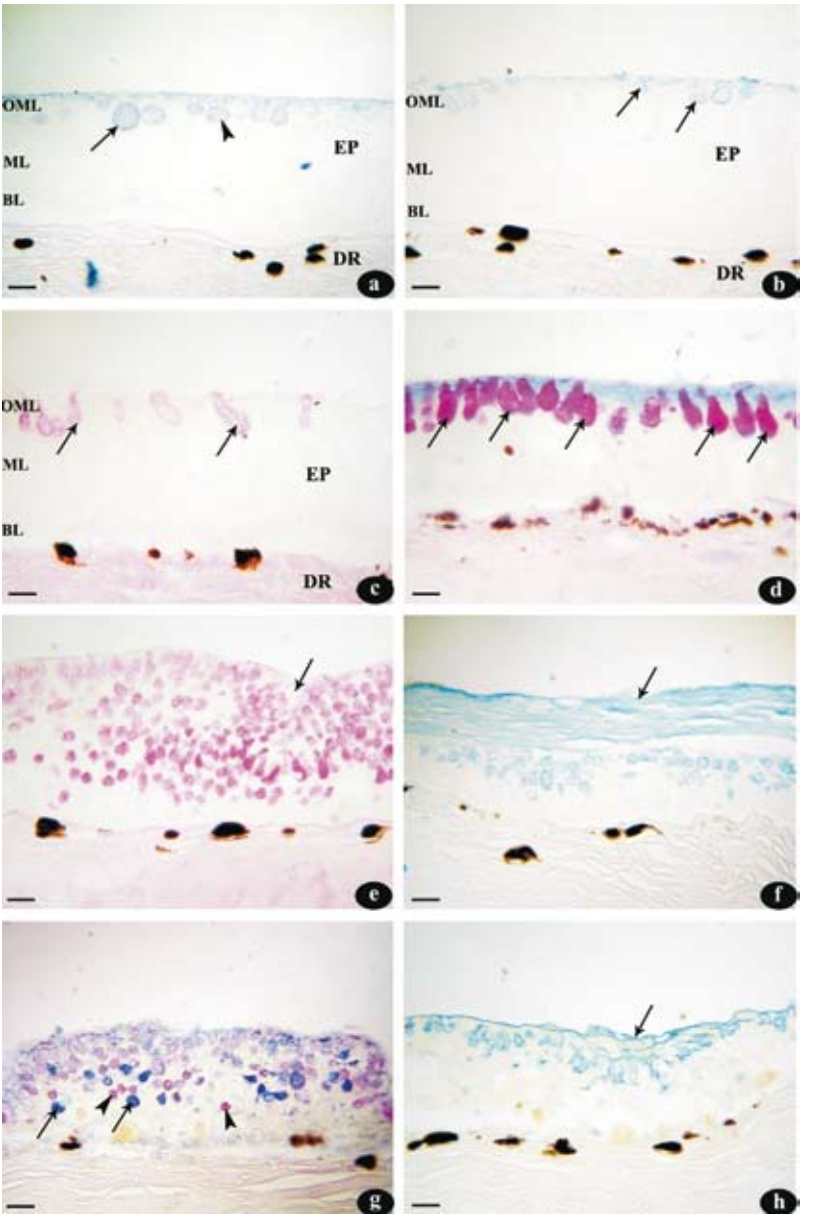
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Fig. 4a. Vertical section of the skin of control fish *Clarias batrachus* (L.) showing the carbohydrates staining of its epidermis. Note the affinity of AB staining of the MCs (arrow) and ECs and PAS (arrow head) at the OML (AB 2.5/PAS) (scale bar = 20 μm)

Fig. 4b. MCs (arrows) and ECs at the OML of the control fish showing positive reaction for sulphated mucopolysaccharides. (AB 1.0) (scale bar = 20 μm)

Fig. 4c. MCs (arrows) at the OML of the control fish showing positive reaction for 1, 2 glycols of carbohydrates (PAS) (scale bar = 20 μm)

Fig.s 4d-4h. Vertical sections of the skin of the fish *C. batrachus* (L.) showing histochemical alterations at different periods of exposure to sodium arsenate solution.

Fig. 4d. Hyperplasia of the PAS positive MCs (arrows) at OML after 24h of exposure. Note alcian blue positive (blue) reaction for acidic glycoproteins shown by the ECs at the OML. (AB 2.5/ PAS) (scale bar = 20 μm)

Fig. 4e. Extensive hyperplasia of the MCs occupying almost the entire area of epidermis after 14d of exposure. Note a pit like depression (arrow) in the epidermis. (PAS) (scale bar = 20 μm)

Fig. 4f. Laying down of a thick layer of slime (arrow) on the surface of skin epidermis after 21d of exposure. (AB 1.0) (scale bar = 20 μm)

Fig. 4g. Hyperplasia of the MCs after 45d of exposure. Note the different type of staining shown by the MCs ranging from magenta (arrow heads), dark blue (arrows) to violet coloured. (AB 2.5/PAS) (scale bar = 20 μm)

Fig. 4h. MCs staining strongly for sulphated mucopolysaccharides. Note the pouring of their contents by the MCs in a pit like depression (arrow) at the OML after 60d of exposure. (AB 1.0) (scale bar = 20 μm)

Symbols and Abbreviations: AB: alcian blue, BL: basal layer, d: day (s), DR: dermis, E: eosin, ECs: epithelial cells, EP: epidermis, h: hour, H/E: haematoxylin/eosin, MCs: mucous cells, ML: middle layer, OML: outermost layer, PAS: periodic acid Schiff.

Discussion

Following exposure, the MCs secreted a thick layer of slime on the skin surface in an effort to protect the skin from the toxic stress of the arsenic salt. To meet the additional requirement of slime due to continued exposure, the density of the MCs increased substantially, following regeneration of large numbers of new MCs, whose dimension also increased simultaneously (Fig. 1). Secretion of the copious amount of slime is perhaps a common phenomenon in the skin and other respiratory organs of many other air-breathing fishes exposed to heavy metal salt (RAJAN and BANERJEE, 1991, 1994; HEMLATHA and BANERJEE, 1997a and 1997b; CHANDRA and BANERJEE, 2003; DEVI, 2004) including *C. batrachus* (L.) (present study). The MCs exhibited varying intensities of PAS and/or AB 2.5, AB 1.0 and AF reactions (Table 1) indicating synthesis of slime containing sulphated, acidic or a mixture of neutral and acidic/sulphated glycoproteins at the same or different stages. ZACCONE et al. (1989) also found that stressed fish contained a mixture of neutral and acidic complex carbohydrates, the latter including *O*-acetylated sialic acids. IGER et al. (1994) revealed the presence of mucosomes of different electron density in the same MCs.

In the initial stages the MCs showed stronger AB 1.0 and AF reactions indicating the incorporation of the additional sulphate moieties into their secretion material. The sulphated mucin is known to bind arsenic molecules, perhaps to keep the toxicant away from the surface of the skin, at least temporarily. Metals bind to proteins in biological systems by their histidine and cysteine residues, histidine binding through its imidazole nitrogen and cysteine through its thiol groups. It was found that the mucus covering of the skin is mainly composed of glycoproteins that have electronegative charges (as shown by AB, AF and BB positive reactions) (Table 1) at neutral pH. However, the newly synthesized contents of the MCs of arsenic exposed *C. batrachus* (L.) often lost their AB 1.0/AF staining at many stages and exhibited increased PAS reaction, indicating the presence of the neutral carbohydrates mostly. This is perhaps due to the acute requirement of sulphated slime to combat the toxicity of the arsenic salt. This extensive demand for sulphated mucin created a marked gap between its demand and synthesis by the epidermis and its MCs.

During the present study it was also noticed very often that the secretion of the ECs also contributed actively to laying a protective but thin covering of slime on the outer surface of the epidermis (Fig. 4f and Table 1). Like the MCs, the ECs also showed periodic fluctuation in their staining properties. Hence the slimy secretions of the epidermis contained a variety of carbohydrates secreted by the ECs as well as MCs at different stages of exposure. In the later stages of exposure, the ECs also showed decreased AB 1.0 and AF reactions, indicating the failure to incorporate sulphate moieties by the slime due to hyperactivity to meet the additional demand for sulphated slime. The protective role

played by the slimy coating however did not last long, perhaps due to extensive loss and the altered nature of the slime following prolonged exposure. This led to wear and tear and sloughing of the superficial cells.

A survey of Fig. 2 showed that the RNA, along with the protein contents of the skin, started to decrease and remained subnormal at most of the stages after 24h and onwards of exposure. This (Fig. 2) indicated that the RNA content fluctuates independently to that of protein contents at different stages of exposure. This was perhaps due to the synthesis, accumulation and sloughing of the slime within the MCs, because a substantial quantity of RNA is known to be shed along with the slime. The decrease in the RNA content was most prominent after 21d, when the protein content showed a significant increase (over the previous stage). This is because changes in the cell size and water content (often caused by xenobiotics) alter the RNA concentration independently of any changes in protein synthesis (DAHLHOFF, 2004). Increase in the RNA quantity with a decrease in the total protein content at certain stages of exposure may perhaps be due to activation of protein degrading enzymes by the arsenic salt (EARNSHAW et al., 1999). Similarly the decrease in RNA quantity with the increase in protein concentration may perhaps be due to activation of RNAse by the stress of the arsenic salts.

The precise role played by the CCs is still a matter of great debate. However the protective role played by these protein rich cells against the stress of various physical, as well as chemical hazards has been well accepted (BANERJEE and MITTAL, 1976; RAJAN and BANERJEE, 1991; HEMLATHA and BANERJEE, 1997a and 1997b; CHANDRA and BANERJEE, 2003; DEVI, 2004). The CCs of exposed *C. batrachus* thus also helped to prevent quick penetration of the toxicants via the skin and withstand the stress of xenobiotics more effectively, even though the CCs were also badly damaged by the ambient arsenic salt. Damage to the ECs of the surface layer of epidermis also resulted in the breaking open of the membranous boundaries of the CCs, especially those in the outer layer that was also simultaneously affected by massive degenerative changes and squeezing out their contents, leaving large empty spaces. In the latter stages of exposure (e.g. after 21 d), the contents of the CCs, along with the sloughed ECs, formed a thick layer on the surface of the skin, which temporarily provided a protective barrier layer at certain stages.

Often a large number of fine black granular deposits were also noticed in the condensed contents of CCs (Figs. 3f and 3g). These are perhaps the arsenic salt deposits. Similar deposits of metal granules have not yet been reported in the contents of the CCs of fishes exposed to various other heavy metal salts (BANERJEE and MITTAL, 1976; RAJAN and BANERJEE, 1991; HEMLATHA and BANERJEE, 1997a and 1997b; CHANDRA and BANERJEE, 2003; DEVI, 2004). The remaining portion of most of the CCs often remained empty or partially filled with a fuzzy substance. Degeneration of the CCs led to their

decreased density. To compensate for the loss of CCs, a large number of ECs developed and occupied the space vacated by the CCs. This helped to maintain the thickness of the epidermis and the protective role played by the skin. RAJAN and BANERJEE (1991) noticed extensive loss of CCs from the epidermis of mercury exposed fish, *Heteropneustes fossilis* and most of the space left by the CCs was occupied by quickly multiplying ECs. Certain new CCs regenerated from the inner layer, even when the toxic stress of the arsenic salt continued. This caused increased density of CCs at certain later stages.

Continuation of exposure often led to further destruction, followed by uncontrolled regeneration of the epidermis, causing significant alteration in its histomorphology and cellular architecture.

Due to extensive regeneration, the density of the MCs further increased significantly, especially at the OML of the epidermis, where they often formed a linear layer. Later (e.g. after 14 d) they occupied the entire spaces of the OML and ML making it difficult to differentiate them from the ECs, even with carbohydrate histochemical stainings. This is because the ECs also stained positively for carbohydrates.

From the above study it was noticed that arsenic caused damage to the structure and chemical composition of the epidermis. Such damage was not noticed in other Indian air breathing fishes (*Heteropneustes fossilis, Channa striata*) exposed to certain other toxic heavy metal salts (such as mercury, lead and zinc).

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SAŽETAK

Istraženi su toksopatološki učinci subletalne koncentracije (1 ppm) natrijeva arsenata na pousminu soma dvodihalice *Clarias batrachus* (L.). Na koži, koja u te ribe ima ulogu akcesornoga dišnoga organa, izravni dodirni stress uzrokovan toksinom očituje se u obliku jakoga oštećenja, uključujući obilno propadanje i ljuštenje epitelnih stanica s degeneracijom vrčastih stanica čiji se sadržaj istisnuo na površinu tijela. Sluznične stanice pokazivale su jaku hiperplaziju i hipertrofiju većim dijelom tijekom izloženosti. Njihova sposobnost primanja boje također se periodično mijenjala iskazujući veći afinitet za sulfatne dijelove. Debeli sloj sluzi zaštićivao je kožu. Epiderma (pousmina) također je pokazivala povremene i neovisne promjene u sadržaju svojih bjelančevina, RNA i DNA. To se pripisuje periodičnoj sintezi, nakupljanju i odbacivanju sluzi s degeneracijom i posljedičnom regeneracijom različitih staničnih sastojaka, osobito u ranom stupnju obrade.

Ključne riječi: akcesorni dišni organ, *Clarias batrachus*, patohistologija, koža, natrijev arsenat