Bull. Environ. Contam. Toxicol. (1988) 41:880–887

Environmental Contamination and Toxicology

Long–Term Effect of Ammonium Sulfate Fertilizer on Histophysiology of Adrenal in the Teleost, *Channa punctatus* (Bloch)

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In the last decade, water pollution used to mean the widespread contamination of water due to direct discharge of industrial wastes and effluents as well as indiscriminate application of biocides in agriculture which find their way into water systems through surface runoff during monsoon period. During recent years,with the advent of agricultural developments, the use of various kinds of chemical fertilizers has further aggravated the problem of water pollution. Often some amount of the fertilizer used in the agricultural fields is ultimately washed into the riverine and lacustrine systems, contributing to their pollution and causing adverse effects in aquatic animals. One such commonly used, readily degradable nitrogenous fertilizer is ammonium sulfate, $(NH_4)_2SO_4$, which liberates ammonia through its direct dissociation in water and by the natural process of ammonification (Mukhopadhyay 1977; Thurston and Russo 1983). Ammonia is widely recognized as a ubiquitous common water pollutant, and it enters natural water system from several sources including industrial effluents, sewage wastes, agricultural inputs such as nitrogenous fertilizers, animal feedlots, and energy development processes, e.g. oil-shale retorting, coal gasification, and liquefaction (Thurston and Russo 1983). Moreover, in aqueous medium, ammonia assumes an equilibrium between ionized $(NH_4)^+$ and unionized (NH_3) chemical forms, and it is the unionized ammonia base which causes toxic effects to fish and other aquatic organisms due to its distinct penetrative properties (Hemens 1966), whereas the ionized form is considered to be nontoxic or comparatively less toxic as it can not pass the tissue barriers (Tabata 1962). Literature concerning the acute and the chronic toxicity of ammonia in fishes is well documented (Willingham et al.1977; Thurston and Russo 1983; Hasan and Macintosh 1986). Although, some data are also available on the ammonia induced alterations in biochemical and physiological processes of fishes (Schenone et al.1982; Chatterjee

and Bhattacharya 1983); nevertheless information on the physiopathologic effects of ammonium sulfate fertilizer on different endocrine organs is relatively scanty (Sathyanesan et al.1978; Ram and Sathyanesan 1987a,b). In teleosts, adrenal (interrenal) gland is known to control the several physiometabolic processes by synthesising the corticosteroid hormones and catecholamines. Therefore, in the present study, an attempt has been made to investigate the histopathological changes in adrenal components and pituitary corticotrophs of a freshwater air-breathing teleost, Channa punctatus, in response to prolonged exposure of six months to safe and sublethal doses of ammonium sulfate fertilizer.

MATERIALS AND METHODS

Over thirty adult C. punctatus, weighing 46+5 g and 14.0+1.5 cm in length, procured locally were acclimated to laboratory conditions for a fortnight prior to the experiment. They were divided into three equal groups of 10 each and kept in 60-L glass aquaria containing chlorine free well-water of pH 7.2, hardness 154 ppm (as $CaCO_3$), alkalinity 68 ppm (as $CaCO_3$), dissolved oxygen 7.2 ppm, and conductivity 0.56 mMho. The water temperature and the photoperiod in the aquaria were not controlled. However, these conditions were similar in both the experimental and control aquaria. For the dose selection, fish were exposed to various concentrations of the $(NH_4)_2SO_4$ fertilizer for 96 h following the method of Basak and Konar (1977), and on the basis of observed mortality levels two different concentrations, 100 and 500 ppm, were chosen. These concentrations were given in terms of the commercial grade fertilizer and identified as toxicologically 'safe' (100 ppm) and 'sublethal' (500 ppm) dose, which caused no mortality but fish appeared to be under stress in latter concentration. Specimens of group I and II were exposed to 100 ppm and 500 ppm concentrations of the fertilizer, respectively, and group III was kept as untreated control. The experiment was started in the first week of January and terminated after a continuous exposure of six months in the last week of June. On alternate days, after feeding the fish with minced goat liver ad libitum, aquaria water was changed and the predetermined quantity of the fertilizer was added to the water of each experimental aquarium.

At the end of the experiment, all specimens were sacrificed at the same time by decapitation, and adrenals and pituitaries, intact with brain, were fixed in aqueous Bouin's fluid and Bouin's sublimate, respectively. Paraffin sections were cut at 5 μm thickness, and the adrenals were stained with haemotoxylin using eosin as counter

stain (HE). Pituitaries were stained with lead haemotoxylin-periodic acid-Schiff-Orange G (PbH-PAS-OG) for the differential localization of corticotrophs, which took specifically the bluish-black colour of lead haemotoxylin.

RESULTS AND DISCUSSION

The adrenal (interrenal) components of C.punctatus are composed of the cortical and the medullary (chromaffin) cells, and found in the form of cell cords arranged in several rows around the post cardinal vein and its head kidney. In control fish, the cortical cells, which are responsible for corticosteroids synthesis, were elongated in shape with distinct cell boundaries, prominent rounded nuclei and median nucleoli.On the otherhand,the catecholamines secreting chromaffin cells were larger than cortical cells, and their cytoplasm was almost chromophobic when stained with haemotoxylin-eosin.These cells were not only found close to the wall of vein but also distributed sporadically among the cortical cells (Fig. 1). In both of the experimental groups, chronically exposed 100 ppm safe (Fig. 2) and 500 ppm sublethal (Fig.3) concentrations of ammonium sulfate fertilizer, the cortical cells revealed extensive hyperplasia, degranulation, involution and exhaustion, probably after prolonged hyperactivity. In the consequence, these cells lost their definite pattern of arrangement and distinct cell boundaries, and showed atrophic and deleterious changes as evidenced by the presence of groups of pyknotic cells in different stages of degeneration and darkly stained specks of necrotic nuclei. Moreover, in sublethal dose treated fish, these histopathological changes were more pronounced than that of safe dose experimentals. On the otherhand, the chromaffin cells in safe dose treated fish were showing slight hypertrophy (Fig.2), whereas in those exposed to sublethal dose these cells were exhibiting atrophic changes characterized by reduction in size, pyknosis and nuclear necrosis leading to cellular involution (Fig.3).

In teleosts, the adrenal cortical activity is known to be under control of the corticotrophs of the pituitary. In C. punctatus exposed to sublethal dose of $(NH_4)_2SO_4$ fertilizer, the corticotrophs were showing the corroborative changes including marked hyperplasia, hypertrophy and degranulation (Fig.5) than controls (Fig.4). The comparable changes, but less pronounced, were also seen in the corticotrophs of safe dose treated fish. These histological alterations in the pituitary corticotrophs of experimental fish may be compensatory to the changes in adrenal cortical cells suggesting the impairment of their histophysiological function.

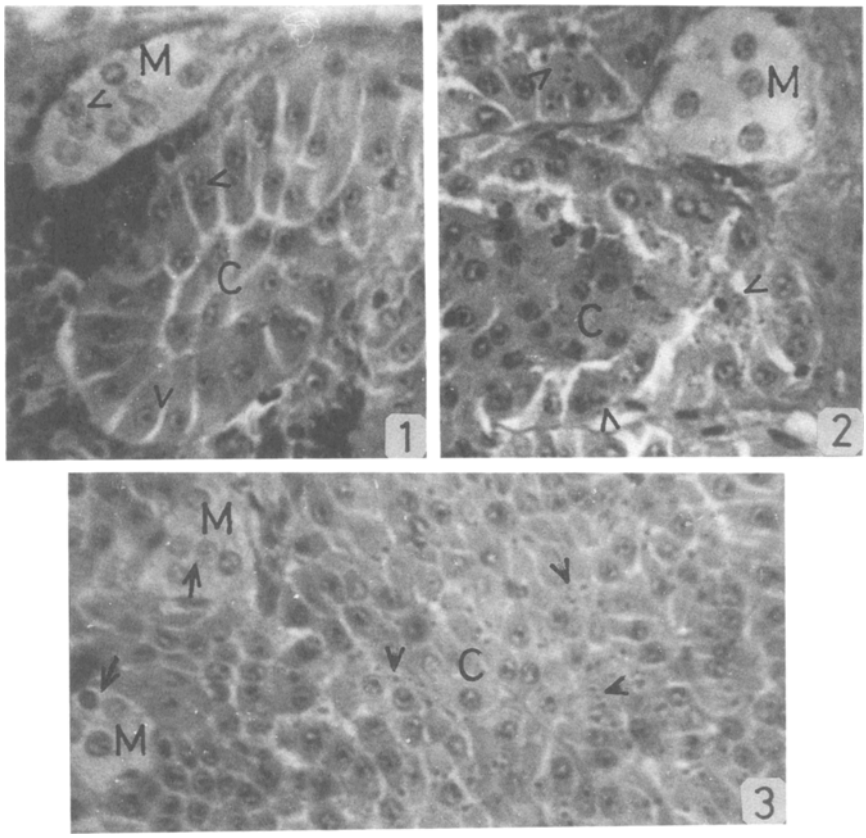

Figure 1. Control fish adrenal, showing cord of elongated cortical cells (C) and chromaffin cells (M). Arrow heads indicate their large rounded nuclei with prominent nucleoli. HE, X450.

Figure 2. Adrenal of safe dose (100 ppm) $(NH_4)_2SO_4$ fertilizer-treated fish, showing the compact mass of hyperplastic cortical cells (C) with groups of degenerating cells (arrow heads), and slightly hypertrophied chromaffin cells (M). HE, X450.

Figure 3. Adrenal of sublethal dose (500 ppm) treated fish, showing the compact mass of hyperplastic degenerating cortical cells (C) with darkly stained specks of necrotic nuclei (arrow heads). The atrophied chromaffin cells (M) with necrotic nuclei (arrows) can also be seen. HE, X450.

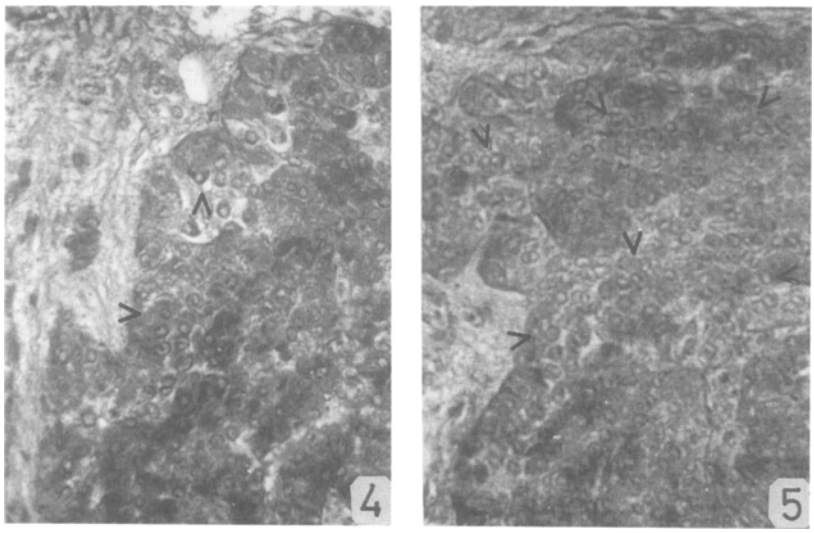

Figure 4. Rostral pars distalis (RPD) of control fish pituitary, showing less number of corticotrophs (arrow heads). PbH-PAS-OG, X600.
Figure 5. RPD of sublethal dose treated fish, showing relatively more number of hypertrophied corticotrophs (arrow heads). PbH-PAS-OG, X600.

Schenone et al.(1982) have suggested that acute ammonia toxicity mechanism in fish is comparable to that of mammals in causing severe physio-metabolic alterations in the central nervous system involving the impairment of cerebral energy metabolism pathway. The basic stress effector system in mammals is thought to be the neuro-endocrine pathway from the hypothalamus via the pituitary to the adrenal gland; so called 'pituitary-adrenal-system' (PAS) which is responsible for the control of physiological changes (Selye 1950). In the teleosts also, comparable to the mammalian PAS, the pituitary-interrenal (adrenal) - axis (PIA) have been suggested to perform the similar function (Bentley 1976). In freshwater teleost fishes, ammonia is excreted but a slight increase in the ambient ammonia concentration results in ammonia stress (Chatterjee and Bhattacharya 1983). The above authors have also reported that in C. punctatus, a 72 ppm ammonia exposure for 48h resulted in more than 50% depletion of the hepatic glutathione pool, which plays a major role in reducing the chemical toxicity induced by xenobiotics. In fish, Tilapia mossambica, ammonia stress due to exposure to ammonium acetate causes variable changes in the total body weight; tissue somatic index of liver, brain, gill, and muscle; percentage tissue water content; and protein and lipid

levels (Begum et al.1984). They have also suggested that at low dose and short exposure, ammonia acts as a 'stimulant', whereas at high dose for a prolonged period it works as 'toxicant'.

Gill tissue damage is common among fish exposed to ammonia. The type of lesion varies, depending upon the concentration and duration of exposure. Acute exposure results in degenerative lesions which include hypertrophy and necrosis of epithelial cells, and separation of epithelium due to edema, whereas chronic exposure induces the deleterious proliferative lesions (Thurston et al.1984). In our earlier studies on C.punctatus,we have found that a prolonged exposure of 6 months to safe (100 ppm) and sublethal (500 ppm) concentrations of $(NH_4)_2SO_4$ fertilizer caused the extensive cellular degeneration resulting in the impairment of physiological functions in liver and thyroid (Ram and Sathyanesan 1987a), and degeneration of the maturing oocytes in ovary (Ram and Sathyanesan 1986a,b) and spermatogenic elements including interstitial cells in testis (Ram and Sathyanesan 1987b) leading to significant retardation of gonadal growth, which might be attributed due to cumulative toxicity of this fertilizer during chronic exposure. In both of the experimental groups of C. punctatus exposed to safe (100 ppm) and sublethal (500 ppm) concentrations of $(NH_4)_2SO_4$ fertilizer, the cortical cells exhibited apparent hyperplasia, degranulation, cellular pyknosis, and nuclear necrosis leading to involution and degeneration of the cells, probably after prolonged hyperactivity, with compensatory changes in the pituitary corticotrophs suggesting the action of this fertilizer as 'adrenal inhibitor'. On the otherhand, in safe dose treated fish, the chromaffin cells were showing slight hypertrophy, whereas in those exposed to sublethal dose these cells revealed atrophic changes characterized by reduced cellular size, pyknosis and nuclear necrosis. Moreover, in the adrenal of sublethal dose treated fish, the observed more pronounced deleterious histopathological alterations might be due to dose-dependent cumulative toxicity of this fertilizer. In Clarias batrachus, exposed for one year to 0.01% concentration of $(NH_4)_2SO_4$ fertilizer, Sathyanesan et al. (1978) have also shown hypertrophy of cortical and medullary cells of the adrenal gland with correlative histological changes in the pituitary corticotrophs.

Thus, on the basis of these results, it can be suggested that ammonium sulfate fertilizer, which is washed into the water systems in small quantities, is capable of inhibiting adrenal function and inducing cellular degeneration either by direct action on the adrenal itself or indirectly via the hypothalamo-pituitary-adrenal axis in this species, in a dose-dependent manner.

Acknowledgments. RNR is grateful to the University Grants Commission of India for the award of Research Associate which made this work possible.

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Received October 12, 1987; accepted July 13, 1988.