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Ammonium Sulfate Induced Nuclear Changes in the Oocyte of the Fish, *Channa punctatus* (Bl.)

Raj Narayan Ram and A. G. Sathyanesan

Department of Zoology, Banaras Hindu University, Varanasi-221005, India

One among the common pollutants present in our reverine and locustrine system is the commonly used fertilizer ammonium sulfate [$(NH_4)_2SO_4$]. Although the unionized ammonia (UIA) base in the water is responsible for the toxicity due to its distinctive penetrative properties but the ionic form can not pass the tissue barriers (Milne et al. 1958). Bhagowati and Rath (1982) reported the retardation of growth and deterioration in the calorific value of fish due to interference of ammonia in aquatic medium. Prolonged exposure of the fish Clarias batrachus to $(NH_4)_2SO_4$ causes endocrine changes (Sathyanesan et al. 1978). However, the deleterious effect of this fertilizer on the reproduction of fishes is not well recorded. In this study, $(NH_4)_2SO_4$ induced degenerative changes in the nucleus of early vitellogenic oocytes of C. punctatus are described.

MATERIALS AND METHODS

Over twenty adult C. punctatus weighing 45 ± 4 g were bought from the local fish market. They were acclimated to the laboratory conditions for 10 days before starting the experiment. They were divided into two groups of 10 each and kept in 40-L glass aquaria having 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 mMhos. The aquaria were kept in natural light and temperature conditions. The average water temperature from January to June was 20, 23, 27, 31, 32 and 35°C, respectively. Group-I was exposed to 100 ppm of $(NH_4)_2SO_4$ fertilizer for 6 months and Group-II served as control. This dose was identified as toxicologically 'safe concentration' for $(NH_4)_2SO_4$ fertilizer in which the fish lived apparently normal. The aquaria water was changed every alternate day after feeding the fish with goat liver. The experiment was started in first week of January when the ovary was in the resting phase and terminated in the last week of June when the control fish exhibited spawning phase ovary with matured vitellogenic stage-IV oocyte. All the specimens were sacrificed at the same time by decapitation and gonads were fixed in Bouin's fluid for histological studies. Paraffin sections were cut at 5 µm thickness, and the ovary was stained with haemotoxylin using eosin as counter stain. The ovarian gonadosomatic index (G.S.I.) was calculated using the formula :

$$\frac{\text{Total Ovary Weight}}{\text{Total Body Weight}} \times 100$$

P- value for G.S.I. was calculated by Student's t-test.

RESULTS AND DISCUSSION

In C. punctatus exposed to 100 ppm $(NH_4)_2SO_4$ for six months, the ovarian growth was inhibited which is characterised by the presence of large number of stage-I, less number of stage-II and few stage-III oocytes whereas the control ovary was fully matured having large number of stage-IV vitellogenic matured oocyte, few stage III, II and I oocytes. The experimental fish did not show any external manifestation of the ammonium toxicity. In the stage-III oocytes of treated fish, the nucleus exhibited degenerative changes which are evidenced initially by the liquefaction of the perinuclear cytoplasm becoming less dense (Fig. 2) followed by the condensation of nucleus rendering it much smaller (Fig. 3) than the control (Fig. 1). In the next stage the nuclear membrane disappears (Fig. 4) and the nucleoli clumped togather and ultimately disappear. Such degenerative changes take place in the stage-III early vitellogenic oocyte. The vacuole-like yolk platelets appear initially in the periphery and in the older ones they migrate towards the centre. As they migrate they become large by deposition of more yolk and by fusion with other platelets (Wallace 1985). In treated fishes after the desolation of the nuclei, the oocytes become atretic and yolk material is resorbed by the proliferating thecal and granulosa cells of the follicular layer. Due to the absence of mature oocytes the G.S.I. (mean $\pm$ S.D.) in the treated fish was significantly lowered (1.13 $\pm$ 0.20, $P < 0.01$) as compared with control (1.80 $\pm$ 0.22).

Chatterjee and Bhattacharya (1983) reported that in

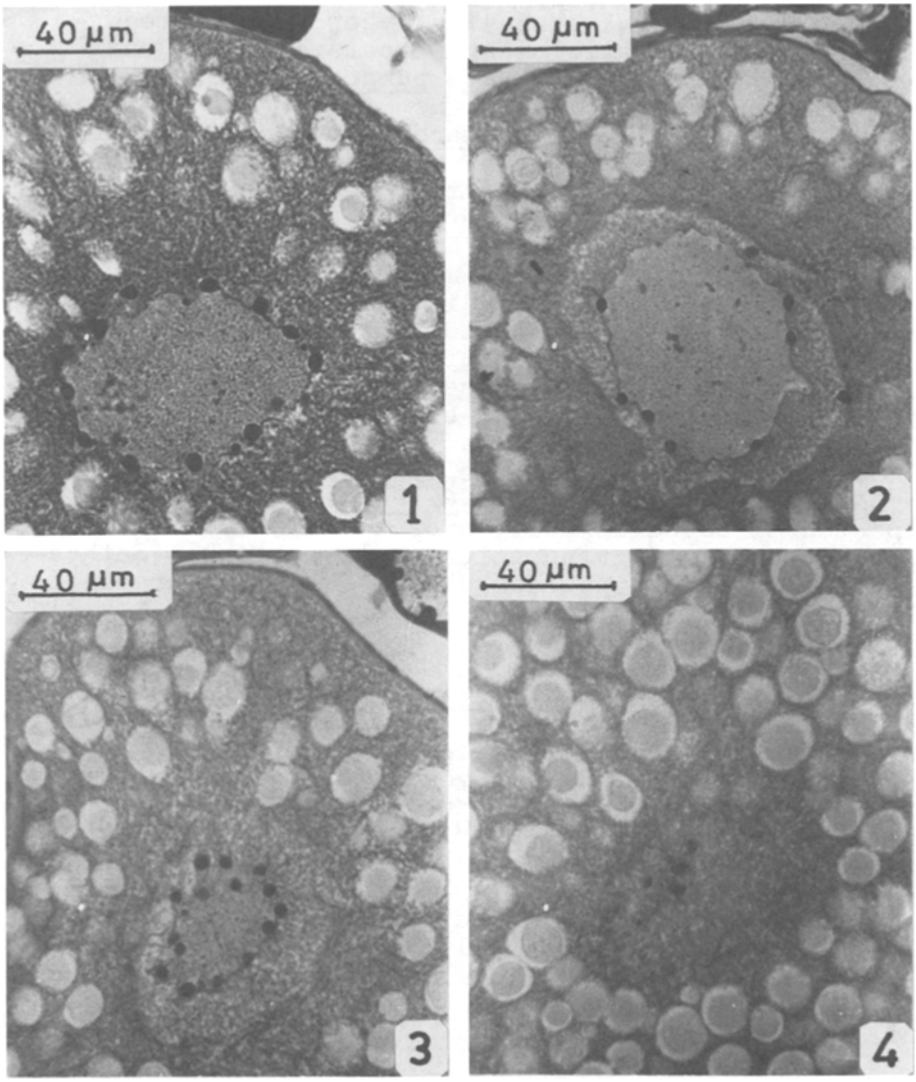

Figure 1. Control stage-III oocyte showing nucleus with large number of peripheral nucleoli.
Figure 2. Stage-III oocyte of $(NH_4)_2SO_4$ treated fish showing perinuclear liquefaction of cytoplasm.
Figure 3. Stage-III oocyte of treated fish showing condensation of nucleus.
Figure 4. Stage-III oocyte of treated fish showing the disappearance of nuclear membrane and clumping up of nucleoli.

C. punctatus, 72 ppm ammonia exposure for 48 hours resulted in more than 50% depletion of the hepatic glutathione pool which plays a major role in reduction of chemical toxicity by xenobiotics. It has been also reported that in *C. punctatus* ammonia exposure caused necrosis of the liver (Mukherjee and Bhattacharya 1975). *C. batrachus* subjected to prolonged exposure to sublethal dose of $(NH_4)_2SO_4$ exhibited marked hypertrophy of the cortical and medullary cells, and the corticotrophs of pituitary (Sathyanesan et al. 1978). Such changes are comparable to those exposed to the metopirone a known adrenal inhibitor. Neural disturbances were reported in the teleosts comparable to that of mammals after intra-peritoneal injection of UIA (Wilson et al. 1969). In teleosts UIA exposure causes the impairment of the cerebral physio-biochemical processes involved in energy metabolism pathway, resulting in a functional alteration in the central nervous system (Schenone et al. 1982). In *C. batrachus* $(NH_4)_2SO_4$ is reported to cause inhibition of thyroid function, and hypertrophy and degranulation of thyrotrophs (Sathyanesan et al. 1978). These changes were comparable to those treated with thiourea, which is a known thyroid inhibitor.

In the experimental *C. punctatus* as the nucleus of the early vitellogenic oocytes degenerates, the ovary is totally devoid of mature oocytes during the spawning phase. However, it is yet to study whether $(NH_4)_2SO_4$ directly acts on the ovary or through the hypothalamo-hypophyseal-ovarian axis.

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REFERENCES

Bhagowati AK, Rath BK (1982) Biochemical composition and nutritional value of three species of hill-stream fish belonging to the genus *Garra* from Northeastern India. Proc Indian Natl Sci Acad 48:67-72

Chatterjee S, Bhattacharya S (1983) Ammonia - induced changes in the hepatic glutathione level of an air-breathing freshwater teleost *Channa punctatus* (Bloch). Toxicol Lett 17:329-333

Milne MD, Scripner BH, Crowfor MA (1958) Non-ionic diffusion and the excretion of weak acids and bases. Amer J Med 24:709-729

Mukherjee S, Bhattacharya S (1975) Histopathological lesions in the hepatopancreas of fish exposed to industrial pollutants. Indian J Exp Biol 13:571-574

Sathyanesan AG, Joy KP, Kulkarni RS (1978) Endocrine changes in fishes in response to pollutants. Quart.J Surg Sci 14:67-77

Schenone G, Arillo A, Margiocco C, Melodia F, Mensi P (1982) Biochemical bases for environmental adoptation in goldfish (Carassius auratus L.): Resistance to ammonia. Ecotoxicol Environ Safety 6:479-488

Wallace RA (1985) Vitellogenesis and Oocyte growth in nonmammalian vertebrates. In: Browder LW (ed) Developmental biology, vol 1. Plenum Publishing Corporation, New York, p 127

Wilson RP, Anderson RO, Bloomfield RA (1969) Ammonia toxicity in selected fishes. Comp Biochem Physiol 28:107-118

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