EFFECT OF CADMIUM CHLORIDE ON THE OVARY OF THE FROG RANA TIGERINA

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Cadmium present in the environment as a contaminant affects all forms of life, including those in aquatic environment. Cadmium is of great concern today because it is highly toxic and its toxicity is cumulative. Some of the prominent effects of this metal are pulmonary and testicular lesions, renal dysfunction, poor bone mineralization, anemia, liver damage, retarded growth, disturbed carbohydrate metabolism and inhibition of drug-metabolizing enzymes. There are no studies on the effect of heavy metals such as cadmium on the amphibian ovary.

The present work deals with the effect of low dose of cadmium chloride on the ovary of the Indian bullfrog, Rana tigerina, an edible frog living in ponds, tanks, streams, etc, which may become exposed to pollutants involving the heavy metals such as the cadmium due to the increased industrialization in recent years.

Adult females of R. tigerina (312-480 g) collected around Karwar in the last week of April were acclimatized for 5 days before use. They were given injections as follows: Group 1. 0.25 ml distilled water (control). Group 2. A total of 600 μg cadmium chloride per frog (each frog received 3 ip injections of 200 μg each at 10-day interval).

The frogs were fed with minced beef on alternate days. They were autopsied on 31st day (10 days after the last injection) and the weights of body, ovary and oviduct were recorded. Ten frogs were used in each group. Pieces of ovary were fixed in Bouin's fluid, embedded in paraffin wax, sectioned at 8 μm thickness and were stained with hematoxylin and eosin for histological observations. The diameter of 20 largest oocytes was measured from the sections taken from different regions of each ovarian sample, and the mean was calculated.

The control frogs were gravid and their ovaries contained fully grown yolky oocytes with clear demarcation between animal and vegetal poles (figure 1). The mean diameter of these yolky oocytes measured 1169 ± 15 μm. The nucleus of these oocytes situated at the animal pole showed highly lobulated nuclear membrane and prominent nucleoli. Zona pellucida was distinct. Several small healthy previtelligenic oocytes were also present. Besides, a few large previtelligenic and early vitelligenic oocytes were seen undergoing atresia. In cadmium-treated frogs the ovaries contained large number of yolky atretic follicles. There was a significant (P < 0.05) decrease in the GSI value (table 1). However, small previtelligenic oocytes less than 300 μm in diameter were normal. The ovaries of 4 out of 10 frogs showed fibrous atretic follicles with multilayered theca (figure 3) and disintegrated granulosa cells. A few sparsely distributed hypertrophied granulosa cells showed pyknotic nuclei. In these oocytes there was a dissolution of the nuclear membrane. The ovaries of the remaining 6 frogs showed many advanced atretic follicles. These atretic follicles showed absence of yolk elements and there was disappearance of the granulosa cells, leaving only the dark brown pigment mass within the thecal elements (figure 4). Improper incorporation of the yolk material was also apparent in some of the atretic follicles (figures 2 and 3). There was a significant decrease in the weights of the oviducts in the cadmium-treated frogs (table 1).

The present findings show for the first time that in amphibians heavy metals such as cadmium severely affect the vitelligenic growth of oocytes (figures 2-4). The improper incorporation of yolk in some follicles due to cadmium (figures 2, 3) may be due to the altered permeability of the blood vessels supplying yolk to ovarian follicles. In amphibians yolk precursors are produced in the liver and then transported to the oocytes. Cadmium is known to cause liver dysfunction and therefore it may interfere with the production of yolk precursors. In addition, the oocytes became atretic accompanied by a marked fibrosis of the thecal layer due to cadmium (figure 3). Fibrous atretic follicles occur rarely in the normal ovaries of the frog. The fibrosis of the thecal layer may be due to the accumulation of cadmium in their cells.

The significant decrease in the weight of oviducts

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<th>Table 1 Effect of cadmium chloride on the ovaries and oviducts of R. tigerina during the pre-breeding period (May)</th>
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<td>Group</td>
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Values are Mean ± S.E of observations from 10 specimens. P values between control and CdCl₂ treated groups are calculated by using student's t test.
Figures 1–4. 1. Cross-section of ovary of control frog showing normal yolk oocytes. 2–4. Cross-sections of ovary of CdCl₂-treated frogs showing an early atretic yolk oocyte with unequal distribution of yolk between animal (A) and Vegetal (V) poles (figure 2), fibrous (arrow) atretic follicles (figure 3) and atretic follicles (AF) in various stages of degeneration (figure 4). (Scale lines = 200 μm.)
solution and the tissues overlying the brain cleared away. Methylene blue and Delafield's haematoxylin techniques were applied on the dissected area. Methylene blue technique proved to be more suitable for the study of smaller nerves. The dissections were carried out in distilled water under a stereoscopic binocular microscope.

The stomodeal nervous system of *H. furcifer* comprises the frontal ganglion and its nerves, the recurrent nerve, the retrocerebral complex and the inguinal ganglion and its nerves. The retrocerebral complex consists of median hypocerebral ganglion, paired corpora cardiaca and corpora allata.

The small and oval frontal ganglion lies on the anterodorsal surface of the pharynx, in front of the brain and is connected with it by a pair of frontal ganglion connectives. These connectives arise from the anterolateral side of the ganglion which runs laterally outwards and then curve medially to meet the basal side of the tritocerebral lobe of the brain. Anteriorly the frontal ganglion gives rise to a median, long and slender nerve, the frontal nerve or the procurent nerve. The frontal nerve runs on the basal side of the pharynx innervating the walls of the labrum. Three short and slender nerves (*N*₁, *N*₂, *N*₃) emerge from each anterolateral side of the frontal ganglion; out of these *N*₁ is slightly longer than the rest. The various nerves emerging from the frontal ganglion, supply the pharyngeal muscles and the labral muscles. From the posterior end of the frontal ganglion arises a long and thin nerve which runs posteriorly to connect the frontal ganglion with the hypocerebral ganglion. This nerve is known as the recurrent nerve. On its way to the hypocerebral ganglion the recurrent nerve gives rise to five pairs of short and thin nerves which innervate the corresponding areas of the oesophagus. The frontal ganglion is connected with the protocerebrum by a slender median nerve, the nervous connectives arising from the posteroventral side of the frontal ganglion.

Cazal pointed the term retrocerebral complex for the ensemble of retrocerebral glands, their nerves and the hypocerebral ganglion. The paired portion of the retrocerebral complex according to Willey includes the anterior corpora cardiaca and the posterior corpora allata.

Hypocerebral ganglion appears as a swelling of the recurrent nerve and remains completely hidden by the dorsally situated corpora cardiaca. It is a very small and elongated structure, lies immediately behind the brain on the posteroventral aspect of the oesophagus. Anterolaterally it is connected with the corpora cardiaca by a pair of short and thick nerves while