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[1]. 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[2]. 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 $\pm$ 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 previtellogenic oocytes were also present. Besides, a few large previtellogenic and early vitellogenic 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 previtellogenic 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 pycnotic 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).

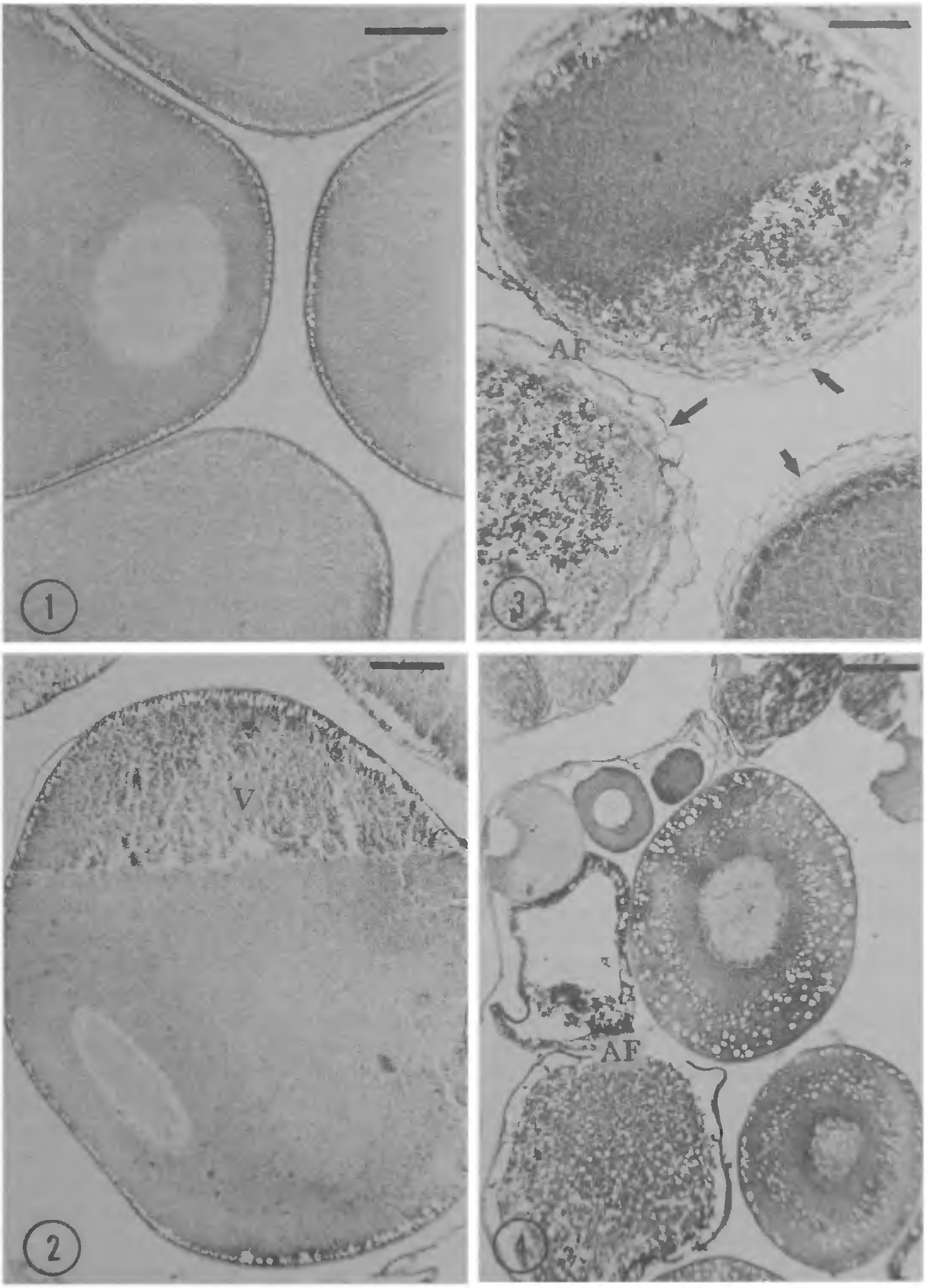
The present findings show for the first time that in amphibians heavy metals such as cadmium severely affect the vitellogenic growth of oocytes (figures 2–4). The improper incorporation of yolk in some follicles due to cadmium (figures 2, 3) may be due to the altered permeability of the blood vessels supplying yolk to ovarian follicles. In amphibians yolk precursors are produced in the liver and then transported to the oocytes[3]. Cadmium is known to cause liver dysfunction and therefore it may interfere with the production of yolk precursors[2]. 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[4]. 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

Table 1 *Effect of cadmium chloride on the ovaries and oviducts of R. tigerina during the pre-breeding period (May)*

Group	Wt of ovary/ 100 g body wt (g)	Wt of oviduct/ 100 g body wt (g)
Control	14.2 ± 1.2	2.8 ± 0.12
$CdCl_2$	10.2 ± 0.9	1.8 ± 0.33
	$P < 0.05$	$P < 0.02$

Values are Mean $\pm$ S.E of observations from 10 specimens. P values between control and $CdCl_2$ treated groups are calculated by using student's t test.



Figures 1–4. **1.** Cross-section of ovary of control frog showing normal yolky oocytes. **2–4.** Cross-sections of ovary of $CdCl_2$-treated frogs showing an early atretic yolky 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 βm.)

(table 1) following cadmium treatment may be attributed to the low titers of estrogens following loss of large follicles (the follicular cells of which are the source of estrogens) in the ovaries of treated frogs.

It is suggested that cadmium even in low doses severely affects the vitellogenic growth of oocytes due probably to the dysfunction of the liver, altered metabolism and possible reduction in the blood and nutrient supply to the growing follicles thereby drastically reducing the fecundity of the female frog *R. tigerina* which is commercially an important species.

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STOMODEAL NERVOUS SYSTEM OF *HIEROGLYPHUS FURCIFER* (ACRIDIDAE—ORTHOPTERA)

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JANET proposed the name stomodeal nervous system in insects. It innervates the organs arising from the stomodaeum and forming a seat of endocrine activity[1]. The investigations of Koestler[2], Bordas[3] and Nabert[4] are more informative. Later, studies on retrocerebral complex of various insect groups were made[5–8]. Many investigators have also described in detail the stomodeal nervous system in a series of selected species of insects[5, 9–12]. The present study deals with the stomodeal nervous system of an Orthopteroid insect, *Hieroglyphus furcifer*.

The specimens for the present study were collected locally during September and October. The heads of freshly killed insects were dissected in insect Ringer solution and the tissues overlying the brain cleared away. Methylene blue[9] and Delafield's haematoxylin techniques[10] 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 ingluvial ganglion and its nerves. The retrocerebral complex consists of median hypocerebral ganglion, paried 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[13] or the procurrent nerve[14]. 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 connectivus arising from the posterodorsal side of the frontal ganglion.

Cazal[7] 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[10] 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 posterodorsal aspect of the oesophagus. Anterolaterally it is connected with the corpora cardiaca by a pair of short and thick nerves while