Bull. Environm. Contam. Toxicol. 30, 24–27 (1983)

Effect of Mercuric Chloride on the Reproductive Cycle of the Teleostean Fish *Channa punctatus*

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Although extensive work has been done on the toxicity of mercury and its compounds, very little is known about their effect on the reproductive cycle. The available investigations largely pertain to mammals (LAMPERTI & PRINTZ 1973, 1974). In pregnant mice, radioactive phenylmercuric acetate is found to accumulate in several areas of the brain including the hypothalamus (BERLIN & ULLBERG 1963). In hamster, LAMPERTI & NIEWENHUIS (1976) suggested the possibility of mercury acting through the hypothalamo-pituitary-gonad axis.

More and more mercuric compounds are entering the aquatic system and large carnivorous fishes are known to accumulate the highest concentration of methylmercury (UNEP/WHO 1976). Hence, in fisheries development point of view, it is important to know the effects of mercury on the reproductive cycle of fish. As such observations on the reproduction of fishes are negligible, this investigation was undertaken to study the changes in the gonadal cycle in response to prolonged exposure to small quantities of mercuric chloride (MC). In this paper, histological changes occurring in the ovary, testis, and pituitary of Channa punctatus in response to continued exposure of 0.01 ppm of MC for six months are described.

MATERIALS AND METHODS

In this investigation, more than 40 adult C. punctatus weighing 40 to 50 g and measuring 8.5 to 10.5 cm in length bought from the Varansi fish market were used. They were acclimated to laboratory conditions for 10 days before starting the experiment. They were divided into two groups of 20 fish each and constituted the experimental and the control groups. The experimental group was kept in a large glass aquaria having 0.01 ppm of MC which was changed every alternate day after feeding. This experiment was started on January 1, 1982 and terminated on June 30, 1982. They were sacrificed by severing the head. The gonads and the pituitary intact with the brain were fixed in Bouin's fluid and Bouin's sublimate, respectively. Paraffin sections were cut at 5μm thickness, and the gonads were stained with Mayer's haemalum using eosin as counter stain. Pituitaries were stained with lead haematoxylin-periodic acid-schiff-Orange G (PbH-PAS-OG), Alcian Blue (AB)-PAS-OG and Mallory's triple stain. The gonadosomatic index (G.S.I.) was calculated using the formula:

0007-4861/83/0030-0024 $01.00

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

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

RESULTS

The gonadal cycle of C. punctatus is divided into five growth phases, namely stages I, II, III, IV, and V which are typical of the teleosts. They denote resting, recrudescence, preparatory, mature, and depleted phases, respectively. These fish were exposed to 0.01 ppm of MC in January when the gonads were in the resting stage, and the experiment was terminated in June when the gonads of the control fish were in the mature phase. The ovary of the exposed fish had only stage I oocytes (Fig. 2). In the control fish, the majority of the oocytes were in the preparatory and mature stages (Fig. 1). The testes of the MC-exposed fish were in the recrudescence stage. Their tubules were filled with secondary spermatogonial cells. The involuted inactive interstitial cells were grouped together at the intertubular junction (Fig. 3) In the control testes, active spermatogenesis was evident, and sperm were also seen in the lumen of the tubules (Fig.4). The interstitial cells exhibited activation in comparison to the experimental fish, characterised by large rounded nuclei with prominent nucleoli(Fig. 4). G.S.I. of the ovary of the control and experimental fish calculated in June are 1.81 ± 0.23 (mean ± SD) and 1.22 ± 0.25 (mean ± SD), P=0.034) whereas that of the testes are 0.16 ± 0.03 (mean ± SD) and 0.088 ± 0.021 (mean ± SD), P=0.06, respectively. Although 0.01 ppm of MC could inhibit the growth of gonads in spite of the favourable environmental conditions of the breeding season, no obvious sign of degeneration was evident in them.

In C. punctatus the gonadal growth cycle could be correlated with the changes in the gonadotrophs of the pituitary. In the control fish having mature gonads, the proximal pars distalis (PPD) is dominated by the actively secreting hypertrophied vacuolated gonadotrophs (Fig. 5). In the experimental fish, the gonadotrophs are small, inactive and fewer in number (Fig. 6), which compares well with the resting phase condition in the gonadal cycle. In the treated fish as in the resting phase the somatotrophs predominate in the PPD but they are in a state of involution characterised by small irregularly shaped nuclei and scanty cytoplasm (Fig. 6) which is very unlike the controls (Fig. 5).

DISCUSSION

In hamster, mercury causes inhibition of follicular maturation, morphological prolongation of the corpora lutea, and fluctuation in the levels of progesterone in the plasma and corpora lutea (LAMPERTI & PRINTZ 1973). According to the above authors, mercury may have a direct effect on the ovary, pituitary, or hypothalamus. Mercury is known to react with the sulfhydryl groups on the cell membrane and impair its function (ROTHSTEIN 1959; PASSOW et al. 1961). NORSETH (1968) has reported accumulation of mercury in lysosomes and mitochondria, which inhibits active transport and cell function.

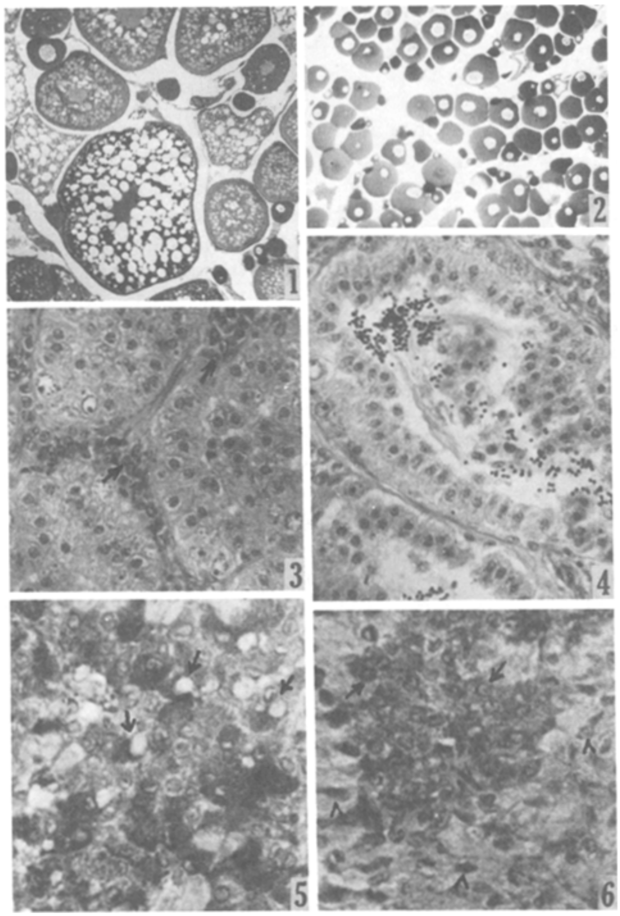

Fig. 1. Control ovary showing large number of stages III and IV oocytes. X50.

Fig. 2. Ovary of mercuric chloride treated fish showing only stage I oocytes. X50.

Fig. 3. Testis of treated fish showing tubules filled with spermatogonial cells. Arrows show inactive interstitial cells. X 50.

Fig. 4. Control testis showing active spermatogenesis and presence of sperms. Arrows show active interstitial cells. X 50.

Fig. 5. Proximal pars distalis of the control fish showing groups of large vacuolated gonadotrophs (arrows). Arrow heads indicate somatotrophs. PbH-PAS-OG. X 750.

Fig. 6. PPD of treated fish showing groups of small gonadotrophs (arrows) and somatotrophs with small irregular shaped involuted nuclei (arrow heads). PbH-PAS-OG. X750.

As mercury is selectively taken up by some neurons of the hypothalamus (CASSANO et al. 1966; NORDBERG & SERENIUS 1969) including the arcuate nucleus (LAMPERTI & PRINTZ 1974) which is responsible for the synthesis of gonadotropin releasing hormone. Its involvement in inhibiting gonadal growth through hypothalamo-hypophyseal gonadal axis is also suggested (LAMPERTI & NIEWENHUIS 1976). MC induced changes in some brain enzymes are reported in Heteropneustes fossilis (SASTRY & SHARMA 1981). Although the pituitary-gonadal relationship in the fishes is well established (review PICKFORD & ATZ 1957; SAGE & BERN 1971; HOLMES & BALL 1974), our knowledge about the different hypothalamic nuclear centres controlling varied endocrine functions in this species is incomplete. In the control C. punctatus, the presence of the mature oocytes could be correlated with the presence of large number of actively secreting gonadotrophs in the proximal pars distalis. In those exposed to MC the gonadotrophs are inactive and less in number which corresponds with the resting phase condition. The involuted state of the somatotrophs in the MC exposed fish indicates an inhibition of the growth process in general.

Progressive spermatogenesis and formation of sperm were noticed in the testes of the controls; whereas in those exposed to MC, only secondary spermatogonia were seen filling up the tubular lumen. The intertubular interstitial cells of the controls showed signs of activity whereas those of the experimentals were involuted. The inhibition of the gonadal growth is reflected on the G.S.I. also. This study shows that continuous exposure to 0.01 ppm of MC inhibited gonadal growth probably through the pituitary-gonadal axis without any obvious sign of degeneration.

Acknowledgements. We are grateful to the Council of Scientific and Industrial Research of India for providing financial assistance.

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