Changes induced by manganese in fish testis

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Summary. Exposure of *Colisa fasciatus*, a freshwater teleost, to 2500 mg/l manganese sulphate for 90 h caused decreased spermatogenic activity and hemorrhage in the testes.

Manganese is released into the freshwater ecosystem by industries such as steel and mining and those which manufacture textile dyes, paints and varnishes, fertilizers, feed additives, ceramics and fungicides. Oshima[1] and Iwao[2] found the toxicity of $MnCl_2$ and $MnSO_4$ for fish in fresh water to be relatively low; their results indicated 24-h lethal concentration limits of 5500 and 3400 mg/l, respectively. The toxic effects of manganese on the CNS, gills, liver mitochondria, and hematology of fish have been studied[3-5]. Degenerative changes in the seminiferous tubules develop after chronic exposure of laboratory mammals to manganese poisoning[6-8], but there are no reports on testicular effects of manganese in fish. The aim of this work was to study the testicular tissue of a freshwater teleost, *Colisa fasciatus* after acute exposure to a sublethal concentration of $MnSO_4$ and to correlate damage, if any, with response profiles in the testis of mammals.

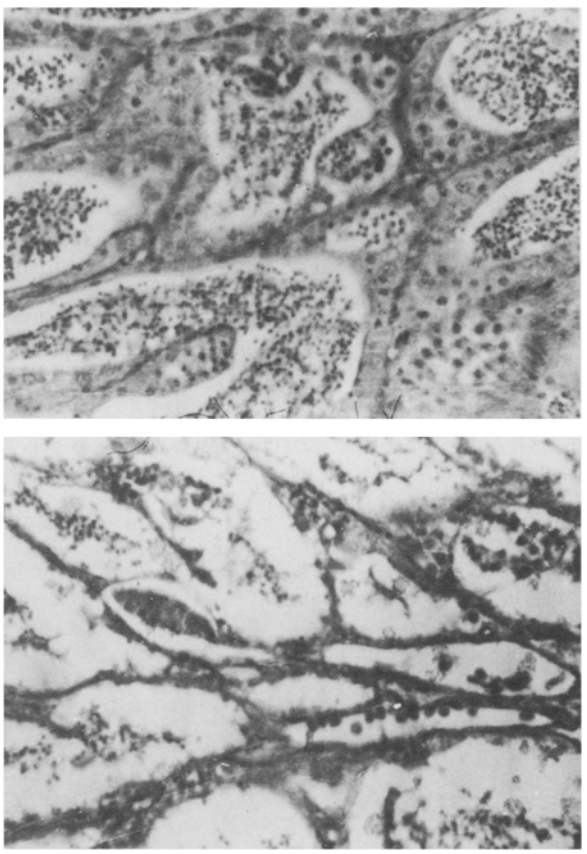
Material and methods. Adult mature males of *C. fasciatus* (weight 5.62 ± 0.34 g) were acclimatized for 10 days in tap water at ambient temperature (25 ± 1 °C) and under natural photoperiod. They were fed daily with dried ground shrimp ad libitum; food was withheld 12 h before and during the experiments. The properties of the test water were: pH 7.3; electrical conductivity 574 µm/h/cm; dissolved oxygen content 6.4 mg/l; hardness 120 mg/l (as $CaCO_3$).

The 96-h LC_{50}-value, based on measured concentrations[9], for $MnSO_4$ was 2850 ppm. Twenty fish were exposed to 2500 ppm $MnSO_4$ for 90 h; the average mortality during this period was 30%. A parallel group of fish not treated with the metal acted as control. Fishes of both the groups were anesthetized with 1 g/3 l MS222 (tricaine methanesulfonate; Sigma Chemical Co., St. Louis) for 2 min and testicular tissue samples were fixed in Bouin-Hollande solution, dehydrated, cleared in xylene, embedded in paraffin wax, sectioned at 5 µm and stained with hematoxylin-eosin stain for histological observation.

Results. The testes of the fish used in experiments were in the prespawning phase, where sperm cells at all stages of development were present in the testis' lobules, since spermatogenesis was still continuing. The paired testes of *Colisa fasciatus* are closely attached to the dorsal abdomen with the help of mesentery. Histologically the testis of the fish consists of lobules of various shapes which are connected with each other by thin connective tissues. The interstitial cells are observed in between the testis lobules. During the spermatogenic process, the sperm mother cell in the germinal epithelium multiplies and produces the primary and secondary spermatocytes, spermatids, and sperms. Distinct blood vessels are present (fig.).

The histology of the testes of manganese-treated *C. fasciatus* showed disorganization of the testis lobules. Most of the lobules had either collapsed or ruptured; the germinal epithelium showed degeneration and desquamation and, in places, was reduced to a sheet of fibrous tissue. The spermatogenic activity was decreased, with a reduction in the number of the primary and secondary spermatocytes and spermatids. Hemorrhage of blood vessels and infiltration by a large number of erythrocytes was observed in the testicular lobules (fig.).

Discussion. This study with *C. fasciatus* demonstrates that the testes in fish are highly sensitive to the deleterious effects of manganese through injury to the vasculature and decreased spermatogenic activity of the testes. Decreased spermatogenic activity has also been observed in rats[6] and rabbits[7,8] during experimental manganese toxicity, but the metal did not cause any vascular lesions. Cadmium, which is physicochemically similar to manganese, produces extensive damage to the blood vessels in the testes of the brook trout, but no detectable damage to the primordial germ cells[10]. In rats, however, Cd can adversely affect the spermatogenic process, possibly through primary injury to the vasculature of the testis[11]. Our experimental results with manganese give no conclusive evidence about whether the damage to the primordial germ cells in the fish testes is direct or secondary to the changes in the vasculature. The mechanism of action of both Cd and Mn requires further investigation in fish. Nevertheless, discharge of manganese in aquatic ecosystem should be regulated as it may affect the reproductive potential of fish.



Top figure shows normal mature testis of control fish. Testis lobules are full of spermatozoa enclosed by the germinal epithelium. Note the normal appearance of blood vessel at the middle right of the photomicrograph. Bottom figure shows the testis of a mature fish exposed to 2500 mg/l $MnSO_4$ for 90 h. Blood vessels are extremely dilated or ruptured. There is invasion of the nucleated erythrocytes into the lobules. Note the disorganization of the lobules and marked decrease in spermatogenic activity. (×400; hematoxylin-eosin.)

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The effect of low doses of X-ray irradiation on cAMP level in Chinese hamster fibroblasts

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Summary. The effect of a stimulating dose of 0.15 Gy on the cyclic adenosine 3′,5′ monophosphate system has been studied. A rapid change is shown in intracellular level of cAMP and in the response of the system to a β-adrenoagonist.

In recent years biological effects of low-level radiation have been the subject of an increasing number of papers. Despite some contradictory early results, the main idea of a stimulating effect is now widely accepted. The latter has been tested on a wide range of subjects at different biological levels[1-3]. Kalendo et al.[4,5] reported in several successive papers on the stimulation of DNA synthesis and mitotic activity after 0.10 Gy of 137_{Cs} radiation. Manzygin et al.[6] found stimulation of cellular proliferation when a synchronized culture was irradiated in the G_1 phase. The above experiments were carried out on Chinese hamster fibroblasts.

Our own experiments show a stimulation of the initial adhesion to substrate of cultured cells after X-ray irradiation at low doses[7]. A maximum effect of approximately 140% (compared to the control) was obtained with a dose of 0.15 Gy. The same dose was used in our experiments described below.

It is well known that cAMP plays a significant role in the regulation of cellular proliferation[8,9]. It also seems clear that the cAMP level correlates positively with cell-to-substrate adhesiveness at least in cells that have become attached[10]. That is why we have studied the direct effect of a stimulating dose of X-ray irradiation on the cAMP level. The ability of the cAMP system to respond to treatment with β-adrenoagonist was studied simultaneously.

Materials and methods. Chinese hamster fibroblastic cells BAB-II-d-ii-FAF-28, aneuploidal clone 431, were grown in flasks containing 250 ml of growth medium Eagle+199 (1:1) supplied with 20% bovine serum and antibiotics (penicillin 100 units/ml and streptomycin 100 mg/ml). Cells in late log phase were washed twice with Hanks' solution, incubated for several minutes with 0.02% EDTA at 37 °C and collected, also in Hanks' solution, by simple shaking. Samples for cAMP determination contained 10^7 cells; at least 3 samples were used for each point in an

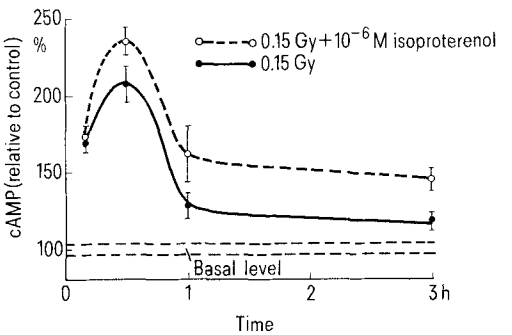

Figure 2. Changes of the intracellular cAMP level after low-dose X-ray irradiation with and without subsequent isoproterenol treatment. The basal cAMP concentration (unirradiated, not isoproterenol treated cells) is 3.35±0.27 pmoles/10^6 cells.

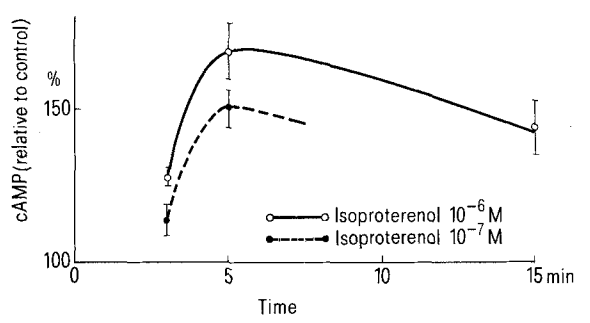

Figure 1. Activation of cAMP production after treatment with isoproterenol. The abscissa shows the incubation time. Concentrations of 10^{-5} M isoproterenol and higher were found to be toxic.

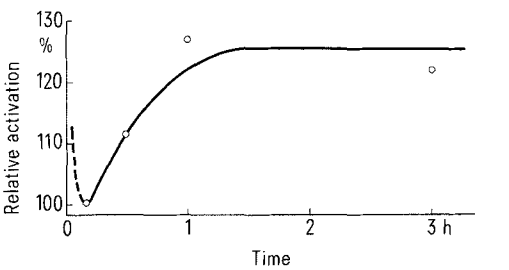

Figure 3. Changes in the relative activation – the ratio between the intracellular cAMP levels in irradiated cells with and without subsequent isoproterenol incubation (0.15 Gy+10^{-6} M isoproterenol): 0.15 Gy. For unirradiated cells the activation of cAMP production with isoproterenol is approximately 170%.