This study implies that in vivo only cholinergic nerve fibres can influence the seminal vesicle epithelium of the guinea-pig. Therefore, if neuronal stimuli do modulate the secretory rate in this tissue, it could only be through the parasympathetic system and not through the sympathetic system.

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Toxic effect of cadmium nitrate on the liver of *Channa punctatus*

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Summary. Exposure of a fresh water fish, *Channa punctatus*, in a medium containing as low as 0.01 ppm of cadmium nitrate, resulted in the necrosis of hepatic cells. A temporary recovery of these cells was however observed when the animals were exposed to lower concentrations.

The advancement of civilization has resulted in an increasing technological use of heavy metals in industries. Cadmium for instance is widely used these days in the manufacture of alloys, pigments for paint and several other industries. This metal is known to pollute the aquatic environment and cause injury to the animals. The target organs often include the liver, the gastrointestinal tract, the respiratory tract and the kidney[2].

Recently Cherian et al.[3], Valberg et al.[4] and Hidalgo et al.[5] reported on the ill effects induced by cadmium on the renal tissue, the gastrointestinal tract and protein synthesis in liver respectively in rodents. Benoit et al.[6], working on the effect of cadmium on 3 generations of brook trout *(Salvelinus fontinalis)*, found that the growth of juvenile of second and third generation offspring was significantly retarded. However, information available on the toxic effects of cadmium on fishes is quite meagre. The present investigation was therefore undertaken to study toxicity induced by cadmium nitrate on the liver of an economically important freshwater fish, the snake-headed fish, *Ch. punctatus*.

Materials and methods. Only adult, healthy fishes of more or less the same size were selected as experimental animals. A number of glass aquaria each of 100:1 capacity were used for experimental work. One aquarium was maintained solely for normal fishes used as control. A stock solution of 1000 ppm of cadmium nitrate was prepared and from this, different quantities were added to other aquaria so as to bring the concentration of cadmium nitrate in the medium, respectively, to 0.01 ppm, 0.03 ppm and 0.05 ppm. About 30–35 laboratory acclimatized specimens of fishes were introduced into each aquarium. All aquaria were properly maintained and the fishes were fed regularly. After specific test periods, i.e. 1, 2, 4, 10, 17, 27, 39, 46, 51 days etc., a few fishes were sacrificed every time and pieces of liver from these animals were immediately fixed in neutral formalin for histopathological studies. The liver from a control fish was also fixed simultaneously. Following the standard technique, paraffin blocks were prepared. Sections of 2–3 µm thickness were taken and double stained with haematoxylin eosin. Some sections of the fresh frozen liver tissue were also stained with Sudan black B for the detection of the fat. The experiments were repeated thrice at random during different periods and a minimum of 3 animals were sacrificed every time.

Observations. Lipid in the form of droplets was observed only when the sections were specifically stained with Sudan black B in both the normal and treated animals. This was done to ascertain that the vacuolization in some sections was not due to the deposition of fat.

The histology of the liver of the normal fish. The liver is comprised of a continuous mass of large hexagonal cells forming laminae or cords. Suspended in the hepatic labyrinth of the laminae are seen a number of blood sinusoids. 2 cells thick wall separates adjacent laminae (figure 1).

The histology of the liver of the fish exposed to cadmium nitrate. On 24 h of exposure, even with the lowest concentration of cadmium nitrate, both the cytoplasmic and nuclear material started precipitating, resulting in the partial vacuolization of the cell. Gradually thereafter, a further reduction of the cytoplasmic material and shrinkage of the nuclei were noticed. After 13 days of exposure to 0.01 ppm and 0.03 ppm concentrations most of the hepatic cells became practically devoid of cytoplasm and vacuolization was more or less complete (figure 2). By this time the nuclei were observed to be at various stages of disintegration and closely pressed towards the border. Whatever was left of the nuclear material in the nuclei, it was seen precipitated towards the border.

Quite interestingly, the regeneration of the protoplasmic material was observed to have started by day 17 in fishes exposed to 0.01 ppm and by day 20 in fishes exposed to 0.03 ppm concentration. With the former concentration, both the cytoplasmic and nuclear material appeared normal by day 28 (figure 3). With 0.03 ppm concentration, the cell structure became normal by day 40. Very soon thereafter, the degeneration of the cellular contents started once again. By day 51, the protoplasmic material had degenerated more or less completely and the decomposed material was seen distributed at random throughout the cell remains.

With higher concentration, i.e. with 0.05 ppm of concentration, the degeneration of the tissue was severe and rapid. By day 20, most of the cells became devoid of protoplasmic contents (figure 4). By day 29, coagulate vacuolization was

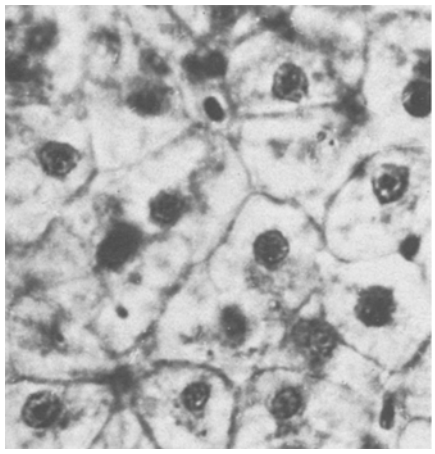

Fig. 1

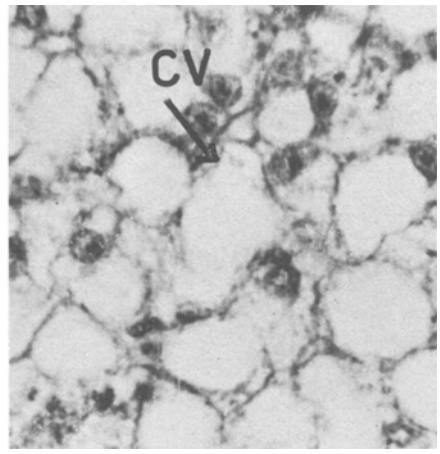

Fig. 2

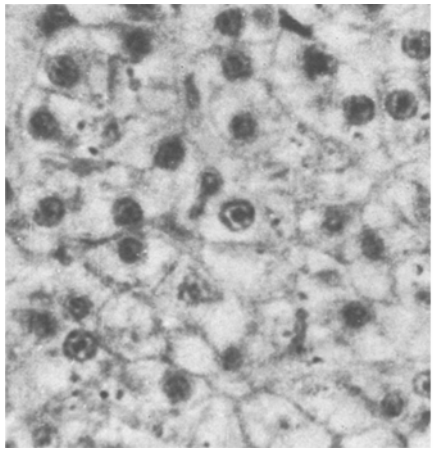

Fig. 3

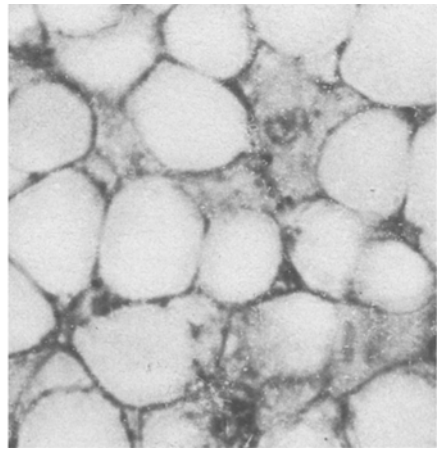

Fig. 4

Fig. 1. The histology of the normal liver of *Channa punctatus* showing cords of hepatic cells. × 1600.

Fig. 2. The liver of the fish exposed to 0.01 ppm concentration for 13 days showing severe damage with most of the hepatic cells completely vacuolated (CV). × 1600.

Fig. 3. The liver of the fish exposed to 0.01 ppm concentration for 28 days showing complete recovery. × 1600.

Fig. 4. The liver of the fish exposed to 0.05 ppm concentration for 20 days, with most of the hepatic cells practically devoid of cytoplasm. × 1600.

observed. No regeneration was noticed except for some negligible quantity of cytoplasm by day 40.

Discussion. During the present studies, different concentrations of cadmium nitrate were observed to induce hepatopathy. Concentration as low as 0.01 ppm brought about necrosis of a few hepatic cells within 24 h. A gradual increase in the damage was seen thereafter. However a remarkable recovery of the cells started by day 17 and by day 28, most of the cells appeared normal with 0.01 ppm concentration. But the recovery did not last long and by day 40, the damage started once again and the effect was more severe.

When the fishes were maintained in higher concentrations of the pollutant, the damage started much earlier and the subsequent recovery which had started became slower and lasted for a less number of days. For instance, compared with fishes exposed to 0.01 ppm concentration, those exposed to 0.03 ppm of the pollutant in the medium recovered rather slowly and for a shorter duration. At a further higher concentration of 0.05 ppm of cadmium nitrate in the medium, the process of degeneration was fast and practically no recovery was noticeable. It is interesting to note here that experiments carried out by Eisler[7] have shown that 10 mg/l of cadmium chloride added in flowing coastal water in which adult killi fish *(Fundulus heteroclitus)* were maintained, no visible effect on the exposed organism was marked. Fat was found to have been deposited both in the livers of the normal animals as well as those exposed to the pollutant. In fact more fat was deposited in the livers of exposed animals. During the present studies, no fat was detected as such in the sections of the liver which were stained with haematoxylin eosin.

It has been reported that the mechanism of cadmium toxicity is probably through combination and with inhibition of sulfahydryl containing enzymes[8]. It is also known to have a direct adverse effect on mitochondria[3]. It is therefore likely that cadmium affects the metabolism of the hepatic cells by inhibition of the enzymes and damage to the organelles. The protein synthesis, however, is not completely suppressed (unpublished data) and recovery is visible for some time in the experimental animals when the concentration of cadmium nitrate introduced in the medium is not particularly high.

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