Cadmium effects on bone and dental tissues of rats in acute and subacute poisoning

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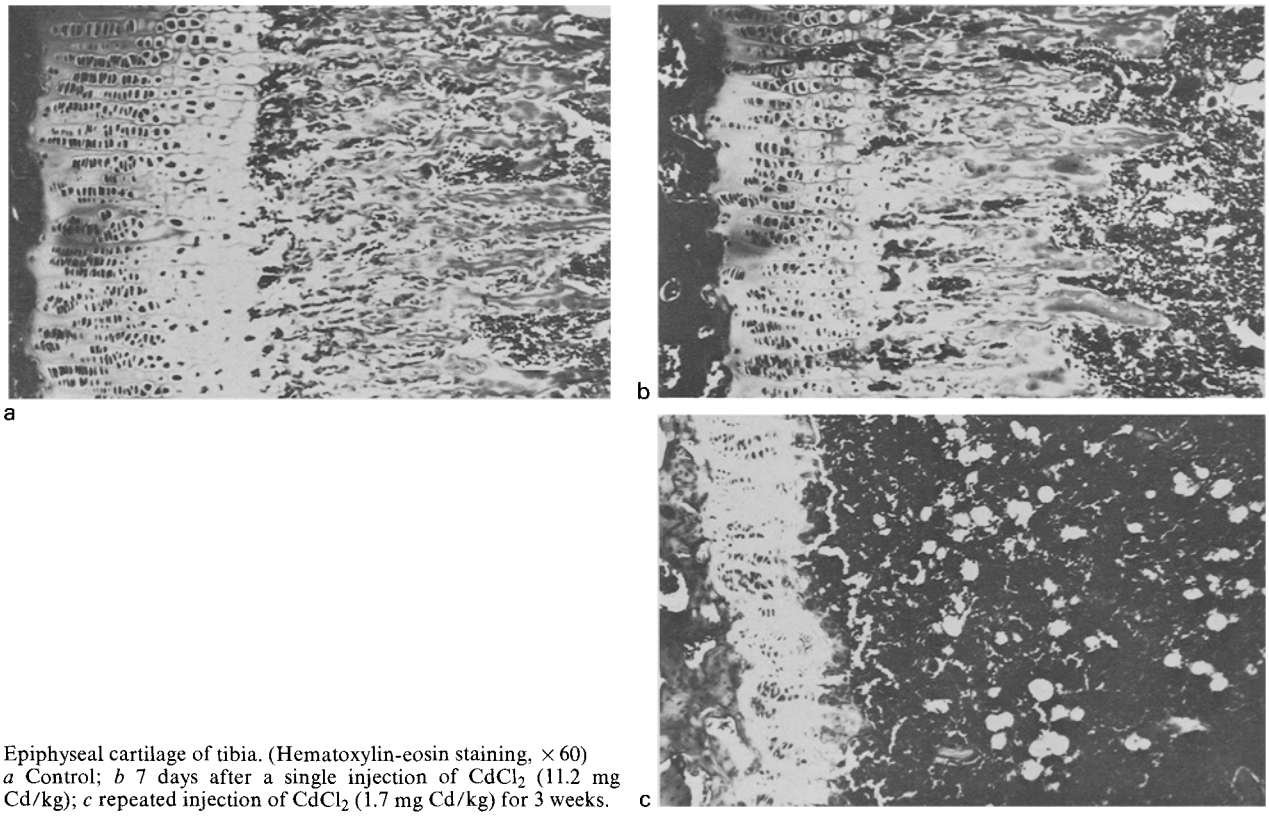
Summary. Injection of cadmium chloride to rats fed by normal calcium diet induced disturbances of tibia and incisor hard tissues metabolism. It is suggested that there may be the possibility of direct cadmium actions on those hard tissues metabolisms.

Although cadmium is known to cause bone lesions in human being and animals, the mechanism by which these lesions are produced remains obscure[2-6]. Many reports have suggested that bone lesions induced by cadmium was coupled with calcium deficiency[7]. Recently, Bawden et al.[8] reported that injected cadmium in young rats was taken up in the bone and dental tissues. The following experiments were undertaken to study the action of injected cadmium on the adult rat tibia and incisor tissues fed normal calcium diet.

Method. Male Wistar rats weighing 250–300 g were used. In the acute experiments, $CdCl_2$ (11.2 mg Cd/kg) was injected s.c. In the subacute experiments, animals were injected s.c. every day for 3 weeks with $CdCl_2$ (1.7 mg Cd/kg). Diet and water were provided ad libitum. In the acute experiment, animals were killed by decapitation after 1 week, and in the subacute experiment, after 3 weeks. Their tibia and incisors were taken immediately for histological and microradiographical studies. In the subacute experiment, Ca^{++} level in serum was estimated by Connerty and Briggs's method[9].

Results. After a single injection of $CdCl_2$, the epiphyseal cartilage zone of tibia was slightly thinner (figure). In the subacute poisoning, this change was more severe (figure); the width of epiphyseal cartilage layer became very narrow and bone trabeculae on the diaphyseal side of the epiphyseal disk have decreased or disappeared. Furthermore, increases of osteoclasts and megakaryocytes in the trabecular zone were observed. The table shows the data on the changes of width of epiphyseal cartilage cells layer. Together with the figure it becomes clear that cadmium acts on the cartilage cells and inhibits their maturation and ossification. Microradiographical studies showed that cadmium caused osteoporotic and not osteomalacic changes in subacute poisoning. As for the dental hard tissues, effects of single injection were very slight, but, in the subacute experiment, odontoblasts have partially disappeared or were deformed in most cases, and dentine-like new tissues appeared between the dentine wall and odontoblast layer. By the microradiographical studies, this new tissues showed a hypercalcified pattern, especially in the zone adjacent to normal dentine. The Ca^{++} level in serum decreased slightly, but it was not statistically significant.

Discussion. Most reports on the cadmium lesions in bones have denied the possibility a direct action of cadmium on bone metabolism and have emphasized that those lesions are secondary to the effects on the systemic calcium metabolism[7]. However, in contrast to other reports, Ca^{++} levels in serum were not significantly different and repeated injection disturbed the dental matrix formation rather than calcification. Suda et al.[10] observed that cadmium administration did not affect the synthesis of vitamin-D of the active form in the rat kidney. Matsue et al.[11] demonstrated that bone lesions occurred before abnormal proteinuria excretion. Bawden et al.[8] reported that injected cadmium



Epiphyseal cartilage of tibia. (Hematoxylin-eosin staining, × 60) *a* Control; *b* 7 days after a single injection of $CdCl_2$ (11.2 mg Cd/kg); *c* repeated injection of $CdCl_2$ (1.7 mg Cd/kg) for 3 weeks.

was taken up strongly in the long bone. In this experiment, cadmium injection to adult rats fed diet with normal calcium levels induced not only the disturbances of the maturation of the epiphyseal cartilage cells, but increased the number of osteoclasts and megakaryocytes in the trabecular area in their tibia. These results may suggest that it is too early to deny the possibility of a direct action of cadmium on the bone metabolism.

Changes of width of proliferative zone induced by repeated

	Body weights at sacrificed (g)	Width (× 10 μm)
Controls	310 ±45.0	21.0±1.0
Cd^{++} group	246.4±66.8	12.4±2.2

Injections s.c. of $CdCl_2$ (1.7 mg Cd/kg) every day for 3 weeks.

1 Acknowledgment. The author wishes to thank Professor Yasumi Ogura for his valuable advices concerning this work.
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Influence of dithiocarb on the biliary excretion of paracetamol and bilirubin in rats

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Summary. In bile-fistula rats, the biliary elimination of conjugated paracetamol and conjugated bilirubin is diminished by simultaneous administration of dithiocarb. This dithiocarb effect could be the result of the interference with the glucuronidation of the compounds.

Dithiocarb was found to be an antidote against paracetamol-induced liver injury in rats and mice[1]. Investigating the mechanism of the antihepatotoxic action, we also studied the effect of dithiocarb on the biliary excretion of paracetamol and bilirubin in bile-fistula rats.

Methods. In male rats (350-450 g) bile duct was cannulated with a polyethylene tube (PE 10) under urethane anaesthesia (1.2 g/kg i.p.) which lasted for the whole experimental period. Body temperature was kept constant at 36.5 °C by using a thermocontroller (Yellow Springs Instruments). Bile sampling was performed for 1-h periods over 8 h after application of 1 g/kg paracetamol p.o. (suspended in 10 ml/kg 1% tylose) and 100 mg/kg dithiocarb i.p.; controls received 10 ml/kg of saline i.p. instead of dithiocarb. Free and conjugated (glucuronide + sulfate) paracetamol was determined by a gas chromatographic method after extraction with ethylacetate and acetylation with acetic anhydride according to Prescott[2]. The amount of conjugated paracetamol was estimated after incubating the bile with glusulase for 16 h. Bilirubin was dissolved in 0.06 N NaOH and injected i.v. (25 mg/kg) into a tail vein, bile sampling was performed for 0.5-h periods over 3 h. Bilirubin was determined with sulfanilic acid as reagent using a commercial kit of Boehringer, Mannheim.

Results. The base line values for bile flow in the control group (1.92±0.10 ml/kg·h) was statistically not different from those in both experimental groups. As shown in figure 1, paracetamol nearly doubled bile flow during the whole time of observation as compared to controls which received 10 ml/kg tylose p.o. and 10 ml/kg saline i.p. The simultaneous treatment with dithiocarb (100 mg/kg i.p.) did not significantly alter the paracetamol-increased bile

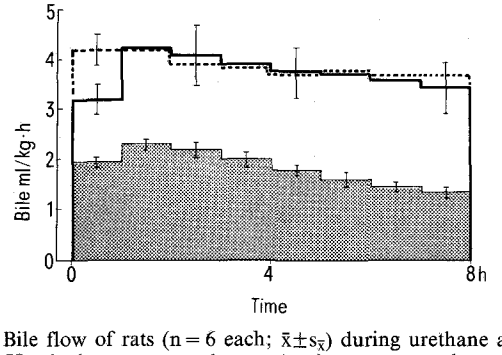

Fig. 1. Bile flow of rats (n = 6 each; $\bar{x}\pm s_{\bar{x}}$) during urethane anaesthesia. Hatched area = controls; —— 1 g/kg paracetamol p.o.; - - - 1 g/kg paracetamol p.o. + 100 mg/kg dithiocarb i.p. The base line value of controls (1.92±0.10 ml/kg·h) was statistically not different from those of the experimental groups.

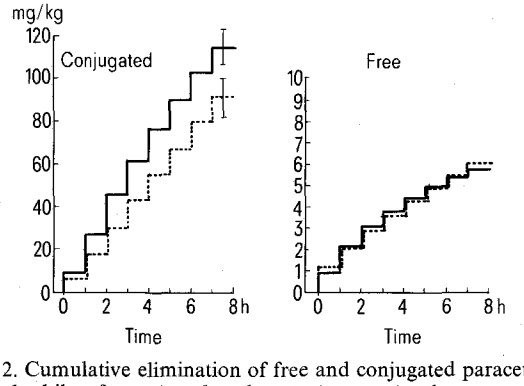

Fig. 2. Cumulative elimination of free and conjugated paracetamol into the bile of rats (n = 6 each; $\bar{x}\pm s_{\bar{x}}$). —— 1 g/kg paracetamol p.o.; - - - 1 g/kg paracetamol p.o. + 100 mg/kg dithiocarb i.p.