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

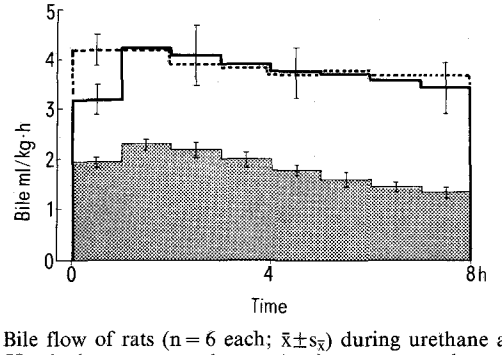

Fig. 1. Bile flow of rats (n = 6 each; $\bar{x}\pm s_{\bar{x}}$) during urethane anaesthesia. Hatched area = controls; —— 1 g/kg paracetamol p.o.; - - - 1 g/kg paracetamol p.o. + 100 mg/kg dithiocarb i.p. The base line value of controls (1.92±0.10 ml/kg·h) was statistically not different from those of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

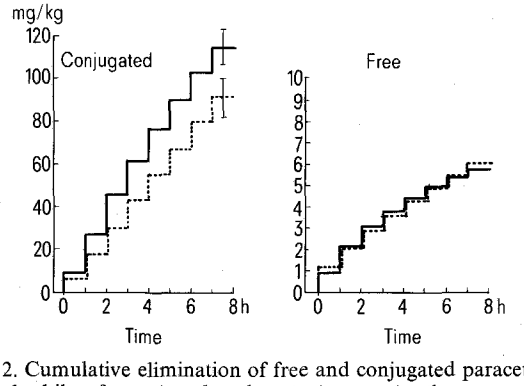

Fig. 2. Cumulative elimination of free and conjugated paracetamol into the bile of rats (n = 6 each; $\bar{x}\pm s_{\bar{x}}$). —— 1 g/kg paracetamol p.o.; - - - 1 g/kg paracetamol p.o. + 100 mg/kg dithiocarb i.p.

flow. The cumulative excretion of free and conjugated paracetamol into the bile of rats is depicted in figure 2. About 12% of the applied dose of paracetamol (1 g/kg p.o.) is eliminated via the bile within 8 h mainly consisting of conjugates (11.5%). Dithiocarb diminished the biliary excretion of conjugated paracetamol, whereas free paracetamol remained unchanged (figure 2).

In further experiments, we investigated the influence of dithiocarb on the biliary excretion of an exogenous bilirubin load because bilirubin is also mainly eliminated via the bile after glucuronidation. As shown in figure 3, nearly 18 mg/kg out of 25 mg/kg bilirubin is excreted into the bile within 3 h, of which 12 mg/kg were conjugated with glucuronic acid. Dithiocarb (100 mg/kg i.p. simultaneously) significantly inhibited biliary elimination of conjugated bilirubin which results also in a reduction of the total amount (figure 3). Bile flow was not altered either by the bilirubin load or by the combined bilirubin-dithiocarb administration (not depicted).

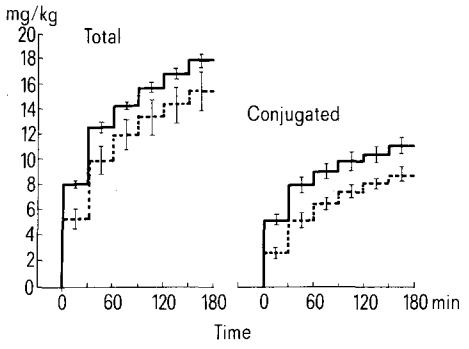

Fig. 3. Cumulative biliary excretion of total and conjucated bilirubin in rats (n = 6 each; $\bar{x} \pm s_{\bar{x}}$). —— 25 mg/kg bilirubin i.v.; - - - 25 mg/kg bilirubin i.v. + 100 mg/kg dithiocarb i.p.

Conclusions. The ability of dithiocarb to reduce the biliary excretion of conjugated paracetamol as well as conjugated bilirubin is not caused by an effect on bile flow, but is rather a consequence of an inhibited conjugation. According to Strömme[3], dithiocarb itself is conjugated to glucuronic acid in the liver and excreted as S-glucuronide. Thus, the effects of dithiocarb on bile excretion of paracetamol and bilirubin are suggested to be the consequence of an interference with the glucuronidation of these drugs. The same interference can be expected for disulfiram also, because this disulfide is hydrolyzed to diethyldithiocarbamate in the organism[3].

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The effect of phenylthiocarbamide (PTC) on mouse brain development

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Summary. PTC - when dispensed during the whole fetal development - causes a disturbance of brain development in new born mice. This disturbance is manifested by a reduction of the cell number, a reduced protein content and a reduced activity of acetylcholinesterase in the brain.

Phenylthiocarbamide (PTC), a substance frequently used in human genetics, was investigated for its acute toxicity and teratogenic effects[1-3], and the chemical similarity of PTC with thiourea suggests that PTC might also have a thyreostatic effect[2,4]. It was shown that after treatment of the mother animals with PTC the weight and nitrogen content of the brains from new-born mice were lowered[2]. Many authors have demonstrated that brain development is considerably impaired by thyroxine deficiency[5-9]. This effect was due to the fact that under hypothyreotic conditions the synthesis of RNA and subsequently protein formation are reduced[10,11]. A lowered cholinergic activity is considered as a further indicator of disturbed brain development due to thyroxine deficiency, because a reduced acetylcholine content[12] or an inhibited activity of acetylcholinesterase[6] were found in brains of hypothyreotic young animals.

In order to confirm a few of these correlations with PTC, pregnant mice were given only PTC in their drinking water, and the contents of DNA, RNA and protein, as well as the activity of acetylcholinesterase, in brains from resulting new-born animals were determined.

Methods. During the whole pregnancy, mice (strain JCR albino) were given only drinking water containing PTC (40 mg/l). The solution was freshly prepared every 2-3 days. The brains of the new-born young were isolated 24-48 h after birth for histological and biochemical analysis.

On 10-µm tissue sections stained according Mallory-Heidenhain, the nuclei were counted over ependymal fields (8 mm^2) of the diencephalon. The total DNA was determined by the method of Zamenhof et al.[13], slightly modified. The total RNA was measured according to Fleck and

Table 1. The effect of PTC on the number of nuclei in the ependyma of the diencephalon from 1-day-old mice. In each tissue section, nuclei were counted in 2 fields both 8 mm^2 in area

Treatment	Number of nuclei per 8 mm^2	%
Controls	189 ± 12	100
PTC-treated	127 ± 13*	67

Number of mice/group: 10. The values are given as the mean ± SEM. *$p < 0.01$ (t-test).

Table 2. The effect of PTC on the DNA, RNA and protein content of the brain from 1-day-old mice

Treatment	DNA (mg/g tissue)	RNA (mg/g tissue)	Protein (mg/g tissue)
Controls	3.7 ± 0.1 (19)	5.9 ± 0.4 (8)	115 ± 2.7 (14)
PTC-treated	3.9 ± 0.1 (23)	6.4 ± 0.4 (8)	97 ± 2.3* (16)

The values are given as the mean ± SEM. *$p < 0.01$ (t-test), in brackets: number of brains.