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).

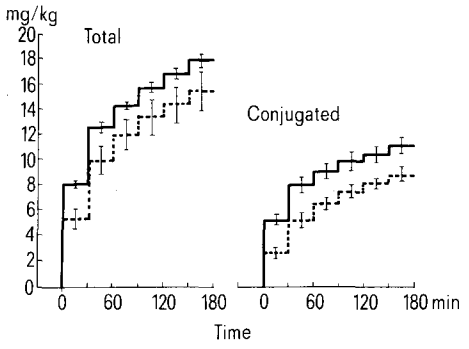

Fig. 3. Cumulative biliary excretion of total and conjucated bilirubin in rats (n = 6 each; $\bar{x} \pm s_{\bar{x}}$). —— 25 mg/kg bilirubin i.v.; - - - 25 mg/kg bilirubin i.v. + 100 mg/kg dithiocarb i.p.

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].

1 O. Strubelt, C.-P. Siegers and A. Schütt, Arch. Tox. *33*, 55 (1974).
2 L.F. Prescott, J. Pharm. Pharmac. *23*, 111 (1971).
3 J.H. Strömme, Biochem. Pharmac. *14*, 393 (1965).

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.

Begg[14]. The protein content was determined by the method of Lowry et al.[15] using bovine serum albumin as a standard. The activity of acetylcholinesterase (EC 3.1.1.7) was measured by the method of Fonnum[16], slightly modified. For all the biochemical analyses, the brains were immediately frozen (−30 °C) and thawed directly prior to analysis.

Results. The number of nuclei in the ependyma of the diencephalon from 1-day-old mice is significantly reduced when the animals have absorbed PTC via the placenta during the whole intrauterine development (table 1). The brains of 1-day-old mice having absorbed PTC during the whole intrauterine development contain less protein than those of control animals. This is valid for the fresh weight as well as DNA or RNA content (table 2). The content of DNA or RNA of the brains from new-born mice treated in this manner remained unchanged compared with control animals (table 2). The activity of acetylcholinesterase is significantly reduced in the brains of likewise pretreated new-born mice (table 3).

Discussion. The central nervous system of new-born mammals is not fully differentiated and biochemically immature. For the completion of brain maturation, euthyreotic conditions are necessary. If, during this critical period, the function of the thyroid gland is impaired, further brain development is retarded. For instance, hypothyreotic young rats show a reduced brain weight[17] and in addition fewer synapses are formed in their cerebellum[18].

The reduction of the number of nuclei in the ependyma of the diencephalon in new-born mice treated with PTC indicates an impaired brain development. However, the histological structure of the thyroid of such mice scarcely differs from that of control animals (unpublished results). Possibly PTC in the concentration used is not strong enough to cause visible changes in the thyroid structure.

The suspected inhibitory effect of PTC on protein synthesis in the brain[2] is confirmed by our demonstration of a reduced protein content after PTC-treatment. A similar effect was described in juvenile rat brains after treatment with propylthiouracil[19]. A change in total DNA or RNA in the whole brain of PTC-pretreated mice was not observed in our experiments, suggesting a relatively weak toxicity of this substance.

Thyroxine also controls the biochemical maturation of neurotransmitter systems. For instance, the content of acetylcholine in 1-day-old rat brains is only 73% of the values from adult brain, referred to g fresh weight[12]. Furthermore, the activity of acetylcholinesterase in young rat brains is reduced under hypothyreotic conditions[7,20]. PTC also causes a reduction of activity of this enzyme in the brain of new-born mice. This is a further indication that PTC causes an inhibition in the brain development by affecting the thyroid gland.

The described influence of PTC on the brain of new-born mice, possibly via an inhibition of thyroxine, is also supported by the observation, that PTC inhibits the metamorphosis of Xenopus larvae (unpublished results).

Table 3. The effect of PTC on the activity of acetylcholinesterase in brains of 1-day-old mice

Treatment	Experiment A	Experiment B
Controls	2.86±0.08 (12)	3.48±0.16 (10)
PTC-treated	1.99±0.05* (10)	2.54±0.09* (10)

The values are presented as μmoles acetylcholine hydrolyzed per min per g brain (mean±SEM). *p=0.02 (Man-Whitney-test), in brackets: number of brains.

1 S.H. Dieke, G.S. Allen and C.P. Richter, J. Pharm. exp. Ther. *90*, 260 (1947).
2 H. Nydegger and H. Stadler, Rev. Suisse Zool. *77*, 775 (1970).
3 P.E. Wheatcroft and Thornburn, Nature New Biol. *235*, 93 (1972).
4 C.P. Richter and K.H. Clisby, Arch. Path. *33*, 46 (1942).
5 R. Balazs, S. Kovacs, P. Teichgräber, W.A. Cocks and J.T. Eayrs, J. Neurochem. *15*, 1335 (1968).
6 S.E. Geel and P.S. Timiras, Endocrinology *80*, 1069 (1967).
7 S.E. Geel and P.S. Timiras, Brain Res. *22*, 63 (1970).
8 J. Nievel, N. Robinson and J.T. Eayrs, Experientia *24*, 677 (1968).
9 S. Talanti and V. Pasanen, Life Sci. *7*, 1245 (1968).
10 H. Stadler, Z. Zellforsch. *117*, 118 (1971).
11 J.R. Tata, Sci. Basis Med. Ann. Rev. *9*, 112 (1969).
12 H. Ladinsky, S. Consolo, G. Peri and S. Garattini, J. Neurochem. *19*, 1947 (1972).
13 S. Zamenhof, L. Grauel, E. Van Marthens and R.A. Stillinger, J. Neurochem. *19*, 61 (1972).
14 A. Fleck and D. Begg, Biochim. biophys. Acta, *108*, 333 (1965).
15 O.H. Lowry, N.J. Rosenbrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
16 F. Fonnum, Biochem. J. *115*, 465 (1969).
17 B.G. Cragg, Brain Res. *18*, 297 (1970).
18 J.L. Nicholson and J. Altman, Science *176*, 530 (1972).
19 A.J. Patel, A. Rabie, P.-D. Lewis and R. Balazs, Brain Res. *104*, 33 (1976).
20 R.D. Rastogni, R.L. Singhal and P.D. Hrdina, Neuropharmacology *14*, 747 (1975).

Interaction between azapropazone and warfarin

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Summary. In vitro studies, using 2 separate techniques, have shown that the anti-inflammatory agent azapropazone caused displacement of warfarin from its plasma albumin binding and it is therefore suggested that such a displacement mechanism may be involved in the reported clinical interaction between these 2 drugs.

A number of non-steroidal anti-inflammatory agents, notably phenylbutazone[1], have been shown to potentiate the anticoagulant effect of warfarin. This type of drug interaction is particularly dangerous since fatal haemorrhage may occur when a patient previously stabilized on warfarin is treated with a drug having phenylbutazone-like properties.

2 possible mechanisms may be implicated in such an interaction: a) a displacement of warfarin from plasma albumin binding sites[2] and b) an effect on warfarin R and S isomer metabolism and elimination[3]. Both mechanisms probably play a role. Preliminary findings[4] from in vitro experiments have suggested that binding of warfarin to