analogous manner to that described for (IIIa) then furnished (IVb) (DMPD) as a crystalline maleic acid salt [mp 131–132][14].

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Summary. A new tricyclic agent with an allenyl side chain experimentally shows antidepressant activity similar to amitriptyline and imipramine but also exerts marked CNS depression. Such dual activity should be of clinical interest for treatment of mixed anxiety and depression.

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Inhibition of Drug Metabolizing Enzymes by Diazepam in Rat Liver

Hypnotics and sedatives are known to stimulate or inhibit the drug-metabolizing enzymes in liver[1,2]. Benzodiazepines are widely used and are frequently prescribed in combination with other drugs. Chlordiazepoxide has been suggested to be an enzyme inducer[3–6]. In one study[7] it was shown that diazepam decreased the pentobarbital sleeping time and serum concentration, but the inducing effect could not be confirmed in another study[6]. Chlordiazepoxide has been shown to stimulate its own metabolism in rat[3], and a tolerance to diazepam has been described in cat[8]. Ethanol seems to enhance the action of diazepam[9] but the mechanisms of these effects of diazepam are not known. We investigated microsomal metabolism of hexobarbital, N-methylaniline and *p*-nitrobenzoic acid as well as the content of cytochrome P-450 in rat liver after diazepam treatment. Further we studied whether diazepam can stimulate its own metabolism and thus explain the developing tolerance. We also compared diazepam to two well-known stimulators of microsomal metabolism, phenobarbital and 3,4-benzpyrene.

Materials and methods. 4 groups of 6 male Sprague-Dawley rats weighing about 200 g were kept on a standard diet. 1 group (controls) received vehicle solvent, 2nd group diazepam 100 mg, 3rd group phenobarbital 80 mg and 4th group 3,4-benzpyrene 20 mg/kg of body weight. All drugs were given by mouth once a day for 6 days in a vehicle solvent consisting of tween 20 and carboxymethyl cellulose (1:4).

Preparation of livers. Animals were decapitated 24 h after the last dose. The livers were removed and rinsed with ice-cold 0.1 *M* phosphate buffer (pH 7.4). All subsequent manipulations were carried out at 2–4 °C. The 20% liver homogenates were prepared in the same phosphate buffer with a Potter-Elvehjem type homogenizer. The homogenate was centrifuged at 12,000 *g* for 20 min. Part of the supernatant was then centrifuged at 105,000 *g* for 1 h. The microsomal pellet was suspended in the phosphate buffer so that 2.5 ml suspension corresponded to 1 g of liver tissue.

Enzyme assays. The 12,000 *g* supernatant was used to assay the hexobarbital hydroxylation, *p*-nitrobenzoic acid reduction, N-methylaniline demethylation and diazepam metabolism. The microsomal suspension was used to measure the cytochrome P-450 content. The incubation conditions for assays of the metabolism of hexobarbital, N-methylaniline and *p*-nitrobenzoic acid have been described by VORNE and ARVELA [10], and that of diazepam by SCHWARTZ and POSTMA[11]. The amounts of the sub-

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Table I. Effect of diazepam (DZP), phenobarbital (PB) and 3,4-benzpyrene (BP) treatment on cytochrome P-450 content and the rates of metabolism of hexobarbital, *p*-nitrobenzoic acid and N-methylaniline in rat liver microsomes

Group	Cytochrome P-450 content (nmol/g liver)	(%)	Rate of metabolism of substrates (nmol/g liver/h)					
			Hexobarbital	(%)	*p*-Nitrobenzoic acid	(%)	N-methylaniline	(%)
Control	8.3 ± 1.6	100	6.95 ± 0.70	100	2.51 ± 0.72	100	1.57 ± 0.29	100
DZP	7.5 ± 0.8	90	4.60 ± 0.93[b]	67	1.49 ± 0.23[a]	59	1.50 ± 0.34	95
PB	19.1 ± 3.4[b]	230	11.10 ± 0.67[b]	160	5.79 ± 2.30[a]	230	4.49 ± 0.58[b]	285
BP	10.6 ± 3.6	128	5.07 ± 1.15[a]	73	2.44 ± 0.43	97	1.43 ± 0.34	91

Mean value ± SD of 6 rats. [a] $p < 0.01$; [b] $p < 0.001$.

Table II. Effect of diazepam (DZP), phenobarbital (PB) and 3,4-benzpyrene (BP) treatment on the metabolism of diazepam in rat liver microsomes

Group	Substrate and metabolites recovered after incubation (nmol)		
	Diazepam	N-methyloxazepam	N-demethyldiazepam
Control	28.10 ± 0.62	2.89 ± 0.62	2.83 ± 0.62
DZP	28.31 ± 0.73	2.92 ± 0.53	2.46 ± 0.28
PB	14.92 ± 1.53[a]	10.35 ± 2.19[a]	8.25 ± 1.89[a]
BP	29.62 ± 0.72	2.84 ± 0.39	2.30 ± 0.34

Mean value ± SD of 6 rats. [a] $p < 0.001$.

strates, the amounts of liver tissues and the incubation times were as follows: Hexobartital 2 μmol, 400 mg, 15 min, N-methylaniline 5 μmol, 400 mg, 30 min, diazepam-C^{14} 35 nmol, 60 mg, 60 min, and *p*-nitrobenzoic acid 3 μmol, 400 mg, 60 min.

The rate of metabolism of substrates and the content of cytochrome P-450 were measured as described previously[12–16]. Student's *t*-test was used in statistical analysis.

Results. Effect of diazepam on some drug-metabolizing microsomal enzymes. The content of cytochrome P-450 and the activities of hexobarbital, *p*-nitrobenzoic acid and N-methylaniline-metabolizing enzymes in rat liver are shown in Table I. Diazepam had no significant effect on the content of cytochrome P-450 or N-demethylation on methylaniline. On the other hand, the oxidation of hexobarbital and reduction of *p*-nitrobenzoic acid were significantly inhibited by diazepam.

Lack of diazepam to change its own metabolism. The amounts of diazepam and its metabolites after incubation can be seen in Table II. Diazepam had no effect on its own metabolism as compared with controls. It is conspicuous that neither 3,4-benzpyrene had any effect on the diazepam metabolism. Oxazepam, one of the metabolites of diazepam, was not found although phenobarbital increased markedly the rate of metabolism of diazepam.

Discussion. In the present study, diazepam delayed the rates of metabolism of hexobarbital and *p*-nitrobenzoic acid, whereas it had neither inhibiting nor stimulating effect on its own metabolism. The cause of tolerance to diazepam remained unsolved in our study. However, one possibility is that some metabolite of diazepam acts as a competitive inhibitor of diazepam, possibly N-desmethyldiazepam as suggested earlier[8]. Another possibility, not excluded in our study, is that there may be changes in the minor pathways of diazepam metabolism, not detected in our methods.

Phenobarbital strongly induced the rate of metabolism of diazepam, which is in good agreement with other studies[11,17]. No oxazepam was noted even after phenobarbital treatment. This agrees well with the study of MARCUCCI et al.[17] made by the same strain of rats as ours, whereas it differs from the study of SCHWARTZ and POSTMA[11] who used another strain of rats in their study. This suggests that the metabolic pathways of diazepam differ in different strains of rats.

The effects of diazepam on drug metabolism differed entirely from those of phenobarbital, but they were to some extent similar to the polycyclic hydrocarbon 3,4-benzpyrene.

Diazepam inhibited some enzyme activities but had no effect on some other enzymes. This finding agrees well with some earlier results[6]. Chlordiazepoxide has been suggested to be an enzyme-inducer in rat[3,6,18] and man[4], whereas nitrazepam in rat[6] and the effect of prazepam is similar to diazepan[19]. In the present study, diazepam had no effect on its own metabolism, which is similar to the finding with prazepam[20] but differs from that of chlordiazepoxide which has been suggested to stimulate its own metabolism[3]. Therefore it is likely that the benzodiazepines differ from each other in regard to their effect on drug metabolizing enzymes.

Zusammenfassung. Nachweis, dass Vorbehandlung von Ratten mit Diazepam den Stoffwechsel von Hexobarbital und *p*-Nitrobenzoesäure in der Rattenleber in vitro hemmt, ohne den Gehalt des Cytochroms P-450 oder den eigenen Metabolismus zu verändern.

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