increased utilization of thiamine due to the higher carbohydrate content in the diet. It is known that carbohydrates tend to increase thiamine consumption and enhance the development of neurologic dysfunction in thiamine-depleted animals[15].

Mice that were fed the different forms of Nutrament diet (groups A, B, and C) for 11 days had more or less the same TPP contents in the cerebellum (table 2) and the values were not significantly different from those seen in mice that were fed ordinary food pellets and water (group D).

We estimated from the amount of diet consumed, mean body weights and thiamine content of the diet, that the daily intake of thiamine by rats fed the Lieber-DeCarli diet was 180–210 µg/kg b.wt. Since the daily requirement of thiamine for rats is 1.25 mg/kg food[16], it can be estimated that the minimal daily thiamine intake for rats is 110–190 µg/kg b.wt. Therefore the rats that were fed the Lieber-DeCarli diet had a daily thiamine intake just above the recommended daily requirement. In the case of Nutrament diet for mice, we estimate the daily thiamine intake from the diet to be 1400–2000 µg/kg b.wt. This is about 3 times in excess of the estimated recommended minimal daily intake[16], namely, 480–600 µg/kg b.wt.

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Effect of praseodymium on drug metabolism in rat liver smooth and rough endoplasmic reticulum

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Summary. A small i.v. dose (3 mg/kg) of a light lanthanon, praseodymium, impairs the drug metabolizing capacity of both the smooth and rough fractions of rat liver endoplasmic reticulum. This decrease in the activity of drug metabolizing enzymes and in the amount of cytochromes P-450 and b_5 is more pronounced in the rough endoplasmic reticulum fraction.

Lanthanons include 15 elements (at. No. 57–71) which are very similar in their chemical and physical character. According to their biological effects these elements can, however, be divided into light (at. No. 57–62) and heavy (at. No. 63–71) lanthanons. The light lanthanons, including praseodymium (Pr) (at. No. 59), are highly hepatotoxic agents[1]. When administered i.v. they cause changes in the ultrastructure of liver cells and several investigations show that the primary attack of lanthanons occurs on the endoplasmic reticulum[2]. Electron microscopic pictures show that after i.v. administration of relatively small amounts of light lanthanons the rough endoplasmic reticulum (RER) decreases while the smooth, vesicular type of endoplasmic reticulum (SER) increases.

In the present study the effect of the light lanthanon, Pr, on the drug metabolism mainly associated with SER, has been compared in these two subcellular fractions of rat liver.

Materials and methods. Adult female Wistar rats (160–180 g) were used. They were given a standard diet (Altromin®) and tap water ad libitum, but 24 h before killing the food was withdrawn. Praseodymium was administered i.v. as a nitrate salt, $Pr(NO_3)_3$. 5 H_2O, in 0.9% saline solution corresponding 3 mg cation/kg. The animals were killed after 1 or 2 days, livers removed and homogenized in 0.1 M phosphate buffer, pH 7.4. To separate the SER and RER subfractions the supernatants obtained after 12,000 × g were submitted to a discontinuous gradient centrifugation according to Fleischer and Kervina[3]. The purity of these fractions was checked by electron microscopy.

To test the drug metabolizing capacity of these fractions the activity of aryl hydrocarbon hydroxylase (AHH) was measured according to Kuntzman et al.[4] and that of aniline hydroxylase (AH) as described by Kato and Gillette[5]. The cytochromes P-450 and b_5 were determined according to Omura and Sato[6] and protein was estimated by the method of Lowry[7].

Results and discussion. In accordance with our earlier findings[8] the administration of a light lanthanon decreases

Table 1. Effect of Praseodymium (3 mg/kg i.v.) on the activities of aryl hydrocarbon hydroxylase (AHH) and aniline hydroxylase (AH) in rat liver smooth (SER) and rough (RER) endoplasmic reticulum and whole microsomes 48 h after injection

	AHH activity (relative fluorescence units/mg protein)	Activity (%)	AH activity (µg p-aminophenol/mg protein/20 min)	Activity (%)
Microsomes (control)	2533 ± 66*	100.0	0.331 ± 0.054	100.0
Microsomes (Pr 3 mg/kg)	729 ± 226	28.8	0.169 ± 0.047	51.1
SER (control)	2450 ± 166	100.0	0.277 ± 0.033	100.0
SER (Pr 3 mg/kg)	720 ± 106	29.4	0.195 ± 0.037	70.4
RER (control)	2569 ± 24	100.0	1.506 ± 0.115	100.0
RER (Pr 3 mg/kg)	344 ± 166	13.4	0.722 ± 0.317	47.9

* ± SD.

Table 2. Effect of praseodymium (3 mg/kg i.v.) on the amounts of cytochromes P-450 and b_5 in rat liver smooth (SER) and rough (RER) endoplasmic reticulum and whole microsomes 24 and 48 h after injection

	P-450 (nmoles/mg protein)	Amount (%)	b_5 (nmoles/mg protein)	Amount (%)
Microsomes				
Control	1.02±0.13*	100.0	0.76±0.11	100.0
24 h	0.80±0.12	78.4	0.56±0.10	73.7
48 h	0.58±0.13	56.8	0.44±0.12	57.9
SER				
Control	1.11±0.15	100.0	0.76±0.16	100.0
24 h	1.02±0.16	91.9	0.62±0.12	81.6
48 h	1.18±0.10	106.3	0.70±0.08	92.1
RER				
Control	1.11±0.16	100.0	0.73±0.15	100.0
24 h	0.52±0.12	46.9	0.34±0.16	46.6
48 h	0.26±0.12	23.4	0.26±0.12	35.6

* ±SD.

the level of both cytochrome P-450 and b_5 and the activities of drug metabolizing enzymes such as AHH and AH in rat liver. A similar trend was also seen in these enzyme activities in both SER and RER subfractions (table 1). When compared to their corresponding controls the enzyme activities in the RER were more affected. This can be due to the different distribution pattern of light lanthanons between these subfractions. When another light lanthanon, cerium, was injected as Ce-144 i.v. into male rats, nearly all the radioactivity in liver microsomes was found in the RER subfraction (unpublished observation).

The fate of the cytochromes P-450 and 5_5 is shown in table 2. Interestingly, no significant decrease in the cytochrome level was observed in the SER subfraction whereas in the RER a marked decrease was seen. The distinct behaviour of the enzyme activities and the changes in both cytochrome levels may partly be due to changes in the phospholipid content of these fractions after Pr-treatment[9]. Pr was found to increase the phospholipid content in the SER but to decrease the phosphatidylcholine content in the RER. Because the microsomal electron transport chain is known to be composed of 3 main components[10]: a flavoenzyme, phosphatidylcholine and cytochrome P-450 the lack of one of these components, phosphatidylcholine, may be the main reason for the pronounced impairment of drug metabolism especially in the RER subfraction.

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Inhibition of uridinediphosphate glucuronyltransferase caused by furosemide

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Summary. The diuretic agent, furosemide, inhibits liver microsomal uridinediphosphate glucuronyltransferase (EC. 2.4.1.17), in monkey and rat. Inhibition is of the non-competitive type.

Furosemide (4-chloro-N-furfuryl-5-sulfamoylanthranilic acid) is a widely used saluretic in the treatment of edema and hypertension. Many attempts have been made in order to clarify its metabolic fate in the body. Furosemide is mainly eliminated by the kidneys where it is secreted by the proximal tubule cells[3]. Recent investigations[4–9] have revealed that furosemide and its metabolites are excreted via the bile route, too. We could demonstrate that one of the metabolites of the diuretic is the glucuronide[10].

The present paper is concerned with the question whether furosemide has an inhibitory effect on microsomal uridinediphosphate glucuronyltransferase (EC. 2.4.1.17) of liver microsomes in vitro, as this is the enzyme which is responsible for the formation of furosemide glucuronide.

Materials. The following chemicals were used in this study: Ammonium acetate, acetylacetone, guaiacol, salicylic acid, p-nitrophenol, saccharose (from Merck AG, Darmstadt), 4-methylumbelliferone (from EGA-Chemie, Steinheim bei Heidenheim/Brenz), aminopyrine, acetylsalicylic acid, 2-hydroxy-2-ethylbutyryl-N,N-diethylamide (HOE 17,879), furosemide (from Hoechst AG, Frankfurt/Main), calcium chloride, acetic acid, trichloroacetic acid (from Riedel-De Haën, Seelze bei Hannover), and glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, nicotinamide adenine dinucleotide phosphate, uridinediphosphate glucuronic acid (from Boehringer, Mannheim).

Preparation of microsomes. Microsomes were prepared from rat (Wistar) and guinea-pig livers by the calcium chloride precipitation method[11–13] and from rhesus monkey livers by differential centrifugation at 10,000 and 100,000×g. The microsomal pellets obtained by these methods were suspended in isotonic potassium chloride solution in such a way that 1.0 ml of the suspension was equivalent to 100 mg of liver fresh weight.