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STARVATION AND PHENOBARBITAL TREATMENT EFFECTS ON DRUG HYDROXYLATION AND GLUCURONIDATION IN THE RAT LIVER AND SMALL INTESTINAL MUCOSA

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Abstract—Drug hydroxylation and glucuronidation enzyme levels were measured in the liver and small intestinal mucosa of male rats after starvation for 3 days and after starvation combined with phenobarbital treatment (80 mg/kg, 3 days). After simple starvation liver microsomal cytochrome P-450 content and NADPH cytochrome *c* reductase activity were unaffected, while *p*-nitroanisole demethylase activity was increased. Specific activities of the UDPglucose dehydrogenase, total β-glucuronidase and 3-hydroxyacid dehydrogenase were increased, UDPglucuronosyltransferase was unaffected and glucuronolactone dehydrogenase was decreased. When activities were calculated per whole liver, all enzymes tested were decreased during starvation due to the reduction of the liver weight. In the small intestinal mucosa specific enzyme activities were lower in the starved animals, with the exception of UDPglucuronosyltransferase which was not changed. The excretion into the urine of D-glucaric and L-ascorbic acid, two final products of the glucuronic acid pathway in the rat, was decreased by fasting. Phenobarbital treatment proved more effective in inducing several enzymes in the starved animals than in those fed *ad lib*. This compensated for the reduction of total activities due to the loss of liver weight. Despite fasting, the excretion into the urine of D-glucaric and L-ascorbic acid was enhanced after treatment with phenobarbital, and D-glucaric acid reached the levels found in normally fed rats. These findings suggest that starvation impairs drug-metabolism in the rat liver and small intestinal mucosa. Inducibility of the drug-metabolizing enzymes, however, is not depressed by this condition, but on the contrary it is markedly enhanced.

Fasting has been reported to promote regression of the liver weight and remarkable changes in the ultrastructure of the hepatic cell, the most prominent being decrease of the glycogen and extensive proliferation of the smooth endoplastic reticulum [1, 2]. Morphological changes of the intestinal mucosa during fasting have also been described [3]. The influence of these changes on drug-metabolism is of great interest, since people malnourished or completely starving are more susceptible to diseases and thus very often in need of medication. For this reason this topic has gained much attention in recent years. Drug hydroxylation and glucuronidation have been studied, however, separately under various experimental conditions and often with controversial results [4, 5].

In the present report, both drug hydroxylation and glucuronidation were studied in the liver and intestinal mucosa of male rats. Furthermore, phenobarbital was used in combination with starvation, to detect possible changes in the induction level of various enzymes involved in drug-metabolism. NADPH cytochrome *c* reductase (EC 1.6.2.4), cytochrome P-450 and *p*-nitroanisole demethylase were taken as representative parameters of drug hydroxylation. UDPglucose dehydrogenase (EC 1.1.1.22), UDPglucuronosyltransferase (EC 2.4.1.17), β-glucuronidase (EC 3.2.1.31), D-glucuronolactone dehydrogenase (EC 1.1.1.70) and 3-hydroxyacid dehydrogenase (EC 1.1.1.45) were measured to clarify the changes in the glucuronic acid pathway. The flow through the pathway was screened by determining excretion into the urine of D-glucaric and L-ascorbic acid, which are products of the free D-glucuronic acid metabolism in the rat. In addition, changes in the protein, total phospholipid and cholesterol contents of microsomes were studied in connection with starvation and phenobarbital administration.

MATERIALS AND METHODS

Animals. Male albino rats were used of the Wistar/Af/Han/Mol/(Han 67) strain, and weighed 200–220 g. This strain was purchased from Møllegaard Avlslaboratorier A/S (Denmark) and represent the third generation outbred with the rotational mating system in the Laboratory Animal Center of our University. During the experiment the animals were kept in stainless steel metabolic cages. Water was provided *ad lib.* and control animals had free access to pelleted rat food (Hankkija Ltd., Finland). Animals consisting the experimental groups were completely deprived of food for 72 hr. Phenobarbital (E. Merck AG, Darmstadt, Germany) was given intraperitoneally in a dose of 80 mg/kg per day, for 3 days both to starved and fed animals.

Preparation of the tissues

Liver. The animals were sacrificed with a blow on the head and bled by cutting the cervical vessels. The

livers were washed with 0·25 M ice-cold sucrose solution and weighed. After mincing with scissors each liver was washed twice in 10 ml 0·25 M sucrose solution, to remove as much as possible of the trapped blood.

Homogenization was performed with a mechanically-driven Potter–Elvehjem homogenizer (400 rev/min) with five pestle strokes, in 3 vol (w/v) 0·25 M ice-cold sucrose. The homogenate was centrifuged at 10,000 *g* for 15 min (Sorvall SS) and the supernatant at 120,000 *g* for 60 min (MSE 50), to obtain the soluble and the microsomal fraction. The soluble fraction was used for enzyme assays after removing the fatty layer with a Pasteur pipette. The microsomes were suspended in 0·15 M KCl to give about 30 mg protein/ml. The treatment of microsomes with trypsin (1 mg/ml, type III, Sigma Chemical Co., St. Louis, U.S.A.) was made as described by Hänninen and Puukka [6]. The reaction was stopped with trypsin inhibitor (type II-O, Sigma) and the microsomal pellet was harvested by centrifuging at 105,000 *g* for 60 min. The trypsin-digested microsomes were resuspended into 1·5 ml 0·15 M KCl per 1 ml original microsomal suspension.

Intestine. A 10-cm segment of the small intestine was dissected aborally from the pylorus, and it was placed in ice-cold 0·25 M sucrose. The mucosa was scraped off with an ampoule file, after cutting the wall longitudinally and cleaning the intestinal content with moistened blotting paper. The samples were homogenized in 4 vol 0·25 M sucrose with a Potter–Elvehjem homogenizer (370 rev/min, 15 sec). After homogenization, the mucosal suspension was centrifuged at 10,000 *g* for 10 min at 4° (Sorvall SS). The 10,000 *g* supernatant fraction was used for enzyme determinations.

Enzyme assays

p-*Nitroanisole-o-demethylase* activity was determined by measuring the formation of *p*-nitrophenol at 420 nm in a Perkin–Elmer 402 spectrophotometer at 38° [7, 8]. The incubation mixture was 1 ml and contained 70 mM Na phosphate buffer, pH 7·8, 1·1 mM *p*-nitroanisole (Fluka AG, Buchs, Switzerland), 5 mM $MgCl_2$, 0·005 mM $MnCl_2$, 2·5 mM Na_3 isocitrate (Fluka), 1 mM NADP and 0·5 I.U. pig isocitrate dehydrogenase (both from Boehringer & Sohne, Mannheim, Germany), and about 1 mg protein from native microsomes.

NADPH cytochrome c reductase activity was determined by monitoring cytochrome *c* reduction at 550 nm in a Perkin–Elmer 402 spectrophotometer [9], at 38°. The assay mixture consisted of 0·1 M phosphate buffer of pH 7·4, 0·3 mM KCN, 50 μM cytochrome *c* (Sigma), 0·15 mM NADPH (Sigma) and about 0·2 mg of microsomal protein.

Cytochrome P-450 concentration was measured as described by Omura and Sato [10] using the extinction coefficient of 91 mM^{-1} cm^{-1}. For the determination, microsomes were diluted with phosphate buffer (final concn 0·1 M, pH 7·4) to give about 2 mg/ml microsomal protein.

UDPGlucose, D-glucuronolactone and 3-hydroxyacid dehydrogenases were measured with a Perkin–Elmer model 402 spectrophotometer by following the formation of reduced NAD at 340 nm. In each measurement, a cuvette without the substrate was used as the blank. The enzyme activity was calculated by using the molar extinction coefficient for reduced NAD, $6·2 \times 10^3$.

UDPGlucose dehydrogenase was determined from the soluble fraction in a final volume of 1 ml containing glycine buffer (0·05 M, pH 8·7), uridine-5-diphosphoglucose (Sigma) (0·15 mM) and NAD (Boehringer) (0·8 mM) at 25° [11].

UDPGlucuronosyltransferase activity was determined from native microsomes by the method of Isselbacher [12] as modified by Hänninen [13] using 0·35 mM *p*-nitrophenol (Merck) as aglycone and a 4·5 mM concentration of uridinediphosphate glucuronic acid (UDPGlcUA) (ammonium salt, 98%, Sigma) in the presence of 10 mM K_2-EDTA (Merck). In order to ensure the linearity of the reaction with time, the preparations were diluted so that no more than about 20–30% of the *p*-nitrophenol added was consumed during 20 min at 38°.

β-Glucuronidase was measured according to the method of Bernfeld *et al.* [14] as modified by Hänninen [13]. Liver and intestinal mucosa were homogenized in 0·01% (v/v) Triton X-100 solution and centrifuged at 10,000 *g* for 15 min. The supernatant was used for the enzyme determination, to obtain the 'total' activity. Another part of the tissue was homogenized conventionally with 0·25 M sucrose and activity was then determined from the microsomal fraction of the liver and the 10,000-*g* supernatant of the intestinal mucosa.

D-*Glucuronolactone dehydrogenase* was measured from the soluble fraction at 38° and in a final volume of 1 ml containing D-glucuronolactone (Fluka) (28 mM), NAD (1·6 mM) and phosphate buffer (0·08 M, pH 7·8) [15].

3-*Hydroxyacid dehydrogenase* was measured from the soluble fraction at 38° in a final volume of 1 ml containing glycine buffer (0·05 M, pH 8·7), sodium L-gulonate (0·01 M), L-cysteine (2 mM) and NAD (0·8 mM). L-Gulonate was prepared from L-gulono-1,4-lactone (Nutritional Biochemicals Co., Cleveland, Ohio) solution incubated with an equimolar amount of sodium hydroxide overnight at 60° [16].

Protein determination was carried out by the biuret method [17] using bovine serum albumin (Sigma) as the reference protein. When the samples were measured, they were decolorized by KCN and the background was determined once more at 555 nm.

The phospholipid content of the preparations was assayed by extracting a 25-μl aliquot of the microsomes with 4 ml chloroform–ethanol (1:1), and separating the precipitated protein by centrifugation. From the solution 1-ml aliquots were dried under a nitrogen stream, hydrolyzed, and the released inorganic phosphate was determined after Bartlett's method [18]. An unhydrolyzed sample was used as the blank. The inorganic phosphate was calculated as lecithin (Sigma type II-E, MW 734) in mg/ml.

Cholesterol determination was carried out according to the method of Abell *et al.* [19] as modified by Anderson and Keys [20]. Samples were first hydrolyzed by alcoholic KOH and total cholesterol was extracted by petroleum ether (b.p. 60–80°, redistilled). After evaporating the solvent, cholesterol was measured colorimetrically at 620 nm.

L-*Ascorbic acid* was measured in the 24-hr urine, which was collected during the third day of fasting in bottles containing 2 ml oxalic acid (8%). The

Table 1. Changes in the body and liver weight promoted by starvation and combination of starvation and phenobarbital administration (80 mg/mg × 3 days)

Experimental groups	Body wt (g)			Liver wt (g)		
	Prior	Post	% Change	Total organ	% Body wt	% Change
Normal diet (5)	227 ± 34			11·4 ± 1·4	5·1	
Starvation (72 hr) (5)	222 ± 17	170 ± 17	23	5·5 ± 0·7	3·2	38
Normal diet + phenobarbital (5)	215 ± 6			12·7 ± 0·9	5·9	
Starvation (72 hr) + phenobarbital (5)	205 ± 5	170 ± 8	17	7·5 ± 0·4	3·6	39

The number of animals is given in parentheses.

method of Owen and Iggo [21] based on the reduction of 2,6-dichlorophenolindophenol was followed by using 0·5 ml urine sample as the starting material.

D-*Glucaric acid* was measured from 24-hr urine collected without any preservative by following the enzymatic procedure described by Simmons *et al.* [22] based on the inhibition of β-glucuronidase after boiling the urine for 1 hr in acidic (2 M Na formate, pH 3·3) and alkaline buffer (1·75 M Tris, pH 9·0). The method, initially applied for human urine, was modified to meet the standards of urinary D-glucaric acid excretion in the rat. β-Glucuronidase (Tyle B-1, Sigma) was used in the amount of 50 I.U. and a standard inhibition curve was obtained by using D-glucaric acid in a range of 5–200 μg in samples treated with formate buffer. Incubation was performed at 38° for 30 min. Under these conditions, 1/5–1/10 of the daily urine produced inhibition corresponding to the linear part of the semilogarithmic standard curve.

To overcome the difficulty of the poor diuresis during fasting, all animals including the controls were given 10 ml saline i.p. in two divided doses.

RESULTS

Changes in the liver and intestine. Complete deprivation of food for 3 days produced a reduction of the body weight (20%), but far more decreased the liver weight (50%). The liver was 3·2% of the body weight in starved animals and 3·6% in starved phenobarbital-treated rats. Phenobarbital administration to fed animals was more effective in increasing the liver to body weight ratio (Table 1).

Apart from these gross changes in the liver, hepatic microsomal protein, phospholipid and cholesterol content were found to differ among the groups of animals used (Table 2). In native microsomes, protein expressed as mg/g wet wt was significantly reduced after 72 hr of fasting. Also, phospholipids were reduced, while cholesterol was increased when calculated per g wet wt and was doubled when expressed in relation to protein. In trypsin-digested microsomes, no significant changes were observed in these parameters.

After phenobarbital administration, the protein content in fed and starved animals was almost the same, but phospholipids were greatly increased in the fasting group. This was also the case in the cholesterol content. As a result, the ratios of phospholipids and cholesterol to protein were increased. Trypsin digestion of microsomes decreased the phospholipid content by the same factor both in fed and starved animals, so that the difference between them remained significant.

Table 2. Hepatic microsomal protein, phospholipid and cholesterol content in starved animals and after phenobarbital treatment during starvation

	No treatment		Phenobarbital treatment	
	Normal diet	72 hr Starvation	Normal diet	72 hr starvation
Native microsomes	(5)	(5)	(5)	(5)
Protein (mg/g wet wt)	27·0 ± 1·4	20·5 ± 0·06†	28·3 ± 1·1	28·8 ± 0·5
Total phospholipids (mg/g wet wt)	8·6 ± 0·3	7·2 ± 0·2*	9·9 ± 0·2	14·4 ± 0·5†
Phospholipid/protein	0·32	0·35	0·35	0·50
Cholesterol (mg/g wet wt)	0·40 ± 0·01	0·47 ± 0·01*	0·32 ± 0·01	0·45 ± 0·01†
Cholesterol/protein	0·01	0·02	0·01	0·02
Trypsin-digested microsomes				
Protein (mg/g wet wt)	16·6 ± 1·3	14·6 ± 0·5	14·1 ± 1·0	15·9 ± 0·4
Total phospholipids (mg/g wet wt)	3·2 ± 0·1	3·0 ± 0·2	3·2 ± 0·1	4·7 ± 0·2†
Phospholipid/protein	0·19	0·21	0·23	0·30

Protein and phospholipids were also determined after tryptic digestion.
No. of animals is given in parentheses.
* $P < 0·01$.
† $P < 0·001$.

Starvation also induced changes in the duodenal mucosa. The mucosal wet weight was significantly less in the starved animals compared to that in the fed controls.

Because of these pronounced changes in the liver and the intestine, enzyme activities have also been calculated per gram wet tissue wt and, in the case of liver, per total organ.

Drug hydroxylation. Simple starvation produced an insignificant increase of the cytochrome P-450 content, when expressed per mg protein and per g wet wt. Total liver content was significantly decreased due to diminution of the size of the organ. NADPH cytochrome *c* reductase was also slightly increased per unit weight, but significantly decreased, when expressed as total liver activity. *p*-Nitroanisole demethylase was greatly increased in the starved animals, even when expressed as total liver activity (Fig. 1).

Phenobarbital treatment increased the cytochrome P-450 content and NADPH cytochrome *c* reductase activity in the starved animals to a much greater extent than in the fed ones. Also, *p*-nitroanisole demethylase activity was greater in the starved animals even when expressed per whole liver.

NADPH cytochrome *c* reductase measured in the postmitochondrial fraction of the intestinal mucosa did not differ significantly between control and experimental animals during starvation. Nevertheless, after administration of phenobarbital, the activity of this enzyme was greater in fasting animals than in fed ones (0·19 ± 0·1 and 0·12 ± 0·02 μmoles reduced cytochrome *c*/min per mg protein, respectively) ($P < 0.005$).

Enzymes of the glucuronic acid pathway. UDPGlucose dehydrogenase was increased in the liver during fasting (Fig. 2). UDPGlucuronosyltransferase was not affected, while D-glucuronolactone dehydrogenase was lower and 3-hydroxyacid dehydrogenase was increased in the starved animals. Phenobarbital

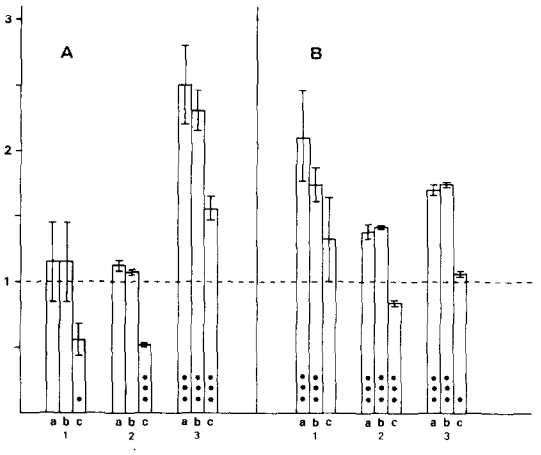

Fig. 1. Relative content of cytochrome P-450 (1) and relative enzyme activities of NADPH cytochrome *c* reductase (2) and *p*-nitroanisole demethylase (3) in the liver microsomal fraction. The mean values (±S.D.) of the experimental animals are compared to the respective controls, considered to be unity. Enzyme levels are expressed as specific activity (a), activity per gram wet tissue wt (b) and activity per whole liver (c). Panel A shows the data from five animals fed *ad lib.* (controls) and five animals which had been starved for 72 hr. In panel B, phenobarbital (80 mg/kg × 3 days) has been given both to fed (controls) and starved animals. Specific activities were as follows—*Panel A* (fed *ad lib.*): (1) 0·53 ± 0·03 nmoles cytochrome P-450/mg protein, (2) 5·0 ± 0·3 μmoles reduced cytochrome *c*/min per mg protein, (3) 0·16 ± 0·05 nmoles *p*-nitrophenol/min per mg protein; *Panel B* (fed *ad lib.* + phenobarbital): (1) 1·26 ± 0·09 nmoles cytochrome P-450/mg protein, (2) 1·8 ± 0·1 μmoles reduced cytochrome *c*/min per mg protein, (3) 0·72 ± 0·04 nmoles *p*-nitrophenol/min per mg protein. (*$P < 0.05$, ***$P < 0.001$).

administration increased the specific activities of the above enzymes to a greater extent in the starved animals than in the fed ones. The same enzymes measured in the duodenal mucosa were found to have

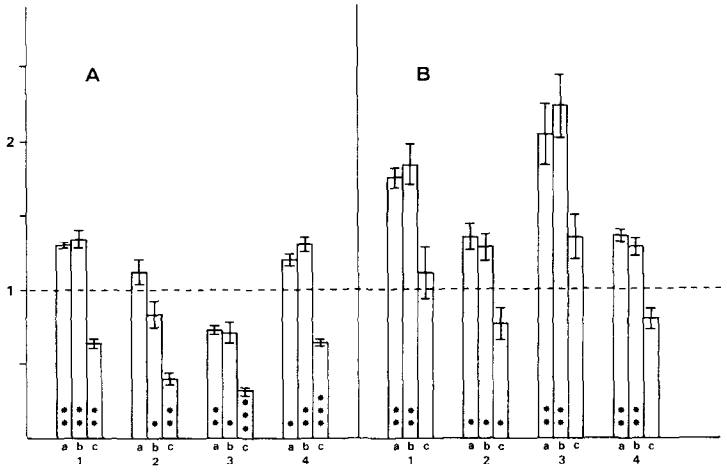

Fig. 2. Relative enzyme activities in the liver of UDPglucose dehydrogenase (1), UDPglucuronosyltransferase (2), D-glucuronolactone dehydrogenase (3) and 3-hydroxyacid dehydrogenase (4). All parameters are expressed as in Fig. 1. Specific activities were as follows—*Panel A* (fed *ad lib.*): (1) 2·7 ± 0·3 nmoles reduced NAD/min per mg protein, (2) 4·2 ± 0·4 nmoles *p*-nitrophenol consumed/min per mg protein, (3) 4·3 ± 0·6 nmoles reduced NAD/min per mg protein, (4) 11·1 ± 1·4 nmoles reduced NAD/min per mg protein; *Panel B* (fed *ad lib.* + phenobarbital): (1) 5·1 ± 0·3 nmoles reduced NAD/min per mg protein, (2) 3·1 ± 0·6 nmoles *p*-nitrophenol consumed/min per mg protein, (3) 10·6 ± 2·8 nmoles reduced NAD/min per mg protein, (4) 10·9 ± 1·2 nmoles reduced NAD/min per mg protein. (*$P < 0.05$, **$P < 0.005$, ***$P < 0.001$).

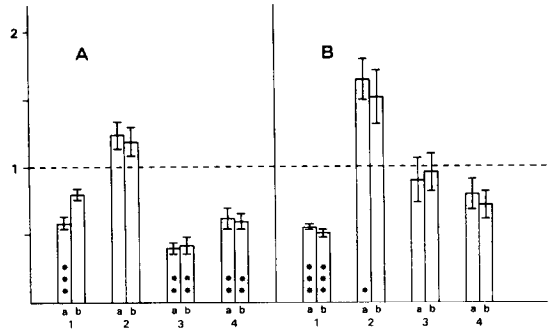

Fig. 3. Relative enzyme activities in the duodenal mucosa of UDPglucose dehydrogenase (1), UDPglucuronosyltransferase (2), D-glucuronolactone dehydrogenase (3) and 3-hydroxyacid dehydrogenase (4). All parameters were calculated as is described in Fig. 1, and are expressed as relative specific activity (a) and relative activity per gram wet tissue wt (b). Specific activities as follows— *Panel A* (fed *ad lib.*): (1) 2·6 ± 0·3 nmoles reduced NAD/min per mg protein, (2) 7·2 ± 1·8 nmoles *p*-nitrophenol consumed/min per mg protein, (3) 1·2 ± 0·3 nmoles reduced NAD/min mg protein, (4) 22·9 ± 4·1 nmoles reduced NAD/min per mg protein; *Panel B* (fed *ad lib.* + phenobarbital): (1) 2·5 ± 0·1 nmoles reduced NAD/min per mg protein, (2) 2·3 ± 0·6 nmoles *p*-nitrophenol consumed/min per mg protein, (3) 1·1 ± 0·08 nmoles reduced NAD/min per mg protein, (4) 12·0 ± 0·5 nmoles reduced NAD/min per mg protein. (*P < 0·050, **P < 0·020, ***P < 0·001).

lower activities in the starved animals even after phenobarbital administration, with the only exception of UDPglucuronosyltransferase which was slightly increased (Fig. 3).

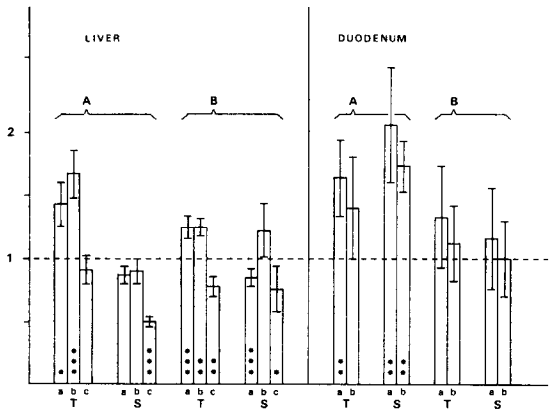

Fig. 4. Relative activity of β-glucuronidase in the liver and the duodenal mucosa of animals fasted for 72 hr, compared to control fed ones (panel A), and of animals fasted for 72 hr and treated with phenobarbital compared to the respective fed controls, also treated with phenobarbital (panel B). In both cases control values were considered as unity. Each group consisted of five animals. Activity has been measured in Triton X-100 (T) and in sucrose (S) homogenized tissues (harvested microsomes in the case of liver) and was calculated as sp. act. (a), activity/g wet tissue wt (b) and activity/total organ (c). Specific activities (nmoles phenolphtalein/min per mg protein) in control animals was as follows—*Liver* (*Panel A*): (T) 196·6 ± 6·6, (S) 86·6 ± 6·6; (*Panel B*): (T) 146·6 ± 3·3, (S) 90·0 ± 3·3. *Duodenum* (*Panel A*): (T) 20·0 ± 3·3, (S) 10·0 ± 1·6; (*Panel B*): (T) 8·0 ± 3·3, (S) 6·3 ± 2·0. (*P < 0·050, **P < 0·025, ***P < 0·005).

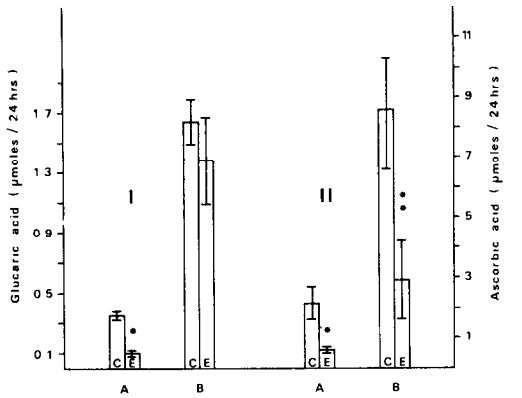

Fig. 5. Excretion of D-glucaric acid (I) and L-ascorbic acid (II) in 24-hr urine. Data for animals fed *ad lib.* (C) and starved for 72 hr (E) are presented without (A) or with (B) phenobarbital treatment (80 mg/kg, 3 days). Each column represents the mean ± S.E.M. from five animals. (*P < 0·02, **P < 0·001).

Activity of β-glucuronidase in the liver tissue homogenized with 0·01% Triton X-100 was increased both in simple starvation and in starvation combined with phenobarbital (Fig. 4). In the sucrose-harvested liver microsomes this enzyme was not affected by starvation and was slightly decreased after phenobarbital treatment. The intestinal mucosa of starved animals had in both Triton X-100 and sucrose homogenates higher activities of β-glucuronidase. This increase fell to the control levels in starved animals treated with phenobarbital.

Both in the liver and intestine, enzyme activities expressed per gram wet wt parallelled well with the respective specific activities (Figs. 1–4). In the case of liver, expression of activities per whole organ showed lower enzyme levels in the starved animals. When starvation was combined with phenobarbital treatment, however, only the total activities of UDP-glucuronosyltransferase and β-glucuronidase were lower in the starved animals, the rest of the enzymes being at the levels found in fed animals treated also with phenobarbital.

Final products of the glucuronic acid pathway. D-Glucaric acid excretion in 24-hr urine after 3 days starvation was about one-third of the control values. This was, however, overcome in the phenobarbital-treated group, where both fed and starved animals had no statistically significant difference. Phenobarbital produced a 4-fold increase in the fed animals and a 14-fold increase in the fasting ones, when compared to the respective controls without drug treatment (Fig. 5). L-Ascorbic acid fell to one-fourth of the values of fed animals. This difference was constant even after phenobarbital administration. In fed animals, phenobarbital produced a 4-fold increase of L-ascorbic acid excretion in 24-hr urine, while in starved animals there was an almost 5-fold increase.

DISCUSSION

Starvation for 3 days was found to promote severe changes in the liver and the activity of the drug-metabolizing enzymes. Microsomal protein was decreased in the starved animals, which is in disagreement with

the results reported by Gigon and Bickel [5]. Although total amount of microsomal phospholipids was decreased in the starved animals, phospholipid content per mg microsomal protein was increased and this was especially marked in the starved animals treated with phenobarbital, which is in agreement with a previous report [23]. In the present study, this change was found to persist despite the tryptic digestion of the microsomes. The cholesterol content of the microsomes has not been determined before. Cholesterol content per mg protein was found to correlate with that of phospholipids, both during starvation and also in starvation combined with phenobarbital treatment.

Starvation did not change the cytochrome P-450 level nor the activity of cytochrome *c* reductase, but greatly increased the activity of *p*-nitroanisole demethylase. Gigon and Bickel [5] and Degkwitz *et al.* [24] have also reported that the cytochrome P-450 level remains unchanged during starvation. On the other hand, all hydroxylation enzymes tested by us were induced to higher levels by phenobarbital during starvation. Despite the decrease of the liver weight, the activity per whole organ was almost the same both in starved and fed animals after a phenobarbital pretreatment.

During starvation, glucose metabolism is significantly altered, in terms of glycolysis, gluconeogenesis and utilization of glucose in the pentose shunt [25, 26]. On this basis, changes could be expected in the intracellular level of UDP-glucose, the active form of glucose under which it enters the glucuronic acid pathway.

UDPGlucose dehydrogenase, the first enzyme of the pathway, was found to be increased in the present study, although Miettinen and Leskinen [27] have reported a decrease under a similar fasting period. This discrepancy might be due to the different preparation of the enzyme used and also to differences in the method of measuring its activity. UDPGlucuronosyltransferase activity in the liver was not changed by simple starvation and this is in agreement with previous studies [27, 28]. In starved animals treated with phenobarbital this enzyme was increased, indicating an overall increase in the glucuronidation capacity.

Total β-glucuronidase activity was increased in the liver and the intestine after a 72-hr starvation. Miettinen and Leskinen [27] have also found an increase of the total hepatic activity in fasted animals. This must be attributed to changes in the lysosomal activity, since microsomal activity was unchanged in the present study. Laborit *et al.* [29] have reported an increased fragility of the lysosomes after fasting. Administration of phenobarbital decreased the microsomal β-glucuronidase activity, while total activity was still higher in the fasted animals, compared to the fed ones which were also treated with phenobarbital. In fed animals, phenobarbital promoted decrease of total β-glucuronidase activity with respect to the untreated controls. This decrease was not, however, significant when activity was expressed per whole organ. Similar results have been reported after treatment with various drug-metabolism inducers [30] including phenobarbital [31].

The duodenal β-glucuronidase activity in fed animals treated with phenobarbital was greatly decreased both in the Triton X-100 and the sucrose homogenized tissue, when compared to the untreated fed controls. The phenobarbital-induced decrease of activity compensated the increase promoted by simple starvation, which had as results a non-significant change in the starved animals treated with phenobarbital.

The significance of the changes in β-glucuronidase activity with respect to the conditions *in vivo* is difficult to interpret. Pyrophosphatase activity has been found to decrease during fasting [27]. UDPGlucuronosyltransferase was not changed in the fasted animals, but the fact that β-glucuronidase was significantly increased suggests that formation of free glucuronic acid during starvation probably takes place via the transferase route. The observed increase of 3-hydroxy-acid dehydrogenase after starvation is in good agreement with that reported by Stirpe and Comporti [32].

D-Glucuronolactone dehydrogenase activity has not been studied before in connection with fasting and administration of phenobarbital during starvation. The depression of this enzyme by starvation and the greater induction by phenobarbital than in the fed controls is difficult to explain. As a result of this, in starved animals treated with phenobarbital, channelling of the free glucuronic acid was significantly changed to the direction of D-glucaric acid formation.

The reduction of D-glucaric acid and L-ascorbic acid excretion in the urine of starved animals cannot be attributed explicitly to the reduction of the liver weight, since it far outstretched it quantitatively. Stirpe and Comporti [32] have reported the decrease of L-ascorbic acid to be due to the reduced activity of L-gulonolactone oxidase. Similarly we found that D-glucuronolactone dehydrogenase, the enzyme responsible for the formation of D-glucaric acid, was significantly lower during starvation. So apart from the reduction of the liver weight, enzymatic factors and perhaps changes in the availability of free glucuronic acid also play a role in D-glucaric and L-ascorbic acid excretion.

Starvation, diabetes and fat-feeding are considered to produce generally the same metabolic changes. On this basis, it has been postulated [33] that D-glucaric acid excretion in starved animals might be enhanced, as this is the case during diabetes. Our results, however, did not prove this hypothesis true. Recently, Sotaniemi *et al.* [34] reported that D-glucaric acid excretion is also decreased in humans during food restriction. The fall of D-glucaric acid and L-ascorbic acid in the urine, and on the other hand the increase of 3-hydroxyacid dehydrogenase activity, make probable that further metabolism of glucuronic acid takes place via the L-xylulose route. This is somehow changed after administration of phenobarbital, which increases the output through the D-glucaric acid pathway.

In the duodenal mucosa, while all other enzymes measured showed a decrease, UDPglucuronosyltransferase was unchanged and even slightly increased when starvation was combined with phenobarbital administration. This suggests that formation of glucuronides is not greatly affected by starvation.

As a rule, starvation has been reported to decrease drug-metabolism *in vivo* of male rats and mice [35–37]. Our findings agree with these observations, since

total hydroxylation and glucuronidation capacity of the liver and the small intestinal mucosa was in general decreased in the starved animals, although specific enzyme activities were occasionally even increased. It has been reported by Kato *et al.* [38] that hypoprotein or aprotein diet diminishes the induction of the microsomal drug-metabolism by phenobarbital. Complete deprivation of food, however, enhanced the elimination of meprobamate from serum and brain and it was even more accelerated when phenobarbital administration was combined with starvation. Furthermore, it has been reported [39] that phenobarbital had an enhanced inducing effect on hydroxylation in fasted castrated female rats. This is in accordance with the present study, where it was shown that the inducibility of several drug-metabolizing enzymes was not deteriorated during fasting. As a matter of fact, induction by phenobarbital was more pronounced in the starved animals than in the fed controls.

The excretion into the urine of D-glucaric acid, although decreased in starved animals, was extremely enhanced in starved animals loaded with a foreign compound. This supports the view of a protecting role of D-glucaric acid synthesis in the detoxifying process of glucuronidation, probably mediated by inhibition of β-glucuronidase activity [40].

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