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EFFECT OF PHENOBARBITAL INDUCTION ON GALACTOSE ELIMINATION CAPACITY IN THE RAT

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Abstract—The capacity to eliminate galactose is used clinically and experimentally as a quantitative test of liver function. Patients with liver disorders often receive drugs which induce changes in hepatic enzymes, and therefore the influence of such drugs on the galactose elimination capacity in rats was investigated.

A paired experiment was performed with 11 pairs of female Wistar rats weighing about 200 g. Each pair received daily intraperitoneal injections of either phenobarbital (50 mg/kg) or isotonic saline for 8 days. The galactose elimination capacity was estimated by intravenous infusion of galactose.

Phenobarbital increased significantly the weight of the liver, the lactate dehydrogenase activity, the microsomal protein, and the hepatic content of cytochrome P-450. The galactose elimination capacity was not significantly changed by phenobarbital and was related neither to the total content of cytochrome P-450 nor to the total lactate dehydrogenase activity.

It is concluded that galactose elimination in rats is independent of enzyme induction by phenobarbital.

THE CAPACITY to eliminate galactose is used clinically as a quantitative measure of "functioning liver mass".[1] It has been shown that during phenobarbital treatment two other liver function tests increase, namely the transport maximum of BSP (bromsulphalein) and the half-time of plasma ICG (Indocyanine Green) in rat and man,[2] probably due to an enhanced biliary flow.[3] Several drugs induce hepatic enzymes[4] and thus might influence the hepatic elimination of galactose. The purpose of the present investigation is to study if phenobarbital induction of the hepatic microsomal enzymes influences the galactose elimination capacity in the rat.

MATERIAL AND METHODS

Female Wistar rats weighing about 200 g at the start of the experiments (Table 1) and fed Altromin® rat pellets *ad lib.* were used.

The experiment was performed in a paired design, consisting of 11 pairs of rats which received daily intraperitoneal injections of 1 ml of either 50 mg/kg of phenobarbital (Chemopol, Prague) or isotonic saline during 8 days.

The galactose elimination capacity[5] was determined 18–20 hr after the last injection. The rats were anaesthezied with thiopental sodium, injected i.p. 100 mg/kg body wt, followed by tracheotomy, intubation and retroperitoneal bilateral nephrectomy. Galactose was administered via a catheter into a jugular vein as a single injection of 280 μmoles (Kabi), followed by continuous infusion of 2·3 μmoles/min during 60 min. After a 20 min period of equilibration blood was sampled every 5 min from a catheter in a carotid artery, and the concentration of galactose was determined enzymatically.[6]

TABLE 1. ELEVEN PAIRS OF RATS TREATED WITH INTRAPERITONEAL INJECITONS OF EITHER PHENOBARBITAL OR ISOTONIC SALINE DURING 8 DAYS

	Phenobarbital (P) (mean ± S.E.M.)	Control (C) (mean ± S.E.M.)	P/C (mean ± S.E.M.)	P (*t*-test, P/C=1)
Initial body wt (g)	195 ± 6	196 ± 7	1·01 ± 0·01	N.S.
Final body wt (g)	201 ± 4	199 ± 6	1·01 ± 0·02	N.S.
Liver wt (g)	9·6 ± 0·2	8·1 ± 0·3	1·20 ± 0·06	<0·01
Lactate dehydrogenase (μmoles/min g liver)	306 ± 58	279 ± 60	1·12 ± 0·05	<0·05
Total lactate dehydrogenase (μmoles/min)	2941 ± 179	2268 ± 175	1·34 ± 0·08	<0·01
Glucose-6-phosphatase* (μmoles/10 min g liver)	1·30 ± 0·64	1·02 ± 0·09	1·30 ± 0·28	N.S.
Microsomal protein (mg/g liver)	28·7 ± 1·1	23·1 ± 0·6	1·25 ± 0·06	<0·001
Cytochrome P-450 (nmoles/mg microsomal protein)	1·26 ± 0·04	1·04 ± 0·02	1·21 ± 0·04	<0·001
Total cytochrome P-450 (nmoles)	348 ± 21	195 ± 8	1·82 ± 0·15	<0·001
Galactose elimination capacity (μmoles/hr)	155 ± 6	143 ± 9	1·14 ± 0·09	N.S.
Galactose elimination capacity (μmoles/hr 100 g rat)	0·77 ± 0·03	0·72 ± 0·05	1·13 ± 0·09	N.S.
Galactose elimination capacity (μmoles/min g liver)	0·27 ± 0·01	0·30 ± 0·02	0·98 ± 0·10	N.S.

* Five pairs of rats only. N.S.—not significant.

The liver was removed immediately after the infussion, placed in a weighed glass containing ice-cold 1·15% KCl and cut with scissors. The glass was weighed again to obtain the liver weight, and the tissue was washed three times with 20 ml of isotonic KCl and homogenized in a Potter–Elvehjem homogenizer. The microsomal fraction was prepared by centrifugation of the liver homogenate in a Sorvall RC-3 centrifuge at 4500 *g* for 60 min and subsequent ultracentrifugation of the pellet at 100,000 *g* for 60 min in a MSE Superspeed 75 ultracentrifuge. The pellet was homogenized in 10 ml 1·15% KCl and the microsomal protein was estimated by the ultraviolet method,[7] cytochrome P-450 by the method of Omura and Sato[8] and glucose-6-phosphatase by assaying inorganic phosphate[9] after incubation with glucose-6-phosphate at pH 6·5. The lactate dehydrogenase activity in the liver homogenate was assayed by following the decrease in absorption of NADH spectrophotometrically at 340 nm at 25°C.

The galactose elimination capacity (GEC) is estimated by

$$\mathrm{GEC} = I - DW\frac{\mathrm{d}c}{\mathrm{d}t} \qquad (1)$$

where I is the infusion rate of galactose, D the relative volume of distribution of galactose (assumed to be 41 per cent of body weight[5]), W the body weight of the animal, and $\mathrm{d}c/\mathrm{d}t$ the slope of the linear regression of the arterial concentration of galactose on time.

RESULTS

Table 1 shows that the initial weight of the rats and the gain in weight during the periods of injections of phenobarbital and saline can be assumed to be identical, whereas the liver weight, the hepatic content of lactate dehydrogenase, and microsomal protein is significantly higher in the phenobarbital group than in the control group. The concentration of glucose-6-phosphatase shows no variation. The amount of cytochrome P-450 is higher in the phenobarbital-treated animals than in the control animals (Table 1).

The galactose elimination capacity (GEC) is not significantly modified by the phenobarbital treatment either per total animal, per g of animal or per g of liver.

The slope of the linear regressions of GEC on the content of cytochrome P-450 (Fig. 1) and on the activity of lactate dehydrogenase is not significantly different from zero (each $P > 0{\cdot}3$).

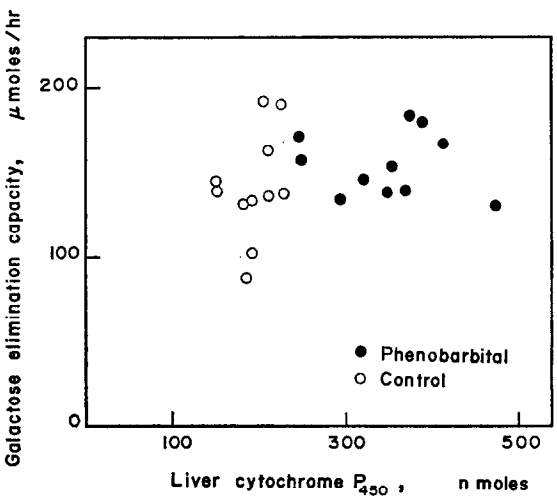

FIG. 1. Relation between galactose elimination capacity and liver content of cytochrome P-450 in phenobarbital and control rats.

DISCUSSION

Conney *et al.*[10] found that daily administration of 100 mg of barbital/kg body wt to rats during 7 days increased the conversion of galactose-1-^{14}C to glucuronic acid pathway intermediates which were excreted in the urine. Probably this is caused by an induction of microsomal enzymes involved in this pathway but they do not report findings concerning the over-all rate of galactose metabolism, so the significance of this pathway for the limitation of galactose elimination can not be evaluated from this work.

Induction of the enzymes of the metabolic pathway of galactose by progesterone has been proposed by Pesch *et al.*[11] They found that progesterone increased the metabolism of galactose-1-^{14}C in three galactosemic patients and decreased the rate of development of cataract in galactose fed rats.

Cuatrecasas and Segal[12] found that galactose feeding to rats increased the galactokinase activity, possible due to substrate induction. Furthermore, they found a higher

activity of galactokinase in newborn than in adult animals, and they suggested that this might explain the greater *in vitro* metabolism of galactose by livers from fetal and newborn than from adult animals, as found by Segal *et al.*[13]

The most important steps in the galactose metabolism are catalysed by cytosolic enzymes (galactokinase,[12] uridyltransferase,[14] and epimerase[15]). Auxiliary pathways, which may be partly microsomal, exist, namely the pathway catalysed by UDP-galactose-pyrophosphorylase,[16] the direct oxidation of galactose to galactonate,[17] and the reduction to galactitol.[18] Microsomal enzymes are involved in the transfer of galactose from UDP-galactose to proteins[19] and lipids,[20] in the glucuronic acid pathway,[10] and in the pathway via galactose-6-phosphate.[21]

In the present study that aspect of the functional capacity of the liver, which is estimated by the galactose elimination capacity (GEC), is not affected by phenobarbital treatment. GEC is of the same order of magnitude as found in other experiments in the rat.[5,22]

The estimation of the galactose elimination capacity includes anaesthesia with thiopental sodium, which might interfere with microsomal oxidation of galactose metabolites, but this would apply to both the control group and the phenobarbital treated rats. It can not be excluded, however, that some phenobarbital may be left in the livers of the phenobarbital treated animals, and this compound might influence the galactose elimination.

The calculation of GEC according to equation (1) involves a correction based on the volume of distribution of galactose (DW), and a systematic difference between both groups with respect to this volume might bias the results. However, the extrapolated blood concentrations at the time of injection, which only depend on the volume of distribution, since the same amount of galactose was given as a priming dose to all animals, are not significantly different ($P > 0{\cdot}3$, paired t-test).

Kunz *et al.*[23] and Platt and Cockrill[24] found that not only microsomal, but also several cytosolic enzyme systems are increased by enzyme induction, although they found a minor, but significant decrease in the lactate dehydrogenase activity.

In our study (Table 1) phenobarbital increases significantly the hepatic microsomal enzyme activity (with cytochrome P-450 as an indicator) 1·82 times, and the cytosolic enzyme activity (with lactate dehydrogenase as an indicator) 1·34 times. The 1·14 times increase of GEC is significantly lower than the increase of cytochrome P-450 ($P < 0{\cdot}001$) but it is not significantly different from the increase of lactate dehydrogenase ($0{\cdot}1 < P < 0{\cdot}2$). Our results are therefore consistent with the view that galactose elimination is limited by the capacity of cytosolic enzymes, e.g. galactokinase, whereas it is extremely unlikely that the activity of a microsomal enzyme is rate limiting. This means that drug induction of the microsomal enzymes presumably does not influence galactose elimination, and that its use as a liver function test is not invalidated by previous administration of enzyme inducing drugs.

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