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Effect of experimental cirrhosis on cholephilic dye metabolism and excretion in the rat

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The metabolism and hepatic excretion of a large number of drugs is significantly impaired in cirrhotic patients and animals with experimental cirrhosis [1, 2]. Perturbations in hepatic enzyme activity, hepatic hemodynamics and drug binding are believed to be responsible for changes in drug elimination. The relative importance of these factors, however, is unknown and the problem is further complicated by the variable effects of cirrhosis on the pathways of liver metabolism.

The cholephilic dye sulfobromophthalein (BSP) has been found to be a useful tool for studying hepatobiliary function, as its biliary transport maximum (T_{max}) is known to be modified in various experimental and pathological conditions. Both alterations in the conjugation capacity [3, 4] or in the transport mechanisms *per se* [5] have been found to affect the biliary excretion of the dye. In the present study we have explored the conjugation and hepatobiliary transport of BSP in rats rendered cirrhotic

by chronic exposure to phenobarbital and carbon tetrachloride. To further evaluate the influence of metabolism or transport dependent changes we also studied the effect of cirrhosis on the hepatic transport of dibromosulphthalein (DBSP), a substance which shares the same transport system with BSP but does not require metabolism for biliary excretion [6].

Materials and Methods

Induction of cirrhosis. Male Wistar rats (Charles River, Barcelona, Spain) were housed in cages in a room maintained on a 12 hr light/12 hr dark cycle and at 22–23°. The rats were allowed free access to pelleted food and water throughout. Cirrhosis was induced by chronic exposure to phenobarbital in the drinking water and by gassing with CCl_4 as described by McLean *et al.* [7]. Treatment was carried out for 11 consecutive weeks and was ceased 14 days before the study. Control animals were kept under the same conditions without any treatment.

Experimental procedures. All surgery was carried out under pentobarbital anaesthesia (50 mg/kg body wt i.p.). Rectal temperature was monitored via a thermistor probe and maintained at 37°. After catheterization of the right jugular vein and right carotid artery, a midline abdominal incision was made and the bile duct was cannulated with polyethylene tubing (PE-50).

After collecting two baseline samples of bile, both control and cirrhotic animals received either BSP or DBSP. The dyes (dissolved in 0.145 M NaCl) were administered as an i.v. injection of 2.15 μmol/100 g body wt followed by a 60 min infusion at 215 nmol/min. 100 g body wt. Bile samples were collected under ice at 10 min intervals. At the end of experiments a blood sample was obtained from the carotid artery catheter, the animals were killed by exsanguination and the livers excised and weighed. A specimen of each liver was fixed in 4% buffered formalin for histological examination.

Analytical procedures. Bile flow was measured gravimetrically assuming a bile density of 1.0 g/mL. The concentration of BSP and DBSP in plasma and bile was determined spectrophotometrically at 580 nm after appropriate dilution with 0.05 N NaOH. Liver BSP and DBSP concentrations were measured by the method of Whelan and Combes [8]. Free and conjugated BSP were separated by paper chromatography using as solvent system butan-1-ol/acetic acid/ethanol/water (120/1/20/40). The spots were cut, eluted with water and read at 580 nm after alkalinization. Bile acid concentration in bile was determined by the method of Paumgartner *et al.* [9]. Determination of total glutathione content of liver homogenates was measured as described by Tietze [10]. Glutathione *S*-transferase activity in liver was determined using BSP as substrate [11]. Both glutathione and glutathione *S*-transferase activity were measured in control and cirrhotic animals without administration of cholephilid dyes. Alkaline phosphatase activity in serum was determined by a commercially available enzyme assay kit (Boehringer Mannheim, Germany).

Statistical methods. Means and SEMs were calculated for all data. Significant differences were determined using the Mann–Whitney U test. Values lower than 0.05 were considered to be significant.

Results and Discussion

All animals termed cirrhotic had cirrhosis by gross inspection and histological examination of the liver. Body and liver weight were not significantly altered by experimental cirrhosis, averaging 428 ± 19 and 15.3 ± 1.3 g vs 449 ± 13 and 16.1 ± 1.0 g in the controls. Serum alkaline phosphatase was significantly increased in cirrhotic animals (175 ± 21 units/L vs 122 ± 12 units/L in the controls; $P < 0.05$).

Table 1 shows bile flow and bile acid secretion during the basal period prior to administration of the cholephilic dyes. No significant differences were found between control and cirrhotic animals. Following BSP administration both its maximal biliary concentration and excretion were significantly reduced in cirrhotic as compared to control rats (−27% and −35%, respectively) (Fig. 1). Cumulative excretion of the dye was also significantly lower in cirrhotic than in control animals (18.9 ± 2.1 μmol vs 27.7 ± 3.2 μmol; $P < 0.05$). The decrease in excretion corresponded both to conjugated and unconjugated BSP (Table 1) and was accompanied by a smaller bile flow increase (Fig. 1) as compared to the controls. In cirrhotic rats the serum concentration of BSP at the end of the experiments was significantly higher than control values (+53%) (Table 1). The liver BSP concentration did not significantly differ in both groups of animals (Table 1). Both glutathione concentration and the activity of glutathione *S*-transferase were significantly reduced in the liver of animals with experimental cirrhosis (Table 1).

Bile flow and the biliary concentration and excretion of

Table 1. Bile, serum and liver parameters in control and cirrhotic rats

	Control	Cirrhosis
Basal values		
Bile flow (μL/min. g liver)	1.70 ± 0.34	1.65 ± 0.13
Bile acid (nmol/min. g liver)	66.0 ± 6.9	64.9 ± 5.7
Infusion of BSP		
Serum BSP (mmol/L)	0.28 ± 0.03	0.43 ± 0.08*
Liver BSP (μmol/g liver)	0.26 ± 0.03	0.25 ± 0.05
Unconjugated BSP in bile (nmol/min. g liver)	4.3 ± 0.4	3.4 ± 0.3*
Conjugated BSP in bile (nmol/min. g liver)	36.6 ± 4.0	22.7 ± 1.9*
Infusion of DBSP		
Serum DBSP (mmol/L)	0.42 ± 0.07	0.59 ± 0.06*
Liver DBSP (μmol/g liver)	0.13 ± 0.01	0.11 ± 0.01
Liver glutathione and glutathione *S*-transferase		
Glutathione (μmol/g liver)	5.37 ± 0.24	4.27 ± 0.43*
Glutathione *S*-transferase (μmol/min. g liver)	2.15 ± 0.12	1.56 ± 0.45*

Values are means ± SEM from six animals. After a priming dose of 2.15 μmol/100 g body wt BSP or DBSP were infused for 60 min at 215 nmol/min. 100 g body wt. Means ± SEM from six animals. Biliary BSP data correspond to 40–60 min after beginning of infusion. Serum and liver BSP were obtained at the end of experiments.

* $P < 0.05$ significantly different from control value.

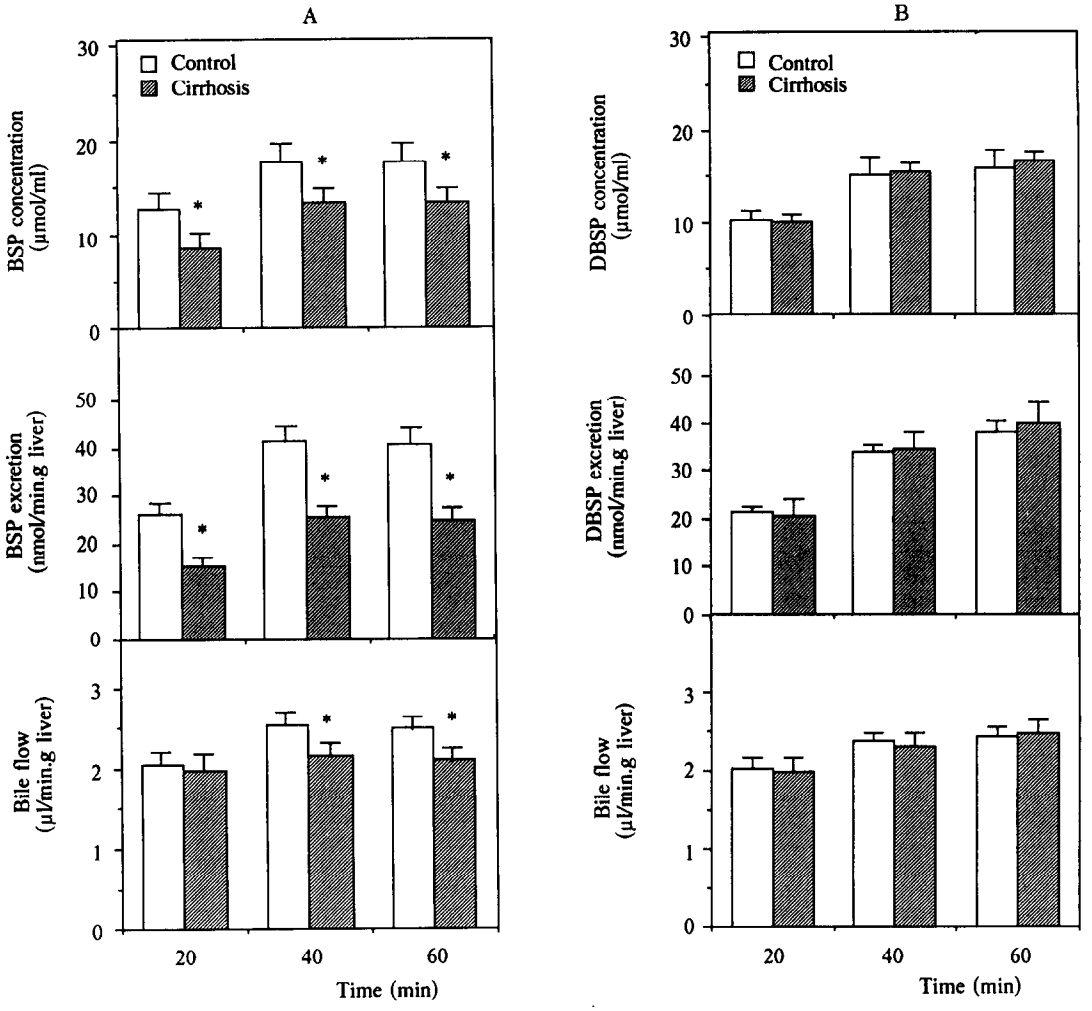

Fig. 1. Effect of experimental cirrhosis on: biliary concentration and excretion of BSP and on bile flow (A); biliary concentration and excretion of DBSP and bile flow (B). After a priming dose of 2.15 μmol/100 g body wt BSP or DBSP were infused for 60 min at 215 nmol/min. 100 g body wt. Means ± SEM from six animals. * $P < 0.05$ significantly different from control value.

DBSP following its intravenous administration in control and cirrhotic rats are shown in Fig. 1. No significant differences were found between both groups of animals. Serum concentration of the dye, in contrast, was significantly higher in cirrhotic rats than in the controls (+40%) and the liver concentration of DBSP tended to be lower in cirrhotic animals (−15%), although the difference did not attain statistical significance (Table 1).

Our results clearly show that experimental cirrhosis causes a marked decrease in the T_{max} and cumulative excretion of BSP in rats. The elimination of BSP from plasma depends on a series of processes, including the transfer from plasma to liver, conjugation and canalicular secretion of the dye. Alterations of any of these could change the rate of BSP excretion.

The higher serum levels of BSP observed in cirrhotic animals after dye infusion could be due to the diminished BSP transport from liver to bile but also to a depressed primary uptake. This is supported by the increased serum concentration found for DBSP, which shares the same transport system in the liver, even in the absence of significant changes of its biliary excretion. The fact that the amount of BSP retained in the liver remains unchanged in cirrhosis would be explained assuming that both uptake and elimination into bile were reduced. In any case, it is known that under conditions of maximal transport, saturation is an intracellular phenomenon and uptake is not a rate limiting step [12]. Therefore, even though it could be proposed that cirrhosis causes decreased uptake from plasma, this would not be responsible for the reduced transport into bile of BSP.

The decreased biliary excretion of the organic anion is not related to reduced bile flow and/or bile acid secretion, since these did not change between control and cirrhotic rats during the basal period prior to administration of the dye. Our data confirm the previous report that bile flow and the secretion of bile acids are maintained in cirrhotic rats, a phenomenon that apparently supports the intact cell hypothesis in cirrhosis of the liver [13]. In any case, even if bile flow and bile acid secretion were diminished, this could only explain a lowered excretion of unconjugated BSP but not that of the conjugated dye [14].

The fact that the rate of DBSP excretion into bile was identical in cirrhotic and control animals, suggests that the reduced BSP excretion could be related to alterations in the metabolic rate of this organic anion. BSP is conjugated in the liver with reduced glutathione (GSH) by means of glutathione *S*-transferases. The conjugation of the dye could have been affected by the reduction in the co-substrate concentration found in cirrhotic animals. In humans it has been demonstrated that cirrhosis is associated with decreased concentrations of circulating GSH, supposedly a consequence of decreased hepatic synthesis [15]. A recent study also indicates that, although liver glutathione concentration progressively returns to normal following cessation of treatment, it is significantly reduced after 14 weeks of CCl_4 administration [16]. Another possibility would be that of changes in glutathione *S*-transferase activity. Different studies have reported that following inhibition of enzyme activity there is a reduced T_{max} of BSP [3, 4]. This has been explained on the basis of the reduced amount of conjugated BSP, with a higher rate of excretion than the parent compound, available for excretion and of the favoured inhibition of BSP–glutathione excretion induced by BSP [17]. Since enzyme activity was reduced in our experiments, this could also contribute to the impairment in the biliary excretion of BSP.

In summary, the results of this study show that in rats rendered cirrhotic by phenobarbital and CCl_4, the decreased T_{max} of BSP into bile is most probably related to changes in the metabolic rate of the organic anion.

Department of Physiology
Pharmacology and Toxicology
University of León
24071 León
Spain

PILAR LOPEZ
MARGARITA DE LAMO
PAQUITA GONZALEZ
MARIA JESUS TUÑON
JAVIER GONZALEZ*

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* Author for correspondence: Dr Javier González, Departamento de Fisiologia, Farmacologia y Toxicologia, Universidad de León, Campus Universitario, 24071 León, Spain.