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Biliary Excretion of Bromosulfthalein in Rabbit

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The effect of bromosulfthalein (BSP) infusions at different doses on bile secretion was investigated in anaesthetized rabbits. The patterns of biliary excretion were found to be similar to other mammals, maximal biliary excretion being achieved by an infusion of 0.50 mg $\times$ kg^{-1}body weight $\times$ min^{-1}. Bromosulfthalein administered at low doses under the maximum hepatic transport capacity resulted in a clear increase of bile flow which was mainly due to an osmotic mechanism. Supramaximal doses produced no effect on bile flow.

The transfer of bromosulfthalein (BSP) from hepatocyte to canaliculi is the limiting step of its biliary excretion, since COMBES (2) was able to show that a considerable amount of dye is localized in the liver even when it is excreted at maximum rates. This point has been confirmed by KLAASEN and PLAA (13) in several species. At times, however, the concept of maximum excretion is difficult to apply, such as in the case of the rat (1, 3) or sheep (11), where the high correlation between maximum transport and bile flow seems to indicate a maximum BSP concentration in bile rather than maximum excretion.

The effect caused by the infusion of BSP on bile flow shows large variations both as a function of doses and of the species of animal studied; thus, maximum doses have a choleretic effect in the dog (23), while supramaximal doses do not affect flow. In the rat, however, very high doses may cause marked decreases in bile flow (4).

The aim of the present work was to investigate the plasma clearance rates and biliary excretion of BSP in the rabbit, placing special interest in the infusion dose at which maximum transport is attained and to the variations taking place in bile flow.

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Materials and Methods

Animals and Experimental Procedure. Castillian-breed rabbits with a weight range between 1.5-2.5 kg were used in the study after anesthesia with ethylurethane administered through the marginal ear vein at a dose of 30 mg/kg body weight. Prior to the study, the animals fasted for 24 hours though water was provided *ad libitum*.

In all cases a routine tracheotomy was carried out and after mid-ventral laparotomy, the pylorus was tied off and the gallbladder was excluded by ligation of the cystic duct. Bile was collected through a polyethylene catheter (PE 3) placed in the choledocus near its opening into the duodenum. The left femoral artery was also fitted with a catheter for the collection of blood samples and for monitoring arterial pressure with a «Stathan» transducer connected to a polygraph (Harvard Apparatus). A third catheter in the femoral vein allowed the administration of infusions using a calibrated peristaltic pump. In all cases a careful control of rectal temperature was made in order to avoid alterations in hepatic function as a result of hypothermia. All samples were taken in darkness in an ice bath to avoid decomposition of the bile pigment.

After a period of equilibrium of 30 min, collection of bile samples was begun. These were taken during intervals of 20 min for a period of 4 h. Except in the control group, BSP was infused from fourth sample up to the end of experiments at doses of 0.05, 0.1, 0.5, 1.0 and 1.5 mg $\times$ kg^{-1} body weight $\times$ min^{-1}, according to the experimental group.

The bromosufthalein (Bromotalein; Merck) was dissolved in a 0.9 % solution of NaCl to which a few drops of 0.1 M phosphate buffer had been added to mantain the pH neutral.

Analytical methods. Bile flow was determined by the difference in weight, before and after collection, of previously weighed tubes. BSP concentrations were determined by the spectrophotometric method of SELIGSON *et al.* (20). Bile bilirubin concentration by the method of MALLOY and EVELYN (17) and bile biliverdin by the method of LARSON *et al.* (15). The concentration of bile salts in bile was measured by the method of LEVIN *et al.* (16) for deoxycholate, the most abundant salt in rabbits (6). Sodium and potassium in bile were evaluated by phlame photometry (Klina Automatic, Beckman) and chloride concentration by potentiometric volumetry with silver nitrate. Results were expressed as means of the values $\pm$ the standard error of the mean (SEM). Linear regression analysis was performed using the least squares method and Student's «t» test was used to study the significance of differences between the means.

Results

There were no significant differences in blood pressure or in rectal temperature during the course of experiments.

In the control group a decrease of 40 % took place in bile flow from the beginning of the assays. On infusing the lowest dose of BSP used in this study (0.05 mg $\times$ kg^{-1} body weight $\times$ min^{-1}) the drop of flow noticed in the control group was avoided (table I). At higher doses (0.1 and 0.5 mg $\times$ kg^{-1} body weight $\times$ min^{-1}), the choleretic effect is less appreciable (table I) and may only be seen comparing the decreases in flow in percentage form, as shown in figure 1, that avoid the influence of oscilations in the initial bile flow. At doses of 1.0 and 1.5 mg $\times$ kg^{-1} body weight $\times$ min^{-1}, there is no choleretic effect at all, though no cholestatic effect took place (fig. 1). All, in control and in experiments with BSP infusion there were similar decreases in bile salt concentration, being no significant diferences between them (table I).

Table I. *Effect of BSP infusion on bile flow and bile bilirubin, biliverdin, deoxycholate, chloride, sodium and potassium concentrations.*
Each value is mean ± SEM of four animals.

Infusion mg×kg^{-1}×min^{-1}	Time h	Flow µg×kg^{-1}×min^{-1}	Bilirubin mg %	Biliverdin mg %	Deoxycholate mEq/l	Chloride mEq/l	Sodium mEq/l	Potassium mEq/l
Control	1	78±12	17.2±3.4	9.3±0.6	5.2±0.4	98±5	157± 8	3.7±0.3
	2	59±8	16.0±4.0	9.5±1.4	5.0±1.3	102±5	166± 8	3.9±0.2
	3	44±3	14.0±4.1	8.5±0.9	5.0±0.6	102±4	166±10	4.4±0.6
	4	40±2	11.0±2.5	7.9±0.4	4.8±0.5	100±3	169±13	4.7±0.4
0.05	1	75±9	16.8±3.8	9.7±1.5	7.8±1.0	86±4	141± 9	3.2±0.2
	2	70±7	15.4±3.3	5.9±1.4	5.5±0.7	95±9	144± 8	3.5±0.2
	3	63±7	11.3±3.5	5.5±1.5	5.3±0.8	98±3	149± 8	3.7±0.1
	4	64±5	7.2±2.1	4.5±0.8	4.6±0.7	100±4	149± 5	3.9±0.1
0.10	1	86±5	15.1±0.9	6.4±1.3	8.8±0.7	84±4	109±16	2.8±0.4
	2	64±5	14.4±1.5	6.7±1.8	7.6±0.6	95±3	123±14	3.0±0.5
	3	51±4	14.0±2.4	7.1±1.8	7.2±0.4	100±3	128± 7	3.6±0.4
	4	48±6	11.2±2.8	6.5±0.8	6.9±0.7	104±2	145± 9	4.6±0.4
0.50	1	59±9	23.9±1.5	13.6±3.1	8.2±0.7	92±5	160±12	4.6±0.5
	2	43±5	25.8±1.1	13.0±2.2	6.3±0.9	96±7	147±13	4.5±0.5
	3	40±4	20.5±1.5	12.0±2.3	5.7±0.9	103±5	158±15	5.8±0.7
	4	38±4	16.8±1.2	10.7±2.5	5.6±1.0	102±5	161±18	6.6±0.7
1.00	1	105±17	16.9±3.9	10.6±3.1	6.3±0.7	85±8	156±10	3.6±0.4
	2	75±17	17.2±4.0	10.0±3.4	5.3±0.7	93±3	170±18	4.7±0.6
	3	64±12	13.8±3.7	10.1±3.0	4.9±0.8	91±5	172±15	5.6±0.6
	4	51±6	11.3±2.8	9.8±2.3	4.6±0.7	93±5	170±13	6.2±0.8
1.50	1	76±10	14.0±3.0	8.1±1.0	6.5±0.5	91±2	153±11	3.9±0.5
	2	53±7	16.1±4.7	7.7±1.2	5.3±0.9	93±3	192±16	6.1±0.9
	3	46±8	13.0±2.6	9.7±1.1	5.1±0.9	92±3	180± 6	7.0±0.6
	4	—	—	—	—	—	—	—

Table II. *Biliary BSP concentration and excretion values for the different infusion doses.*
Each value is mean ± SEM of four animals.

		Infusion mg × kg^{-1} × min^{-1}				
		0.05	0.10	0.50	1.00	1.50
BSP concentration, mg/ml	1st h	0.21±0.05	0.39±0.10	4.93±0.60	3.33±1.07	4.20±0.72
	2nd h	0.39±0.06	0.81±0.16	6.70±0.65	4.53±1.12	5.93±0.36
	3rd h	0.38±0.09	0.94±0.21	6.26±0.72	4.10±0.94	—
BSP excretion µg×kg^{-1}×min^{-1}	1st h	15.1±3.4	22.9±5.1	203.3±16.6	207.0±47.6	215.9±23.6
	2nd h	24.5±5.6	39.8±4.7	261.1±26.3	261.3±50.7	275.9±34.6
	3rd h	24.0±5.9	42.4±4.6	241.7±36.2	219.8±47.6	—

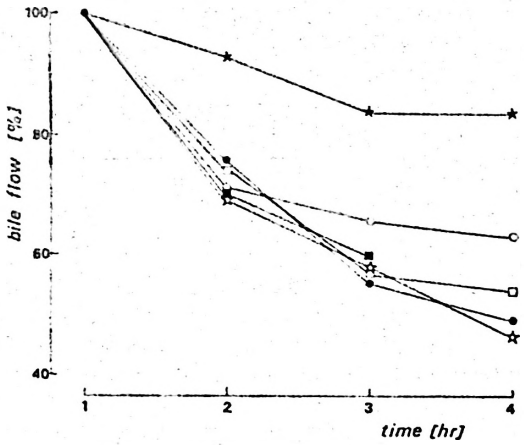

Fig. 1. *Influence on bile flow of BSP infusion at doses of 0.05 (★—★), 0.10 (□—□), 0,50 (○—○), 1.00 (☆—☆) and 1.50 (■—■) mg × kg⁻¹ body weight × min⁻¹.*

Regarding bile pigments, there was a drop in their concentration and output throughout the assays which was attenuated with the infusion of BSP from a dose of 0.1 mg × kg⁻¹ body weight × min⁻¹ and at higher doses a slight increase could be appreciated at the beginning of infusion (table I). At doses of 1.0 and 1.5 mg × kg⁻¹ body weight × min⁻¹ increases in sodium and above all potassium concentrations could be seen; at lower doses there was no appreciable variation with respect to the control (table I).

When BSP was infused its concentration in bile increased gradually until a stable value was reached after 40 min (table II). The excretion of the dye suffered different changes. At low doses of infusion (0.05 and 0.1 mg × kg⁻¹ body weight × min⁻¹) excretion values remained almost unchanged. At higher doses (0.5, 1.0 and 1.5 mg × kg⁻¹ body weight × min⁻¹) BSP excretion decreased slightly and progressively as infusion went on (table II). The highest dose sometimes gave rise to neurotoxic (ataxia) and hepatotoxic (with sharp falls in flow and biliary excretion of BSP) symptoms which eventually caused the death of the animal before the experiment had been completed.

Plasma concentrations of BSP during infusion at doses of 0.05 and 0.1 mg × kg⁻¹ body weight × min⁻¹ remained constant with no significant changes throughout experiments (fig. 2). At higher doses (0.5 mg × kg⁻¹ body weight × min⁻¹), plasma BSP concentration began to increase noticeably, which was even more marked at doses of 1.0 and 1.5 mg × kg⁻¹ body weight × min⁻¹. In all cases constant values of dye excretion were found, being those close to 260-270 μg × kg⁻¹ body weight × min⁻¹ (table II). All these results clearly show that maximum hepatic

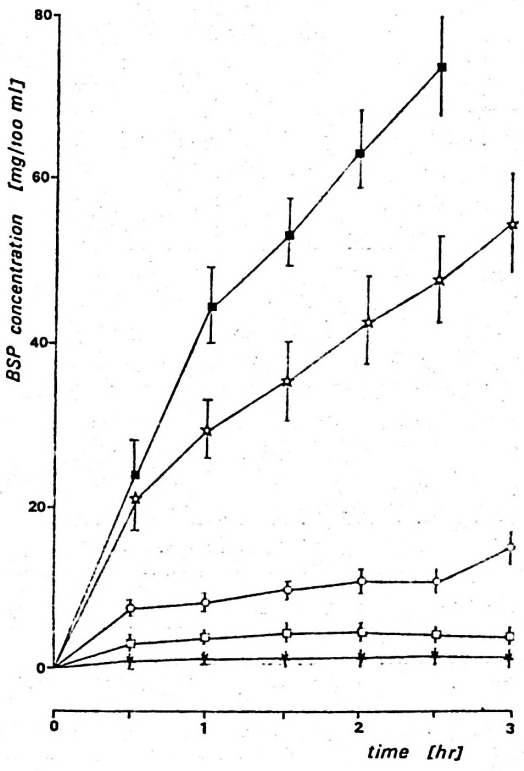

Fig. 2. *Plasma BSP levels during its infusion at doses of 0.05 (★—★), 0.10 (□—□), 0,50 (○—○), 1.00 (☆—☆) and 1.50 (■—■) mg × kg⁻¹ body weight × min⁻¹.*

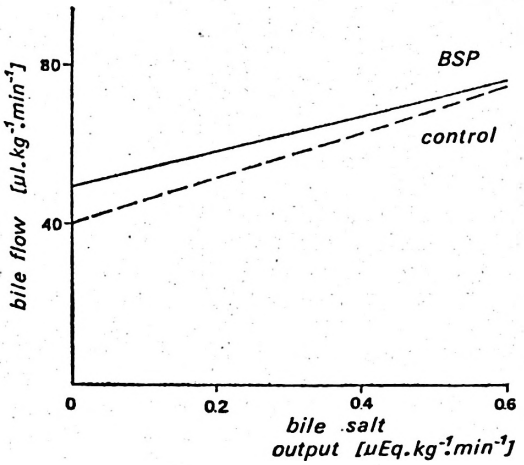

Fig. 3. *Relationship between bile flow and bile salt output during BSP infusion at a dose of 0.05 mg × kg⁻¹ body weight × min¹⁻.*

Control	BSP infusión
$y = 63x + 40$	$y = 51x + 49$
$\bar{y} = 75$	$\bar{y} = 66$
$r = 0.5854$; $p < 0.001$	$r = 0.3621$; $p < 0.05$
BSIF = 53 %	BSIF = 74 %

transport of BSP from plasma to bile had been reached. Biliary recovery of the dye, which for the first three doses was about 50 % of the amount infused, clearly fell at the two higher doses, reinforcing the idea that it is precisely from the dose of 0.5 mg × kg⁻¹ body weight × min⁻¹ that the hepatic transport of BSP has been saturated.

Discussion

The decrease in bile flow observed in the control group is similar to that obtained by our own team in unanesthetized rabbits (7) or pentobarbital- anesthetized rabbits (8) and may be attributed to a decrease in bile salts when their enterohepatic circulation has been interrupted.

The choleretic effect of BSP, quite apparent at a dose of 0.05 mg × kg⁻¹ body weight × min⁻¹, might be explained, according to our present results and those described in the literature, by the following hypothesis: at low doses, BSP is excreted in a conjugated and monomolecular form, thereby exercising an osmotic effect which would attract water and electrolytes towards the canaliculum, causing an increase in flow. Moreover, on using the method of ERLINGER *et al.* (6) an increase in the bile salt independent fraction appeared when BSP was infused (figure 3). This choleretic effect has already been described for other species (11, 23) and for other excretable anions in bile (9, 12).

At progressively higher doses, even larger amounts of non- conjugated BSP would start to appear, possibly due to the depletion of glutathion of hepatocytes (5). The non- conjugated form would tend to form micellar aggregations owing to their lipophilic nature, thus decreasing their osmotic activity and in turn their choleretic effect (22). At even higher doses, above maximum transport level, an intrahepatic accumulation would take place, leading to toxic effects, probably at the mitochondrial level (10) which could induce cholestasis. This does not appear in our study, contrary to what has been described in the rat (3), and is possibly due to the fact that the rabbit, compared whith the rat, excretes BSP in urine in sizeable amounts. SMITH (21) reports considerable differences in the amount of biliary excretion of organic compounds and suggests that urinary and biliary excretion play complementary roles and that the rabbit is among the animals which use the biliary route to a limited extent.

It is necessary to point out that the behaviour of BSP in relation to flow clearly differs from that shown by other organic anions in the rabbit, as are the cases whith indocyanine green (14) or rose bengal (4) in which there are choles-

tatic effects related to a decrease in the BSIF. This could mean that the excretory process for BSP and other organic anions does not necessarily have to be similar.

The results of our experiments show that the excretion rate of BSP in the rabbit is similar to that of other mammals, there being a saturation in the hepatic transport of this substance whith the appearance of maximum values which are not surpassed even when BSP accumulates progressively in blood. The doses after which maximum transport is reached in the rabbit is similar to that used in man, dog or pig, but lower than that in sheep and rat (18). In our study, maximum biliary excretion is less than that described by other authors (13), though this could be due to racial differences, as has been reported in the case of the rat (14). It should be noted that maximum transport values fall slightly throughout the assay; this has been studied in depth by DHUMEAUX *et al.* (3), though no definite conclusions have been put forward.

The behaviour of the bile pigments, whose concentration and output suffer even slight increases (table I), is at first sight unexpected, since some workers have proposed transport mechanisms which are shared between BSP and bile pigments (10). This could be explained on the basis of the formation of micelles, in agreement with what has been proposed above; such a phenomenon might facilitate the excretation of pigments. It is not surprising that this does not take place in the case of the bile salts, since as WARE *et al.* (22) have pointed out, micellar interaction between BSP and glycodeoxycholate is minimal.

Regarding electrolytes, our results are partly in agreement with those of RUTISHAUSER and STONE (19) who, like ourselves, find clearly differentiated increases in sodium and potassium concentrations, whith no changes in those of chloride after the administration of high doses of BSP.

Resumen

Se estudia el efecto de la infusión de bromosulfoftaleína (BSP) a diferentes dosis sobre la secreción biliar en conejos anestesiados. Las pautas de excreción biliar resultan similares a las ya descritas para otros mamíferos, alcanzándose una excreción biliar máxima a partir de una dosis de infusión de 0.50 $mg \times kg^{-1}$ peso corporal $\times min^{-1}$. La bromosulfoftaleína administrada a dosis por debajo de la máxima capacidad de transporte hepático produce un claro incremento en el flujo de bilis debido principalmente a un mecanismo osmótico. Las dosis de infusión supramáximas no tienen ningún efecto sobre el flujo de bilis.

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