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IMPAIRMENT IN THE HEPATIC CLEARANCE OF [^{35}S]-BROMOSULPHOPHTHALEIN IN PARACETAMOL-INTOXICATED RATS

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1 The overall functional capacity of the liver was evaluated using [^{35}S]-bromosulphophthalein (BSP, 100 mg/kg, i.v.) in biliary fistulated adult rats pretreated orally with different doses of paracetamol (APAP) for varying time intervals.

2 The maximal hepatic damage occurred between 12–18 h after single doses of APAP (0.5 or 1 g/kg); hepatic excretory function returned to control levels by 48–72 hours.

3 Administration of either 0.5 or 1 g/kg APAP 18 h before BSP caused a dose-dependent inhibition of the choleretic effect of BSP and of the 60 min cumulative excretion of the dye, but conversely, produced a significant increase in the liver and plasma concentrations of ^{35}S.

4 Following acute (0.25 g/kg), or subacute (0.5 g/kg, twice daily for 7 days) treatment with APAP, the total excretion of ^{35}S in bile and the retention of ^{35}S in the liver or plasma remained essentially the same as that for the controls.

5 In rats given single doses of 1 g/kg APAP, the hepatic uptake of the dye was significantly increased during the early stages of intoxication, while the opposite effect was observed at late periods.

6 The bile flow appeared to be inversely related to the excretion of unchanged BSP, and directly related to the excretion of the major BSP conjugate in bile.

7 The hepatic clearance of BSP was more rapid in rats treated subacutely with 0.5 or 1 g/kg APAP, than in those treated acutely with equal doses, suggesting that the intensity of APAP-induced hepatotoxicity became less severe after the repeated administration of this drug.

8 It is concluded that the hepatic uptake, metabolism and excretion of BSP are reversibly impaired following APAP-induced liver injury.

Introduction

Hepatic damage is a well recognized consequence of paracetamol (acetaminophen, N-acetyl-*p*-aminophenol; APAP) over-dosage in humans (Prescott, Wright, Roscoe & Brown, 1971), and in rats (Boyd & Bereczky, 1966; Dixon, Nimmo & Prescott, 1971; Mitchell, Jollow, Potter, Davis, Gillette & Brodie, 1973a). While several biochemical abnormalities indicating hepatocellular damage have been recorded in patients suffering from acute APAP poisoning (Proudfoot & Wright, 1970), information on the overall functional capacity of the liver in the intact organism is lacking. It is generally maintained that the transfer of bromosulphophthalein (BSP) from blood to bile involves three steps: uptake into the liver cells and storage, metabolism, and excretion by the liver. The present study was undertaken to determine which of the hepatic processes concerned with the elimination of BSP from blood to bile would be affected following APAP-induced liver injury.

Methods

Animal treatment

Male Wistar rats (230–280 g) (Woodlyn Farms, Guelph, Ontario) were fed on master laboratory cubes and acclimatized for one week to the laboratory environment before use. They were fasted overnight (12–16 h) before the experiment but had free access to tap water.

Paracetamol was suspended in a 0.25% aqueous solution of gum tragacanth and administered by gavage. The volume of each dose was 2 ml/100 g body weight. Control animals received an equal volume of gum tragacanth vehicle. In the subacute experiment, rats were treated twice daily (09 h 00 min and 20 h 00 min) for 7 days with varying doses of APAP. Control rats received corresponding volumes of the gum tragacanth vehicle over the same period. All rats were weighed daily and received food and water *ad libitum*. The di-sodium salt of [^{35}S]-bromo-

sulphophthalein (BSP), purchased from the Radiochemical Centre, Amersham (sp. act. 33.6 mCi/g), was mixed with unlabelled BSP solution (Hynson, Westcott and Dunning, Inc., Baltimore, Maryland) to give a specific radioactivity of 168 uCi/gram. The radioactive dye was administered as a bolus injection at a dosage of 100 mg/kg body weight.

The common bile duct and femoral vein were cannulated with polyethylene tubing (PX-011) while the rats were under light ether anaesthesia. Since hypothermia is known to reduce bile flow (Roberts, Klaassen & Plaa, 1967), the body temperature was maintained between 37–38°C by means of an electric lamp and the core temperature was monitored with a thermistor inserted about 2.5 cm into the rectum. In order to establish an even biliary flow the first 10 min bile was discarded, then a control bile sample was collected for 30 min before the administration of BSP. Rats were kept in the supine position by light ether anaesthesia throughout the study period.

Collection of body fluids and tissues

Duplicate 10 µl bile and tail blood samples collected at 2, 5, 10, 15, 30, 45 and 60 min after the dye injection were transferred to scintillation vials containing 1 ml of Soluene (Packard Instrument Co., Inc.; Downers Grove, Ill.). Bile was collected for two consecutive 30 min periods. At the end of 1 h, each rat was killed by withdrawing blood from the abdominal aorta in an heparinized syringe, plasma was separated by centrifugation and duplicate 10 µl samples were added to 1 ml of Soluene for measuring the total concentration of BSP. The liver was immediately removed and weighed after blotting off the extraneous blood. Quadruplicate samples (50–100 mg) were taken from different lobes of the liver for estimation of ^{35}S-radioactivity. The bladder was emptied with a syringe, and duplicate 10 µl aliquots of urine were taken for ^{35}S analysis. Bile and urine volumes were measured to the nearest 0.01 ml. The bile samples were kept frozen until analysed for unchanged BSP and metabolites.

Radioactivity determination

Complete solubilization of samples was accomplished by shaking for 24 h at room temperature. The urine and bile samples turned intense blue in the alkaline medium of Soluene. The blue colour was bleached by the addition of 50 µl of glacial acetic acid to the digested samples; it was also added to all other vials including the standards and blanks. Radioactivity was determined with a liquid scintillation counter (Nuclear-Chicago, Isocap-300) after the addition of 15 ml of toluene based scintillation fluid as described earlier (Buttar, Coldwell & Thomas, 1973). Quenching was corrected by the external standard ratios method. The dye concentrations were estimated by computer processing and the values were expressed as unchanged BSP.

Thin layer chromatography of bile

The status of ^{35}S-radioactivity in bile was ascertained by thin layer chromatography (t.l.c.) using Eastman Chromogram sheets (20 x 20 cm No. 6065) impregnated with cellulose and fluorescent indicator and the analytical procedure described by Whelan & Plaa (1963). The two 30 min bile samples were pooled and 2 µl aliquots from various animals were applied to the sheets at a distance of 2 cm apart. Ascending chromatograms were prepared using the upper phase of an organic solvent system consisting of *n*-butanol: glacial acetic acid: water (5 : 1 : 5). The spots were developed by exposing the sheets to concentrated ammonia fumes, air dried, sprayed with 0.25% ninhydrin in ethanol and heated in an oven at 100°C for 3 minutes.

The developed chromatograms were cut into strips (2 cm wide and 16 cm long) and the amount of ^{35}S in each spot was determined by further cutting these strips into sections, each 1 cm wide, and counting each section in a liquid scintillation counter. The ^{35}S radioactivity associated with spots of different R_F-values was expressed as a percentage of the total radioactivity in the bile.

The bile acids (from bile collected for 30 min prior to the dye injection) were extracted, hydrolysed and analysed by gas-liquid chromatography using the method of Watanabe (unpublished). Total tissue water was determined by weighing liver specimens before and after drying to constant weight in an oven at 100°C. The osmolality of bile was checked with an automatic osmometer (Osmette A, Precision Systems Inc.).

Statistics

The statistical significance between test and control groups was determined by Student's *t* test (two-tailed). The difference between means was considered significant when $P < 0.05$. The results were expressed as means with standard errors.

Results

Effects of paracetamol pretreatment on the disappearance of ^{35}S from blood and appearance in bile

When [^{35}S]-BSP was injected intravenously into rats with cannulated bile ducts, ^{35}S was detectable in bile samples taken 2 min after the injection. The highest levels of ^{35}S were observed in bile samples collected at 15 or 30 min intervals from both the control and

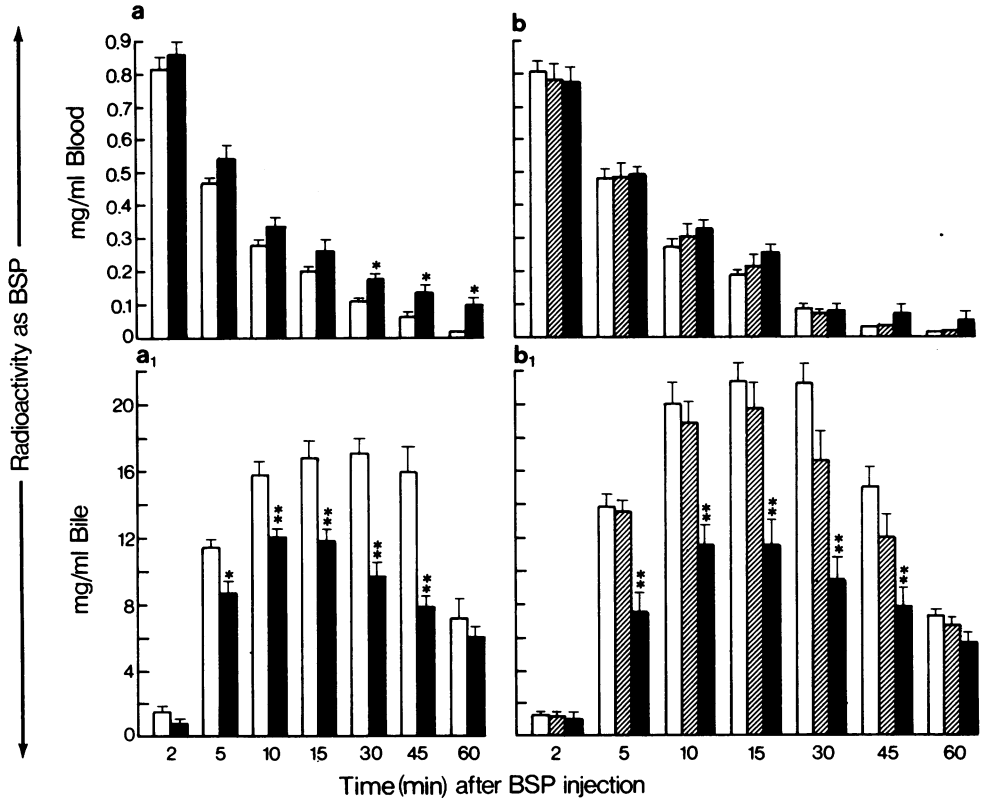

Figure 1 Time course of the disappearance of ^{35}S from the blood and its appearance in the bile during the 60 min period following administration of [^{35}S]-bromosulphophthalein (100 mg/kg, i.v.). (a) and (a_1), Rats were pretreated with single doses of paracetamol (1 g/kg, p.o.) and the hepatic function tests were done at 18 h after dosing. Open columns = Controls; solid columns = rats pretreated with paracetamol. (b) and (b_1) Rats were pretreated twice daily for 7 days with 0.5 and 1 g/kg paracetamol (p.o.) and the hepatic function tests were done 12 h after the last dose of paracetamol. Control rats received corresponding volumes of 0.25% aqueous gum tragacanth solution. Open columns = controls; cross-hatched columns = paracetamol 0.5 g/kg; solid columns = paracetamol 1.0 g/kg. Each column represents the mean value (± s.e. mean) obtained from 4 to 5 rats. * $P < 0.05$; and ** $P < 0.01$ when compared with control values.

APAP-poisoned animals, followed by a decline. At the end of 60 min, although the biliary levels of ^{35}S were not significantly different between the control and drug-treated animals, the blood concentrations of ^{35}S were significantly greater in the rats pretreated with APAP (1 g/kg) either 6, 12 or 18 h before the injection of [^{35}S]-BSP. As an example the results obtained 18 h after the acute administration of 1 g/kg APAP are shown in Figure 1a.

When the animals were treated with repeated doses of APAP (1 g/kg) over 7 days, there was again a significant impairment of the excretion of ^{35}S into the bile, but the rate of decline of radioactivity in the blood was similar to that observed in control animals (Figure 1b).

Excretion into bile and retention of ^{35}S in liver of paracetamol pretreated rats

Administration of BSP to control rats resulted in hypersecretion of bile. The choleretic responses were stronger during the 30–60 min than during 0–30 min periods (Table 1). In contrast, significant diminutions in biliary flow were recorded in each of the two 30 min samples following BSP administration to rats treated 6, 12 and 18 h previously with single doses of APAP

(1 g/kg). This effect of APAP persisted up to 24 h, and thereafter the choleretic response to BSP was again observed. The percentage change in biliary flow was calculated as defined by Guarino & Schanker (1968):

$$\frac{\text{ml of bile excreted during 30 min period after BSP injection}}{\text{ml of bile excreted during 30 min period before BSP injection}} \times 100.$$

In rats pretreated for 6, 12 and 18 h before testing the 60 min cumulative recoveries of ^{35}S in bile were 1.5 to 1.9 times smaller, and the quantity of ^{35}S remaining in the liver was 2.9 to 8.5 times greater than the control values (Table 1). These APAP-induced effects disappeared 24 h after dosing.

The urinary excretion of [^{35}S]-BSP-derived radioactivity was negligible both in control (range 0.39–0.48%) and in drug treated animals (range 0.26–1.51%).

Effects of single versus multiple doses of paracetamol on bile flow, biliary and renal excretion of [^{35}S]-bromosulphophthalein and the retention of ^{35}S in the liver

Other tests of liver function such as the activities of serum transaminases (GOT, GPT), the accumulation of triglycerides in liver and histological abnormalities (unpublished observations) had shown that the peak hepatotoxic effects of APAP were induced within 12 to 18 h after dosing; hence 18 h after dosing was chosen as the time at which to study further the effects of APAP on the hepatic clearance of BSP and on its retention in the liver or plasma.

Administration of single doses of 0.25 g/kg APAP did not affect the hepatic clearance of BSP. However, when radioactive dye was injected into rats given single doses of either 0.5 or 1 g/kg of APAP, there was a dose-related inhibition of the choleretic effect of BSP, which was accompanied by a significant decrease in the 60 min cumulative excretion of the dye. At the same time, the retention of ^{35}S in the liver was increased significantly (Table 2).

None of the above mentioned variables was markedly altered in rats receiving 0.5 g/kg APAP for 7 days (Table 2). On the other hand, administration of BSP to rats dosed twice daily for 7 consecutive days with 1 g/kg APAP was followed by a significant reduction both in the biliary flow and in 60 min cumulative excretion of dye, while the retention of ^{35}S in the liver remained essentially unaltered. Rats treated for 7 days showed a 5- to 18-fold greater excretion of [^{35}S]-BSP-derived radioactivity in the urine.

The acute or subacute administration of 0.5 g/kg APAP did not produce any significant changes in the retention of ^{35}S in plasma. However, 60 min after the administration of BSP to rats previously treated with 1 g/kg APAP, the mean plasma concentration of ^{35}S was 5.5 times higher (162.5 ± 27.5 *vs* 29.5 ± 4.1 μg/ml) in the acutely treated rats, and 4 times higher (87.0 ± 15.7 *vs* 21.3 ± 2.3 μg/ml) in subacutely treated rats, than the corresponding control values. Thus, the relatively low levels of ^{35}S in both the liver and plasma of subacutely treated rats suggest that the hepatic clearance of BSP is more rapid in 7 day-treated animals than in animals given single equivalent doses of APAP.

Table 1 Time course of changes in bile flow and in biliary excretion of ^{35}S-radioactivity after the intravenous injection of [^{35}S]-bromosulphophthalein (BSP) (100 mg/kg). Rats were pretreated with single doses of paracetamol (APAP, 1 g/kg, p.o.) and the dye clearance tests were done at different time intervals after dosing

Time BSP injected after dosing with APAP (h)		*Change in bile flow (%)[c] after injection of BSP*		*^{35}S recovered in bile (% of dose)*			*^{35}S remaining in whole liver after 60 min (% of dose)*
		0–30 min	*30–60 min*	*0–30 min*	*30–60 min*	*Total*	
Controls	(15)[b]	133.3 ± 2.6[a]	175.6 ± 6.3	32.8 ± 1.9	41.9 ± 1.8	74.6 ± 1.7	3.5 ± 0.2
6	(5)	62.5 ± 11.6**	78.3 ± 16.6**	22.4 ± 3.2*	24.3 ± 2.6**	46.7 ± 7.5**	30.0 ± 2.7**
12	(4)	54.2 ± 6.8**	64.6 ± 6.4**	18.9 ± 3.3**	22.2 ± 3.9**	41.1 ± 7.0**	24.8 ± 3.2**
18	(5)	88.1 ± 8.1**	112.3 ± 4.8**	20.4 ± 2.7**	21.9 ± 3.1**	42.2 ± 5.6**	10.4 ± 1.6**
24	(4)	168.3 ± 12.1**	125.2 ± 8.8**	51.3 ± 8.7**	19.6 ± 3.6**	70.9 ± 10.0	3.4 ± 0.7
48	(3)	165.9 ± 9.4**	159.4 ± 7.5	53.6 ± 7.7**	22.6 ± 2.4**	76.2 ± 6.1	3.1 ± 0.4
72	(4)	156.3 ± 11.1**	165.5 ± 10.8	51.0 ± 6.9**	27.6 ± 2.9**	78.6 ± 6.7	3.0 ± 0.3

[a]Values represent the mean ± s.e. mean. [b]Figures in parentheses indicate the number of rats in each group. Control data were obtained during each test period, but the variations between the control rats were so small that all the control data were pooled for the final analyses. [c]For the calculation of per cent change in bile flow see results section.

* $P < 0.05$; ** $P < 0.01$ when compared with control values.

Table 2 Effect of acute and subacute oral administration of paracetamol (APAP) on bile flow and the biliary and urinary excretion of ^{35}S-radioactivity after the intravenous injection of [^{35}S]-bromosulphophthalein (BSP) (100 mg/kg)

Exptl. condition and dose (g/kg)	*Change in bile flow (%)[d] after injection of BSP*		*^{35}S recovered (% of dose)*				*^{35}S remaining in whole liver after 60 min (% of dose)*
	0–30 min	*30–60 min*	*Bile 0–30 min*	*Bile 30–60 min*	*Urine 0–60 min*	*Total*	
Acute[a]							
Control	138.9 ± 7.0[c]	168.3 ± 11.0	38.8 ± 7.4	41.6 ± 4.2	0.36 ± 0.10	80.7 ± 3.9	3.4 ± 0.4
0.25	141.7 ± 4.5	153.6 ± 5.6	46.3 ± 4.2	36.1 ± 2.6	0.36 ± 0.09	82.7 ± 2.2	3.4 ± 0.6
0.50	103.9 ± 3.5*	129.9 ± 8.2*	32.9 ± 4.1	33.6 ± 4.5	0.33 ± 0.06	66.8 ± 5.9*	7.2 ± 1.4*
1.0	88.1 ± 8.1*	112.3 ± 4.2*	20.4 ± 2.7*	21.9 ± 3.1*	0.28 ± 0.08	42.5 ± 5.5*	10.4 ± 1.6*
Subacute[b]							
Control	148.8 ± 5.0	156.4 ± 3.2	48.3 ± 3.7	36.2 ± 2.9	0.22 ± 0.01	84.7 ± 1.9	2.8 ± 0.4
0.50	160.2 ± 10.8	139.0 ± 11.4	56.1 ± 3.2	23.2 ± 4.0	1.06 ± 0.20*	80.6 ± 3.0	3.0 ± 0.4
1.0	120.0 ± 10.5*	114.2 ± 9.3*	34.8 ± 6.9	21.3 ± 3.1*	3.93 ± 1.18*	59.9 ± 6.4*	5.6 ± 1.1

[a]Rats were given single doses of paracetamol (p.o.) and the liver function tests were done 18 h after dosing. [b]Rats were given paracetamol (p.o.) at the dose indicated twice daily for 7 days and the liver function tests were done 12 h after the last dose of paracetamol. Control rats received corresponding volumes of 0.25% aqueous gum tragacanth solution twice daily for 7 days. [c]Values represent the mean ± s.e. mean from groups of 4 to 5 rats. [d]Per cent changes in bile flow were calculated as indicated in the text.
* Indicate those values which are significantly different from the control group at $P < 0.05$.

Time-related effects of paracetamol pretreatment on ^{35}S levels in the liver, bile and plasma

The results summarized in Table 3 show that both liver and plasma concentrations of ^{35}S 60 min after intravenous injection of radioactive dye were significantly greater than control values at 6, 12 and 18 h in rats given 1 g/kg APAP. There was also a decrease in the biliary concentration of ^{35}S and this was statistically significant 18 h after dosing. By 24 h, the liver, bile and plasma concentrations of ^{35}S had returned to control levels.

The bile/plasma concentration ratios of ^{35}S were significantly smaller than their respective control values at 6, 12 and 18 h in 1 g/kg APAP-treated rats, returning to control levels 24 h after dosing (Table 3). The liver/plasma concentration ratio was significantly greater during the first 6 h, while at 18 and 24 h the values of this ratio became significantly smaller than that of the controls, returning to control values by 48 hours.

Effects of paracetamol pretreatment on liver weight, biliary flow rate, bile pH, bile osmolality and bile acids

The liver weight and biliary flow rate were both significantly increased after administering single or multiple doses of 0.5 and 1 g/kg of APAP. The increase in liver weight could not be attributed to the increased amounts of cellular water, because the liver water content remained virtually unchanged in APAP-pretreated rats (Table 4). A significant loss of body weight (not shown) occurred in rats dosed twice daily for 7 days. However, no statistically significant

Table 3 Effect of acute single dose treatments of paracetamol (APAP, 1 g/kg) on liver, bile and plasma levels of ^{35}S-radioactivity 60 min after the intravenous injection of [^{35}S]-bromosulphophthalein (BSP) (100 mg/kg)

Time BSP injected after dosing with APAP (h)		*^{35}S Concentration*			*Concentration ratios*	
		Liver (μg/g)	*Bile (μg/ml)*	*Plasma (μg/ml)*	*Bile/Plasma*	*Liver/Plasma*
Controls	(15)[b]	110.1 ± 7.7[a]	7910.0 ± 318.1	26.4 ± 1.3	303.5 ± 9.9	4.4 ± 0.3
6	(5)	1038.5 ± 77.3**	7262.5 ± 646.4	117.8 ± 19.8**	66.4 ± 11.5**	9.3 ± 1.2**
12	(4)	846.0 ± 90.2**	6700.0 ± 711.3	216.7 ± 33.5**	32.5 ± 3.8**	3.9 ± 0.5
18	(5)	251.3 ± 37.1*	6027.5 ± 437.9*	162.5 ± 27.5**	39.3 ± 4.5**	1.6 ± 0.07**
24	(4)	79.5 ± 13.0	6646.7 ± 698.7	26.3 ± 2.9	268.1 ± 36.2	2.7 ± 0.4*

[a]Values represent the mean ± s.e. mean. [b]Figures in parentheses indicate the number of rats in each group.
* $P < 0.05$; ** $P < 0.01$ when compared with control values.

Table 4 Effect of acute and subacute administration of various dosages of paracetamol on liver weight and biliary flow rate

Exptl. condition and dose (g/kg)	*Liver wet weight (g/kg body wt.)*	*% liver water content*	*Bile flow rate (ml 30 min^{-1} kg^{-1})*	*Total bile acids (per unit bile vol)*
Acute[a]				
Controls	30.6 ± 1.2[c]	70.3 ± 0.25	1.68 ± 0.04	467.5 ± 35.6
0.25	29.8 ± 0.8	71.0 ± 0.19	2.06 ± 0.16	299.3 ± 19.9*
0.50	39.1 ± 3.1*	70.8 ± 0.20	2.47 ± 0.20*	320.0 ± 37.0*
1.0	41.5 ± 1.2*	72.1 ± 0.22	2.62 ± 0.18*	228.3 ± 66.3**
Subacute[b]				
Controls	31.8 ± 1.6	70.4 ± 0.18	1.67 ± 0.06	530.3 ± 41.9
0.50	41.3 ± 2.6*	71.5 ± 0.20	2.06 ± 0.07*	272.8 ± 36.5**
1.0	44.0 ± 1.9**	71.6 ± 0.24	3.07 ± 0.37*	290.3 ± 46.8*

[a]Rats were given single doses of paracetamol (p.o.) and its effects on various parameters were determined 18 h after dosing. [b]Rats were given paracetamol (p.o.) twice daily for 7 days and the effects on various parameters were determined 12 h after the last dose of paracetamol. Control rats received corresponding volumes of aqueous solution of gum tragacanth (0.25%) twice daily for 7 days. [c]Values represent the mean ± s.e. mean from groups of 4 to 5 rats.
* $P < 0.05$; ** $P < 0.01$ when compared with control values.

changes in liver weight or biliary flow rate were observed in 7 day-treated rats when calculations were based on the body weight recorded before the initial administration of APAP. The excretion of bile acids was significantly suppressed following the acute or subacute administration of various dosages of APAP, and returned to normal within 48–72 h after administration of the highest dose. No apparent changes were noted either in bile pH (control range 8.09–8.71, APAP-treated range 7.45–8.77), or in bile osmolality (control range 293–321, APAP-treated range 298–331 mOsm/kg H_2O) in the acutely or subacutely treated rats.

Nature of ^{35}S radioactivity in the bile

The t.l.c. of bile showed two major and one minor metabolite of BSP. Standards from radioactive dosing solution of BSP always gave a single peak at a mean R_F-value of 0.74 (range 0.69–0.81). Results in Table 5 show that following the acute administration of 1 g/kg of APAP the biliary excretion of unmetabolized BSP (R_F 0.73) was significantly increased during the first 18 h, returning to control values after 24 hours. In contrast, this treatment significantly reduced excretion of the first major metabolite (R_F 0.33), and correspondingly increased the excretion of

Table 5 Time course of changes in the biliary excretion of unchanged bromosulphophthalein (BSP) and metabolites following the injection of [^{35}S]-BSP (100 mg/kg, i.v.) to paracetamol (APAP, 1 g/kg, p.o.) pretreated rats

Time BSP injected after dosing with APAP (h)		*Metabolites* R_F *0.17 (0.13–0.19)*	*Metabolites* R_F *0.33 (0.31–0.38)*	*Metabolites* R_F *0.51 (0.50–0.56)*	*Unchanged BSP* R_F *0.73 (0.69–0.81)*
Controls	(12)[b]	2.6 ± 0.19[a]	49.6 ± 4.1	27.4 ± 2.3	19.8 ± 1.6
6	(3)	3.8 ± 1.30	30.9 ± 9.6	30.3 ± 7.4	35.1 ± 15.7*
12	(3)	1.5 ± 0.30*	16.0 ± 5.3**	38.3 ± 3.3**	44.3 ± 8.6**
18	(3)	2.2 ± 0.15	22.1 ± 1.9*	46.9 ± 1.8**	28.8 ± 1.3*
24	(4)	1.5 ± 0.40*	42.7 ± 2.5	37.6 ± 1.6	18.9 ± 1.6

[a]The radioactivity associated with different R_F-values (shown as means and ranges) is expressed as a percentage of total ^{35}S present in 1 h bile. Values represent mean ± s.e. mean. [b]Figures in parentheses indicate the number of bile samples analysed.
* $P < 0.05$; ** $P < 0.01$ when compared with control values.

Table 6 Biliary excretion of unchanged bromosulphophthalein (BSP) and metabolites during 1 h following intravenous administration of [^{35}S]-BSP (100 mg/kg) to rats pretreated with different doses of paracetamol

Exptl. condition and dose (g/kg)		*Metabolites* R_F *0.16 (0.13–0.19)*	*Metabolites* R_F *0.35 (0.31–0.38)*	*Metabolites* R_F *0.52 (0.50–0.56)*	*Unchanged BSP* R_F *0.75 (0.69–0.79)*
Acute[a]					
Controls	(8)[d]	2.2 ± 0.23[c]	50.8 ± 6.5	26.8 ± 3.6	18.4 ± 1.1
0.25	(4)	2.9 ± 0.25	37.2 ± 1.3	39.8 ± 1.4*	20.1 ± 0.9
0.50	(4)	2.9 ± 0.27	33.9 ± 6.3	42.9 ± 5.1*	20.2 ± 1.6
1.0	(3)	2.2 ± 0.15	22.1 ± 1.9**	46.9 ± 1.8*	28.8 ± 1.3**
Subacute[b]					
Controls	(5)	3.1 ± 0.30	62.7 ± 6.6	17.7 ± 4.1	16.4 ± 1.5
0.50	(4)	1.6 ± 0.32*	57.4 ± 1.4	31.4 ± 1.0*	10.0 ± 0.9*
1.0	(4)	0.5 ± 0.01**	42.2 ± 8.9	43.1 ± 6.2*	14.2 ± 4.6

[a]Rats were given single doses of paracetamol (p.o.), and BSP clearance tests were done 18 h after dosing. [b]Rats were dosed twice daily for 7 days, and BSP clearance tests were done 12 h after the last dose of paracetamol. Control rats received corresponding volumes of 0.25% aqueous solution of gum tragacanth twice daily for 7 days. [c]The radioactivity associated with different R_F-values (shown as means and ranges) is expressed as a percentage of total ^{35}S-radioactivity present in 1 h bile. Values represent mean ± s.e. mean. [d]Figures in parentheses indicate the number of bile samples analysed.
* $P < 0.05$; ** $P < 0.01$ when compared with control values.

the second major metabolite (R_F 0.51) of BSP at 12 and 18 hours. Significantly less of the minor metabolite (R_F 0.17) was detected at 12, 24 and 48 h in acutely dosed rats.

As shown in Table 6, increasing doses of acute APAP caused a corresponding increase in the excretion of the metabolite associated with R_F 0.52, and produced a corresponding decrease in the proportion of the metabolite associated with R_F 0.35. Significantly higher amounts of unchanged BSP were excreted in bile of rats given single doses of 1 g/kg of APAP, as compared with values observed in controls; on the other hand, the excretion of unchanged dye remained similar in gum tragacanth-treated controls and in 7 day APAP (1 g/kg) treated animals. The recovery of unmetabolized BSP was 2-fold smaller in the bile of rats given 0.5 or 1 g/kg of APAP twice daily for 7 days, compared to the amount recovered from the bile of animals given single equivalent doses of this drug. Furthermore, in contrast with the acutely treated rats, the formation of the major BSP metabolite was 1.7-fold and 1.9-fold greater following the subacute administration of 0.5 and 1 g/kg of APAP, respectively (Table 6).

Discussion

The results of this study show that the hepatic disposition of BSP is markedly but reversibly impaired following APAP-induced rat liver damage, and that the hepatotoxic effects depend upon the doses administered. In humans the clinical signs of acute APAP poisoning appear after a latent period of 24 h or more following ingestion and death is usually due to liver failure (Matthew, 1973). A time lag was also noticed in the present study for the full appearance of the hepatotoxic effects of APAP, maximal hepatic damage occurring 12–18 h following the acute administration of this drug, while the hepatic excretory function returning to control levels by 48–72 h (Table 1).

The hepatic clearance of [^{35}S]-BSP-derived radioactivity appeared to be more rapid in rats treated subacutely with APAP, than in those given equal doses on an acute basis (Table 2). Moreover, in contrast with the acutely treated rats, the biliary excretion of unchanged dye was 2-fold smaller, while the production of the major metabolite was 1.7- and 1.9-fold greater after the subacute administration of 0.5 and 1 g/kg of APAP, respectively (Table 6). These observations suggest that the intensity of APAP-induced hepatotoxicity becomes less severe after the repeated administration of this antipyretic/analgesic drug. While the mechanism of adaptation to this hepatotoxin remains unknown, unpublished results with ^{14}C-labelled acetaminophen indicate that the renal elimination of APAP-derived ^{14}C is faster in subacutely treated rats than in their acutely treated counterparts.

The bile to plasma concentration ratio is generally used as a basis for determining the active transport of compounds from plasma to bile. According to Plaa (1971) BSP conjugates are actively transported from plasma to bile. The significantly low bile/plasma concentration ratios observed during 6, 12 and 18 h in animals pretreated with 1 g/kg APAP (Table 3) suggest that the active transfer of BSP and/or its metabolites from liver cells into the bile is impaired following treatment with toxic doses of this drug. Another indicator of overall hepatic excretory function is the liver/plasma BSP concentration ratio. This ratio was significantly high at 6 h, and returned to control levels after 12 h, and at 18 and 24 h the values became significantly smaller in APAP-pretreated rats than that of the controls (Table 3). These results imply that hepatic uptake of the dye is increased during early periods, but is decreased during late periods of APAP intoxication. The reason for this dualistic change in hepatic uptake of BSP in APAP poisoned rats is not immediately clear.

Dyes such as phenol red, fluorescein (Sperber, 1959), and BSP (Klaassen & Plaa, 1968) which are actively transported across the hepatic membrane into the bile generally cause a considerable increase in bile flow due to their osmotic effect. On the other hand, the findings of Priestly & Plaa (1970) suggest that a diminution of bile flow occurs when inhibition of BSP conjugation with glutathione lead to elevated biliary concentration of unconjugated BSP. The results of this study substantiate these previous observations. The administration of BSP to rats treated previously with APAP caused a dose-dependent inhibition of the choleretic effect of BSP (Table 2), and this occurred at a time when increased excretion of unchanged BSP and reduced excretion of the major BSP conjugate were both seen (Tables 5 and 6). It seems unlikely that the temporarily deranged secretion of bile salts would account for the reduced excretion of BSP. Schulze & Czok (1974) have shown that the biliary excretion of BSP remains essentially unchanged in bile salt-depleted and taurocholate-substituted rats.

There is a strong evidence that in rat, man and other mammalian species BSP is mainly conjugated with glutathione (GSH) (Combes, 1964). It has been shown that factors such as the administration of iodomethane (Priestly & Plaa, 1970), or a protein-free diet (Combes, 1965), cause an interference in the hepatic conjugation and subsequently in the biliary excretion of BSP by depleting GSH stores in rat liver. Our unpublished results on the time-related effects of APAP (1 g/kg, p.o.) indicate that the hepatic GSH levels are reduced to a minimum within 3–6 h and restored to normal 24 h after the treatment. Recently Mitchell and coworkers (1973b) have demonstrated that APAP administration causes a dose-dependent

depletion of GSH content of mouse liver and pretreatment with cysteine, a GSH precursor, prevents hepatic necrosis. Additional studies have shown that the severity of APAP-induced liver necrosis in various species correlates directly with the rate of hepatic GSH depletion (Davis, Potter, Jollow & Mitchell, 1974). These observations suggest that the reduced formation of the major BSP conjugate and the simultaneously increased excretion of unconjugated BSP found in the present study, are associated with the APAP-caused depletion of hepatic GSH. Other possibilities, such as an APAP-induced defect in the transport of BSP-GSH conjugate from the hepatocytes into the biliary canaliculi, or a non-specific inhibitory action of this drug on the S-aryl glutathione transferase system responsible for BSP conjugation, cannot be excluded.

The results in Table 5 and 6 indicate that the increased biliary excretion of unchanged BSP and the reduced excretion of its major metabolite were always accompanied by an increased excretion of the second major metabolite of this dye. These observations suggest a shift in the primary metabolic pathway of BSP following APAP-induced liver injury. Combes & Stakelum (1960) have suggested that apart from its preferred conjugation with GSH, minor quantities of BSP can be excreted in the form of cysteine, cysteinylglycine and glutamic acid conjugates in rat bile. It may be that when the supply of GSH is reduced, BSP conjugates with the aforementioned amino acids, which are part of the tripeptide glutathione, before its excretion into the bile.

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