BILIARY EXCRETION OF DRUGS IN THE RAT DURING LIVER REGENERATION*

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Abstract Alterations in rat liver function during liver regeneration were studied by measuring bile flow and the biliary excretion of bile salts, bromosulfophthalein (BSP), its glutathione conjugate (BSP-GSH), phenol-3,6-dibromophthalein disulfonate (DBSP) and tartrazine (TZ). Immediately after partial hepatectomy, bile flow and bile salt secretion, expressed per g of liver, were significantly increased. Thereafter bile flow remained high through the third day. The concentration of bile salts on the first day was significantly lower than that of controls and remained low through the fourth day, suggesting an increased osmotic potency. Plasma clearance of BSP was proportional to residual liver mass. The excretion of BSP-GSH after injection of BSP was decreased in parallel to the decrease in BSP-GSH transferase activity during liver regeneration. In contrast, the excretion of unconjugated BSP and unknown conjugates was actually increased. Biliary excretion of injected BSP-GSH was significantly decreased up to the third day postoperatively. These results suggest that some of the decreased BSP-GSH excretion after injection of BSP is attributable to the decreased activity of BSP-GSH transferase and to competition by unconjugated BSP. After partial hepatectomy the excretion of injected BSP-GSH and DBSP, expressed per g of liver, was about twice that of controls and thereafter rapidly returned to control levels. In contrast to the other dyes, the excretion of TZ decreased in proportion to liver mass following partial hepatectomy. On the second and third day, the marked sex differences seen in controls were not observed. These findings suggest that TZ may be transported by mechanisms different from those controlling the excretion of BSP and related compounds.

This investigation was undertaken to study the biliary excretion of drugs during liver regeneration. When two thirds of a rat liver is removed surgically, growth of the remaining lobes is markedly stimulated so that nearly 100 per cent of the original weight is restored in 1–2 weeks. Many cytological and biochemical changes occur at this time which reflect the primary objective of the regenerating liver, i.e. replacement of the original liver mass [1, 2]. During this period, some hepatic processes are temporarily suspended. There is a depression of catabolism of nucleic acids and protein precursors [1] and of microsomal drug metabolism [3–6]. In connection with the latter, it was of interest to determine what other changes relating to drug disposition occur during liver regeneration, in particular those associated with bile production and the biliary excretion of drugs. There is a paucity of publications pertaining to biliary function during liver regeneration. Forty-eight hr after partial hepatectomy, mitotic activity of bile duct epithelium increases but returns to normal after 3 weeks [7]. This time course lags well behind that of liver growth and the return of activity is apparently due somewhat to increased size [7]. In residual lobes the membranes bordering the bile canaliculus are unchanged [7]. Bile output, although initially depressed, returns to normal slightly faster than does liver weight [8]. Bilirubin excretion is low at first but gradually approaches normal values [7, 9]. Twenty-four hr after partial hepatectomy a loss of nearly two thirds of the liver mass is associated with only a one third decrease in the rate of biliary excretion of several drugs and a decrease of 10–20 per cent in the rate of bile production [10, 11].

In the present study, we investigated the biliary secretion of bile salts and three organic anions during liver regeneration. These organic anions were chosen on the basis of their excretion characteristics. Sulfobromophthalein (BSP) is largely metabolized before excretion. Phenol-3,6-dibromophthalein disulfonate (DBSP) is rapidly excreted mainly as the unchanged form. Tartrazine (TZ) is also excreted unchanged and rats show a marked sex difference in its excretion [12].

MATERIALS AND METHODS

Animals. Male and female Wistar rats weighing 200–220 g were used throughout. The rats were anesthetized with ether, and the median and left lateral lobes of the liver were removed. These two lobes constituted approximately 70–75 per cent of the total liver mass. In the sham-operated animals (controls) the peritoneum was opened and the wound closed as in the hepatectomized rats. The rats were allowed to recover with access to food and water *ad lib.* for various periods of time prior to the experimental procedure.

Drugs and chemicals. BSP and DBSP were obtained from Hynson, Wescott and Dunning (Baltimore, Md.) and were injected i.v. in saline. A supply of DBSP was also provided by Dr. Norman Javitt of the

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Table 1. Liver weight, bile flow and bile salt secretion during liver regeneration

Time after hepatectomy		Liver wt (% body wt)	Bile flow (ml/hr/kg)	Bile flow (ml/hr/g liver)	Bile salts (μmole/hr/kg)	Bile salts (μmole/ml)	Bile salts (μmole/hr/g liver)
Controls	(7)†	3.98 ± 0.09	4.57 ± 0.28	0.11 ± 0.01	97.7 ± 8.5	21.6 ± 1.1	2.2 ± 0.2
3 hr	(7)	1.24 ± 0.04*	2.76 ± 0.12*	0.23 ± 0.01*	67.2 ± 5.6*	24.4 ± 1.6	5.7 ± 0.4*
1 day	(6)	1.88 ± 0.06*	3.95 ± 0.25	0.21 ± 0.01*	55.4 ± 4.8*	13.9 ± 0.6*	3.0 ± 0.3
2 day	(5)	2.47 ± 0.08*	5.06 ± 0.41	0.21 ± 0.02*	82.3 ± 7.6	16.3 ± 0.9*	3.1 ± 0.2
3 day	(7)	2.97 ± 0.06*	5.22 ± 0.22	0.18 ± 0.01*	87.6 ± 6.8	17.3 ± 0.6*	3.0 ± 0.3
4 day	(5)	3.30 ± 0.06*	5.05 ± 0.28	0.16 ± 0.01	91.1 ± 9.3	16.6 ± 1.1*	2.8 ± 0.3
7 day	(7)	3.58 ± 0.10	4.34 ± 0.13	0.12 ± 0.01	90.5 ± 6.5	20.7 ± 1.2	2.2 ± 0.1

Rats received 1 ml of saline i.v. and bile was collected for 60 min. † Number of animals is shown in parentheses. Results are expressed as the mean ± S.E.M. * Significantly different from the control values: $P < 0.05$.

Department of Medicine, Cornell University Medical College, (New York, N.Y.). TZ was purchased from Allied Chemical (Morristown, N.J.) and injected i.v. in saline. BSP-GSH was prepared synthetically by the method of Whelan, Hoch and Combes [13]. Reduced glutathione (GSH) was purchased from Sigma Chemical Co. (St. Louis, Mo.).

Plasma disappearance of BSP. After the i.v. injection of BSP into anesthetized animals, blood samples were obtained at 1, 3, 5, 7, 10, 15, 20 and 30 min, centrifuged and dye concentration in the plasma determined after dilution with 0.1 N NaOH, as described below.

Biliary excretion. Animals were anesthetized with urethane 1 g/kg, i.p., and the bile duct isolated through a midline abdominal incision and cannulated with PE-10 tubing. Renal pedicles were ligated in rats receiving TZ. During bile collection, body temperatures were monitored through rectal probes and maintained at 38 ± 0.5 . All compounds were injected i.v. and 10-min bile samples were collected in graduated tubes for 60 min.

Analytical methods. For the determination of BSP and DBSP, bile samples were appropriately diluted with 0.1 N NaOH and the absorbance determined at 580 and 575 nm, respectively. BSP and its metabolites in bile were separated by t.l.c. as described by Whelan and Plaa [14]. For the determination of TZ, bile samples were diluted 500-fold with water and the absorbance at 427 nm measured against a blank of diluted bile. Total bile salts were determined by the method of Mroczak and Riegelman [15]. Sodium taurocholate was used as the standard.

Assay of BSP-GSH conjugation. Conjugation of BSP with glutathione was measured as previously described by Goldstein and Combes [16]. The reaction mixtures contained 0.2 μmoles of BSP and 15 μmoles of GSH in a final volume of 3.2 ml, at pH 8.0. The 105,000 *g* supernatant fraction of rat liver was the source of enzyme. Drugs and enzyme solutions were prepared in 0.1 M sodium pyrophosphate buffer adjusted to pH 8.1 with 5 N HCl. The assay was carried out at 25 and changes in absorbance at 330 nm were monitored.

Protein was determined by the method of Lowry *et al.* [17]

Statistical analysis. Data were analyzed by a group comparison Student's *t*-test: $P < 0.05$ was considered significant.

RESULTS

Changes in liver weight, bile flow and bile salt secretion during liver regeneration. In the first set of experiments, we studied liver weight, bile flow and bile salt secretion during regeneration. Animals were injected with 1 ml of saline in these experiments. At various times after partial hepatectomy, bile was collected for 1 hr and the residual liver removed and weighed. Compensatory growth of the residual liver was almost complete within 7 days (Table 1). This corresponds closely with the results of Henderson and Kersten [3]. Three hr post operatively total bile flow was lower than that of controls (Table 1), but thereafter did not differ significantly from that of controls. Bile flow as a function of liver weight (ml/hr/g liver) was significantly increased at 3 hr and remained high through the third day. Total bile salt secretion at 3 hr was approximately one third lower than that of controls but when calculated as a function of liver weight was far greater than that of controls. Figure 1 shows

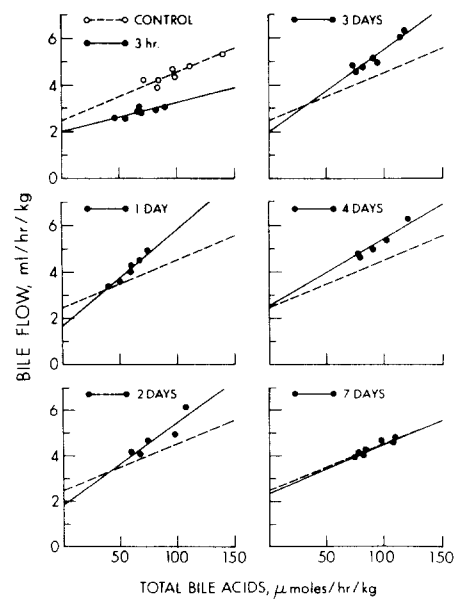

Fig. 1. Relation between total bile salt excretion and bile flow during liver regeneration. Each slope was calculated by the method of least squares. Rats received 1 ml of saline i.v. Bile was collected for 60 min.

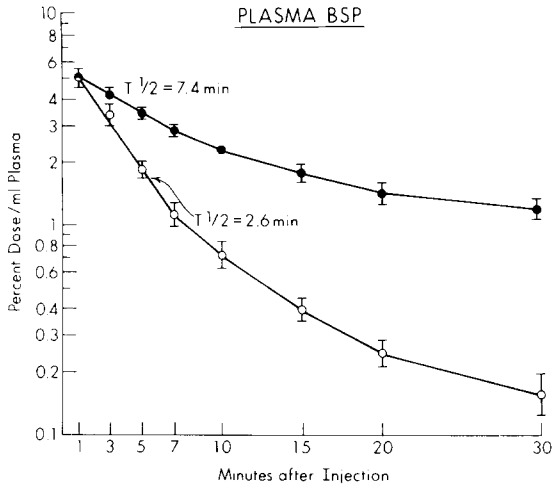

Fig. 2. Plasma disappearance of BSP immediately after partial hepatectomy. Both groups received 10 mg of BSP i.v. Results are given as the mean ± S.E.M. for 5 or more animals.

the relationship between bile flow and bile salt secretion. The slope of the straight line for 3-hr animals was less than that seen in controls and the y-intercept was decreased.

Thus, there appeared to be a decrease in the bile salt independent fraction associated with the decrease of liver mass. Bile salt secretion on the first day after surgery was significantly lower than that of controls (Table 1), although the bile flow at this period was not significantly different from that of control (Table 1). The concentration of bile acids on the first day was also significantly lower than that of controls (Table 1). It can also be seen in Fig. 1 that the slope of the line from the first day's animals was greater than that of controls. It appears that at this time a smaller amount of bile salt produced bile flow equal to that of controls. After the first day, the slope of line and the y-intercept gradually returned to control levels in 7 days (Fig. 1).

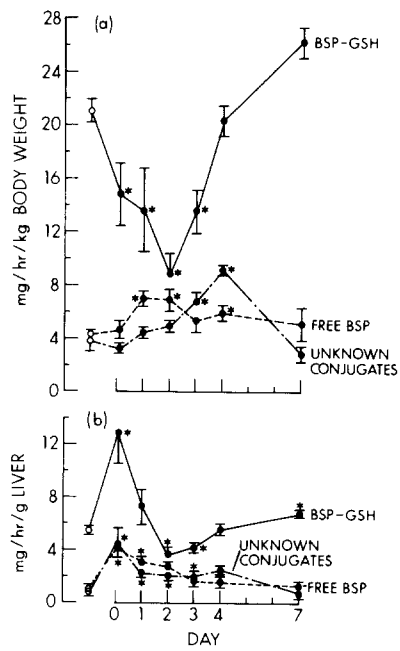

Fig. 3. Biliary excretion of BSP and its metabolites during liver regeneration. Ten mg of BSP was administered i.v. Bile was collected in 10-min periods for 60 min. Each point indicates the mean ± S.E.M. for 3–6 animals. Open circles indicate control values. * Significantly different from the control values; $P < 0.05$.

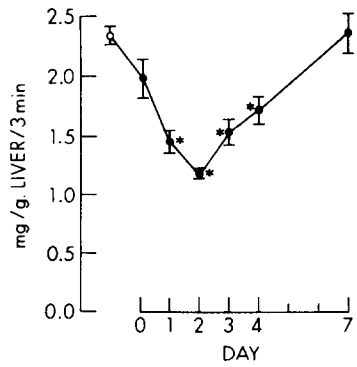

Fig. 4. BSP conjugating activity in 105,000 g supernatant fractions of regenerating liver at various intervals after partial hepatectomy. Each point indicates the mean ± S.E.M. for 3–6 animals. Open circles indicate control values. * Significantly different from control values; $P < 0.05$.

Plasma disappearance of BSP. BSP, 10 mg, was injected into control and partially hepatectomized rats and plasma levels of dye were determined at various times after injection. The plasma disappearance rate was approximately proportional to liver mass. Thus in the experimental group in which two thirds of the liver had been removed, the initial $T_{1/2}$ was nearly three times that of controls (Fig. 2).

Alteration of biliary excretion of BSP during liver regeneration. After the i.v. administration of BSP, it is excreted into the bile of normal rats mainly as the glutathione conjugate (BSP-GSH), plus a small amount of free BSP and three unknown conjugates [14]. In this study, the biliary excretion of BSP-GSH, free BSP and the sum of three unknown conjugates were determined. In Fig. 3a it is shown that the excretion of BSP-GSH was greatly decreased 3 hr after partial hepatectomy, reached a minimum after 2 days, and returned to control levels at 4 days. On the other hand, the excretion of free BSP and unknown conjugates did not diminish at any time. In fact, the excretion of BSP on the first and second days and the excretion of unknown conjugates on the third and fourth days were significantly higher than that of controls. When expressed per g of liver, excretion of BSP-GSH was over twice that of controls at 3 hr and excretion of free BSP and of unknown conjugates four and three times, respectively, that of controls (Fig. 3b).

The decreased excretion of BSP-GSH (Fig. 3A) could be due to decreased BSP-GSH transferase activity and/or a decreased hepatic transport of BSP-GSH. This was investigated in two ways. First, rats were partially hepatectomized and BSP-GSH transferase was subsequently measured in the 105,000 g supernatant fraction of the residual liver. As seen in Figure 4, enzymic activity expressed per

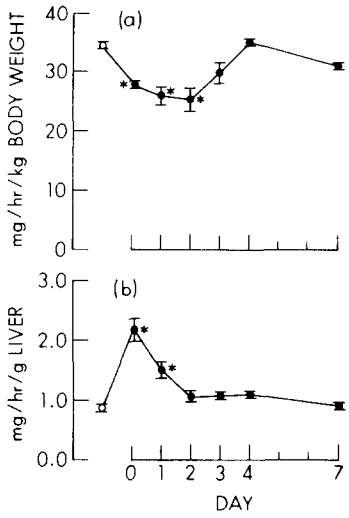

Fig. 5. Dye excretion after administration of BSP-GSH during liver regeneration. BSP-GSH (equivalent to 10 mg of BSP) was administered i.v. Bile was collected in 10-min periods for 60 min. Each point indicates the mean ± S.E.M. for 3–7 animals. * Significantly different from control values; $P < 0.05$. Open circles indicate control values.

g of liver gradually decreased postoperatively and reached a minimum on the second day. Complete recovery of activity was obtained in 1 week. Concentration of protein within the supernatant fraction was not significantly changed during the entire course of the experiment. In a second set of experiments, chemically prepared BSP-GSH (equivalent to 10 mg of BSP) was injected into rats at various times after partial hepatectomy and excretion of dye determined. Excretion was slightly decreased up to the third day postoperatively (Fig. 5a). On the other hand, dye excretion expressed per g of liver was significantly higher between 3 hr and 1 day postoperatively and returned to control levels by the second day (Fig. 5b).

Biliary excretion of BSP after bile drainage. In view of the possible association between the biliary excretion of BSP and bile salts, the excretion of this dye was determined after partial depletion of bile salts. The bile ducts of anesthetized animals were cannulated and bile collected for 4 hr. BSP was then injected and total dye excretion determined. Bile salts were also measured on the bile sample collected just prior to the injection of BSP. In Table 2 it can be seen that in the partially hepatectomized rats dye and bile salt excretion per g of liver were quite high when determined directly after cannulation. However, after 4 hr of bile drainage both dye and bile salt excretion had decreased approximately 50 per cent. On the other hand, in sham-operated animals the bile salt excretion per g of liver fell to rather low levels after several hr of bile drainage while the excretion of BSP was not significantly altered.

Table 2. Relationship between BSP and bile salt excretion after partial hepatectomy

Conditions	Dye excretion (%dose/hr/g liver)	Bile salt excretion (μmoles/hr/g liver)
Sham operated	7.64 ± 0.14	2.29 ± 0.14
Sham operated + bile drainage*	7.18 ± 0.51	0.17 ± 0.14
Hepatectomized	19.4 ± 2.0	6.20 ± 0.45
Hepatectomized + bile drainage*	9.4 ± 1.1	3.39 ± 0.18

* In these animals bile was allowed to drain for 4 hr prior to BSP injection. Bile salts were determined on bile obtained during the fourth hr. Values are the mean ± S.E.M. for 4 or more animals.

Alterations of biliary excretion of DBSP during liver regeneration. In Fig. 6a it is shown that dye excretion after administration of DBSP (10 mg) was markedly decreased between 3 hr and 4 days after partial hepatectomy. Dye excretion at 3 hr, expressed per g of liver, was twice that of controls and returned to control levels in one day (Fig. 6b). Accordingly, it appears that DBSP excretion is affected only during the early periods of regeneration as has been demonstrated for BSP-GSH (Fig. 5).

Alteration of biliary excretion of TZ during liver regeneration. In view of a marked sex difference in the biliary excretion of TZ in rats [12], we studied the biliary excretion of TZ in male and female rats after partial hepatectomy. After the i.v. injection of 26.7 mg the excretion in control female rats was about three times that seen in males (Fig. 7). Immediately after partial hepatectomy, the excretion of TZ in males and females fell to 25 and 20 per cent, respectively, of controls levels, while the liver weight of male and female rats was about 30 and 26 per cent, respectively of controls. Therefore, the decrease in TZ excretion was approximately proportional to the decrease in liver mass in the both sexes. This is also apparent from Fig. 7b in which TZ excretion is expressed per g liver. TZ excretion at 3 hr was slightly decreased

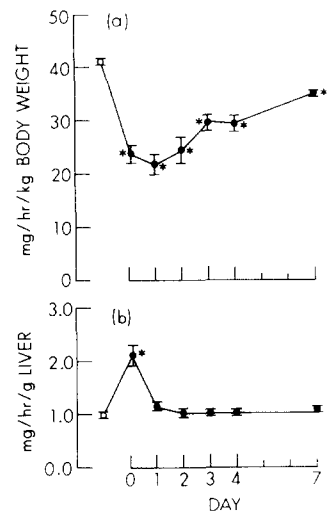

Fig. 6. Biliary excretion of DBSP during liver regeneration. Ten mg of DBSP was administered i.v. Bile was collected in 10-min periods for 60 min. Each point indicates the mean ± S.E.M. for 3–7 animals. Open circles indicate control values. * Significantly different from control values; $P < 0.05$.

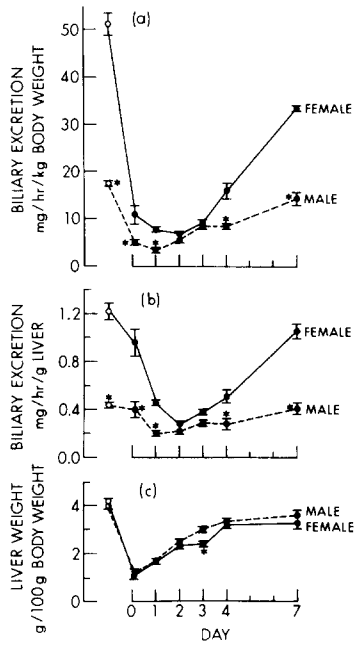

Fig. 7. Alteration of TZ excretion and of liver weight in male and female rats during liver regeneration. Rats with ligated renal pedicles received 26.72 mg (50 μmoles) of TZ i.v. Each point indicates the mean ± S.E. for 3–7 animals. Open circles indicate control values. * Significantly different from the values of female rats; $P < 0.05$.

from control levels in both sexes but the difference was not statistically significant. Excretion of TZ reached a minimum on the first day after partial hepatectomy in males and on the second day in females (Fig. 7a and b). On the second and third days, TZ excretion in males and females was not significantly different, while thereafter the sex differences returned. It appears that the excretion mechanism for TZ undergoes marked changes in both sexes during regeneration. Moreover, these results suggest that there is a large difference between the excretion mechanisms of TZ and BSP or DBSP.

DISCUSSION

Following partial hepatectomy on rats restoration of original liver weight occurs in 1–2 weeks (Table 1) [1, 3, 6], depending on the age of the animals. Henderson and Kersten [3] observed that the compensatory growth of the residual liver was almost complete within 1 week using rats, weighing 150–180 g. Our results with rats weighing 200–220 g were similar (Table 1). Slower growth was observed in experiments using male rats weighing 300–375 g [18]. Here 90–95 per cent or the preoperative liver mass was restored in 15 days. These differences may reflect rates of mitosis [19] and DNA synthesis [20] which vary with the age of the animal.

Three hr after partial hepatectomy bile secretion was 60 per cent that of controls, although 70 per cent of the liver had been removed (Table 1), as has been observed by others [11, 18]. At this time excretion of total bile salts was reduced approximately 30 per cent but the concentration of bile salts was essentially unchanged (Table 1). Here too, it is seen that at 3 hr bile salt excretion per g of liver was more than twice that of controls. The results in Fig. 1 suggest that after partial hepatectomy, bile salt independent flow may be somewhat diminished. This may contribute to the overall decrease in total bile production. Probably only a small proportion of the total bile salt pool is normally contained within the liver. Much of it is within the intestinal tract and perhaps other extra-hepatic areas [21]. Therefore, the total amount of bile salts potentially available for hepatic transport is only slightly less in hepatectomized animals compared to controls. Furthermore, blood flow per g of liver in the rat has been reported to be 30–80 per cent greater than that of controls between 4 and 16 hr following partial hepatectomy [22]. This may account for the relatively small diminution of bile salt excretion (30 per cent) and bile flow (40 per cent) compared to a loss of 65–70 per cent of the liver mass. Klaassen [11] has speculated that under normal conditions many hepatocytes are quiescent, but may become active under stress conditions such as partial hepatectomy. This could contribute to the increase in both bile salt (Table 1) and dye (Figs 3, 5, 6) excretion calculated per g of liver. However, the results in Fig. 1 suggest a diminished bile salt independent flow, which may be attributable to a change in the composition of bile during regeneration. Changes in bile composition 24 hr after partial hepatectomy have been reported [11]. However, none of these could readily explain the steeper slopes seen in Fig. 1 and a reason for the seemingly greater choleretic effect of bile salts at this time is not yet available.

Partial hepatectomy depressed the rate of plasma clearance for BSP in proportion to loss of liver mass (Fig. 1). The small increase in hepatic blood flow a few hr following partial hepatectomy [22] apparently did not alter the hepatic uptake process. Biliary excretion of dye was also decreased as observed previously [11]. However, when BSP, BSP-GSH and the unidentified conjugates were determined individually in bile considerable differences were seen. The total excretion of BSP-GSH diminished to less than half of control values by the second day (Fig. 3a). After the injection of BSP-GSH itself, dye excretion was also decreased but to a lesser extent (Fig. 5a). Total excretion of free BSP, however, was not decreased after partial hepatectomy (Fig. 3a). In fact, excretion rates exceeded those of controls on the first and second days. Evidence has been presented that BSP itself may inhibit the biliary transport of BSP-GSH [23]. Thus higher levels of free BSP transport after partial hepatectomy may partially account for the marked drop in BSP-GSH transport (Fig. 3a). Another contributory factor may be the drop in hepatic BSP conjugating activity. The time course of change for this enzyme (Fig. 4) parallels that of BSP-GSH excretion (Fig. 3) very closely and is typical of a number of drug metabolizing enzymes [3, 6, 24]. Glutathione S-transferase B has been shown to be identical to the hepatic anion binding protein, ligandin [25]. Diminished ligandin concentration may be associated with decreased storage capacity for BSP and consequently a more rapid biliary excretion.

When biliary excretion of each of the drugs (BSP, BSP-GSH, DBSP) is measured per g of liver, an increase is apparent in every case immediately following

partial hepatectomy (Figs 3b, 5b, 6b). This is particularly evident for unconjugated BSP where excretion per g of liver is 370 per cent that of controls 3 hr after surgery (Fig. 2b). In addition to the above explanation, the apparent increase in free dye transport may be associated with the increase in bile salt excretion, which, when measured per g of liver 3 hr after surgery is 240 per cent that of controls (Table 1). This is inferred from recent evidence that the increased unconjugated BSP transport seen during bile salt administration is attributable to a direct stimulating effect of the bile salt on BSP excretion rather than to an increase in bile flow alone [26–28]. In support of this idea are the experiments in which bile was allowed to drain for 4 hr after hepatectomy. Bile salt excretion was considerably less than that in animals without bile drainage and there was a proportional decrease in dye excretion (Table 2). In control animals the loss of bile salts during biliary drainage was not accompanied by lower dye excretion. It is inferred that the lower rate of dye excretion in control animals is not dependent on concomitant excretion of bile salts. Excretion of unknown BSP conjugates was also enhanced on the second and fourth day following partial hepatectomy (Fig. 2a and b). This suggests that unlike BSP-GSH biosynthesis (Fig. 4) mercapturic acid formation may not be depressed during the regenerating period. However, there have been no specific investigations on these enzyme systems during regeneration.

A profound decrease in TZ excretion, both total and per g of liver, was observed after partial hepatectomy in both males and females, although excretion of TZ in controls is much greater in females than in males (Fig. 7). This sex difference has been reported previously [12] and it appears to be related to sex hormones [29]. However, there is no direct evidence for the mechanism by which the hormones influence the biliary excretion of TZ. The sex difference disappears during the second and third day after surgery but subsequently returns on the fourth day (Fig. 7a and b).

Functional heterogeneity of hepatocytes within the lobule is known and the turnover of cells during regeneration begins near the portal area and progresses toward the central lobular zone [30]. It can be speculated that such heterogeneity may also apply to biliary transport. If the major contribution to the biliary excretion of bile salts and BSP is normally made by periportal cells, then their rapid proliferation might account for the higher excretion per g of liver seen in the early stages of regeneration. In such a situation one could imagine that TZ is handled by central lobular cells, which reproduce more slowly. This would explain the depressed excretion of TZ during regeneration.

Until recently it had been thought that organic anions were excreted into bile through a common process [31, 32]. However, recent reports suggest that more than one hepato-biliary pathway may function in the transport of organic anions [33–35]. We have previously shown that the hepatic transport of several organic anions but not taurocholate is inhibited in rats by pretreatment with the hypolipidemic drug, nafenopin [36, 37]. In contrast, this drug markedly enhances the hepatic transport of chlorothiazide (T. Uesugi and W. G. Levine, unpublished results). BSP excretion in the rat can be enhanced by infusion of taurocholate [38] but is inhibited by several anions [31]. The results reported in this paper imply that BSP-GSH and DBSP are transported into the bile by a similar mechanism while the mechanism for TZ transport is considerably different. It can be seen that direct information on biliary excretion mechanisms may be obtained by investigating the changes that occur during liver regeneration.

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