Hepatobiliary Excretion of Bile Acids and Rose Bengal in Streptozotocin-Induced and Genetic Diabetic Rats

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ABSTRACT

Divergent opinions regarding the effect of streptozotocin (STZ)-induced diabetes on bile flow rate may be due to the differing lengths of time after STZ administration at which bile flow was measured. Also, the biliary excretion of bile acids can influence the canalicular transport of several organic anions. Therefore, the hepatic clearance of the bile acid-dependent organic anion rose bengal was studied over a 30-day period in STZ-induced insulin-dependent Sprague-Dawley diabetic rats with elevated bile acid pools and in fatty noninsulin-dependent diabetic and lean Wistar rats. Excretion of total bile acids and rose bengal was higher in diabetic rats than in Sprague-Dawley control or lean or fatty Wistar rats. Depletion of bile acids for 10 hr in the 30-day STZ rat prevented the increased excretion of rose bengal. Bile flow rates in fassy and lean Wistar rats were similar to that in Sprague-Dawley controls. Increased bile acid excretion 7 and 14 days after STZ was not accompanied by the expected significant increase in bile flow, reflecting decreased bile acid-independent bile flow, regardless of method of calculation of bile flow (per g liver or per kg body weight). By 30 days, there were significant increases in bile acid excretion and bile flow. The increased clearance of rose bengal 7 days after STZ indicates that pathophysiological changes in the hepatocyte begin soon after the initiation of diabetes. Studies of taurocholate uptake into liver plasma membrane vesicles indicated that the maximal velocity of transport across the basolateral membrane was increased with no change in $K_m$. This change was not observed in vesicles from insulin-treated diabetic rats. Therefore, studies employing STZ need to allow time for STZ toxicity to be overcome and for the pathology of diabetes to become established, to accurately reflect the diabetic condition.

For medications metabolized and/or excreted primarily by the liver, changes in drug clearance and elimination are guides to dosage (Bass and Williams, 1988). Diabetes may alter the pharmacodynamics and pharmacokinetics of pharmaceutical agents, thereby increasing the risk of drug toxicity and side effects, or, conversely, decreasing drug efficacy (Nakashima et al., 1992; Barrientos et al., 1993; Watkins and Sanders, 1995). For the precise prescribing of pharmacological agents for diabetic patients, it is important that the long-term effects of diabetes (untreated as well as insulin-treated) on drug disposition be studied in animal models accurately reflecting the diabetic state.

STZ is widely used to develop an animal model of insulin-dependent diabetes (Büyükevrim, 1994; Kolb and Kröncke, 1993; Shafir, 1990), and it is important to differentiate between true diabetic effects and the toxic effects of STZ (Weiss, 1982). STZ-induced diabetes produces pathological changes as late as 3 mo after STZ in endocrine cells of the pancreatic and bile duct system (Park and Bendayan, 1994), changing the number of hormone secreting cells as well as their distribution, with an as yet undetermined net effect on bile flow. Biliary structure as well as hepatic function are affected by diabetes (Watkins and Sanders, 1995).

Some investigators have determined that STZ-induced diabetes is cholestatic (Andrews and Griffiths, 1984; Carnovale and Rodriguez Garay, 1984; Carnovale et al., 1986, 1987, 1991; Garcia-Marin et al., 1986, 1988), whereas others have reported normal bile flow or even choleresis (Badawy and Evans, 1977; Kirkpatrick and Kraft, 1984; Villanueva et al., 1990; Watkins and Dykstra, 1987; Watkins and Noda, 1986; Watkins and Sherman, 1992). Examination of these conflicting data leads one to hypothesize that the effect of STZ on bile flow rate is a function of time after STZ administration, i.e., bile flow is diminished immediately to several days after STZ injection, whereas flow is normal 1 mo after STZ. In addition, the organic anion rose bengal causes cholestasis in normal rats but an increase in flow in diabetic rats 4 wk after STZ (Watkins and Noda, 1986). Because bile production is partly dependent on bile acid excretion and the biliary excretion of rose bengal is bile acid-dependent, it was suggested that the higher bile acid excretion in the diabetic rat contributes to the excretion of rose bengal. Thus, the purpose of our

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ABBREVIATIONS: STZ, streptozotocin; SD, Sprague-Dawley; mRNA, messenger RNA; cLPM, canalicular liver membrane vesicles; blLPM, basolateral liver membrane vesicles; $V_{max}$, maximal velocity rate.
Materials and Methods

Chemicals. Rose bengal, 3α-hydroxy steroid dehydrogenase, STZ, sodium taurocholate and urethane were all purchased from Sigma Chemical Co. (St. Louis, MO). [6-3H]-Taurocholic acid (2.10 Ci/mmol) was obtained from DuPont/New England Nuclear (Boston, MA). All other chemicals were of the highest quality commercially available. Deionized water was used in all studies.

Animals. Male SD rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) and the lean and fatty Wistar rats (graciously provided by Dr. R. G. Peterson, Diabetes Research and Training Center, Indiana University School of Medicine, Indianapolis, IN) were housed in stainless steel cages in groups of four in temperature controlled (20–26°C) animal quarters with a 12-hr light/dark cycle. All animals were fasted overnight. Water and food were withheld from animals until experimentation. All experiments were performed within 4 to 7 wk after STZ administration.

Biliary excretion studies. After the rats were anesthetized with halothane (1.5%). After a midline abdominal incision, the liver was perfused with ice-cold 250 mM sucrose in 1 ml EDTA buffered with 10 mM HEPPES/Tris (pH 7.4) through the hepatic portal vein. Subsequent isolation of the lateral and sinusoidal portion (bLPM) of the hepatocyte membrane from the apical surface was adapted from Inoue et al. (1982). Rat liver vesicles containing predominantly cLPM were isolated by methods described in Prpic et al. (1984). Immediately after isolation, the membranes were suspended in 250 mM sucrose buffered with 10 mM HEPPES/Tris (pH 7.4) at concentrations of 10 mg protein/ml. Protein concentrations were measured according to Lowry et al. (1951) using bovine serum albumin as the standard. All membrane isolations were performed within 4 to 7 wk after STZ administration.

All marker enzyme activities were determined from frozen membrane vesicles that were thawed on the day of assay. ATPase activities (Na+,K+-) and Mg2+- of the isolated vesicles were measured (Scharschmidt et al., 1979), using ouabain as an inhibitor of Na+,K+-ATPase. Alkaline phosphatase activity was determined using p-nitrophenyl phosphate as substrate (Scharschmidt and Keefe, 1981). γ-Glutamyltranspeptidase activity was measured with L-(g-glutamyl-p-nitroanilide (Meister et al., 1981). Succinic dehydrogenase activity was determined by the method of King (1967), and benzphetamine N-demethylase was measured according to Lu et al. (1972). These enzyme marker assays indicated that there was substantial enrichment of γ-glutamyltranspeptidase and alkaline phosphatase in cLPM above homogenate values in normal, diabetic and insulin-treated diabetic rats, with little enrichment in the prepared bLPM. Vesicular enrichment of Na+,K+- and Mg2+-ATPase was similar in normal, diabetic and insulin-treated diabetic rat liver membranes. Both bLPM and cLPM vesicle preparations were de-enriched in mitochondrial and microsomal markers.

Transport studies. All transport assays were performed using freshly prepared membrane vesicles. Uptake of 3H-taurocholate was measured via a rapid filtration technique modified from the method described by Inoue et al. (1982). Briefly, the membranes were vesiculated by repeated passage (10×) through a 25-gauge needle and kept on ice until use. Membrane suspensions of 10 μl containing 100 μg of protein were preincubated for approximately 5 min at 25°C and uptake was initiated by addition of 90 μl of incubation solution: 50 mM sucrose, 100 mM NaCl, 0.2 mM CaCl2, 10 mM MgCl2, 10 mM HEPPES/Tris, pH 7.4, and various concentrations of 3H-taurocholate. After 10 sec of incubation at 25°C, uptake was terminated by addition of 3.5 ml ice-cold stop solution (1 mM unlabeled taurocholate + incubation solution without 3H-taurocholate) to the test tube. The
diluted samples were immediately filtered through presoaked (with cold stop solution to decrease nonspecific binding to the filters) 0.45 µm HAWP filters (Millipore/Continental Water Systems, Bedford, MA). Stop solution (3.5 ml) was again used to rinse the test tube and then the filter. Filters were placed in scintillation vials, 5 ml Bio-Safe II (Research Products International, Mount Prospect, IL) was added as scintillant, and bound radioactivity was analyzed using a Beckman (Fullerton, CA) LS 8000 scintillation counter. All values were corrected for the amount of radioactivity bound to filters in the absence of membrane vesicles. An aliquot of 10 µl incubation solution was used to determine the total radioactivity in the assay.

ATP-dependent transport of taurocholate in canalicular membrane vesicles was determined for radioactive taurocholate (1–300 µM) using the rapid filtration technique described above. The pH 7.4 incubation solution was enriched with 0, 0.1 and 1.0 mM ATP with an ATP-regenerating system (3 mM creatine phosphate, 3.6 µg creatine phosphokinase) as described in Adachi et al. (1991).

Values of the apparent \( K_m \) and the apparent \( V_{max} \) were obtained using an Eadie-Hofstee analysis. \([V]/[S]\) was graphed using linear regression; the \( y \)-intercept signified \( V_{max} \), and the line's slope represented \(-K_m\).

**Statistics.** Means and S.E. were calculated for all data. Significant differences were determined using an analysis of variance followed by Duncan's test to compare the means. \( P < 0.05 \) was judged to be significant.

**Results**

Table 1 shows that body weights for normal and STZ-treated SD rats were similar, but Wistar fatty rats were 40% larger than Wistar lean counterparts. Liver weight was higher in 30-day STZ diabetic SD and Wistar fatty rats. When calculated per gram liver, basal bile flow is significantly decreased (to 70% of normal) 7 and 14 days after STZ, but returns to normal by 30 days after STZ. When calculated per kg body weight, bile flow remains normal at 7 and 14 days after STZ, but is significantly elevated (to 130% of normal) 30 days after STZ. Bile flow was decreased in fatty rats as compared to lean Wistar rats. Serum glucose concentrations were approximately 6-fold higher in all STZ-treated rats than in normal SD rats, whereas glucose levels were only slightly increased in Wistar fatty vs. lean animals. Normal SD and Wistar lean rats were euinsulinemic, STZ-treated rats had insulin levels that were 5% of normal and Wistar fatty rats had marked hyperinsulinemia with values 7.8-fold more than those in lean rats.

The serum concentration of rose bengal decreased with time in all four groups of rats (fig. 1, top). Rose bengal serum concentration was significantly lower than normal in 7-day diabetic rats from 90 to 120 min. From 20 to 120 min, rose bengal concentration in serum was significantly lower in 14-day diabetic rats than in normal SD rats. Rose bengal serum concentration was lower than normal for 30-day STZ diabetics from 15 to 120 min. Table 2 indicates that total and biliary clearances were increased 3- and 7-fold, respectively, above normal in 30-day STZ-treated rats. Steady-state volume of distribution and elimination half-life were decreased significantly in 14- and 30-day STZ-treated rats. Thus, rose bengal is cleared more quickly in diabetic rats than in the controls.

The second panel of figure 1 indicates that biliary excretion of rose bengal was increased above normal in 14- and 30-day diabetic rats. Excretion was elevated in 7-day STZ-treated rats in the 15- and 45-min collection periods. Cumulative and maximal excretion and biliary clearance of rose bengal in 30-day diabetic rats were increased to 315, 300 and 540% above control, respectively. The third panel of figure 1 illustrates that basal bile acid excretion increases significantly 7, 14 and 30 days after STZ treatment.

Basal bile flow rates in \( \mu l/min/kg \) body weight were within normal range at 7 and 14 days after STZ, but significantly increased by 30 days (fig. 1, bottom). After rose bengal injection, bile flow in normal rats decreased as expected. By 7 days after STZ, there was some decrease evident after rose bengal, although at 14 days, bile flow rates reached levels comparable to basal levels after rose bengal. By 30 days after STZ, bile flow remained elevated after rose bengal injection.

In contrast, pronounced alterations in rose bengal elimination were not evident in the Wistar fatty rats (fig. 2). Serum concentrations were higher in fatty rats for the first 30 min of the experiment than in lean litter mates. Biliary excretion of both rose bengal and endogenous bile acids was not different between the two groups, but bile flow was diminished by rose bengal in fatty rats to a greater extent than in lean rats. Rose bengal produced cholestasis in both lean and fatty animals; a similar response was seen in normal rats (fig. 1). Higher blood concentrations are indicative of a decrease in total clearance and an increase in elimination half-life (table 2) in fatty rats. Biliary clearance of rose bengal was decreased 35% in Wistar fatty animals. No significant change in volume of distribution was determined.

Because rose bengal excretion is influenced by bile acids, bile was collected from additional 30-day STZ-treated rats for 10 hr to deplete the rats of bile acids (fig. 3). However, in contrast to figure 1 rose bengal excretion and bile flow rates

<table>
<thead>
<tr>
<th>Body Weight (g)</th>
<th>Liver Weight (g)</th>
<th>Bile Flow</th>
<th>Glucose</th>
<th>Insulin</th>
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<tbody>
<tr>
<td>Normal</td>
<td>285 ± 8</td>
<td>10.7 ± 0.59</td>
<td>58.2 ± 2.5</td>
<td>1.79 ± 0.12</td>
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<td>7-day STZ</td>
<td>234 ± 10</td>
<td>10.6 ± 0.40</td>
<td>54.7 ± 4.0</td>
<td>1.21 ± 0.09(^b)</td>
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<tr>
<td>14-day STZ</td>
<td>266 ± 10</td>
<td>12.6 ± 0.73</td>
<td>60.5 ± 4.1</td>
<td>1.28 ± 0.09(^b)</td>
</tr>
<tr>
<td>30-day STZ</td>
<td>295 ± 12</td>
<td>14.1 ± 0.47(^b)</td>
<td>83.3 ± 4.5(^b)</td>
<td>1.83 ± 0.15</td>
</tr>
<tr>
<td>Wistar lean</td>
<td>387 ± 10(^c)</td>
<td>13.0 ± 0.41(^b)</td>
<td>53.8 ± 1.7(^b)</td>
<td>1.60 ± 0.06</td>
</tr>
<tr>
<td>Wistar fatty</td>
<td>505 ± 14(^b)</td>
<td>18.2 ± 0.76(^b)</td>
<td>49.8 ± 2.6(^b)</td>
<td>1.38 ± 0.07(^b)</td>
</tr>
</tbody>
</table>

ND, Not determined.

\(^a\) Values are means ± S.E. for six to eight rats.

\(^b\) Values are significantly different from Normal at \( P < .05 \).

\(^c\) Values are significantly different from Wistar lean at \( P < .05 \).
The serum disappearance of rose bengal, biliary excretion of bile acids and rose bengal and bile flow rates in normal, 7-day diabetic, 14-day diabetic and 30-day diabetic rats. Values represent means ± S.E. of six to eight rats. * Indicates the value is significantly different from normal control rats at P < 0.05. For clarity, the asterisk has been omitted from the top panel at time points less than 20 min.

Fig. 1. The serum disappearance of rose bengal, biliary excretion of bile acids and rose bengal and bile flow rates in normal, 7-day diabetic, 14-day diabetic and 30-day diabetic rats. Values represent means ± S.E. of six to eight rats. * Indicates the value is significantly different from normal control rats at P < 0.05. For clarity, the asterisk has been omitted from the top panel at time points less than 20 min.

Discussion

STZ-induced diabetes results in an increase in the bile acid pool (Carnovale et al., 1987; Garcia-Marin et al., 1988; Kirkpatrick and Kraft, 1984; Villanueva et al., 1990; Watkins and Dykstra, 1987; Wey et al., 1984). Cholesterol and other steroid hormones are known to bind to receptors and affect transcription of specific mRNAs. Similarly, bile acids (modified cholesterol compounds) also influence transcription for a variety of proteins. For many years, bile acids were suspected to stimulate synthesis of specific carriers involved in both bile acid-dependent (Adler et al., 1977) and bile acid-independent (Wannagat et al., 1978) bile flow, as well as basolateral transporter biosynthesis (Simon et al., 1982). Recent studies demonstrated that bile acids exert feedback inhibition on cholesterol 7-α-hydroxylase via a bile acid-regulated element in the promoter (Hoekman et al., 1993; Twisk et al., 1993), as well as suppress sterol 27-hydroxylase mRNA and transcriptional activity of the corresponding gene in cultured rat hepatocytes (Twisk et al., 1995a). Physiological levels of insulin down-regulate both cholesterol 7-α-hydroxylase and sterol 27-hydroxylase gene transcription, with a resultant suppression of bile acid synthesis (Twisk et al., 1995b). This finding helps explain the lack of increased bile acid pool in hyperinsulinemic Wistar rats, and the increased bile acid pool size in STZ-induced insulin-deficient diabetic animals.

Rapid advances are being made in understanding the molecular mechanisms for hepatic uptake and biliary excretion. Both Na⁺-dependent and Na⁺-independent bile acid transporters as well as other organic anion transporters have been demonstrated on the basolateral surface (Frimmer and Ziegler, 1988; Jacquemin et al., 1994; Oude Elferink et al., 1995; Steiger et al., 1994). In addition, rat canalicular membranes contain four ATP-dependent transport processes including the multispecific organic anion transporter, as well
as ATP-independent organic anion transporters, which are distinct from the bile acid transporter(s) (Arias et al., 1993; Oude Elferink et al., 1995; Pikula et al., 1994a, 1994b; Sippel et al., 1994; Zimniak and Awasthi, 1993). The finding of an increased maximal velocity for the taurocholate transporter in the diabetic bLPM and that insulin treatment normalized this result (fig. 4) suggests that changes in diabetic rat liver brought about in the vectorial transport of taurocholate, either at the basolateral or canalicular domain, result from a greater free bile acid pool. Thus, a higher bile acid pool increases secretory rates and the total amount of free bile acid contained in enterohepatic circulation. Free bile acids display detergent-like properties and are thus detrimental to cellular membranes. They can potentially damage the tight junctions that separate the sinusoidal and lateral domains of the hepatocyte membrane from the canalicular domain.

These possibilities, coupled with an increase in the bile acid pool in the diabetic, argue that the taurocholate trans-

**Fig. 2.** The serum disappearance of rose bengal, biliary excretion of bile acids and rose bengal and bile flow rates in lean and fatty Wistar rats. Values represent means ± S.E. of six to eight rats. * Indicates the value is significantly different from normal control rats at $P < .05$.

**Fig. 3.** The serum disappearance of rose bengal, biliary excretion of bile acids and rose bengal and bile flow rates in bile acid depleted and unmanipulated 30-day diabetic rats. Values represent means ± S.E. of six to eight rats. * Indicates the value is significantly different from normal control rats at $P < .05$.

<table>
<thead>
<tr>
<th></th>
<th>$C_l$</th>
<th>$V_{\text{dis}}$</th>
<th>$t_{1/2}$</th>
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<tbody>
<tr>
<td>Normal</td>
<td>1.22 ± 0.21</td>
<td>0.26 ± 0.05</td>
<td>183 ± 26</td>
</tr>
<tr>
<td>7-Day STZ</td>
<td>1.74 ± 0.27</td>
<td>0.47 ± 0.09</td>
<td>107 ± 11b</td>
</tr>
<tr>
<td>14-Day STZ</td>
<td>2.51 ± 0.47b</td>
<td>1.09 ± 0.15b</td>
<td>68.5 ± 9p</td>
</tr>
<tr>
<td>30-Day STZ</td>
<td>3.73 ± 0.41b</td>
<td>1.96 ± 0.41b</td>
<td>91.1 ± 8b</td>
</tr>
<tr>
<td>Wistar lean</td>
<td>2.15 ± 0.14</td>
<td>0.50 ± 0.09</td>
<td>108 ± 6</td>
</tr>
<tr>
<td>Wistar fatty</td>
<td>1.56 ± 0.09bc</td>
<td>0.32 ± 0.05c</td>
<td>109 ± 17</td>
</tr>
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</table>

* Values are means ± S.E. for six to eight rats.

b Significantly different from control at $P < .05$.

c Values in fatty rats are significantly different from lean rats at $P < .05$. 

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a higher bile acid pool increases secretory rates and the total amount of free bile acid contained in enterohepatic circulation. Free bile acids display detergent-like properties and are thus detrimental to cellular membranes. They can potentially damage the tight junctions that separate the sinusoidal and lateral domains of the hepatocyte membrane from the canalicular domain.

These possibilities, coupled with an increase in the bile acid pool in the diabetic, argue that the taurocholate trans-
porter is modified at either the basolateral or canicular domain. Increased bile acids in the blood facing the sinusoids of the hepatocytes cause either an increased number of receptors, an increase in the turnover number (reflected by an increased $V_{max}$), or a change in the affinity (an alteration in the $K_p$) of the carrier protein for taurocholate. Diabetic bLPM significantly increased its average maximal transport velocity from 2.9 to 5.2 nmol TCA/mg protein/10 sec. It is somewhat surprising to observe differences in the bLPM and not in the cLPM of the diabetic, since during normal hepatic transport, canicular bile acid secretion is the rate-limiting step (Blitzer and Boyer, 1978). In fact, Icarte et al. (1991) found that 14 or 24 days after alloxan administration to rats, the maximum taurocholate secretory rate (i.e., bile salt receptor sites at the canicular surface) increased significantly in response to an expansion of the bile acid pool. In contrast, reports that amino acid, glucose and bile salt Na$^+$-dependent transport and uptake are enhanced in chronically diabetic rats at the enterocyte and hepatocyte membrane surfaces (Caspary, 1973; Fedorak et al., 1989; Samson et al., 1980) are consistent with the changes found in the bLPM in this study. Thus, it is reasonable to infer that the sinusoidal domain of the hepatocyte responds to an increased bile acid pool in the blood by producing more transporters or by changing the responsiveness of existing transporters by allosteric interaction or phosphorylation as shown for canicular transport (Pikula et al., 1994a, b). Once in the cell, bile acids likely bind with other proteins that aid in propelling the bile acid to the canicular surface. After biliary secretion, the bile acids form mixed micelles with phospholipids and cholesterol (Hofmann, 1994) and no longer exist in a free state that can damage the membrane. Our study examined taurocholate transport using a Na$^+$-dependent pathway. However, transport across the basolateral portion of the hepatocyte membrane may also use a chloride-dependent sodium-independent transporter. Also, a Na$^+$-independent process is used to secrete taurocholate from the canicular domain. Inoue et al. (1984) have reported that saturation for cLPM uptake is reached in the absence of sodium and the presence of a strong electrical potential difference across the membrane. Future studies need to determine whether the diabetic state causes alterations in the maximal driving forces for transport of taurocholate across LPM.

In normal SD rats (Watkins and Noda, 1986), in Wistar fatty rats with normal endogenous bile acid concentrations (fig. 2) and in bile acid-depleted STZ rats (fig. 3), rose bengal reduces bile flow. Presumably, this results from rose bengal binding to the ATP site of the Na$^+$K$^+$-ATPase (in a manner similar to eosin, Skou and Esmann, 1988), thereby decreasing the magnitude of the sodium gradient, decreasing Na$^+$-dependent bile acid uptake into the hepatocytes, and thus decreasing the amount of bile acids available for excretion into the canaliculus. The net result is reduction in the bile acid-dependent portion of the bile flow.

Taking these findings into consideration, we postulate that the increased bile acid pool in STZ-treated rats is stimulating transcription of mRNA(s) for Na$^+$-independent anion transporter(s) on the basolateral membrane, capable of transporting both bile acids and rose bengal, producing the increase in serum clearance seen with rose bengal in the diabetic animals in this study. Similarly, increased transcription of organic anion canicular transporter(s) capable of transporting bile acids and/or rose bengal may be simultaneously occurring. If the increased bile acid pool induces Na$^+$-independent transporters as diabetes progresses, then the initially observed decreased bile flow due to inhibition of Na$^+$K$^+$-ATPase upon administration of rose bengal in control rats would begin to be overcome by the enhanced Na$^+$-independent fraction of bile acid transport. Indeed, this is what is observed in 7-, 14- and 30-day diabetic rats: rose bengal is removed from the serum more quickly, bile acid excretion increases and bile flow is more elevated the longer diabetes is allowed to develop.

The increased clearance of rose bengal from the circulation and the higher excretion of bile acids into the canaliculus argue against STZ toxicity playing a role in bile flow changes by 7 days post-STZ. We suggest that rose bengal is interacting with Na$^+$K$^+$-ATPase and thus is slower in leaving the hepatocyte in 7- and 14-day rats. By 30 days, the bile acid pool is greatly increased, and the increased canicular transport of bile acids may exert "osmotic drag" and remove rose bengal from the hepatocyte, preventing interaction with (and inhibitory effect upon) Na$^+$K$^+$-ATPase. Studies directed to the effect of rose bengal on Na$^+$K$^+$-ATPase are needed to clarify this point.

Insulin increases Na$^+$K$^+$-ATPase activity (Gelehrter et al., 1984). A deficit in insulin might therefore be expected to decrease Na$^+$-dependent uptake of bile acids. Garcia-Marin

![Graph showing initial uptake of taurocholate across cLPM and bLPM](image-url)
et al. (1988) concluded that cholestasis observed in 6- and 20-day post-STZ diabetic rats was a result of hyperglycemia and hypoinsulinemia decreasing the bile acid-independent fraction of bile flow in these animals. At 1 day after STZ, bile acid excretion and bile flow are significantly lower than normal (Carnovale and Rodriguez Garay, 1984; Carnovale et al., 1986; Garcia-Marin et al., 1988). By 6 or 7 days, bile acid excretion is above normal levels, and bile flow is only slightly depressed (fig. 1), indicating that it is the bile acid-independent fraction that is affected. If oxidative stress due to hyperglycemia is occurring (Mukherjee et al., 1994), resulting in decreased glutathione excretion into the bile (Ballatori and Truong, 1992), then one might expect a decrease in bile flow until such time as the bile acid-dependent flow increased to the point that the reduced bile acid-independent fraction of bile flow is overwhelmed. The present data are consistent with that hypothesis.

In considering the different conclusions in the literature about the effects of diabetes on bile flow, it is therefore apparent that one reason for the divergent opinions may be differing lengths of time after STZ administration at which bile flow was measured. The progression of the disease creates abnormal physiological conditions with concomitant changes in functioning at 30 days. Interestingly, Villanueva et al. (1990) also studied bile flow up to 28 days after STZ injection, but concluded that bile flow had not recovered for the three rats they maintained in that period of time. Their 28-day diabetic rats were similar in body weight, bile acid levels and bile flow rates to our 14-day STZ diabetic rats. However, there was a significant difference in reported glucose levels (345 vs. 639 mg/dl). This suggests a difference in endogenous insulin levels between the two groups, which (as discussed above) accounts for the variance in bile acid levels with resulting effects on bile acid-dependent bile flow.

A second reason for the conflicting conclusions can be attributed to the method of calculation of bile flow. Pharmacokinetics and administration of rose bengal and streptozotocin are all determined according to body weight, and the calculation of basal bile flow has been done in this study both per g liver and per kg body weight to allow comparison between the two methods (table 1). When calculated per g liver on 7- and 14-day diabetic rats, bile flow is significantly decreased (also observed by other investigators), but returns to normal levels in the 30-day diabetics. Calculation of bile flow per kg body weight suggests that bile flow remains normal at 7 and 14 days after STZ but significantly increases by 30 days after STZ.

Therefore, the discrepancy in the literature as to whether STZ-induced diabetes produces cholestasis or choleresis can be explained by the two methods of calculation of bile flow, by different serum glucose levels in diabetic rats (reflecting different insulin levels) and by the length of time after STZ administration at which the bile flow rate is determined. Initial STZ toxicity may decrease bile flow until cellular repair occurs and cellular function returns to normal, but by 7 days after STZ, organic anion uptake from the circulation and biliary excretion of bile acids are both increased over that of the normal animals, indicating that STZ toxicity is no longer an important factor affecting bile flow. The increase in bile acids seen in diabetes results in increased clearance of anionic substances from the serum, possibly by stimulating the transcription of sinusoidal Na\(^+\)-independent organic anionic transporters. In conclusion, studies using STZ to produce animal models of diabetes need to allow time for STZ toxicity to be overcome and for the pathology of diabetes to become established to accurately reflect the diabetic condition. The \textit{in vitro} data obtained in our study suggest that one of the basolateral taurocholate transporters is modified by diabetes resulting in elevated biliary clearance of the bile acid. The increase in the \(V_{\text{max}}\) for taurocholate transport in the bLPM fraction may reflect introduction of more bile acid receptor sites or an increased turnover number of the transporter in the basolateral membrane via a process of adaptive regulation in response to the increased bile acid that accumulates in hepatic sinusoidal blood during enterohepatic circulation. This results in a higher \(V_{\text{max}}\) for the bLPM of uncontrolled diabetics. Finally, diabetic rats treated with insulin behaved almost analogously to normal rats in this regard and displayed normal kinetic parameters for the vectorial transport of taurocholate.

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References


Biliary Excretion in Diabetic Rats


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