Biliary secretion in elasmobranchs. II. Hepatic uptake and biliary excretion of organic anions

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Boyer, James L., Joseph Schwarz, and Neil Smith. Biliary secretion in elasmobranchs. II. Hepatic uptake and biliary excretion of organic anions. Am. J. Physiol. 230(4):974-981. 1976. - [35S]Bromosulfophthalein ([14C]BSP), [14C]sodium taurocholate ([14C]NaTC), and 10 mg of unlabeled BSP and of phenol-3,6-dibromophthalein disulfonate (DBSP) per kilogram body weight were injected in the caudal artery of free-swimming dogfish sharks (Squalus acanthias) and small skates (Raja erinacea). Twenty-four hours later, 85.8 ± 15.7% of [35S]BSP was recovered in bile and liver in dogfish and 78.4 ± 9.9% in skates. Similar results were obtained for [14C]NaTC. Unlabeled BSP or DBSP (10 mg/kg body wt) were also selectively excreted in bile over a 4-day period and at comparable rates in both species. More than 85% of [35S]BSP, BSP, and DBSP in bile was in unconjugated form. Selective hepatic clearance of BSP occurred despite nonselective binding to liver homogenates and very low concentrations of binding proteins in liver cytosol. Analysis of the organic anion plasma disappearance curves suggest that the clearance of anions into bile in elasmobranchs is delayed disproportionately relative to hepatic uptake. Albumin-BSP infusions did not prevent selective hepatic uptake of [35S]BSP, although biliary excretion was delayed further. These studies demonstrate that transport systems for biliary excretion of organic anions evolved prior to migration of marine life from the sea and relatively independently of intrahepatic conjugation and organic anion-binding proteins.

Squalus acanthias; Raja erinacea; bromosulfophthalein; phenol-3,6-dibromophthalein; sodium taurocholate; erythritol; liver; bile

PRELIMINARY STUDIES from our laboratory have demonstrated that bromosulfophthalein (BSP) is selectively removed from plasma and excreted into bile by the liver of two elasmobranchs, Squalus acanthias and Raja erinacea (7, 8). These observations are at variance with present concepts of hepatic organic anion excretion in marine species that suggest that these compounds might be preferentially excreted by the gills or kidneys (11, 31). This view is based largely on evidence that hepatic microsomal enzyme activities are low and that liver cytoplasmic organic anion-binding proteins (ligandin), which facilitate the hepatic uptake of these compounds in mammals, are either absent or found in low concentrations in fish (28). In order to study this process in more detail, we have utilized a technique for hepatic bile collection in the free-swimming elasmobranch that is described in the preceding report (9). In the present study we used this technique to study the process of hepatic uptake and biliary organic anion excretion in these species, comparing the biliary excretion of BSP with phenol-3,6-dibromophthalein disulfonate (DBSP), a BSP analogue that is excreted in unconjugated form in the bile of mammals (21, 24), and sodium taurocholate (NaTC), a conjugated bile acid that is not prevalent in elasmobranch bile (19). The role of protein binding was also assessed by studying the effects of albumin infusion on the hepatic uptake and biliary clearance of [35S]BSP in vivo and by comparing the binding of [35S]BSP to tissue homogenates and liver cell supernatant in vitro.

METHODS

All studies were performed in male dogfish sharks and small skates of both sexes from Raja species obtained at the Mount Desert Island Biological Laboratory in Salsbury Cove, Maine, as previously described (9). Bromosulfophthalein sodium salt was obtained from Hynson, Westcott & Dunning, Inc., Baltimore, and phenol-3,6-dibromophthalein disulfonate was kindly provided by Dr. H. A. B. Dunning, Jr. of the same company; [35S]BSP and [14C]erythritol were obtained from Amersham/Searle Corp. and [14C]sodium taurocholate was purchased from California Biochemical Company; bovine serum albumin (fraction V) was obtained from the Metrix Division of Armour Pharmaceutical Company; Scintisol Complete came from Isolab, Inc.; and cellulose F thin-layer plates were obtained from J. T. Baker Chemical Co., Phillipsburg, N.J.

Experimental model. Bile was collected in free-swimming dogfish sharks and skates by inserting a cannula (Clay Adams PE-260) into the lumen of the gallbladder and tying it in place after ligating the common duct and withdrawing the gallbladder fluid. The cannulas were then externalized through an abdominal incision and small balloons were attached to obtain bile samples. The technique of bile collection is described in detail in the preceding paper (9). Studies of bile composition before and after cannulation indicated that the technique was effective in collecting hepatic bile that was not modified by transport function of the gallbladder (9).

[35S]BSP plasma disappearance, hepatic uptake, biliary excretion, and effect of albumin infusions. Thirty to 50 μCi of [35S]BSP containing 23 μCi/mg BSP in saline were injected into 19 dogfish and 4 skates. The syringes were weighed before and after injection to determine the
volume administered. Plasma samples were obtained in four skates and six dogfish at 15, 30, 60 min, 2 h, 4 h, 8 h, and 24 h; bile was collected from 0 to 4, 4 to 8, and 8 to 24 h after injection of the isotope into the caudal artery or vein. In another experiment, \[^{35}S\]BSP was also mixed with 15 ml of 10% bovine serum albumin and injected into dogfish to determine the effect of increasing plasma protein binding on BSP excretion. All experiments were terminated at 24 h and livers were rapidly removed and weighed. Duplicate 50-mg samples of liver were digested in 0.5 ml 2 N NaOH overnight at 80°C, neutralized with 0.1 ml glacial acetic acid, and placed in 12 ml of Scintisol Complete for determination of hepatic BSP content; 100-\(\mu\)l samples of plasma and 100-\(\mu\)l aliquots of the administered solutions were also analyzed and all specimens were counted in a Nuclear-Chicago Mark I liquid scintillation spectrometer. Corrections for quenching were determined by external standard ratios. Plasma \[^{35}S\]BSP disappearance curves were analyzed into two components by curve peeling of log plots of the activity expressed as disintegrations per minute per 100 \(\mu\)l of plasma (32). Fractional disappearance rates \(k_1\) and \(k_2\) were determined by the formula \(k_{1,2} = \frac{\ln 2}{t_{1/2}}\).

In a separate experiment, the tissue distribution of \[^{35}S\]BSP was determined in dogfish 2 h after injection, when the majority of activity had disappeared from plasma, but prior to evidence of significant biliary excretion. These results were compared with other studies in which \[^{35}S\]BSP was administered via the caudal artery together with 15 ml of a 10-g/100 ml solution of bovine serum albumin. No untoward effects of the albumin infusion were noted as the fish continued to swim actively. In these studies, fish were sacrificed 2 h after injection of the label. The livers and kidneys were removed from each fish and weighed and a sample of muscle was obtained. Muscle mass was assumed to equal 43% of total body weight as previously determined by Burger (12). Samples of each tissue were digested and counted as described above.

**Hepatic uptake and biliary excretion of \[^{14}C\]sodium taurocholate.** The biliary excretion of \[^{14}C\]sodium taurocholate was examined over a period of 24 h as described for \[^{35}S\]BSP.

**Biliary excretion of unlabeled BSP and DBSP.** In other experiments, the biliary excretion of unlabeled BSP was compared with its analogue DBSP after intraarterial injection of each compound (10 mg/kg body wt) into separate groups of fish. Bile was collected daily for periods of 4 days in these studies and the volume was measured. The BSP and DBSP were then quantitated in samples of bile by the method of Seligson, Marino, and Dodson (35) and expressed as a percentage of the administered dose.

**Bile plasma \(B/P\) ratios of \[^{35}S\]BSP, \[^{14}C\]taurocholate, and \[^{14}C\]erythritol.** Determinations of \(B/P\) ratios of these solutes were obtained after adjustment of the plasma value for an approximation of the time lag imposed by the biliary dead space on the appearance of the label in bile collected in the balloons. Bile/plasma \(^{35}S\) and \(^{14}C\) ratios were compared with values obtained for \[^{14}C\]erythritol, a nontransported, diffusible solute that was administered to dogfish and skates in a similar manner.

**Determination of BSP and DBSP metabolites secreted into bile.** Samples of hepatic bile (2.5-15 \(\mu\)l) containing BSP, DBSP, or \[^{35}S\]BSP were placed on precoated cellulose F thin-layer chromatographic plates and separated in solvent system A, consisting of n-butanol:glacial acetic acid:H\(_2\)O (40:10:50) as previously described by Whelan and Plaa (36). The \[^{35}S\]BSP metabolites were also separated in solvent system B (kindly provided by A. M. Guarino at the National Cancer Institute), consisting of ethanol:n-butanol:3 M NH\(_4\)OH (11:40:19). Separation was achieved within 2-4 h in both systems. In some specimens, trailing occurred with solvent system B and these biles were then lyophilized and extracted with anhydrous methanol prior to separation by thin-layer chromatography. Controls consisted of BSP, DBSP, or \[^{35}S\]BSP added to fish bile. Rat bile containing BSP and metabolites was also plated as an additional control. Spots were identified after exposure to ammonia fumes or fluorescent light. Spots for quantitative recovery were scraped, eluted as previously described (6), and counted in 10 ml dioxane with a Nuclear-Chicago Isocap liquid scintillation spectrometer. Recovery of \[^{35}S\]BSP standards from the cellulose plates (10 studies each) averaged 91 ± 2.5% with solvent system A and 91 ± 3% with solvent system B. The BSP metabolites were combined and the data expressed as percent free or percent conjugated.

**Plasma protein electrophoresis.** Five-\(\mu\)l samples of plasma or standard (100 mg/ml bovine albumin) were applied to 6-inch cellulose polyacetate electrophoresis strips and separated in a Gelman electrophoresis chamber for 1 h at 200 Volts with barbital buffer, pH 8.6, ionic strength 0.075. Protein bands were stained with phenol blue.

**Binding of \[^{35}S\]BSP to tissues and plasma.** Tissue was obtained for binding studies from both dogfish sharks and skates. After severing the spinal cord, 5- to 10-g samples of liver, kidney, and muscle were rapidly removed; the tissue was placed on ice, weighed, minced with scissors in ice-cold phosphate buffer, and homogenized at 4°C in 3 vol 0.1 M sodium phosphate buffer, pH 7.6. Oil was removed from the liver homogenate of dogfish sharks and skates when necessary by centrifugation at 1600 g for 20 min (International Refrigerated Centrifuge). The homogenates were frozen at −20°C for subsequent analysis. Protein concentration in matched samples of plasma and homogenates of liver, kidney and muscle were determined by the method of Lowry et al. (30). Protein concentration ranged from 41.2 to 53.5 mg/g in dogfish liver and from 121 to 146 mg/g in skate liver after correction for lipid content (lipid averaged 32% of liver weight in male dogfish and 7% in skates). Each sample was prepared in duplicate and adjusted to a final concentration of 10 mg protein/ml in a volume of 2.5 ml with 0.1 M PO\(_4\) buffer, pH 7.4. Then \[^{35}S\]BSP (0.1 \(\mu\)Ci) was added together with 0.5 mg of unlabeled BSP and incubated at room temperature for 30 min. The BSP-protein solution was added to dialysis bags that had been presoaked for 3 days at 4°C in 0.1 M phosphate buffer, pH 7.4, containing 0.005 M EDTA. The bags
were then suspended in conical 25-ml tubes and centrifuged at 1,800 rpm (Sorvall table model centrifuge) to obtain a small quantity of filtrate. Determination of percent BSP bound was calculated by expressing the activity (disintegrations per minute) in the filtrate as a ratio of the activity in the bag after correcting for non-specific binding to the cellophane (79 ± 2% of the activity filtered through the dialysis tubing in 20 control experiments that did not contain protein).

**Determination of organic anion-binding proteins (ligandin).** Four milliliters of a 105,000-g supernatant from plasma curves were made with [W]NaTC (Fig. 3B), this anion appeared in this secretion. Although fewer observations could be detected in bile from the dogfish, liver of both species 24 h after their administration (Table 3).

Excretion of BSP and DBSP was also determined in dogfish sharks (Squalus acanthias) and small skates (Raja erinacea). Ordinate is represented as disintegrations per minute per 100 µl plasma plotted as the log: abscissa is represented in hours. Fractional disappearance rate (k₁ and k₂) and half-life are given for each of the 2 major exponentials (arrows).

### TABLE 1. Analysis of plasma disappearance curves after injection of [³⁵S]BSP and [¹⁴C]NaTC

<table>
<thead>
<tr>
<th></th>
<th>Initial Exponential</th>
<th>Final Exponential</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t₁/₂, min</td>
<td>k₁, %/min</td>
</tr>
<tr>
<td>[³⁵S]BSP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dogfish (6)</td>
<td>15.8</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>± 2.7</td>
<td>± 0.8</td>
</tr>
<tr>
<td>Skate (4)</td>
<td>16.1</td>
<td>4.45</td>
</tr>
<tr>
<td></td>
<td>± 2.6</td>
<td>± 1.0</td>
</tr>
<tr>
<td>[¹⁴C]NaTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dogfish (2)</td>
<td>21.75</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>± 0.075</td>
<td>± 0.095</td>
</tr>
</tbody>
</table>

Data are expressed as mean or mean ± SD; number of experiments given in parentheses.
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Fig. 2. Tracing of cellulose acetate paper electrophoresis of plasma proteins in dogfish plasma (left), bovine serum albumin standard (middle), and plasma obtained 15 min after injection of albumin (right). Large arrow represents direction of current; small arrows, the origin. Bands were stained with phenol blue.

Table 2. Distribution of $^{35}$S]BSP in tissues of dogfish 2 h after injection and effect of albumin infusion

<table>
<thead>
<tr>
<th></th>
<th>Liver</th>
<th>Plasma</th>
<th>Kidney</th>
<th>Muscle</th>
<th>Recovery of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (5)</td>
<td>72 ± 14</td>
<td>3.8 ± 1.95</td>
<td>11.6 ± 3.8</td>
<td>4.3 ± 1.8</td>
<td>92 ± 15</td>
</tr>
<tr>
<td>Albumin infused (4)</td>
<td>71 ± 14</td>
<td>6.8 ± 2.0</td>
<td>2.6 ± 1.0</td>
<td>4.5 ± 1.1</td>
<td>85 ± 12</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD; number of experiments given in parentheses. NS, no significance (P > 0.05).

Although binding to liver homogenates in dogfish was slightly greater than to plasma, no differences were noted in the skate. Surprisingly, kidney homogenates demonstrated the highest BSP binding per milligram of protein in both species. These observations are in con-
difficult because plasma values for the dye were quite low.

An analysis of $^{35}$S]BSP and unlabeled BSP and DBSP metabolites in bile from three sets of experiments revealed that these organic anions were excreted essentially in the free unconjugated form (Table 5). Several minor metabolites of BSP could be identified by thin-layer chromatography, one migrating with BSP-glutathione from rat bile.

Because the tissue distribution of organic anions like BSP is thought to be dependent in part on the binding affinity of these compounds for plasma and tissue proteins, we compared the binding of $^{35}$S]BSP to plasma and tissue homogenates from liver, kidney, and muscle containing equivalent concentrations of protein (Table 6).

Table 3. Bile and hepatic content of $^{35}$S]BSP and $^{14}$C]NaTC at 24 h

<table>
<thead>
<tr>
<th></th>
<th>Bile</th>
<th>Liver</th>
<th>Both</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{35}$S]BSP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dogfish (7)</td>
<td>53.5 ± 17.7</td>
<td>32.2 ± 11.7</td>
<td>85.8 ± 15.7</td>
</tr>
<tr>
<td>Skate (3)</td>
<td>48.3 ± 20.9</td>
<td>30.0 ± 17.4</td>
<td>78.4 ± 9.9</td>
</tr>
<tr>
<td>$^{14}$C]NaTC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dogfish (2)</td>
<td>75.0</td>
<td>5.4</td>
<td>80.4</td>
</tr>
<tr>
<td>Skate (1)</td>
<td>74.5</td>
<td>2.1</td>
<td>76.6</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD; number of experiments given in parentheses.
FIG. 4. Biliary excretion of BSP and DBSP in dogfish shark over a 4-day period after injection of a 10-mg/kg dose into caudal artery. Eight studies were performed for each anion, and data are expressed as mean ± SD of percent of administered dose. Differences were not significant (NS).

FIG. 5. Biliary excretion of BSP and DBSP in small skate over a period of 4 days after intracaudal injection of 10 mg/kg body wt. Four studies were performed with each anion, and data are expressed as mean ± SD of percent of administered dose. Differences were not significant (NS).

TABLE 4. Bile/plasma ratios

<table>
<thead>
<tr>
<th>Anion</th>
<th>Solvent System A</th>
<th>Solvent System B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Free</td>
<td>% Conjugated</td>
</tr>
<tr>
<td>BSP</td>
<td>Dogfish (6)</td>
<td>87.5 ± 4.08</td>
</tr>
<tr>
<td></td>
<td>Skates (3)</td>
<td>86.1 ± 3.56</td>
</tr>
<tr>
<td>DBSP</td>
<td>Skates (4)</td>
<td>94.4 ± 2.2</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD of % free or % conjugated BSP or DBSP in bile for each solvent system tested; number of studies given in parentheses.

TABLE 5. Percent free and conjugated [35S]BSP, BSP, and DBSP in bile

<table>
<thead>
<tr>
<th>Anion</th>
<th>Solvent System A</th>
<th>Solvent System B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Free</td>
<td>% Conjugated</td>
</tr>
<tr>
<td>BSP</td>
<td>Dogfish (9)</td>
<td>95.2 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>Skates (5)</td>
<td>85.9 ± 7.6</td>
</tr>
<tr>
<td>DBSP</td>
<td>Skates (4)</td>
<td>94.4 ± 2.2</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD of % free or % conjugated BSP or DBSP in bile for each solvent system tested; number of studies given in parentheses.

DISCUSSION

Bile is the major route of excretion in mammalian species for most organic anions with molecular weights exceeding 300–500 and is a complex process involving removal from plasma proteins (primarily albumin), uptake across the hepatic sinusoidal membranes, intracellular binding to proteins within the cytosol, conjugation to more water-soluble products, and finally transport across the bile canalicular membrane against a large concentration gradient into bile (14). The mechanisms and relative importance of these transport steps have not been fully elucidated, partly because it has been difficult to dissociate the influence of one process from another.

In the present study the ability to collect bile over several days in free-swimming elasmobranchs (9) has enabled us to examine organic anion excretion in lower vertebrates where selective hepatic uptake and biliary excretion might not be expected to occur because plasma albumin, intrahepatic conjugating mechanisms, and binding proteins (ligandin) are either absent or low in activity (11, 28). However, a few isolated reports have indicated that marine species are capable of excretion of organic anions such as bilirubin, BSP, and other dyes from plasma in control studies. Albumin was demonstrated in plasma 15–30 min after intra-arterial injection (Fig. 2) and resulted in a small increase in plasma BSP and a substantial decline in binding to kidney tissue (Table 2). However, BSP content in the liver was essentially unchanged 2 h after infusion of the albumin-[35S]BSP mixture. Despite negligible effects on hepatic uptake of BSP in this study, a 24-h collection of bile indicated that the biliary excretion of BSP was significantly delayed (Fig. 7).

TABLE 6. Percent binding 0.1 µCi [35S]BSP to plasma and tissue homogenates

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solvent System</th>
<th>% Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>Solvent System A</td>
<td>Solvent System B</td>
</tr>
<tr>
<td></td>
<td>% Free</td>
<td>% Conjugated</td>
</tr>
<tr>
<td></td>
<td>Dogfish (6)</td>
<td>69.4 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>Skates (3)</td>
<td>66.6 ± 5.8</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD; number of experiments in parentheses. * Values significantly different from plasma. † Values significantly different from liver.
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(12, 20). Whether or not bile is the major route of excretion for these anions has not been previously established, and it had been suggested that the kidney or gills were preferential routes of excretion. Nevertheless, the present study indicates that despite these theoretical considerations several different organic anions, which have been studied extensively in mammalian species, also undergo selective hepatic uptake and biliary excretion in marine elasmobranchs.

These observations help clarify several concepts concerning the excretion of organic anions into bile. First, despite the lack of albumin (33), BSP was able to bind to other proteins in elasmobranch plasma (Table 6) (33), so that albumin was not essential either for transport of this organic anion in plasma nor for interaction with the hepatic sinusoidal membrane for hepatic uptake. Even when a BSP-albumin solution was introduced into the circulation, hepatic uptake was relatively unaffected, although biliary excretion of the dye was delayed. This suggests that plasma protein binding influences the overall clearance of BSP in elasmobranchs as previously found in mammalian studies including man (1, 5, 10, 15) presumably by increasing the efflux of the organic anion from liver back into plasma. In the present studies, dogfish and skate liver homogenates bound BSP only slightly more than plasma (per milligram of protein) and significantly less than kidney tissue in contrast to studies in the rat, where binding is greatest in the liver (10). As previously reported by Levine et al. (28), organic anion-binding protein (ligandin) (29) was essentially absent in elasmobranchs (Fig. 6). In the present study, only small amounts of BSP were bound to proteins in the 44,000- to 46,000-mol wt range of ligandin. Despite the relative absence of specific hepatic binding proteins, the livers of both dogfish shark and skate removed the majority of administered BSP, DBSP, and NaTC fairly rapidly and eventually preferentially eliminated these anions into bile. Thus, ligandin does not seem to play a role in the selective hepatic uptake of organic anions in elasmobranchs as has been claimed for mammals (27, 34). Mechanisms for selective hepatic uptake and biliary excretion of organic anions therefore appear to have evolved prior to and independently of the development of high concentrations of hepatic ligandin, which occurred after verbetrate migration from the sea (28). Recently, ligandin has been shown to be identical to the enzyme glutathione transferase B (17, 23). Although glutathione transferase has been detected in cell supernatant from elasmobranch liver (3), the enzyme assays in fish were performed with 1,2-dichloro-4-nitrobenzene, which is predominantly a substrate for transferase A and C (18).

In mammalian species, BSP is excreted primarily as the glutathione conjugate. The importance of conjugation has been studied in detail and is suggested as the rate-limiting step for BSP excretion in rat and guinea

![Figure 6](http://ajplegacy.physiology.org/)

**FIG. 6.** Sephadex G-75 column chromatography of 4 ml of a 105,000 g liver supernatant and BSP (2.5 mg) from representative experiments in rat, dogfish, and skate. Protein elution curves were determined by measurement of O.D. at 280 nm and are represented by open circles and solid lines; BSP binding was expressed as O.D. at 580 nm after alkalinization (see METHODS) and is represented by solid dots and dotted lines. Note that there are 2 prominent BSP peaks in rat, which correspond to ligandin (Y protein) and Z protein, respectively but only negligible BSP binding is observed with proteins of similar molecular size in liver cell supernatant from dogfish and skate.

![Figure 7](http://ajplegacy.physiology.org/)

**FIG. 7.** Effect of albumin infusions on biliary excretion of [14C]BSP in 3 dogfish compared with 7 controls. There was both a delay in appearance of BSP in biliary cannula and a reduced percentage of administered dose excreted in bile by 24 h in albumin-infused fish. Data expressed as mean ± SD.
they represented less than 15% of the excreted compounds. Bromosulfophthalein was excreted at an identi-
cal liver and is readily excreted unaltered into bile (21,
branchs and although several metabolites are formed
not undergo conjugation with glutathione in mamma-
tional turnover rates for BSP in man average 0.14 (2,4,
patic uptake as it is in mammalian species. Initial frac-
tionately slow in elasmobranchs in contrast to their
relatively rapid rate of hepatic uptake. Since the major-
ary excretion in elasmobranchs. This question will need
further study by comparing the relative rates of excre-
tion of BSP and conjugated BSP.

Biliary excretion of these organic anions is dispropor-
tionately slow in elasmobranchs in contrast to their
relatively rapid rate of hepatic uptake. Since the major-
ity of injected BSP is present in the liver at 2 h, the
initial fast-decaying component of the plasma disappear-
ance curves (Fig. 1) is primarily a function of he-
aptic uptake as it is in mammalian species. Initial frac-
tional turnover rates for BSP in man average 0.14 (2, 4,
with doses that approximate 5 mg/kg body wt. Al-
though the plasma disappearance curves in the present
study were determined with [35S]BSP at doses of approx-
imately 1.0 mg/kg body wt, the initial fractional turn-
over of BSP in elasmobranchs was only 3 times slower,
de spite a very slow cardiac output (estimated to be 1.5
liters/kg body wt per h in dogfish sharks) (22), and a
much smaller sinusoidal surface area imposed by a two-
cell-thick hepatic plate. Thus, when differences in he-
aptic blood flow and liver anatomy are considered, the
hepatic uptake of organic anions is relatively slow in
elasmobranchs. Biliary excretion, however, is inordi-
nately slow. For example, a 10-mg/kg dose of BSP or
DBSP was not completely eliminated by 4 days, yet
would be excreted in most mammalian species within
an hour. The discrepancy in the rate of hepatic uptake
and biliary excretion is also reflected by the k1/k2 ratio
from the plasma BSP disappearance curves. When this
ratio is calculated from published reports of BSP disappear-
ance curves in man (2, 4, 32), for example, it ranges
from 1:3 to 1:4, in contrast to the very low k1/k2 ratio of
1:46–1:56 seen with BSP in elasmobranchs in the present
study. Thus, the transport of organic anions into bile is
delayed disproportionately in the elasmobranchs, in
keeping with their very slow rate of bile formation (9,
13). Conceivably, less polar solutes such as unconjuga-
gated BSP might be sequestered within the lipid of the
hepatocyte, which is extensive in elasmobranchs, or
might diffuse back out of the bile across the lipid cell-
lar membranes and thus undergo a bilihepatic circula-

It also is not clear why bile/plasma ratios of BSP and
NaTc achieved values that were higher than previously
reported in mammalian species. These ratios might be
increased through concentration of the solutes if water is
also reabsorbed from bile during its passage down the
biliary tree. However, bile/plasma ratios of [14C]eryth-
ritol, a diffusible, nontransported solute, which pre-
munely enters bile at the hepatocyte, were close to 1 (Table 4).
The extraordinarily high bile concentrations of
frasers in elasmobranch bile in the present study
support the observation that bile may be a very useful
fluid to sample environmental contaminants in the
marine biosphere (25, 26).

Parts of this work were supported by the L. L. Sinton Trust Fund.
J. L. Boyer is the recipient of Public Health Service Academic Career Development Award in Digestive Diseases AM 70218.

Received for publication 23 June 1975.

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