Biliary secretion in elasmobranchs. I. Bile collection and composition

JAMES L. BOYER, JOSEPH SCHWARZ, AND NEIL SMITH
Liver Study Unit, Department of Medicine, University of Chicago, Chicago, Illinois 60637, and Mount Desert Island Biological Laboratory, Salsbury Cove, Maine 04672

FEW STUDIES HAVE BEEN PERFORMED on biliary function in fish (3, 7) in part because of the slow rates of flow and the difficulties in collection of bile in marine species. In elasmobranchs, bile will not flow readily into cannulated common ducts, and ligation of the gallbladder is impossible without injury to hepatic tissue because the gallbladder is embedded within the hepatic parenchyma. Therefore, in the present study we utilized a technique for collecting bile directly as it entered the gallbladder lumen. Comparison of the composition of cannula and gallbladder bile indicated that hepatic bile could be collected by this technique in both species without effective contact with gallbladder epithelium. The low concentrations of HCO₃⁻ and Cl⁻ and the high bile salt levels in dogfish gallbladder bile indicate that mechanisms for concentration and acidification of bile in the gallbladder developed early in vertebrate evolution. These results indicate that gallbladder cannulation in free-swimming elasmobranchs is a useful technique for the study of bile secretory and excretory function in marine species.

METHODS

All in vivo studies were performed at the Mount Desert Island Biological Laboratory in Salsbury Cove, Maine, during July–August of 1971 and 1973. Male spiny dogfish sharks (2.2 ± 0.3 kg body wt) were caught by trawl in Frenchman’s Bay, placed in circulating seawater tanks aboard the laboratory launch, and transferred to large saltwater tanks at the laboratory. Male and female skates (0.94 ± 0.35 kg body wt) were netted in Southwest Harbor, Maine. All fish were utilized within several days of capture. On the morning of study, fish were restrained in a trough for approximately 10–15 min while their gills were perfused with well-oxygenated seawater at a rate of 1.5 liters/min. Plasma samples were obtained from the caudal artery in heparinized syringes. A small ventral abdominal incision was performed, the common duct was identified and ligated near the entrance of the hepatic ducts, and bile was carefully drained through a small incision in the distal tip of the gallbladder. Specimens of bile were usually frozen at −20°C for subsequent analysis. A large-bore polyethylene cannula (Clay Adams PE-240-260) was then introduced through the distal tip of the gallbladder and passed into the proximal portion of the lumen where hepatic bile entered. A suture was placed around the cannula, sealing off the distal tip of the gallbladder, and the cannula was then externalized through the abdominal wall and the wound closed with 4-0 silk sutures.

After the operative procedure, small balloons were attached to rubber plungers that were obtained from 10.0-ml disposable plastic syringes and placed over the end of the cannula. The fish were then allowed to swim freely in the large tanks that were continuously supplied with 15°C seawater pumped directly from Frenchman’s Bay. Bile could be collected easily by briefly restraining the fish and removing the balloons, and fish could be maintained in a free-swimming state for periods ranging from 4 to 7 days. Bile would not flow through the cannulas if the distal end was pressed against the proximal portion of the gallbladder wall and it was occasionally necessary to withdraw the cannula by several millimeters to correct this problem.

The composition of plasma and gallbladder bile obtained at the time of cannulation was compared with cannula bile secreted during the first 24 h. The initial 2- to 4-h sample that was presumed to be contaminated with residual bile from the gallbladder was discarded.

Bile and plasma composition studies. Calculation of percent solids in bile was performed by evaporation of a 1- to 2-ml weighed sample to dryness in a 100°C oven for 24 h. Osmolarity was determined in 0.2-ml samples by freezing-point depression with an Osmette A automatic...
osmometer (Precision Instrument, Waltham, Mass.) and pH was measured on freshly collected samples in a Corning pH meter, model 10 (Scientific Instruments). Concentrations of Na⁺ and K⁺ were measured by flame photometry and magnesium and calcium by atomic absorption spectrometry (Perkin-Elmer model 107); Cl⁻ was measured in a Cotlove chloridometer, and HCO₃⁻ was kindly determined by Dr. Thomas Maren with a Kopp-Natelson microgasometer, model 600 (10). A miniaturized Van Slyke technique was used that was modified from the Scholander manometric method. Estimates of bile acids or alcohols and other 3-hydroxysteroids were assayed with the steroid dehydrogenase method used previously in this laboratory (1).

RESULTS

Free-swimming cannulated dogfish sharks and small skates secreted bile slowly and at variable rates that reached peak values during the first 24 h in dogfish (1.77 ± .89 ml/kg) and in the second 24-h period in small skates (2.66 ± .89 ml/kg). Thereafter, the rate of secretion declined (Table 1). Liver-body weight ratios averaged 0.11 ± 0.02 for 18 male dogfish but were considerably lower in 24 male and female skates (0.03 ± 0.01) in which livers contained less lipid.

Composition studies of bile and plasma are represented in Tables 2 and 3 for the two species. Dogfish bile was more concentrated, averaging 13.36 ± 9.86% dry wt, than skate bile (5.25 ± 1.36% dry wt). Plasma osmolality and electrolyte composition compared favorably with values previously reported for these species (8, 11). The slightly lower values for skate plasma osmolarity correlated with a decline in osmolarity of the seawater in Frenchman's Bay during the second summer of these experiments, when studies with Raja species were performed.

*Squalus acanthias* gallbladder bile was dark green and differed markedly in composition from specimens of hepatic cannula bile, which were usually light brown or pale green. The Na⁺, K⁺, and Ca²⁺ values in *Squalus* gallbladder bile were significantly increased above their respective values in cannula bile, and a much larger increment (15-fold) was noted in the concentration of 3-hydroxysteroids (Table 2). In contrast, the gallbladder concentrations of HCO₃⁻ and Cl⁻ were diminished and the pH was acidic (6.39 ± 0.22). Despite gross differences in concentrations of electrolytes and bile salts, the osmo- larities of cannula and gallbladder bile were identical. Also, though cannula bile was similar in composition to plasma with respect to Na⁺, Cl⁻, and HCO₃⁻, slightly increased concentrations were observed for K⁺, Mg²⁺,

### TABLE 1. Bile flow in dogfish and skates

<table>
<thead>
<tr>
<th>Dogfish</th>
<th>1.77 ± 0.89</th>
<th>1.8 ± 0.59</th>
<th>1.02 ± 0.62</th>
<th>0.80 ± 0.27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skates</td>
<td>1.99 ± 1.10</td>
<td>2.66 ± 0.89</td>
<td>1.80 ± 1.04</td>
<td>2.03 ± 0.78</td>
</tr>
</tbody>
</table>

*Values are means ± SD; number of fish in parentheses.

### TABLE 2. Composition of plasma and hepatic and gallbladder bile in dogfish shark

<table>
<thead>
<tr>
<th>Solids, % dry wt</th>
<th>pH</th>
<th>Osmolarity, mosM</th>
<th>Bile Acids, mM</th>
<th>Na⁺, mM</th>
<th>K⁺, mM</th>
<th>Cl⁻, mM</th>
<th>HCO₃⁻, mM</th>
<th>Mg²⁺, mg/100 ml</th>
<th>Ca²⁺, mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>984 ± 26</td>
<td>6.39 ± 0.22</td>
<td>258 ± 15</td>
<td>3.8 ± 0.7</td>
<td>204 ± 14</td>
<td>3.3 ± 0.6</td>
<td>4.6 ± 1.6</td>
<td>15.4 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Skates</td>
<td>922 ± 64</td>
<td>5.8 ± 0.7</td>
<td>271 ± 14</td>
<td>6.6 ± 0.9</td>
<td>224 ± 21</td>
<td>5.6 ± 3.5</td>
<td>9.0 ± 2.0</td>
<td>18.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Gallbladder bile</td>
<td>134.4 ± 8.9</td>
<td>6.39 ± 0.22</td>
<td>293 ± 69</td>
<td>6.5 ± 0.7</td>
<td>79 ± 49</td>
<td>0.8 ± 0.3</td>
<td>10.5 ± 1.5</td>
<td>58.7 ± 9.6</td>
<td></td>
</tr>
</tbody>
</table>

*Values are means ± SD; number of fish sampled in parentheses. Analyzed by hydroxysteroid dehydrogenase method. Plasma or gallbladder constituents were significantly different from hepatic bile. All gallbladder constituents were significantly different from plasma where measured.

### TABLE 3. Composition of plasma and hepatic and gallbladder bile in small skate

<table>
<thead>
<tr>
<th>Solids, % dry wt</th>
<th>pH</th>
<th>Osmolarity, mosM</th>
<th>Bile Acids, mM</th>
<th>Na⁺, mM</th>
<th>K⁺, mM</th>
<th>Cl⁻, mM</th>
<th>HCO₃⁻, mM</th>
<th>Mg²⁺, mg/100 ml</th>
<th>Ca²⁺, mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Time 0</td>
<td>1056 ± 14</td>
<td>6.39 ± 0.22</td>
<td>265 ± 13</td>
<td>4.3 ± 0.6</td>
<td>261 ± 13</td>
<td>4.4 ± 0.5</td>
<td>2.5 ± 0.2</td>
<td>16.4 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>946 ± 15</td>
<td>6.39 ± 0.22</td>
<td>265 ± 13</td>
<td>4.3 ± 0.6</td>
<td>261 ± 13</td>
<td>4.4 ± 0.5</td>
<td>2.5 ± 0.2</td>
<td>16.4 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Cannula (hepatic) bile</td>
<td>1010 ± 28</td>
<td>6.39 ± 0.22</td>
<td>230 ± 12</td>
<td>6.2 ± 2.0</td>
<td>15.4 ± 2.1</td>
<td>6.2 ± 2.0</td>
<td>32.8 ± 4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-24 h</td>
<td>7.90 ± 5.21</td>
<td>230 ± 12</td>
<td>13.0 ± 2.1</td>
<td>6.2 ± 2.0</td>
<td>32.8 ± 4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallbladder bile</td>
<td>5.25 ± 1.36</td>
<td>7.52 ± 0.21</td>
<td>920 ± 9</td>
<td>5.1 ± 1.7</td>
<td>7.3 ± 0.3</td>
<td>20.7 ± 3.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values are means ± SD; number of studies from separate fish in parentheses. Significantly greater than plasma (P < 0.01). Significantly less than plasma (P < 0.01).
and Ca++. Altogether, these findings suggested that the cannulation procedure was successful in preventing contact of bile with gallbladder epithelium and that cannulation through the gallbladder was an effective technique for obtaining hepatic bile in the dogfish shark.

Skate bile was slightly different in composition. Gross inspection of bile in these species revealed that the green color of gallbladder bile was much lighter and nearer to plasma than in dogfish. This conclusion was also supported by electrolyte studies that showed identical values in gallbladder bile and plasma for Na+, K+, Cl-, HC03-, Mg++, and Ca++ when samples were obtained simultaneously from 10 fish (Table 3). Furthermore, gallbladder bile pH (7.52 ± 0.21) was nearly identical to reported values for plasma pH (8). Although the concentration of 3-hydroxysteroid derivatives in the gallbladder bile was 5 times greater than in hepatic bile, it was low compared with the values obtained from Squalus acanthias. Cannula bile from the skate, in contrast to gallbladder bile, demonstrated a significant increase in respect to plasma in both osmolarity and concentrations of K+, HC03-, Mg++, and Ca++.

DISCUSSION

Previous studies of bile secretion and composition in elasmobranchs are few; however, Burger (3) reports an average value for bile flow in dogfish of 1.3 μl/min per kg body wt, which is roughly comparable to the results in the present study (3), and approximately 100 times slower than in a rodent such as a rat. It is essential that the fish be maintained in the free-swimming state for adequate bile production, as bile flow quickly deteriorates within a few hours if fish are restrained in small tanks, even though they are supplied with well-oxygenated 15°C seawater. Under these conditions, hepatic and renal blood flow and blood pH decline (3). Although neither species eats in captivity, the present study demonstrates that both dogfish sharks and small skates can be maintained easily in the free-swimming state with externalized bile fistulas for periods of at least 4-5 days without serious deterioration. During this time the fish continue to secrete bile (Table 1) although the rates of flow progressively diminished in the dogfish shark. Bile flow was more erratic in the skate, possibly because of greater technical difficulty in locating the biliary cannula in a position where it would not intermittently obstruct bile flow. After 4-5 days, the abdominal wound often became necrotic and bile secretion diminished.

A comparative study of the composition of cannula and gallbladder bile in the two species suggests that this technique is quite satisfactory in the dogfish for the continuous collection of hepatic bile and that the transport functions of gallbladder are effectively bypassed, since HC03- and Cl- concentrations did not diminish and bile salts were not highly concentrated in the hepatic cannula bile. These studies also indicate that dogfish gallbladder epithelium functions in a fashion comparable to higher vertebrates by transporting Cl- and HC03- and concentrating bile acids in micelles without an alteration in osmolarity (4). However, in skates the composition of electrolytes in gallbladder bile was similar to plasma, suggesting that gallbladder transport function may be less active in this species. Differences in solid content of gallbladder bile from the two species also indicate that the concentrative function of the gallbladder is less in the small skate than in dogfish, although it is also possible that bile may have been in contact with dogfish gallbladder epithelium for longer periods of time prior to collection of the sample than in the skate. The higher osmolarity and concentrations of electrolytes in the skate cannula bile suggest that the cannula may have stimulated duct secretion in this species since HC03- and electrolytes are secreted from mammalian ducts (12, 13) and result in a rise in bile osmolarity (5).

High values were observed for bile salts in gallbladder bile of the dogfish. The major bile salt constituents in elasmobranch bile (6), C17 bile alcohols (Scyomn sulfate) are detected by the 3-hydroxysteroid dehydrogenase assay. However, we observed an excess of anions in dogfish gallbladder bile, and it is possible that other neutral steroids were measured by the 3-hydroxysteroid assay. Bile salts can also be estimated indirectly by determining the differences between the sum of bile Na+ and K+ and the sum of Cl- and HC03- (9, 14). With this method, a bile salt value of 292 mM rather than 366 mM is calculated for dogfish gallbladder bile. Similar calculations in skate bile result in an anion excess of only 46 mM, which is close to the estimate with 3-hydroxysteroid dehydrogenase (35 mM). Thus, the apparent anion excess is limited to dogfish bile and would appear to be an unidentified neutral 3-hydroxysteroid.

The mechanism of bile formation is entirely unknown in marine species. However, since both the electron-microscopic appearance of bile canaliculi (unpublished observations) and the electrolyte constituents of bile of elasmobranchs are similar to those in mammalian species, it is likely that analogous mechanisms exist. Nevertheless, the rate of bile secretion is inordinately slow in elasmobranchs compared to mammalian species, which may in part be related to the effects of both low body temperature and high plasma osmolarity on bile production. Further studies will be necessary to elucidate bile secretory function in elasmobranchs. In the accompanying report (2) the role of bile as an excretory route for organic anions in these species is examined.

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REFERENCES


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