When 10 μM dibutyryl cAMP was administered to the perfusate (2 studies only) bile secretion was significantly inhibited to flow rates that were 30% and 50% of control values. However, there also was no significant recovery following perfusion with dibutyryl cAMP-free ringers. The effect of forskolin and dibutyryl cAMP are opposite to that observed in mammalian systems where similar concentrations of dibutyryl cAMP have been shown to stimulate bile secretion (Graf, J., In: Stimulus Secretion Coupling in the Gastrointestinal Tract, ed. by R.M. Case and H. Goebell, pub. University Park Press, Baltimore, p. 301, 1976).

Two types of membrane bound adenosine receptors have previously been identified in many tissues including the liver. The activation of these receptors enhances or reduces intracellular levels of cyclic AMP (Londos, C., et al., Proc. Nat'l. Acad. Science U.S.A., 77:2551, 1980). The elasmobranch (dogfish) rectal gland appears to contain adenosine receptors both stimulating and inhibitory for adenylate cyclase (Forrest, J.N., et al., Bull. MDIBL 20:152-155, 1980; Forrest, J.N., et al., Bull. MDIBL 22:51, 5-15, 23, 1982). In three out of five experiments in which we administered 1 μM 2-chloro adenosine (the slower metabolized analogue of adenosine) bile secretion was significantly greater than control (257 ± 151%). Furthermore, the simultaneous addition of 10 μM theophylline, which competes for the adenosine receptor, reduced secretion in each of the experiments where stimulation was observed (47 ± 23.5% of the stimulated rate). In one experiment, both stimulation and inhibition of secretion could be reproduced in the same liver (Fig. 2).

These preliminary studies indicate that cAMP may play a role in regulating the secretion of bile in the small skate. Further studies are required to define the sensitivity of this system to hormones and the mechanisms involved. Since these effects are observed in an isolated portal vein perfused liver system, the site of the cyclase effect is likely to be the hepatic parenchymal cell. However, these agents may have altered transport systems in the bile ducts although these structures are primarily perfused via the hepatic artery which is not functional in this system.

Supported by USPHS, Grant No. 25636.

THE SECRETION PATTERN OF TETRAPYRROLES IN THE SMALL SKATE (RAJA erinacea)

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Unconjugated bilirubin, the conjugates of bilirubin, and biliverdin have been detected in gallbladder bile of the small skate (Raja erinacea) (Chowdhury et al., Comp. Biochem. Physiol. 66B:523-528, 1980; McDonagh and Palma, Comp. Biochem. Physiol. 73B:501-507, 1982). The question then arises as to the source of the biliverdin appearing in Elasmobranch bile. The biliverdin could be an end product of heme metabolism or it could be formed by the oxidation of bilirubin in the gallbladder. This question was investigated in the following studies by comparing the tetrapyrrole composition of the gallbladder bile and hepatic bile of the small skate.

Methods

Animals--Forty-two small 1 kg male skates (Raja erinacea) were obtained by net in waters off Southwest Harbour, Maine during the summer of 1983, and maintained in well-oxygenated tanks at Mount Desert Island Biological Laboratories until they were used. Gallbladder bile was obtained by aspiration and hepatic bile was obtained in free swimming fish from a cannula inserted into the gallbladder as previously described (Boyer et al., Am. J. Physiol. 230:970-973, 1976).

Analytical Procedures--The volume of bile was measured, and ascorbic acid (5 mg/ml) was added to the samples which were protected from light and stored frozen until analyzed. Extraction, detection and quantitation of the tetrapyrroles of bilirubin and biliverdin were carried out as described in the literature (McDonagh et al. Comp. Biochem. Physiol. 73B:501-507, 1982; Gordon et al. Can. J. of Biochem. 60:1050-1058, 1982).

Results

Bile Pigment Content of Small Skate Gallbladder Bile--After 1 day in captivity the total volume of bile in the gallbladder of eleven skates was 1.9 ± 0.8 ml and it did not change significantly after 5 days in captivity (Table 1). In contrast, by 5 days the bile pigment content in the gallbladder increased two to three-fold. A large

Table 1.--The Effect of Captivity on the Tetrapyrrole Content in the Small Skate's Gallbladder Bile+

Days in Tank	No. of Skates	Total Volume (ml)	Bilirubin (μmoles)	Biliverdin (μmoles)	Total Tetrapyrroles (μ moles)
1	11	1.9 ± 0.8	111 + 78	91.0 + 50	202 ± 129
2	11	2.1 ± 0.5	132 + 64	72.7 ± 98	205 ± 112
3	3	2.5 ± 0.5	140 ± 42	187.9 ± 74	328 ± 116
5	4	2.7 ± 1.1	399 ± 98°	181.6 ± 129°	552 ± 227°

Bile was aspirated from skate gallbladder and bile pigments were quantitated spectrophotometrically.

+The values are means ± standard deviation of the mean °Significance was obtained by group t-test compared to values obtained on day one $p < 0.05$.

variation in secretion rates was observed between skates, so that these differences were not significant until the skates had been in captivity for at least three days. Both the total amount of bilirubin tetrapyrroles and biliverdin had significantly increased by this time.

Bile Pigment Content of Hepatic Bile--The tetrapyrrole content of hepatic bile was obtained by analyzing the freshly secreted bile of the cannulated free swimming skate. The rate of secretion of bile pigments by the 15 skates varied markedly, so that only a representative experiment is illustrated (Fig. 1). At each time period sampled, the amount of biliverdin in the bile exceeded that of bilirubin and its conjugates.

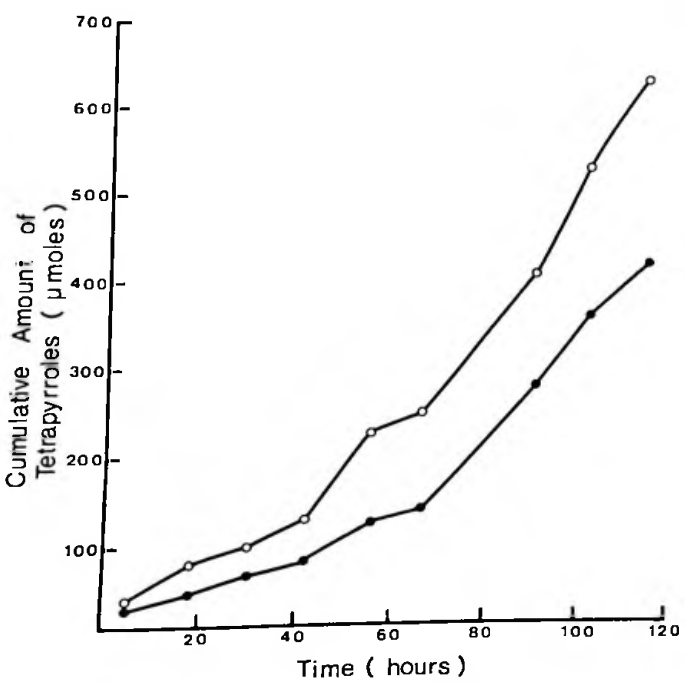

Figure 1.--Representative graph of cumulative amounts of biliverdin (o) and bilirubin and its conjugates (o) secreted into the bile of a free-swimming skate during a 5 day period.

The relative proportions of bilirubin and the conjugates of bilirubin detected in hepatic bile and gallbladder bile of the small skate are summarized in Table 2. As shown, there are no significant differences between the two

Table 2.--The Relative Proportions of Bilirubin and the Conjugates of Bilirubin in Hepatic Bile and Gallbladder Bile of the Small Skate

Source of Bile	Number of Samples	Bile pigments expressed as a % of total pigments[+]		
		Bilirubin	Bilirubin Monoglucuronide	Bilirubin Diglucuronide
Gallbladder	16	5.0 ± .7	69.2 ± 12.1	25.8 ± 10.1
Hepatic Bile[o]	41	2.0 ± .6	63.0 ± 16.0	35.0 ± 16.0

[+]Values are means ± standard deviation of the mean. [o]These values are derived from all bile samples collected from seven free swimming skates. -Data obtained by HPLC and TLC analysis of bile samples.

sources of bile. Bilirubin monoglucuronide accounted for 65% of the total bile pigments secreted, bilirubin diglucuronide for 30%, and bilirubin for 5% or less. In some of the samples, bilirubin diglucoside was detected, but it accounted for less 1% of total bile pigments. There was no evidence in the free swimming skate that the pattern of bile pigments changed over the five day period.

Figure 2 illustrates the effect of a load of biliverdin (5 mg/kg skate) on the secretion pattern of bilirubin

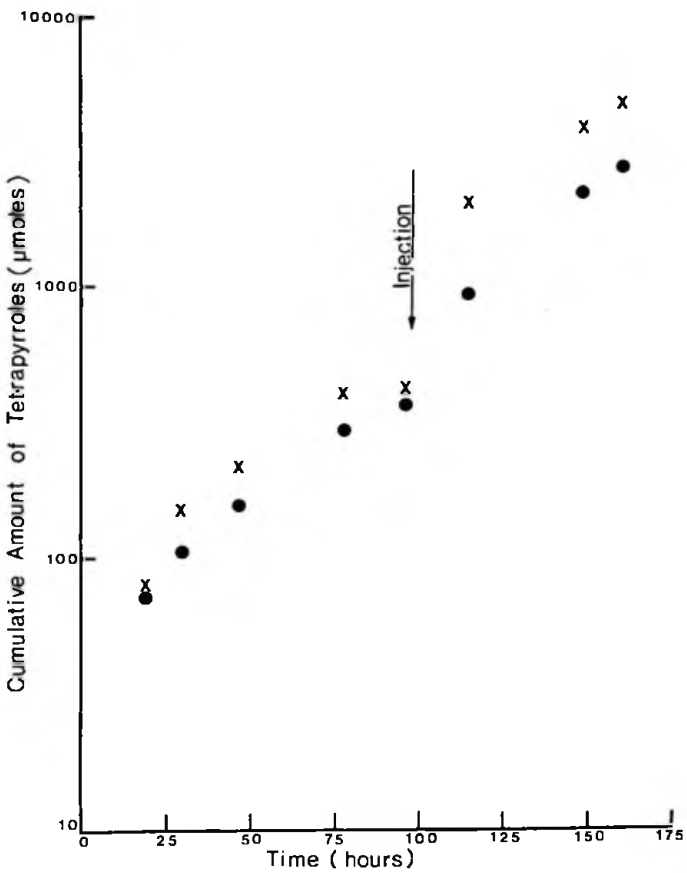

Figure 2.--Representative graph (semi-log scale) of cumulative amounts of biliverdin (x) and bilirubin and its conjugates (o) secreted into bile after the injection of biliverdin.

and its conjugates, and biliverdin in one free swimming skate. In all skates, the biliverdin was injected on the third of fourth day after placement of a cannula in the gallbladder. The average volume of bile produced per day was 1.84 ± 0.43 ml before and 1.61 ± 0.65 ml after the injection. As noted, biliverdin was the major tetrapyrrole secreted before the injection, and the secretion rates and proportion of biliverdin and bilirubin tetrapyrroles in hepatic bile remained relatively constant until the time of injection. After the injection of biliverdin into four

skates a dramatic increase occurred in the secretion of both biliverdin (which changed from 164 ± 63 μmoles/day in the interval before injection to 2565 + 1257 μmoles/day in the interval after injection) and the bilirubin tetrapyrroles (100 ± 58 μmoles/day to 550 ± 274 μmoles/day).

Discussion

The degradation of heme initially results in the formation of biliverdin, which is subsequently reduced to bilirubin. Humans, rats, dogs, and some fish secrete bilirubin and its conjugates into their bile (Chowhury, J.R. et al., Seminars in Liver. 3:11-23, 1983). However, in some elasmobranchs, like Torpedo Californicus, unconjugated biliverdin is the predominant bile pigment, while bilirubin is absent (McDonagh, A., and Palma, L.A.: Comp. Biochem. Physiol. 73B:501-507, 1982). The results of our experiments suggest that biliverdin is a normal constituent of small skate bile and not merely a by-product of bilirubin oxidation in the gallbladder. This conclusion is based on the inability to detect an increase in the percentage of biliverdin in gallbladder bile with time in captivity and the relative smaller percentage of biliverdin in the gallbladder compared to hepatic bile. The injection of biliverdin into four free-swimming skates resulted in a dramatic increase in the secretion of both biliverdin and bilirubin tetrapyrroles. This not only demonstrates that an endogenous mechanism exists for the secretion of biliverdin into bile, but also that biliverdin reductase (the enzyme responsible for the conversion of biliverdin to bilirubin) may be rate-limiting in the skate, unlike mammalian liver.

The HPLC and TLC analysis of both gallbladder bile and free-swimming skate bile samples indicates that the skate also secretes bilirubin, bilirubin monoglucuronide, and bilirubin diglucuronide into its bile. However, the skate secretes these compounds in different relative proportions than previously observed in mammalian systems. For instance in rat and dog bile, bilirubin diglucuronide is the major bilirubin conjugate while in small skate, bilirubin monoglucuronide is the predominant conjugate. This study indicates that bilirubin and its conjugates as well as an intermediary metabolite in heme catabolism, biliverdin, are secreted into the bile of a small skate. This work was supported by USPHS Grant No. AM 25636 and Grant No. AM 32741-01. Michael Grossbard is the recipient of an American Liver Foundation Student Fellowship.

COMPARISON OF SEROSAL-MUCOSAL SULPHATE FLUX IN RABBIT AND WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS) INTESTINE

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The Serosa to Mucosa (s-m) flux of $^{35}S\text{-}SO_4$ was measured across preparations of rabbit ileum and flounder intestine stripped of serosa and outer muscle layers and mounted in modified Ussing chambers (Naftalin & Kleinzeller, 1981, Am. J. Physiol. G392-G400, Naftalin and Smith, 1983, 336P).

Flux values in nM $cm^{-2}Hr^{-1}$ were calculated from sample beta scintillation counts by conventional methods (Table 1.).

Table 1.--Results s-m flux of $^{35}S\text{-}SO_4$, in nM $cm^{-2}Hr^{-1}$

	Flounder	Rabbit
Control	34.7 +/- 2.5 (28)	42.4 +/-2.1 (27)
SITS 50 μM	35.5 +/- 6.6 (5)	38.3 +/-3.9 (20)
Theophylline 10 mM	46.2 +/- 3.5 (28)	57.7 +/- 3.0 (28)
SITS + Theophylline	31.2 +/- 3.2 (21)	30.5 +/-4.2 (13) **

(SITS = 4-acetmido-4' -isothiocyano-2,2' -sulphonic acid stilbene) *indicates a comparison to control, two symbols = $P < 0.005$, three symbols = $P < 0.001$.