with choline transiently increased effluent ^{3}H-Tc concentration, a phenomenon that was never observed with reintroduction of choline. When lithium replaced Na^{+}, a decrease in uptake rate was observed at 3.73 μM Tc in 0.25% BSA but not consistantly at higher Tc concentrations. Increasing BSA concentration from 0.25% to 2.0% did not significantly change the observed uptake rate even though this approximately tripled estimated equilibrium concentrations of bound Tc at each of the 3 Tc concentrations used (Table 3).

Table 3.--Taurocholate uptake rates in skate liver perfused with 0.25% and 2.0% Bovine serum albumin

3.73 μM Taurocholate % BSA	Estimated Bound conc.	$\times 10^{-8}$ moles min^{-1} g^{-1} liver uptake rate
0.25%	1.14 μM	.191 $\pm$ .04
2.0%	2.92 μM	.145 $\pm$ .07
26.1 μM Taurocholate		
0.25%	7.04 μM	.943 $\pm$ .15
2.0%	20.2 μM	.740 $\pm$ .31
74.5 μM Taurocholate		
0.25%	15.6	1.58 $\pm$ .39
2.0%	55.7	1.58 $\pm$ .77

DISCUSSION--Because the Ka for Tc and BSA was not changed with choline substitution, the effect of Na^{+} on Tc clearance could not be attributed to changes in Tc-BSA binding. Measurement of Ka in elasmobranch ringers at 15°C and 37°C gave values nearly identical to published values at 37°C in mammalian ringers, indicating that neither the temperatures nor ionic strengths tested affect this equilibrium. By exclusion, the observed Na– dependence must be attributed to either a membrane carrier for free Tc or a membrane-Tc-Albumin interaction. Because rates did not increase when the bound concentration was increased by increasing BSA (as would be expected if complex concentration facilitated uptake), complex recognition by the sinusoidal membrane seems unlikely in contrast to studies in perfused rat liver. However, a significant increase in Tc clearance rates was observed as total Tc concentration was increased, at fixed BSA concentration. Indicating that the free or total Tc, rather than the albumin bound fraction is a major determinant of the net rate of uptake. These results are most consistent with a Na^{+} facilitated coupled transport of free Tc across hepatic sinusoidal membrane as the anion becomes available by dissociation from albumin. Supported by USPHS Grant #AM25636.

EVALUATION OF A SULFATED BILE ALCOHOL IN THE BILE OF THE SMALL SKATE (RAJA ERINACEA)

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Hepatologists in general agree today that active bile salt secretion plays an important role in bile formation in man, dog, rat and the guinea pig, presumably by producing an osmotic pressure gradient within the canaliculi that promotes the passive movement of water and diffusible solute from sinusoidal blood to the canalicular lumen. To what extent such a mechanism may be similarly operative in lower vertebrates is unknown. The bile acids in the Cyclostomes and Elasmobranchs appear to be largely sulphated bile alcohols that differ with respect to their physiochemical properties from the bile salts commonly found in mammalian bile (Haslewood, GAD, The Biological Importance of Bile Salts, North Holland, Oxford, 1978). Just how these differences may affect the contribution of

bile salts to bile formation in fish and other primitive vertebrates remains obscure. Although fish biles have been extensively studied (op. cit.) no information is available regarding the physiology of bile salt secretion. Scymnol sulphate (5β-cholestane-3α,7α,12α,24,26,27-hexol-26(or 27) sulfate), for example, has been claimed on the basis of chromatography to be the major bile salt in bile of several species of sharks and rays (Tammar A.R. in Chemical Zoology, ed. M. Florkin and B.T. Sheer, Bile salts in fishes 8:595, 1974) but modern methodology is needed to confirm this observation. Moreover, the role of bile alcohols in bile formation and the changes in its biliary concentration during alteration with bile flow or storage in the gall bladder have not been examined. The present study was undertaken to determine if bile alcohol is detectable in the bile of the Small Skate (Raja erinacea) using capillary gas-liquid chromatography - mass spectrometry. The preliminary data presented herewith confirm earlier observations that a sulfated bile alcohol is indeed a major bile salt in skate bile and may be helpful in appraising the interrelation between bile salt excretion and bile formation in this species.

MATERIALS AND METHODS--Experimental procedure--Samples of bile were obtained from the gallbladders and hepatic ducts of isolated perfused livers from three male Small Skates (Raja erinacea) using methods previously described (Reed et al, Am. J. Physiol., 242:G313-G318, 1982). Bile was aspirated from the gallbladder prior to opening it and inserting a cannula in the cystic duct for the collection of hepatic duct bile which was collected continuously at measured intervals thereafter. All samples were frozen immediately and maintained in the frozen state until analysis. Gallbladder bile alone was used in making the measurements reported here.

Analysis of bile alcohols--Fifty μl of bile, to which about 50000 cpm of [7-^{3}H] testosterone was added, were dissolved in 20 ml of 72% methanol which was filtered through SP-Sephadex C25 in H^+ form. The column was washed with 40 ml of 72% methanol and the combined eluates were neutralized to pH 7 with 0.1 M aqueous sodium hydroxide, taken to dryness and redissolved in 20 ml of 72% ethanol. The solution was passed through a column of Lipidex-DEAP in acetate form in 72% ethanol (Setchell, Almé, Axelson and Sjövall, J. Steroid Biochem. 7:615-629, 1976; Karlaganis, Nemeth, Hammerskjöld, Strandvik and Sjövall, Eur. J. Clin. Invest. 12:399-405, 1982). Neutral steroids were recovered with 25 ml of 72% ethanol, and an aliquot was counted for [7-^{3}H] testosterone. Steroid monoglucuronides (if present) were eluted with 35 ml of 0.25 molar formic acid in 72% ethanol. Steroid sulfates were eluted with 20 ml of 0.5 M potassium acetate/hydroxide pH 10 in 72% ethanol. Again, about 50000 cpm of [7-^{3}H] testosterone were added to the sulfate fraction. This fraction was neutralized to pH 7, evaporated to dryness, redissolved in water and extracted with Sep-Pak C_{18}. The cartridge was washed with 10 ml of water and eluted with 10 ml of methanol. The eluate was taken to dryness, and solvolysis was performed: the residue was dissolved in 3 ml of ethanol and 27 ml ethyl acetate equilibrated with 2M aqueous sulfuric acid and 20 μl of concentrated sulfuric acid were added. The solution was incubated overnight at 39°C (Almé, Bremmelgaard, Sjövall and Thomassen, J. Lipid Res. 18:339-362, 1977) and was then neutralized with 2M sodium hydroxide and taken to dryness. The liberated steroids were extracted with SepPak C_{18} cartridges as described above. The methanol eluate was taken to dryness, redissolved in 10 ml of 72% ethanol, and passed through Lipidex DEAP. The effluent and an additional wash with 25 ml of 72% ethanol were taken to dryness and the residue was transferred with methanol to conical test tubes together with 25 nmol of coprostanol as internal standard. Trimethylsilyl ethers were prepared in pyridine-hexamethyldisilazane-trimethylchlorosilane, 3:2:1 (by vol.), for 30 min at 60°C. Samples were taken to dryness under a stream of nitrogen and were immediately dissolved in hexane. Capillary gas-liquid chromatography - mass spectrometry was carried out using a Finnigan 1020 instrument, a 15 meter x 0.3 mm i.d. persilanized glass capillary column (Grob, Grob and Grob, J. High Resolution Chromatogr. and Chromatogr. Commun. 2:677-678, 1979) coated with OV 73 (0.2%) and a fused silica interface 0.2 mm i.d. coated with OV 1. Operating conditions were as follows: injector

temperature 250°C, column temperature 70°/20° per min/260°C, separator temperature 250°C, energy of bombarding electrons 22 eV. Repetitive magnetic scanning was carried out over the mass range 34-800 in 3 sec.

RESULTS AND DISCUSSION--The major component in the sulfate fraction had a retention index of 3740. The mass spectrum of the TMS ether derivative (Figure 1) showed a series of significant peaks at m/z 591, 501, 411

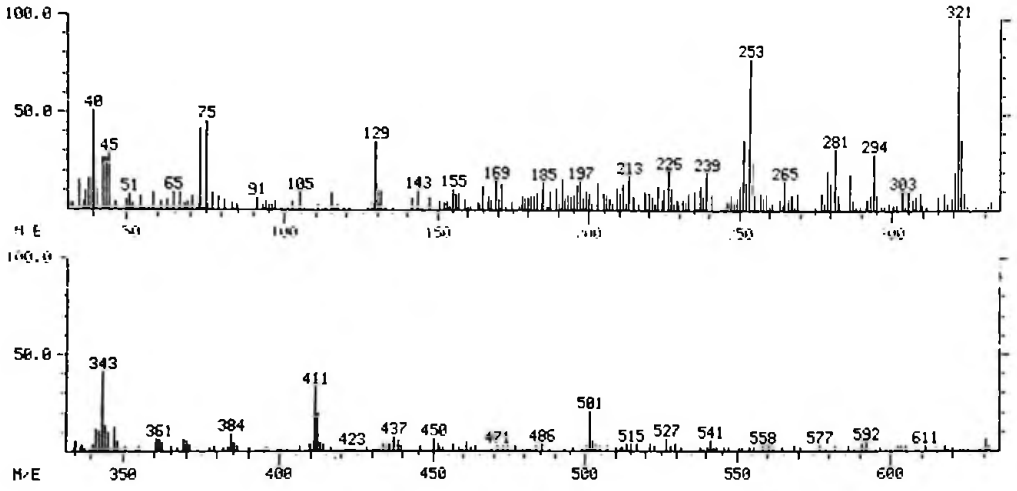

Figure 1.--Computer printout of the mass spectrum of the trimethylsilyl ether derivative of a bile alcohol isolated from skate bile at a retention index of 3740. The numbers shown above the peaks indicate mass fragments. Relevant "diagnostic ions" are reported in the text.

and 321 (base peak) formed by loss of 219 mass units (the 3 terminal carbons and 2 trimethylsilyl groups) and consecutive losses of trimethylsilanol from the molecular ion. In addition, peaks were found at m/z 631 (M-2x90-89), 541 (M-3x90-89), 450 (M-5x90), 360 (M-6x90) indicating a possible molecular weight of 900. Another series of peaks were found at m/z 617 (M-103-2x90), 527 (M-103-3x90); 437 (M-103-4x90) and 347 (M-103-5x90). Peaks at m/z 253 and 343 indicated three hydroxyl groups in the ring system. These findings are consistent with 5β-cholestane-3α,7α,12α,24,26,27-hexol (scymnol) but further studies are necessary for conclusive identification, i.e., microchemical reactions and chemical ionisation mass spectrometery.

In the analysis to date this highly polar sulfated bile alcohol, found for the first time in this species of skate, appears to be the dominant, if not exclusive, bile acid present in the bile. A search for other bile acids in the glucuronide fraction, for example, has proved fruitless. It is possible, therefore, that sulfated bile alcohol plays a major role in fat digestion and bile formation in the Small Skate that warrants further exploration. This investigation was supported by the Swiss National Science Foundation and the Sandoz-Stiftung zur Förderung der medizinisch-biologischen Wissenschaften. We are deeply indepted to Prof. Jan Sjövall for very helpful discussions. The technical assistance of Mr. Th. Weber and the secretarial help of Miss R. Steiner are gratefully acknowledged.

STEROID PRODUCTION BY ISOLATED SKATE OVARIAN FOLLICULAR CELLS

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Elasmobranchs produce steroid hormones (Lance and Callard, The Vertebrate Ovary, Plenum Publishing Corp., 1978) and the ovaries are major sources of these hormones, since circulating levels of androgen and estrogen are markedly decreased after ovariectomy (Jenkins and Dodd, J. Fish Biol., 21:297, 1982). Previous studies have demonstrated steroidogenic capacity by use of radiolabelled precursors (Callard and Leathem, Arch. Anat. Microsc. Morphol. Exp., 54:35, 1965) and by enzyme histochemistry (Lance and Callard, Gen. Comp. Endo., 13:255, 1969). However, the specific cells responsible for steroid biosynthesis have yet to be definitively established. In this report,