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 = $P < 0.001$.

It may be seen, that the flux per unit area of exposed tissue of $^{35}S\text{-}SO_4$ from serosa to mucosa under control conditions, and in the presence of both theophylline and SITS is similar in both species. The addition of theophylline produces a significant ($P < 0.005$) increase in flux, consistant with a raised mucosal border anion conductance (Naftalin and Simmons 1979, J. Physiol. 290:331-350). The reduction in basolateral border SO4 permeability induced by SITS (Smith,Orellana & Field, 1981, J. Membrane Biol. 63, 199), reverses this action of theophylline (ref. Naftalin & Smith, 1983).

The flounder and the rabbit appear to share a common response to the secretagogue theophylline. Following exposure to theophylline, they both exhibit an increased anion flux. Inhibition of this increased flux by SITS demonstrates that it has a transcellular component. Previously, it has been suggested that the failure of flounder intestine to show anion secretion is due to its lack of crypts, this is used as indirect evidence for the role of crypts in mammalian intestinal secretion (Field, Smith and Bolton [1980] 55:157). The data presented here shows that regulation of transcellular anion flux is present in flounder intestine and hence that the presence of crypts is not necessary to the regulation of anion secretion in either flounder or mammalian intestine. This work was supported by grants to R.J.N and P.M.S. from NATO and grants from the Royal Society and the Dale Fund to P.M.S.

THE RELATIONSHIP BETWEEN 2-D-DEOXY-D-GALACTOSE SECRETION AND ION COTRANSPORT ACROSS THE BASO-LATERAL BORDERS OF WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS) INTESTINE

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The Serosa to Mucosa (s-m) flux of ^{86}Rb was measured across preparations of Flounder intestine stripped of serosa and outer muscle layers and mounted in modified Ussing chambers (Naftalin & Kleinzeller, Am. J. Physiol. 240:G392-G400, 1981). These measurements were correlated with transepithelial fluxes of ^{3}H labelled 2-deoxy-D-galactose (2-d-gal).

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

Table 1.--S-M flux of ^{86}Rb nM cm^{-2} Hr^{-1}

		+furosemide (0.5 mM)	
Control	115.0 +/- 8.3 (27)	86.6 +/- 6.9 (15)	**
+Mannitol (10 mM)	203.3 +/- 28.1 (6)	125.6 +/- 38.1 (6)	*
+2-d-gal (10 mM)	172.1 +/- 26.9 (6)	83.2 +/- 13.4 (4)	***
low Na (10 mM)	102.8 +/- 15.0 (8)	95.9 +/- 13.3 (8)	
low Cl (10 mM)	78.5 +/- 13.4 (8)	83.2 +/- 9.2 (8)	

Low Na, Na replaced with choline, low Cl, Cl replaced with gluconate. +Indicates a comparison to control, *indicates comparison plus or minus furosemide. One symbol = $P < 0.05$, two symbols = $P < 0.02$, three symbols = $P < 0.01$.

^{86}Rb s-m flux into a bathing solution containing 1 mM K was reduced by 25% ($P < 0.01$) following the addition of furosemide to the serosal bathing solution. Replacement of Ringer Cl on the serosal side by gluconate led to a similar reduction of ^{86}Rb flux (32% $P < 0.01$). Addition of furosemide to low Cl Ringer did not elicit any further inhibition in ^{86}Rb glux.

While there appears to be a reduction in ^{86}Rb flux in low Na Ringer, the variation within these experiments does not permit any definite conclusions about the requirement for Na to be drawn from this data alone, however the furosemide-dependence of s-m ^{86}Rb flux is absent in low Na Ringer.

The sensitivity of the s-m flux of ^{86}Rb to furosemide indicates that ^{86}Rb crosses the epithelium via a Na-K-Cl