BILIRUBIN GLUCURONIDATION IN FISH. II. BILIRUBIN GLUCURONOSIDE GLUCURONOSYL TRANSFERASE

Namita Roy Chowdhury, Jayanta Roy Chowdhury and Irwin M. Arias, Liver Research Center, Albert Einstein College of Medicine, Bronx, N.Y. and Mt. Desert Island Biological Laboratory, Salisbury Cove, Maine

Bilirubin diglucuronide is the major bilirubin metabolite in most mammals. Formation of bilirubin diglucuronide takes place in two enzymatic steps. UDPglucuronate glucuronyl transferase (EC 2.4.1.17) a microsomal enzyme catalyzes the transfer of one mole of glucuronic acid from UDPglucuronate to bilirubin forming bilirubin monoglucuronide. A second enzyme, bilirubin glucuronoside glucuronosyl transferase (EC 2.4.1.95), is concentrated in plasma membrane-enriched fractions of rat liver homogenates, and converts two moles of bilirubin monoglucuronide to one mole of bilirubin diglucuronide and one mole of unconjugated bilirubin (Jansen et al. 1977, J. Biol. Chem. 252:2710-2716).

In the present study, we analyzed bile pigments in hepatic and gallbladder bile of two elasmobranchs (spiny dogfish, Squalus acanthias and small skate, Raja erinacea) and a teleost (winter flounder, Pseudopleuronectes americans). Subcellular fractions of liver from these fish were studied for UDPglucuronate glucuronyl transferase and bilirubin glucuronoside glucuronosyl transferase activities.

Fish were anesthetized by placing them in aquaria containing sea water at 4°C containing 250 mg/l MS 222 (tricaine methane sulfonate) for approximately 2 min. Anesthesia was maintained by perfusion of gills with ice-cold sea-water containing 25 mg/l MS 222. The gallbladders were exposed and gallbladder bile was aspirated and immediately analyzed. The cystic ducts were cannulated through an opening in the gallbladder, and the common bile duct was ligated immediately distal to it. Cannulae were brought out through puncture wounds in the abdominal wall, and opaque ballons were attached. Fish were maintained in refrigerated sea-water aquaria and hepatic bile samples were collected from the balloons every 24 h for 3 days.

Each bile sample was divided in half. One half was treated with ethyl anthranilate diazo reagent (Heirwegh, et al. 1970, Biochem. J. 120:877-890). Azodipyrroles were quantitated following separation by thin layer chromatography. Diazotized bile pigments obtained from Wistar rat bile were used as reference compounds. The two major bands had the same R_f as the two major bands in rat bile and represent unconjugated and glucuronide azodipyrryl derivatives of conjugated bilirubin. In addition to the two major bands, there were minor bands representing 5-15% of total azopigments. Bilirubin and its conjugates were extracted from the other half of the bile and separated as tetrapyrroles by thin-layer chromatography (Blanckaert, et al, 1978, Biochem. J. 171:203-214). Bile pigments adhering to the silicagel was treated with ethylanthranilate diazo reagent, and the resulting azopigments were quantitated following separation by thin-layer chromatography. Relative proportions of unconjugated bilirubin, bilirubin monoglucuronide, bilirubin diglucuronide and unidentified pigments in the bile of the three fish are shown in Table 1.

TABLE 1

Relative Abundance of Unconjugated Bilirubin and Various Bilirubin Conjugates in Fish Gallbladder Bile. Bile Pigments were Extracted and Separated as Tetrapyrroles, and Quantitated Following Thin-layer Chromatography of their Ethylanthranilate Azoderivatives

	Dogfish (4)	Skate (6)	Flounder (4)
Unconjugated bilirubin	30 ± 4	28 ± 3	20 ± 3
Bilirubin monoglucuronide	60 ± 6	50 ± 5	63 ± 6
Bilirubin diglucuronide	6.6 ± 2.1	13 ± 2.5	16 ± 3
Unidentified monoconjugates	3.5 ± 1.5	9.1 ± 3.3	1 ± 0.4

Skate (30 g), dogfish (100 g) or flounder liver (12 g) was perfused with ice-cold 0.25 M sucrose-Tris HCl buffer (0.005M, pH 8), minced and homogenized in the same buffer (3 ml/g liver). The homogenate was centrifuged at 4°C at 900 x g for 10 min. The pellet was washed once and resuspended in the homogenization buffer ("Nuclear fraction"). The wash was added to the supernatant ("Postnuclear fraction).

UDPglucuronate glucuronyl transferase was assayed as described before (Bilirubin Glucuronidation in Fish. I. this Bulletin). Bilirubin glucuronoside glucuronosyl transferase activity was assayed as described by Jansen et al (J. Biol. Chem. 1977. 252:2710-2716). Protein was measured according to Lowry et al. Distribution of UDPglucuronate glucuronyl transferase and bilirubin glucuronoside blucuronosyl transferase was different in subcellular fractions (Fig. 1).

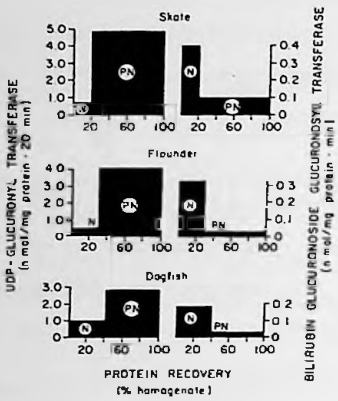

Figure 1. Protein recovery and enzyme specific activities in nuclear (N) and post-nuclear (PN) fractions of skate, flounder and dogfish liver. Protein recovery as percentage of total homogenate protein is shown in the abscissa. UDPglucuronate glucuronyl transferase activity and bilirubin glucuronoside glucuronosyl transferase activity were assayed as described in "Material and methods" and are expressed as nmol/mg protein. 20 min and nmol/mg protein.min, respectively (ordinate).

This suggests that, as in rats (Jansen et al. 1977), the two enzyme activities are concentrated in different organelles of the liver cells.

ROLE OF MEDIUM pH IN THE REGULATION OF CHLORIDE TRANSPORT IN THE INTESTINE OF THE WINTER FLOUNDER, PSEUDOPLEURONECTES AMERICANUS

Philip L. Smith, Stephanie Orellana and Michael Field, The University of Chicago.

Salt absorption in flounder intestine increases as medium pH increases (Field et al, J. Memb. Biol. 41:265, 1978; Field et al, Bull. MDIBL 18:44, 1978). Whether this effect is due to HCO_3 per se or to pH has not been determined. Furthermore, the step or steps in transepithelial Cl transport that are pH (or HCO_3)-dependent have not been elucidated, although preliminary experiments suggest that NaCl co-transport across the brush border is not affected (Field et al, Bull. MDIBL 18:44, 1978). The present study was undertaken to further explore the regulation of intestinal salt absorption by medium pH and [HCO_3].

Methods

Methods for maintaining fish and for in vitro determinations of electrical properties and both transepithelial and trans-luminal border (J_{me}) ion fluxes are those previously described (Field et al, J. Memb. Biol. 41:265, 1978; Frizzell et al, J. Memb. Biol. 46:27, 1979). Ringer solutions employed were the standard 20mM HCO_3-Ringer (Field et al, J. Memb. Biol. 41:265, 1978); 4mM HCO_3-Ringer, which was the same as the standard Ringer except that 8mM each of sulfate and mannitol were substituted for 16mM HCO_3; and HEPES-Ringer (pH 8.2), which was also the same as the standard Ringer except that 20mM N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid titrated with NaOH was substituted for $NaHCO_3$. Solutions were gassed with either 1% or 5% CO_2 in O_2 or, in the case of HEPES-Ringer, with 100% O_2. D-Glucose and D-Mannitol, 10μmol/ml, were always added to serosal and mucosal sides, respectively.