SLOW BILIARY ELIMINATION OF METHYLMERCURY IN THE MARINE ELASMOBRANCHS, RAJA ERINACEA AND SQUALUS ACANTHIAS N. Ballatori and J.L. Boyer, Liver Center, Department of Medicine, Yale University School of Medicine, New Haven, CT.

Methylmercury is a major toxic contaminant of the marine biosphere produced by the bacterial methylation of inorganic forms of mercury (Jensen and Jernelov, Nature 223:753, 1969). Methylmercury is avidly accumulated by multicellular aquatic organisms, and is substantially concentrated as one moves up the aquatic food chains. This bioconcentration of methylmercury is reflected in a biological half-time of the metal in fish which is on the order of months to years. Thus, once ingested, methylmercury remains essentially for the life of the marine animal (Jarvenpaa et al, Suomen Kemistilehti B43:439, 1970). The reason for the avid retention of methylmercury by marine species is not known, but may be related to either, 1) inefficient excretory mechanisms (biliary-fecal, urinary, or secretion across the gills), 2) an unusually high affinity of methylmercury for the proteins in fish muscle, or 3) a combination of these factors. Although the long $t_{\frac{1}{2}}$ for methylmercury in fish precludes the direct determination of its pathways for excretion, indirect evidence obtained in rainbow trout (Giblin and Massaro, Toxicol. Appl. Pharmacol. 24:81, 1973) suggests that the main route of methylmercury's excretion in trout, as in mammals, is through the feces. Since biliary excretion is the primary pathway contributing to fecal elimination in mammals, a low rate of excretion of mercury into fish bile might account in part for the prolonged biological half-times in these species.

In the present studies we quantitated the extent of methylmercury excretion into bile in the elasmobranchs R. erinacea (little skate), and S. acanthias (spiny dogfish shark), and determined several factors that regulate methylmercury excretion into bile in these species. ^{203}Hg-Labeled CH_3HgCl was administered via the caudal vein, and bile collected through exteriorized cannulas in the free swimming fish, as described previously (Boyer et al, Am. J. Physiol. 230:970 and 974, 1976).

RESULTS AND DISCUSSION-- Free swimming cannulated small skates and dogfish sharks excreted a minute fraction of the administered radiolabeled mercury into bile over a 3 day period (Fig 1). Less than 1% of the dose was excreted into bile over the 3-day bile collection interval. Although the overall rate of excretion into bile was low in the skate and dogfish, the concentration of radiolabeled mercury in bile (skate = 4-10 uM, dogfish = 1-2 uM at a dose of 5 umol/kg) was similar to that found in mammalian bile after an equimolar dose of methylmercury. Thus, the slow rate of biliary methylmercury elimination in skate and dogfish was attributed to their unusually low rate of bile formation (Fig 1). That the low biliary excretion of mercury is not related to an impaired hepatic uptake for methylmercury is indicated by the fact that greater than 10% of the administered ^{203}Hg was found in the liver of both skates and dogfish at 3 days after its administration.

The ability of the skate to excrete mercury into bile at a higher concentration and rate than the dogfish may be related to differences in the biliary excretion of glutathione (Fig 1), as previously shown for the rat (Ballatori and Clarkson, Am. J. Physiol. 244:G435, 1983). Hepatic levels of glutathione in the skate and dogfish were 0.62 ± 0.43 (n=14) and 0.36 ± 0.12 (n=8) umoles glutathione equivalents/g, respectively. However, glutathione was detectable only in skate bile (Fig 1). In addition, gel filtration of bile on Sephadex G-75 showed that a major fraction of the

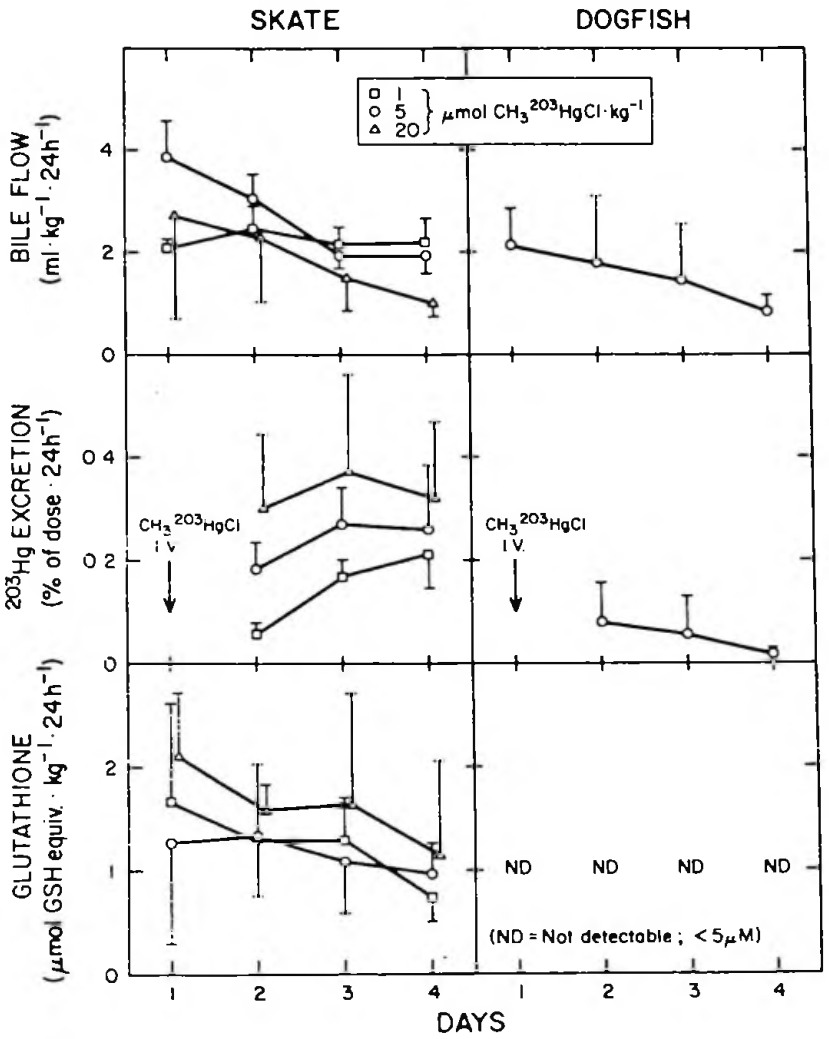

Figure 1. Bile flow, mercury excretion into bile, and glutathione excretion into bile in the skate and in the dogfish. Bile was collected into rubber balloons in free-swimming cannulated fish for a total of 4 days. After a control bile collection period of 24 h, ^{203}Hg-labeled CH_3HgCl was injected into the caudal vein in doses of 1 (n=4), 5 (n=6), and 20 (n=4) umol/kg body weight in the skate, and at a dose of 5 umol/kg in the dogfish (n=8). The CH_3HgCl was dissolved in elasmobranch Ringer's containing 5 mM Na_2CO_3. Values are means $\pm$ standard deviations.

^{203}Hg in freshly collected skate bile eluted in the low molecular weight region (less than approximately 10,000 dalton: $Ve/Vo > 2$), while most of the mercury in freshly collected dogfish bile eluted in the void volume. Furthermore, sulfobromophthalein, a compound known to inhibit the biliary excretion of both glutathione and methylmercury in rats, as well as L-buthionine-S,R-sulfoximine, an inhibitor of glutathione biosynthesis, were able to inhibit glutathione and mercury excretion in the skate (data not shown).

In summary, although there were significant differences between skates and dogfish in their hepatic handling of methylmercury that were partly attributed to differences in glutathione excretion, both species excreted mercury into bile slowly when compared to the rate of biliary excretion by other animals. The impaired hepatic excretory process for methylmercury in skates and dogfish was primarily related to the slow production of bile, and may account for the long biological half-times for methylmercury in marine species.