showed that BCG and phenol red were both effective inhibitors of 2,4-D secretion *in vivo*. However, as shown previously the clearance of 2,4-D by the flounder was high (i.e., 225-450 times the GFR), 3-6 times its clearance in the dogfish. Despite the slower clearance in the dogfish, plasma 2,4-D levels were equal to those in the flounder, suggesting either that absorption from the i.m. site was slower or that a substantial portion of the dose had been sequestered in a depot(s). Since muscle and liver account for 20-40% of the dose during the first 24 hr after 2,4-D injection (Guarino et al., Xenobiotica 7:623-631, 1977), the latter possibility seems likely. Therefore, pending additional studies using 2,4-D taurine, it would appear that the differences between the excretion rate for 2,4-D in the flounder and dogfish may be explained by a combination of lower renal transport capacity in the dogfish and greater availability in the flounder.

Overall, it is clear that the rate of renal tubular transport plays a major role in determining the excretion rate of these foreign organic anions. Secondly, the inverse correlation between extent of taurine conjugation and the rate of excretion does not appear to reflect any causal relationship. The differences between species and between chemicals appear to reflect pharmacokinetic factors, such as storage, and differences in transport of the parent molecules rather than differences between the parent and its conjugate.

HETEROGENEITY OF HEPATIC BENZO(a)PYRENE HYDROXYLASE (ARYL HYDROCARBON HYDROXYLASE) AND 7-ETHOXYRESORUFIN DEETHYLASE ACTIVITIES IN INDIVIDUAL WINTER FLOUNDER, *Pseudopleuronectes americanus*, FROM COASTAL MAINE

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Hepatic microsomal benzo(a)pyrene hydroxylase (AHH) activity is induced in fish that are pretreated with various polycyclic aromatic hydrocarbons, dioxins, polychlorinated biphenyls (PCBs), and polybrominated biphenyls (PBBs). Since all of these compounds have demonstrated or suspected toxicity to a variety of species, including man, the induction of AHH activity in fish has been suggested as a biochemical monitor for pollution of the aquatic environment by selected, toxic chemicals.

Recently, we found that a large percentage of winter flounder (about 85% of the 13 fish studied) had partially induced hepatic microsomal mixed-function oxidase systems and that this induction was identical, or at least very similar, to that caused by the administration of polycyclic hydrocarbons. Our purpose this summer was to perform a similar study on a larger fish population to test for both sex and time-of-year (June to August) effects on the apparent induction phenomenon.

Three enzymatic parameters were measured in the liver of each fish: AHH activity, AHH activity in the presence of 7,8-benzoflavone (BF, 10^{-4} and 5×10^{-4} M) and 7-ethoxyresorufin (7-ERF) deethylase activity. In mammals, and in the little skate, high hepatic AHH activities, the inhibition of these AHH activities by *in vitro* BF, and high 7-ERF activities are associated with the formation of cytochrome P-448 and pretreatment with polycyclic hydrocarbon-like inducing agents.

Winter flounder were collected by drag net and kept in flowing seawater (12-15°). Flounder caught and maintained in this manner appeared healthy. They were held in the tank for at least 2 days and no longer than two weeks before sacrifice. Livers, minus the gallbladders, were removed immediately and placed on ice. They were homogenized in 0.15 M KCl-0.001 M HEPES buffer, pH 7.5, to prepare a 33% w/v homogenate. Hepatic microsomes were obtained, the protein content of whole homogenate and microsomal preparations determined, and AHH activities with whole homogenate (100 µl) or microsomes quantitated as described previously (Pohl, Bend, Guarino and Fouts, Drug Metab. Dispos. 2:545, 1974). Additional AHH incubation mixtures which contained BF (10^{-4} and 5×10^{-4} M) were set up *in vitro*.

Incubation conditions and assay for fluorescent metabolites were identical for AHH reactions with and without BF. 7-ERF activity of flounder hepatic microsomes or homogenate (10-25 µl) was assayed essentially as described by Burke and Mayer (Drug Metab. Dispos. 2:583, 1974) for rats, as modified by our laboratory for fish liver preparations (Bend, Bogar and Foureman, Bull. MDIBL 17:47, 1977).

As previously mentioned, a major aim of this study was to assay selected hepatic mixed-function oxidase activities in a large number of individual flounder and the use of whole homogenate as the enzyme source (as compared to washed microsomes) would be advantageous. However, since our earlier study (Bull. MDIBL 17:47, 1977), was performed with washed hepatic microsomes, initial experiments were conducted to compare AHH and 7-ERF activities, and the effect of *in vitro* BF on AHH activity in washed microsomes and whole homogenate prepared from the same fish. Once again, very large variations were observed in AHH activities of hepatic microsomes (0.55 to 23.1) from flounder, and similar variation was found in AHH activities of whole homogenate (0.17 to 7.36). Similarly, the 7-ERF activity of whole homogenate and microsomes correlated well with AHH activity; the fish with highest 7-ERF activity had highest AHH activity, and the flounder with lowest AHH activity had undetectable 7-ERF activity in both microsomes and whole homogenate. Moreover, BF inhibited AHH activity in both microsomes and whole homogenate of the six flounder with highest AHH activities (indicating fish with induced mixed-function oxidase activities) and stimulated AHH activity in homogenate and microsomes of the remaining two fish. Based on these results we felt justified in using whole liver homogenate as the enzyme source in subsequent experiments, even though specific AHH activities were considerably lower (up to 75%) in whole homogenate preparations than in washed microsomes.

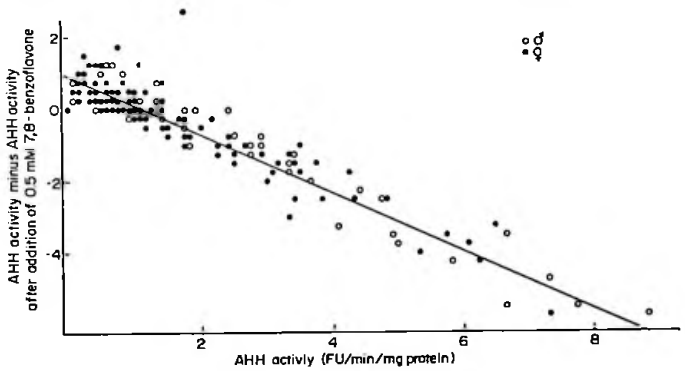

Figure 1. AHH activity vs. difference in AHH activity after "*in vitro*" addition of 0.5 mM 7,8-benzoflavone in male and female flounder hepatic homogenates.

Between June and August 1978, a total of 172 flounder were studied. As an initial step in the analysis of this data, specific AHH activity (in the absence of BF) of each liver homogenate was plotted against the change in AHH activity due to the addition of BF (Figure 1). When expressed in this manner the data closely approximated a straight line, and linear regression procedures indicated that the best fitting line (which is shown in Figure 1) could be described by the following equation: DIFFERENCE IN AHH ACTIVITY DUE TO BF = 0.9753-0.80835 x AHH SPECIFIC ACTIVITY.

Several things are obvious from the data. The higher the AHH activity, the greater was the inhibitory effect of BF, and the lower the AHH activity the greater the stimulation by BF. There was also no major difference between the male and female flounder studied. The calculated AHH activity where BF should have no stimulatory or inhibitory effect is 1.2 FU/min/mg protein. Interestingly, 87% (73 of 84) of the fish with AHH activities below 1.2 showed activation upon addition of BF and 84% (74 of 88) of the flounder with AHH activities above 1.2 showed inhibition in the presence of BF.

Multiple regression techniques indicated a statistically significant association between hepatic AHH activity and sex in flounder. Male fish tended to have slightly higher AHH activities than did

female fish and there also tended to be an inverse relationship between fish weight and hepatic AHH activity. However, these differences were relatively small and the statistical significance was in part a reflection of the large sample sizes employed in the analysis.

There was also a positive correlation between hepatic AHH activity and 7-ERF activity in the individual flounder studied. This is not surprising since cytochrome P-448 from rat hepatic microsomes supports the rapid oxidative metabolism of both benzo(a)pyrene and 7-ethoxyresorufin. This is also the case in hepatic microsomes from flounder, little skates (*Raja erinacea*), and sheepshead (*Archosargus probatocephalus*) that were treated with polycyclic hydrocarbons.

We were also interested in determining whether or not the date of sacrifice (and assay) was related to hepatic AHH activity of flounder. However, a plot of AHH activity *versus* date of assay did not illustrate any clear-cut relationship between these two parameters although it did reemphasize the variability of enzyme activity in the fish studied.

The causative factor for the induction of AHH activity in about half of the flounder examined has still not been clearly elucidated. However, since individual induced fish were found in all groups tested over the course of the summer, it does not appear to be related to spawning. It is also interesting that both male and female fish were affected. These observations are consistent with the enzyme response being caused by an exogenous factor, such as the presence of a polycyclic hydrocarbon-like inducer in the environment. This possibility is further enhanced by the fact that the enzyme responses monitored in the fish with high hepatic AHH activity are identical to those found in flounder that have been pretreated with polycyclic hydrocarbons. However, additional studies are still required before pollutants are definitely associated with the elevated hepatic AHH activities in many (but not all) Maine flounder, and more importantly, before enzyme induction in fish is used as a biochemical monitor for toxic environmental contaminants.

THE EGG CASE OF *Raja erinacea*: MECHANICAL PROTECTION OR OSMOREGULATORY DEVICE?

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The little skate, *Raja erinacea*, is oviparous and lays egg cases throughout the year (Bigelow and Schroeder, Fishes of the Gulf of Maine, p. 69, 1953). Smith (Biol. Rev. 11:49-82, 1936) suggested that the cases (as well as the female uteri in ovoviviparous and viviparous species of elasmobranchs) provide for an osmotically isolated environment until the embryo is capable of urea retention and osmoregulation. However, Reed (Comp. Biochem. Physiol. 24:668-675, 1968) found that encapsulated embryos of the skate *Raja binoculata* do possess the enzymes of the Krebs ornithine-urea cycle and are able to retain near adult levels of both urea and trimethylamineoxide (Reed, Biol. Bull. 135:537-547, 1968). The retention of urea seems to be secondary to an urea-impermeable egg membrane since Needham and Needham (J. Exp. Biol. 7:7-18, 1930) had shown that the egg cases are permeable to urea.

Despite the ability to maintain high urea levels, it appears that osmoregulation in seawater is limited since Libby (Nat. Geogr. Mag. 116:412-420, 1959) found that if embryos of *R. eglanteria* are removed before day 20 of the 64-day developmental period (after day 20 the mucous plug of the egg case is dissolved and seawater enters the egg case), they are unable to survive in seawater.

It, therefore, appears that the full complement of osmoregulatory mechanisms is not developed before approximately 1/3 of the developmental period is over. It is obvious that the data on the ontogeny of elasmobranch (and teleost, for that matter) osmoregulation is sketchy at best. To initiate our investigations of this system we measured the Na^+, K^+ and Cl^- concentration of the intracapsular fluids and the Na^+ efflux with newly-shed egg cases of the little skate, *Raja erinacea*.