Linear regression analysis of chloride cell counts versus short-circuit currents in pooled data from six roof and six opercular epithelia (Fig. 1) showed that the correlation coefficient was 0.92 (significant at $P < 0.001$) and that the regression line essentially passed through the origin. This close correlation between short-circuit current (i.e., active chloride secretion) and the number of chloride cells provides strong evidence that the chloride cell is responsible for chloride secretion in both roof and opercular epithelia. This work was supported by NIH grants GM 24766 (to K.K.) and GM 25002 and EY 01340 (to J.Z.).

FURTHER CHARACTERIZATION OF HEPATIC ARYL HYDROCARBON HYDROXYLASE INDUCTION IN A NATIVE POPULATION OF WINTER FLOUNDER, Pseudopleuronectes americanus, FROM COSTAL MAINE

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Many toxic agents (PCBs, PBBs, halogenated dioxins, and polycyclic aromatic hydrocarbons [PAH]) cause induction of the cytochrome p-450-dependent monooxygenase system involved in oxidative xenobiotic metabolism, and it has been suggested that induction of this enzyme system in fish could predict exposure to such environmental pollutants. However, before using enzyme induction in fish as a sentinel system for certain classes of chemical contaminants, it is necessary to elucidate clearly the causative factors of this enzyme response (including the contribution of different physiological states). The purpose of this study was to further investigate the PAH-type induction of the hepatic monooxygenase system of large numbers of male and female winter flounder captured near Mt. Desert Island, Maine, during the summer months, paying particular attention to factors that might be responsible for, or related to, the enzyme response.

In two previous successive summer seasons, we (Bend et al., Bull. MDIBL 17:47-49, 1977; Bend et al., Bull MDIBL 18:60-62, 1978) had shown marked heterogeneity of hepatic aryl hydrocarbon hydroxylase (AHH) and 7-ethoxyresorufin deethylase (7-ERF) activities in flounder as well as a differential effect of in vitro 7,8-benzoflavone AHH activity (stimulated AHH activity in fish with low AHH and 7-ERF activities and inhibited AHH activity in fish with higher AHH and 7-ERF activities).

Winter flounder sampled during June-August, 1979, using previously described assay procedures (Ibid, 18:60-62, 1978), again showed a marked heterogeneity in hepatic AHH and 7-ERF activities. The percentage of flounder with PAH-type induced hepatic monooxygenase systems, by year, is shown in Table 1. The data demonstrates that this is a

Table 1. Percentage of native winter flounder examined with polycyclic hydrocarbon type-induced[a] hepatic monooxygenase systems

Date	No. of Fish Assayed	% Induced[a]
June - August, 1977	15	85
June - August, 1978	172	49
June - August, 1979	81	72

[a]Fish with relatively high AHH activity in which AHH activity was inhibited by in vitro addition of 7,8-benzoflavone (0.5 mM).

recurring phenomenon in the Maine flounder population we have investigated. The plot of AHH activity vs. (AHH activity in the presence of 7,8-benzoflavone-AHH activity in the absence of this flavone) for both male and female fish sampled during 1979 (Figure 1) showed a high correlation between these two parameters (correlation coefficient 0.95, N = 81).

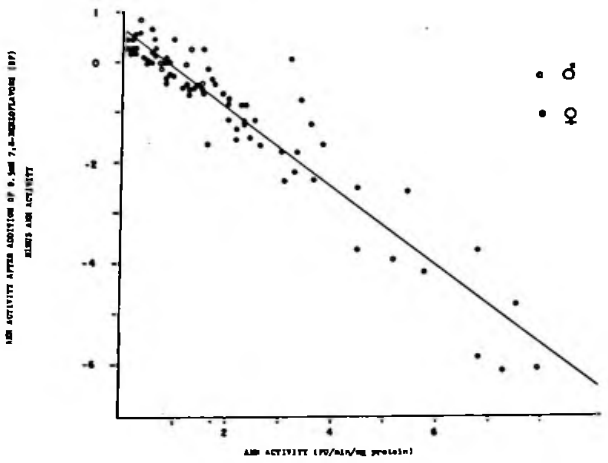

Figure 1. AHH activity vs (AHH activity after in vitro addition of 0.5 mM 7,8-benzoflavone minus AHH activity) in male and female flounder homogenate

Enzyme activities were studied in kidney homogenate from 31 flounder on the assumption that if the hepatic enzyme response was due to exposure to PAH-like inducers occurring in the marine environment, renal activities in the flounder with induced hepatic AHH activities might also be elevated. However, the renal monooxygenase system of only one fish tested appeared to be induced (higher AHH activity inhibited by in vitro 7,8-benzoflavone) even though the hepatic monooxygenase system of 85% of these particular flounder was induced. This strongly suggests that if environmental contaminants are responsible for the hepatic enzyme response, these chemicals are largely sequestered in the liver and unavailable in high enough concentrations in the circulation or kidney to cause PAH-type induction of the renal monooxygenase system.

The enzyme activities that we routinely monitored (AHH and 7-ERF) are selectively induced after exposure to only a few classes of chemicals (PAH, certain halogenated biophenyl and chlorinated dioxin isomers) that occur as pollutants and this induction is related to the de novo synthesis of a new form (or forms) of cytochrome P-450, commonly designated cytochrome P-448 or cytochrome P_1-450. Benzphetamine, on the other hand, is normally a poor substrate (very low turnover) for PAH-induced forms of cytochrome P-450 whereas it is generally a good substrate for control (normal) cytochromes P-450 in fish. Consequently, we measured hepatic benzphetamine demethylase (BZPH) activities as previously described (Bull. MDIBL 12:12-14, 1972) in several winter flounder for comparison to hepatic AHH activities in the same fish. As shown in Figure 2 there was only a 2-fold variation in BZPH activities in the eight fish studies (vs. a 35-fold variation in AHH activities) and BZPH activity appeared to be independent of AHH activity. This data is consistent with the heterogeneity of hepatic AHH and 7-ERF activities in winter flounder being due to the induction of variable amounts of one or more forms of cytochrome P-450 in some fish by exposure to PAH-type inducers in the marine environment. This argument is strengthened by the data presented in Table 2, where the amount of cytochrome P-450, the wavelength of maximum absorption of cytochrome P-450 (in the CO-ligated and reduced form), and the AHH activity in hepatic microsomes from several winter flounder are compared. In the fish with the highest AHH activity and the highest microsomal cytochrome P-450 content, the wavelength of maximum absorption occurs near 448 nm, indicative of the presence of large amounts of cytochrome P-448 in the microsomes. However, in several of the other fish, AHH activity has been induced without an obvious hypsochromic shift in the wavelength of maximum absorption of the cytochrome P-450 spectrum. We have previously observed this phenomenon in fish treated with PAH-

COMPARISON OF BENZPHETAMINE DEMETHYLASE (BZPH) AND ARYL HYDROCARBON HYDROXYLASE (AHH) ACTIVITIES IN LIVER HOMOGENATE OF SEVERAL WINTER FLOUNDER

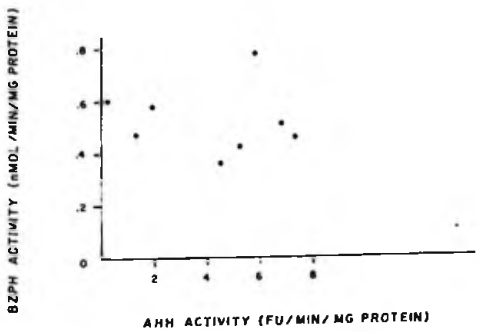

Figure 2.

Table 2. Cytochrome P-450 content and AHH activity of hepatic microsomes from several winter flounder

Cytochrome P-450 (nmol/mg protein)	λ Max (nm)	AHH (Units/nmol cytochrome P-450/min)
0.60	448.4	6.4[a]
0.25	451.0	0.7[b]
0.23	450.4	1.1[a]
0.18	450.2	3.1[a]
0.16	450.8	1.1[a]
0.12	450.2	1.8[a]
0.12	450.0	0.9[a]

[a]AHH activity inhibited by in vitro addition of 7,8-benzoflavone (0.5 mM).

[b]AHH activity increased by in vitro addition of 7,8-benzoflavone (0.5 mM).

ype compounds (i.e., large increases in AHH activity that are inhibited in vitro by 7,8-benzoflavone with no shift n the wavelength of maximum absorption of the microsomal cytochrome P-450 spectrum) and have shown, at least in ittle skate, that this is due to the synthesis of hepatic microsomal cytochrome P-448, although it is not the major form f cytochrome P-450 present in these microsomes (Bend et al., In: Pesticide and Xenobiotic Metabolism in Aquatic Organisms (M. A. Q. Khan, J.J. Lech, and J. Menn, eds). Am. Chem. Soc. Symposium Series 99: Washington, D.C. 979, pp. 297-318). The data in Table 2 also demonstrate why we have routinely used a combination of monooxygenase ctivities (7-ERF activity and AHH activity in the presence and absence of 7,8-benzoflavone) to test for PAH-type nzyme induction in the Maine winter flounder population (rather than the amount of hepatic cytochrome P-450 or ts wavelength of maximum absorption) since marked increases (up to 20-fold) can occur in enzyme activity with no oncomitant increase in cytochrome P-450 content or a definite hypsochromic shift in its spectrum.

It has been previously reported by Stegeman (Fed. Proc. 36:941, 1977) that sexually mature male rainbow trout (Salmo gairdnerii) contain more hepatic microsomal cytochrome P-450 and have higher aminopyrine demethylase activities (about 3-fold) than do females during spawning. This suggested that spawning and age (sexual maturity) of the winter flounder might be related to the wide variations in 7-ERF and AHH activities that we observed in this fish population. Consequently, we compared AHH activity to the gonad wt/body wt. ratio in female fish. Relatively large variations were noted in gonad wt/body wt. ratios (0.8 to 2.3%), consistent with different levels of ovarian function in these fish. Hepatic AHH activities (in homogenate) varied from 0.1 to 7.5 Units/min/mg protein. However, the correlation coefficient for AHH activity vs. gonad wt/body wt. ratio was only 0.34 (N=72), indicating no meaningful relationship between the two parameters. Thus, it appeared that spawning and sexual maturity were making a minor contribution, at best, to the widely divergent AHH and 7-ERF activities, which is also consistent with induction of the hepatic monooxygenase system in these fish by a PAH-type environmental pollutant.

Future work will determine the chemical(s) responsible for the PAH-type enzyme induction observed in Maine winter flounder and the amount of the compound(s) required for minimal and maximal AHH induction. The support of this research by the U.S. Environmental Protection Agency through the Federal Interagency Energy/Environmental Research and Development Program is acknowledged.

PURIFICATION OF A GLUTATHIONE S-TRANSFERASE FROM LITTLE SKATE LIVER, PREPARATION OF AN ANTIBODY TO THIS PURIFIED ENZYME AND IMMUNOLOGICAL SIMILARITY OF THE PURIFIED ENZYME AND HEPATIC AND EXTRAHEPATIC GLUTATHIONE S-TRANSFERASES FROM SEVERAL MARINE SPECIES FROM MAINE

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The glutathione S-transferases (GS-T) are an important family of enzymes involved in the detoxication of electrophiles, including carcinogenic and mutagenic epoxides. We have previously shown these enzymes to be widely distributed in hepatic and extrahepatic tissues of marine vertebrate and invertebrate species (James et al., Chem. Biol. Interactions 25:321-344, 1979). Since little skate was efficient at catalyzing the reaction of benzo(a)pyrene 4,5-oxide, a polycyclic arene oxide, with glutathione (GSH), the GS-T of this species were purified. Antibodies were prepared to the major purified enzyme and were tested for cross-reactivity to cytosolic GS-T from several tissues of various marine vertebrate and invertebrate species to investigate the phylogenetic distribution of GS-T that would react with the antibodies to this particular GS-T.

The following procedure was used for the purification of hepatic GS-T from the little skate. An aliquot (130 ml) of microsomal supernatant fraction pooled from several (6-10) male little skate livers was eluted from a DEAE-cellulose column (29 x 5 cm) with 0-0.1 M KCl linear gradient in 2 liters of 0.01 M potassium phosphate buffer, pH 8.0. Five separate peaks of GS-T activity with 2,4-dinitrochlorobenzene (DNCB) as substrate were obtained, accounting for 82%-106% of the activity applied (Figure 1). After concentrating each eluted peak by ultrafilitration (Amicon, PM-10 membrane), they were applied individually to a column (21 x 1.5 cm) of epoxy-activated Sepharose 6B (Pharmacia) which had been reacted with GSH (0.6 μmol GSH/g Sepharose). After thorough washing with 0.01 M Tris buffer, pH 8.0, the GS-T activity was removed from this column by elution with 15 mM glutathione in 0.05 M Tris (100 ml). This eluate was then successively concentrated and diluted with 1 mM GSH in 0.01 M Tris, pH 8.0. Electrophoresis of these peaks showed that this process had purified 2 of the 5 enzymes, including the major enzyme (peak 4, Figure 1) which accounted for over 60% of the total catalytic activity with DNCB. The enzyme was further characterized using conventional techniques. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicated a molecular weight in the range of 28,500 daltons; electrofocusing showed the protein to have a pI of 5.4.

For antibody production, mg quantities of purified GS-T 4 were emulsified (w/o) in Freund's complete adjuvant (the ratio of antigensolution [0.5-0.9 mg/ml] to adjuvant was 1:1.2). The emulsion was injected subcutaneously at