for the transepithelial electrical potential difference and short-circuit current. Active Cl absorption is mediated by one or more neutral processes but is only minimally affected by the removal of Na from the bathing media. A preliminary "model" consistent with our findings is illustrated in Figure 1 and is proposed simply to serve as a guide for more detailed studies in the future.

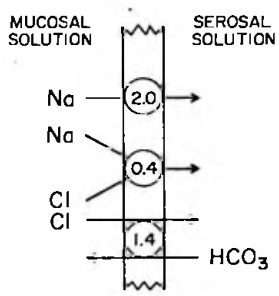

Figure 1

Second, the gallbladder of *Squalus acanthias* is a relatively leaky epithelium (like gallbladders from a variety of other species) and the paracellular conductance is predominantly due to Na. Thus replacement of Na in both bathing media with choline resulted in a 6-fold increase in tissue resistance whereas replacement of Cl in both bathing media with sulfate resulted in only a twofold increase in R_t. This notion is further supported by the fact that the serosa-to-mucosa fluxes of Na are 2-3 times greater than those of Cl. Thus it appears as if the paracellular shunt pathway of this epithelium sharply prefers Na over Cl, a feature that is relatively uncommon in leaky epithelia.

Finally, the results of our preliminary studies indicating that ion transport across this epithelium is not affected by amiloride, cAMP or a very potent carbonic anhydrase inhibitor strongly suggests that the mechanisms responsible for Na and Cl transport warrant further investigation.

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PARTIALLY INDUCED HEPATIC MICROSOMAL MIXED-FUNCTION OXIDASE SYSTEMS IN INDIVIDUAL WINTER FLOUNDER, *Pseudopleuronectes americanus*, FROM COASTAL MAINE

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The induction of hepatic microsomal benzo(a)pyrene hydroxylase (AHH) activity in fish has been suggested as a biochemical monitor for petroleum pollution in the marine environment (Payne, Science 191:945, 1976). However, as chemicals other than polycyclic aromatic hydrocarbons (PAH), including certain halogenated dioxin and biphenyl isomers, occur as aquatic pollutants and also dramatically induce AHH activities in various species of fish (Bend and James, Biochem. Biophys. Perspect. Marine Biol. 4: in press) and since hepatic AHH of some marine species, such as the Atlantic stingray (*Dasyatis sabina*), is not increased by repeated PAH administration (Bend, James, and Dansette, Ann. N. Y. Acad. Sci. 298:505, 1977), caution must be exercised in correlating elevated hepatic AHH activities with pollution by petroleum products.

In fish, as in mammals, dramatic increases in hepatic microsomal AHH activities appear to be related to the formation of cytochrome P-448, although not enough of this new form of the hemoprotein is present to cause

a wavelength shift in the absorption maximum of CO-saturated reduced liver microsomes (i.e., from 450 nm to 448 nm). Our laboratory has recently isolated cytochrome P-448 from solubilized hepatic microsomes of PAH-treated little skates, although it was not the predominant form of the cytochrome (Elmamlouk, Philpot, and Bend, Pharmacologist 19:160, 1977). In vitro α-naphthoflavone (α-NF) significantly inhibited hepatic microsomal AHH activity in induced little skates but stimulated AHH activity in untreated fish (Bend, Hall, and Foureman, MDIBL Bull. 16:3, 1976), which is also consistent with PAH-mediated cytochrome P-448 formation (Wiebel and Gelboin, Biochem. Pharmacol. 24:1511, 1975). Consequently, hepatic microsomal AHH activities were determined, in the presence and absence of α-NF, to test for induction of the hepatic microsomal mixed-function oxidase (MFO) system of several fish. Experiments on fish pretreated with 1,2,3,4-dibenzanthracene (DBA) were performed concomitantly.

Adult male or female little skates (*Raja erinacea*), spiny dogfish (*Squalus acanthias*), and winter flounder were captured near Mt. Desert Island, Maine. Fish were acclimated in pools equipped with continuously circulating seawater or in live-boxes immersed in seawater for at least 24 hr before use. Hepatic microsomes were prepared, the protein content determined, and AHH activities measured as described previously (Pohl, Bend, Devereux, and Fouts, MDIBL Bull. 13:94, 1973) except that additional incubation mixtures containing 10^{-3} M and 10^{-4} M α-NF (in vitro) were assayed simultaneously. 7-Ethoxyresorufin deethylase (7-ERF) activity was assayed essentially as described by Burke and Mayer (Drug Metab. Disp. 2:583, 1974), at a 7-ethoxyresorufin concentration of 2 μM and a final pH of 7.8. Product formation was linear for at least 8 min at incubation temperature (30°) used.

As shown in Table 1, administration of DBA to winter flounder increased hepatic microsomal AHH and 7-ERF activities relative to two control fish, and AHH activity was inhibited by α-NF in all treated fish. These

TABLE 1

Effect of 1,2,3,4-dibenzanthracene (DBA) pretreatment on hepatic microsomal aryl hydrocarbon hydroxylase (AHH) and 7-ethoxyresorufin deethylase (7-ERF) activities in winter flounder (*Pseudopleuronectes americanus*)

	AHH Activity (units/min/mg protein)		7-ERF Activity
	without α-NF	with α-NF (10^{-4} M)	(pmol/min/mg protein)
Control Fish	0.05	0.19	0
	0.40	0.19	23
	1.76	0.83	146
DBA-treated Fish*	1.11	0.63	157
	1.67	1.01	187
	1.84	0.78	261

*Injected I.P. with 10 mg/kg 1,2,3,4-dibenzanthracene (in corn oil) on days 1, 2, and 3; sacrificed on day 10.

data are consistent with the induction of cytochrome P-448 in DBA-dosed flounder. The presence of high AHH and 7-ERF activities in one control flounder, and the inhibition of the AHH activity by α-NF in this animal also suggested that the hepatic microsomal MFO system of this fish was induced. Consequently, several other untreated flounder were studied (Table 2).

There was a tremendous variation in hepatic microsomal AHH activity (over 60-fold) and 7-ERF activity (over 35-fold) in the 13 flounder tested. While this variation in enzyme activity could be due to the heterogeneity of a wild population, it seems more likely to be related to differing degrees of induction of the MFO system in these fish. The flounder were divided into two groups according to whether α-NF

TABLE 2

Aryl hydrocarbon hydroxylase (AHH) and 7-ethoxyresorufin deethylase (7-ERF) activities in hepatic microsomes from untreated winter flounder (*Pseudopleuronectes americanus*)

	AHH Activity (units/min/mg protein)		7-ERF Activity (pmol/min/mg protein)
	without α-NF	with α-NF (10^{-4} M)	
Group A[1]	0.16 ± 0.14 (2)[2]	0.66 ± 0.66 (2)	16.5 ± 14.8 (2)
	(0.05 - 0.26)[3]	(0.19 - 1.12)	(6 - 27)
Group B[4]	1.82 ± 0.98 (11)	0.95 ± 0.55 (11)	137 ± 57 (6)
	(0.28 - 3.15)	(0.19 - 1.63)	(64 - 230)

[1]Those fish where in vitro α-NF stimulated AHH activity; [2]Mean ± SD (N); [3]Range; [4]Those fish where in vitro α-NF inhibited AHH activity.

stimulated (Group A) or inhibited (Group B) hepatic microsomal AHH activity, with the implication that those fish in group B had partially induced MFO systems. The much higher ERF activities in the group B fish support this hypothesis, since ERF activity is preferentially induced by compounds that result in the formation of cytochrome P-448 (Burke and Mayer, Drug Metab. Disp. 2:583, 1974). The fact that 85% of the flounder tested had induced MFO systems prompted us to do similar experiments in dogfish and little skates. In these two species, caught in the same locale as the flounder, none of the untreated fish showed evidence of induction although hepatic microsomes from all DBA-pretreated dogfish and skates had elevated hepatic microsomal AHH activities which were inhibited by α-NF (data not shown).

Whether or not the induction of the hepatic MFO system of most of the winter flounder investigated here is due to exposure of these fish to environmental contaminants, such as the PAH, is still unknown. However, a recent observation made in our Florida laboratory where only 8 of 81 sheepshead (*Archosargus probatocephalus*) caught in the wild had induced hepatic MFO activities and where induction almost totally coincided with the spawning season (April and May) (Bend, Foureman, James, Second Internatl. Symp. Aquatic Poll. Pergamon, in press) suggests that normal physiological processes may also be related to induction of the hepatic MFO system in the flounder. (The flounder were not assayed during spawning season.) Obviously, more detailed investigations are required before induction of the MFO system in fish is routinely used as a biochemical index for chemical pollution of the aquatic environment.

ALANINE SYNTHESIS DURING STARVATION IN SKELETAL MUSCLE OF THE SPINY DOGFISH, *Squalus acanthias*

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The spiny dogfish, *Squalus acanthias* is able to survive for many weeks without food during which period it must rely on endogenous stores to provide both energy and materials for synthesis. The synthesis of urea must continue during starvation, since despite a significant loss of the nitrogenous product to the environment the urea content of plasma decreases only slightly (see below). A second synthesis of likely importance during starvation is that of glucose. In mammals, glucose or glycogen are the obligatory substrates for ATP generation in nervous and anaerobic tissues. It is unlikely that sufficient carbohydrate could be stored in the dogfish to satisfy the needs of prolonged starvation, so that gluconeogenesis is required. Both urea and glucose could be synthesized from a number of amino acids and the most likely source of these