TABLE 2

PERMEABILITY MEASUREMENT OF *Fundulus* INTESTINE

	K_{trans} x $10^{-7}cm^{-1}sec^{-1}$	
	m → s	s → m
^{3}H-Water	549.5±55.3 (12)	791.3±38.4 (12)
^{14}C-Urea	96.0±10.9 (7)	97.9±12.1 (3)

Radioisotopes, ^{22}Na and ^{36}Cl, were then used to measure the ion flux across the intestinal mucosa; ^{3}H-water and ^{14}C-urea were used to measure the permeability constant. The results are summarized in Tables 1 and 2 where it can be seen that there was a net mucosal-to-serosal flux of both Na and Cl ions similar to that obtained from the intestine of winter flounder and catfish. The K_{trans} constant for ^{3}H-water and ^{14}C-urea was of the same magnitude as that observed in both flounder and catfish intestine.

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IN VITRO EPOXIDE METABOLISM IN SOME MARINE SPECIES

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Epoxide metabolites have been implicated as the agents responsible for the carcinogenic effect that aromatic hydrocarbons have when administered to mammals. For a recent review of this subject, see (Oesch, Xenobiotica 3: 305, 1973). Epoxides are generated in vitro by the action of mixed-function

oxidase enzymes on hydrocarbon substrates and can be enzymatically detoxified in mammals by glutathione-S-epoxide transferase to form the hydroxyglutathione conjugate and by epoxide hydrase to form the dihydrodial. Marine species are now known to have mixed-function oxidase enzymes and are thus capable of generating epoxide metabolites so it is of interest to find out if these species can detoxify epoxides enzymatically by glutathione-S-epoxide transferase or epoxide hydrase or both.

The rate at which glutathione-S-epoxide transferase can metabolize epoxides is dependent on the concentration of glutathione as well as the concentration of enzyme for a given concentration of epoxide, so the basal levels of glutathione were also measured in a few fish. In mammals the concentration of free glutathione in liver is known to play an important role in protecting the liver from the toxic effects of several chemicals, most of which are substrates (after epoxidation) for the glutathione-dependent transferases.

We studied glutathione-S-epoxide transferase and epoxide hydrase in vitro using styrene oxide (8-^{14}C) as substrate. Epoxide hydrase was assayed in microsomes according to the method of Oesch et al. (Biochim. Biophys. Acta 227: 685-691, 1971), and glutathione-S-epoxide transferase was assayed in soluble fraction by the method of James et al. (The Pharmacologist 17: 191, 1973). Species used were the large skate, *Raja ocellata*, the little skate, *Raja erinacea*, the dogfish shark, *Squalus acanthias*, and the winter flounder, *Pseudopleuronectes americanus*. The organs tested for activity were liver and in some cases kidney and spiral valve. Centrifugation procedures for preparation of microsomes and soluble fractions were as previously described (Bend, Pohl, Fouts, Bull. MDIBL 12: 12, 1972). For flounder the assays were performed using 10,000 x g supernatant as the source of both enzymes, so

TABLE 1

EPOXIDE METABOLIZING ENZYMES IN 4 SPECIES

nmoles product formed · min^{-1} · mg $protein^{-1}$

SPECIES	LIVER		KIDNEY		SPIRAL VALVE	
	Epoxide Hydrase	Glutathione-S-epoxide transferase	Epoxide Hydrase	Glutathione-S-epoxide transferase	Epoxide Hydrase	Glutathione-S-epoxide thansferase
Little Skate (*Raja erinacea*)	0.47±.11[a] n = 10	2.10±.17 n = 11	0.26 (.17,.35) n = 2	2.43 (2.25,2.57) n = 2	-----	-----
Large Skate (*Raja ocellata*)	1.83±.24 n = 5	2.55±.32 n = 3	0.55 (.48,.62) n = 2	2.43 (2.11,2.75) n = 2	.09±.01 n = 3	1.79±.33 n = 3
Dogfish Shark (*Squalus acanthias*)	7.57±2.39 n = 4	9.3 n = 1	9.61±1.59 n = 4	8.29 n = 1	0.83 n = 1	3.36 n = 1
Winter Flounder (*Pseudoplexronectes americanus*)	1.78[b] n = 1	2.06 n = 1	0.56 n = 1	0.3 n = 1	-----	-----

[a]The values quoted for specific activities are the mean ± S.D. (N) for $n \geq 3$. Otherwise, individual values are given.

[b]Epoxide hydrase is measured in microsomes and glutathione-S-epoxide transferase in soluble fraction except in the case of winter flounder, where 10,000 x g supernatant was assayed for each enzyme using the conditions specified in the text.

the specific activities quoted for flounder are not directly comparable to those found in the other species. Glutathione levels were measured in liver, gill, kidneys, and spiral valve mucosa of a large skate (*Raja ocellata*) using the Ellman procedure. Glutathione levels of several organs of the little skate (*Raja erinacea*) and thorny skate (*Raja radiata*) were measured using the more sensitive fluorometric assay of Lyle and Cohen (Anal. Biochem. 4: 434, 1966).

Table 1 summarizes the mean specific activities of glutathione-S-epoxide transferase and epoxide hydrase assayed at 30°C at a substrate (styrene oxide) concentration of 1mM. Glutathione-S-epoxide transferase was measured in the presence of 10mM glutathione and at pH 7.6, the observed pH optimum. Epoxide hydrase was assayed at pH 9, the optimum of this enzyme. Assays were carried out at 30° because this was the observed temperature optimum for the microsomal mixed-function oxidase enzymes. Above this temperature denaturation of mixed-function oxidases may occur. However both epoxide metabolizing enzymes in skate liver showed increasing activity with increasing temperature up to 37° for glutathione-S-epoxide transferase, and up to 47° (the highest temperature at which assays were performed) for epoxide hydrase. An incubation time of ten minutes and a protein concentration of about 1 mg per 1.5 ml incubation volume were usually used in assaying glutathione-S-epoxide transferase, and the product formation was linear with time up to 15-20 minutes and with protein concentration up to about 2 mg per 1.5 ml incubation mixture for skate liver soluble fraction. Skate liver microsomal epoxide hydrase was linear with time up to 60 minutes incubation time and linear with protein up to 8 mg per ml incubation. Epoxide hydrase was usually assayed using a 20 minute incubation time in the presence of about 3 mg protein per ml.

TABLE 2

GLUTATHIONE (GSH) LEVELS IN SEVERAL TISSUES OF MALE SKATES

	Little Skate[a] (*Raja erinacea*)		Large Skate[b] (*Raja ocellata*)		Thorny Skate[b] (*Raja radiata*)	
	µg glutathione/ g tissue	mg GSH/ whole organ	µg GSH/ g tissue	mg GSH/ whole organ	µg GSH/ g tissue	mg GSH/ whole organ
LIVER	716±334	18.09±8.09	425	42.93	775	33.56
KIDNEY	299±103	0.78±.26	229	1.49	188	1.22
SPIRAL VALVE MUCOSA	374±186	3.66±2.38	393	8.97	330	3.23
GILLS	0, 119	0, 2.28	105	0.68	---	---
BRAIN	583, 186	0.53, 0.18	---	---	260	0.65
SALT GLAND	321, 0	0.13, 0	---	---	---	---
SPLEEN	238, 170	0.84, 0.28	---	---	---	---
PANCREAS	138, 99	0.15, 0.10	---	---	---	---
HEART	94, 265	0.08, 0.23	---	---	---	---
TESTES	94, 106	0.59, 0.61	---	---	---	---

[a]Values quoted are mean ± S.D. (N) for 5 animals in the case of liver, kidney, and spiral valve mucosa. Only 2 animals were used to measure the glutathione content of the other organs.
[b]Values for 1 animal.

Glutathione-S-epoxide transferase was assayed in little skate (*Raja erinacea*), liver, and kidney soluble fractions in the presence of variable concentrations of glutathione at 1mM styrene oxide concentrations. The apparent K_m and V_{max} values for GSH were similar for each organ, K_m about 1.5×10^{-4}M and V_{max} about 2.3 nmoles $\cdot$ min^{-1} $\cdot$ mg $protein^{-1}$. At high concentrations of glutathione (50mM), inhibition of activity was observed using 1mM styrene oxide.

The endogenous concentration of glutathione in the five specimens of *Raja erinacea* tested were 1.1-3.9mM for liver, about 1mM for kidney, and 0.4-1.8mM for spiral valve mucosa. Levels of glutathione in some other organs and species of skate are shown in Table 2.

This work demonstrates the presence of epoxide metabolizing enzymes in four species of fish that are common in Maine. The relatively high levels of epoxide hydrase and glutathione-S-epoxide transferase in the dogfish shark (*Squalus acanthias*) were especially interesting and raise the possibility that endogenous compounds may act as substrates for these enzymes in this species. In the little skate (*Raja erinacea*) the specific activities of glutathione-S-epoxide transferase are similar to those of glutathione-S-aryl transferase (Bend, Fouts, Bull. MDIBL 13: 4, 1973).

The presence of these enzymes and of concentrations of free glutathione in liver and kidney that exceed the apparent K_m for glutathione at a substrate (styrene oxide) concentration of 1mM (higher than would be expected for many environmental pollutants) provide evidence that compounds which act as substrates for the glutathione transferases should be readily detoxified by the skate.