prepared from lobster hepatopancreas or green gland (Pohl, Bend, Devereux and Fouts, Bull. MDIBL 13: 1973).

This study has shown that the lobster hepatopancreas is the principal site of storage for a single, purified polychlorinated biphenyl isomer after parenteral administration. The prolonged period required for excretion of this compound is consistent with the observed presence of lipophilic environmental contaminant residues in hepatopancreas of lobsters in nature. It further suggests that lobster hepatopancreas might be avoided as a foodstuff if one is interested in minimizing his dietary source of organochlorine chemical residues.

1973 #2

GLUTATHIONE S-ARYLTRANSFERASE: DISTRIBUTION IN SEVERAL MARINE SPECIES AND PARTIAL CHARACTERIZATION IN HEPATIC SOLUBLE FRACTIONS FROM LITTLE SKATE *Raja erinacea* LIVER

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The biliary excretion of several xenobiotics (or their conjugated metabolites) appears to be an important step in their elimination by some marine animals (Adamson, Fed. Proc. 26: 1047, 1967; Adamson and Guarino, Comp. Biochem. Physiol. 42A: 171, 1972). Sulfobromophthalein (BSP) is excreted in the bile of the dogfish (Boyer, Bull. MDIBL 11: 2, 1971; Guarino *et al.*, Bull. MDIBL 12: 41, 1972) and of the rainbow trout (Schmidt, Weber, Proc. West. Pharmacol. Soc. 15: 40, 1972). In mammals biliary excretion of BSP is normally associated with its conjugation to glutathione (GSH), catalyzed by the enzyme GSH S-aryltransferase. Therefore, soluble fractions isolated from liver or hepatopancreas of several marine species were assayed for GSH S-aryltransferase activity (towards 1,2-dichloro-4-nitrobenzene). Several properties of this enzyme and its distribution in extrahepatic organs of this species were also examined.

Little skates (650-1,300 g), large skates (1,400-2,400 g), thorny skates (1,400-1,500 g), dogfish (1,280-1,600 g), killifish (approximately 1 g), eels (110-180 g), hagfish (220-250 g), King of Norway (850-1,300 g), winter flounder (205-280 g), mackerel (510-770 g), rock crabs (120-190 g), and lobsters (435-620 g) of mixed sex were captured in local Maine waters or purchased from commercial fishermen. All animals were maintained in live-cars or tanks equipped with flowing seawater at MDIBL for at least 24 hours before use with the exception of the mackerel whose livers were frozen just after death prior to assay the following day. Tissues were homogenized as described previously (Bend, Pohl and Fouts, Bull. MDIBL 12: 12, 1972). Cell debris, nuclei, and mitochondria were sedimented from the homogenate by centrifugation at 10,000 g for 20 minutes. The microsomal supernatant ("soluble fraction") resulting after centrifugation of the 10,000 g supernatant at 177,700 g for 40 minutes (Beckman 60Ti rotor, 50,000 rpm) was used as the enzyme source. Protein concentration of soluble fractions was determined by the procedure of Lowry *et al.* (J. Biol. Chem. 193: 265, 1951).

Glutathione S-aryltransferase activity was measured spectrophotometrically by appearance of the product, S-(2-chloro-4-nitrophenyl) glutathione, essentially as described by Booth, Boyland, and Sims (Biochem. J. 79: 516, 1961). An extinction coefficent of 10,300 M^{-1} cm^{-1} at 350 nm (the wavelength of maximum absorbance) was used for calculation of transferase activity. Assays were performed either with a Gilford model 300-N flow-through microsample spectrophotometer equipped with a model 3017 thermo-cuvette and a model 4008 data lister (at the MDIBL laboratory) or a model 2400 Gilford spectrophotometer in combination with a circulating water bath (at the NIEHS, North Carolina laboratory). Duplicate determinations were performed at 37° C since highest enzyme activity was observed at this temperature with soluble fractions from flounder liver and gill (Table 1) or little skate liver (data not shown).

TABLE 1

EFFECT OF ASSAY TEMPERATURE ON THE GLUTATHIONE S-ARYLTRANSFERASE OF SOLUBLE FRACTIONS FROM FLOUNDER LIVER AND GILL

Assay temperature (°C)	Specific activity (nmoles product formed/ min/mg protein)	
	Gill	Liver
20	0.18*	0.60
26	0.38	0.79
32	0.61	0.94
37	0.75	1.24

* All values reported are means of two separate determinations. Gills and livers were pooled from six and five flounder, respectively.

Typical incubation mixtures contained 5.0 mM GSH, 1.0 mM 1,2-dichloro-4-nitrobenzene (added in 0.10 ml 95% ethanol), 0.75 ml of 0.4 M Hepes-sodium hydroxide buffer (pH 9.0 at 37° C), and 0.5 to 3.0 mg soluble protein in a final volume of 3.0 ml. Reported conjugation rates have been corrected for nonenzymatic product formation (by running a sample without soluble fraction) and for base-line drift (by running a sample without substrate). The actual procedure consisted of introducing an aliquot of the homogeneous reaction mixture (immediately after substrate addition) into the flow-through spectrophotometer and allowing the temperature of the mixture to come to 37° C. As soon as the temperature had reached the desired level (about 30 sec) the optical density was recorded by the data lister at 8 second intervals (timed by stopwatch). The reaction was allowed to proceed for at least 2.4 minutes in each case although initial rates were normally calculated from the first 0.8 minutes.

As shown in Figure 1 the pH optima for little skate liver GSH S-aryltransferase occurred between 8.4 and 9.0. This range tends to be appreciably smaller than that found in mammals such as the rat (Biochem. J. 79: 516, 1961) or rabbit (Bend, Hook and Fouts, Pharmacologist 15:

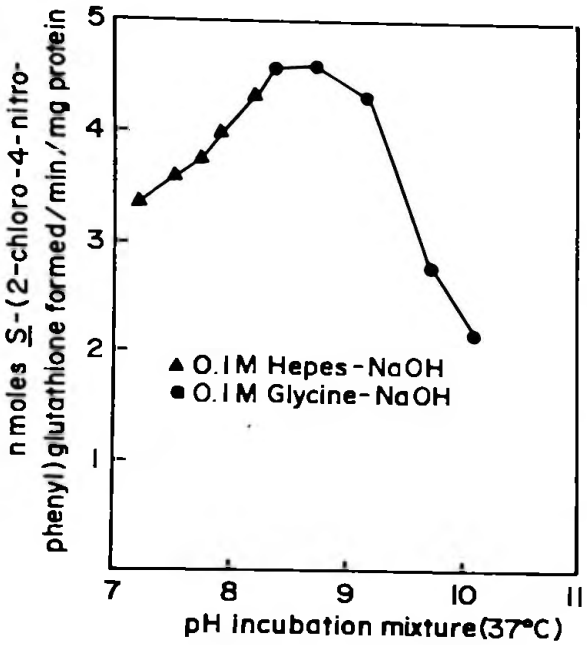

Figure 1. pH Optima of GSH S-aryltransferase in hepatic soluble fraction from the little skate. Soluble fraction was prepared from the pooled livers of three skates.

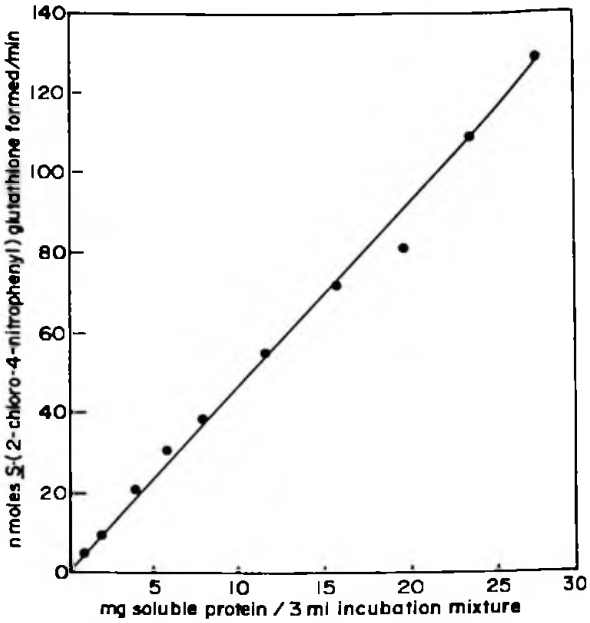

Figure 2. Protein concentration-activity curve of GSH S-aryltransferase in hepatic soluble fraction from the little skate. Soluble fraction was prepared from the pooled livers of three skates.

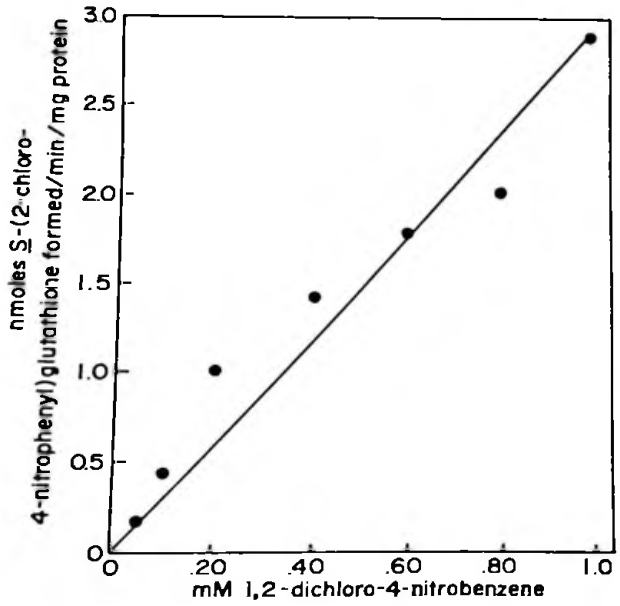

Figure 3. Substrate concentration-activity curve for GSH S-aryltransferase in hepatic soluble fraction from the little skate. Soluble fraction was prepared from the pooled livers of two skates.

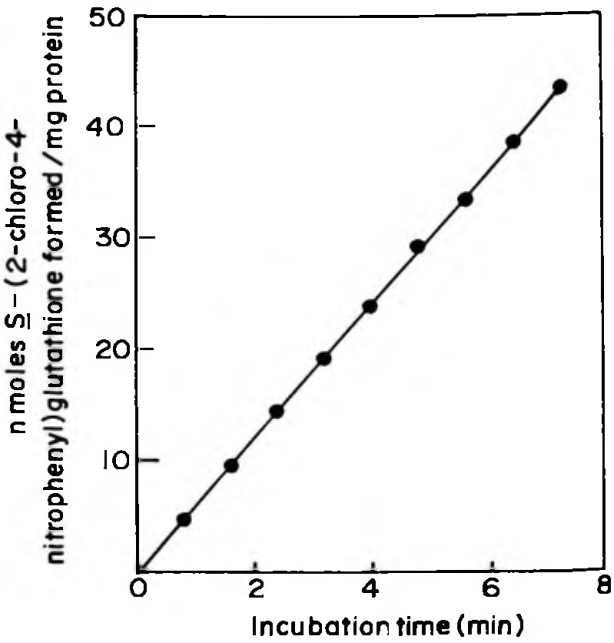

Figure 4. Time course of GSH S-aryltransferase activity in hepatic soluble fraction from the little skate at 37° C. Soluble fraction was prepared from the liver of a single skate.

249, 1973). The final pH of usual incubation mixtures was 8.4. Product formation remained linear with increasing soluble protein (from skate liver) concentrations up to at least 8 mg per 3 ml incubation mixture (Figure 2). The protein concentration of our routine assay system did not normally exceed 1 mg/ml, well within the linear portion of the curve.

At the maximum 1,2-dichloro-4-nitrobenzene concentration that could be maintained in solution (slightly over 1.0 mM), skate liver GSH S-aryltransferase was still not saturated with respect to this substrate (Figure 3). This has been previously observed for the analogous system and liver soluble fractions from rat and rabbit. As illustrated in Figure 4 product formation at 37° C with soluble fraction from little skate liver remained linear for at least three times as long (7.8 min) as the normal incubation period (2.4 min) and almost ten times as long as the timer period over which specific activity was calculated (initial 0.8 min). It is interesting that the temperature optimum of GSH S-aryltransferase was not less than 37° C in flounder liver and gill (Table 1) and skate liver (the highest temperature studied to date) whereas the optimal temperature for several microsomal oxidative xenobiotic-metabolizing enzymes occurs near 30° C in the little skate (Bend, Pohl and Fouts, Bull. MDIBL 12: 12, 1972; Bull. MDIBL 13: 1973). However this is not surprising in view of the relative stability of mammalian GSH S-aryltransferase versus mammalian microsomal oxidative drug metabolizing enzymes.

TABLE 2

GLUTATHIONE S-ARYLTRANSFERASE ACTIVITY IN LIVER OR HEPATOPANCREAS OF SEVERAL MARINE SPECIES

Species	Specific activity [nmoles S-(2-chloro-4-nitrophenyl)glutathione formed/min/mg soluble protein]
Little Skate, *Raja erinacea*	6.58 ± 5.95 (4)*
Large Skate, *Raja ocellata*	3.14 ± 1.66 (3)
Thorny Skate, *Raja radiata*	2.69 ± 1.13 (3)
Dogfish, *Squalus acanthias*	1.45 ± 0.55 (4)
Killifish, *Fundulus heteroclitus*	45.1 ± 14.7 (3)
Eel, *Anguilla rostrata*	6.70 ± 7.77 (3)
Hagfish, *Myxine glutinosa*	5.78 ± 1.45 (3)
King of Norway, *Hemitripterus americanus*	1.99 (1.89-2.09)**
Winter flounder, *Pseudopleuronectes americanus*	1.91 ± 0.69 (3)
Mackerel, *Scomber scombrus*	1.88 ± 0.47 (3)
Lobster, *Homarus americanus*	6.05 ± 2.44 (6)
Crab, *Cancer borealis*	3.55 ± 0.92 (3)

* mean ± S.D. (N)
** mean (range)

Glutathione S-aryltransferase activity was present in the soluble fractions from liver or hepatopancreas of all marine species tested, elasmobranchs, teleosts, and crustaceans (Table 2). The killifish, a small teleost, had the highest specific activity observed; indeed killifish hepatic GSH transferase activity is about five times greater than that of the rabbit (9.00 ± 2.64, mean ± S.D., n = 4; Pharmacologist 15: 249, 1973). Due to the small size of the killifish, livers from 12-20 fish were pooled prior to soluble fraction preparation and the reported value is from three such pools. Lobster and crab hepatopancreas soluble fractions contained relatively high transferase activity although these species possess very low if any xenobiotic oxidative metabolizing activity in subcellular fractions from this organ (Bull. MDIBL 12: 12, 1972).

TABLE 3

DISTRIBUTION OF GLUTATHIONE S-ARYLTRANSFERASE ACTIVITY IN SEVERAL ORGANS OF THE LITTLE SKATE, *Raja Erinacea*

Organ	nmoles S-(2-chloro-4-nitrophenyl)glutathione formed /min/mg soluble protein	/min/g tissue
Spiral valve mucosa	4.62*	209.3
Stomach lining	4.30	177.3
Liver	4.17	181.5
Pancreas	3.59	181.4
Kidney	2.13	93.1
Gill	0.67	19.3
Spleen	0.30	16.0
Heart	0	0

* The various organs of three skates were pooled. Values are means of two separate assays on each tissue.

Glutathione S-aryltransferase activity was widely distributed in extrahepatic tissues of the little skate (Table 3). Soluble fractions prepared from spiral valve mucosa and stomach lining had higher specific activity and more total activity per gram of mucosa than did liver. Pancreas and kidney also contained appreciable levels of activity. Glutathione S-aryltransferase is also widely distributed in extrahepatic organs of the rabbit (Pharmacologist 15: 249, 1973).

This study has demonstrated the wide distribution of a cytosol enzyme, GSH S-aryltransferase, in hepatic tissue of marine species. This enzyme in hepatic (and possibly extrahepatic) tissues is probably involved in the excretion (biliary) of polar conjugates of xenobiotics such as BSP in elasmobranchs and teleosts. Our findings also suggest that mercapturic acids, which are synthesized from GSH conjugates of xenobiotics in mammals, are likely excreted as metabolites in the urine of marine species.