PD is that the relative passive permeabilities of basal and apical surfaces of the oxyntic cell are dissimilar [*Hogben, pp111-130, Gstric Secretion, Eds: Sach, G., Heinz, E. & Ullrich, K.J., Academic Press, '72*]. Given such a dissimilarity of the surfaces, the imposition of a PD change would result in an altered intracellular ionic composition which in turn could explain an altered secretory rate. Where the passive permeabilities of the two survaces differ one would also predict a long half-rime, $t_{1/2}$, in response to a square-wave pulse of current. Many gastric mucosaw have a very long $t_{1/2}$, e.g. 14 seconds for the frog (*Noyes and Rehm, Am. J. Physiol. 219: 184, '70*]. In contrast, the longest $t_{1/2}$ encountered in the isolated gastric mucosa of *Squalus* was approximately 33 milliseconds (unpublished). These features of the electrophysiology of the dogfish gastric mucosa, absence of both a "Rehm effect" and a long time constant, are consistent with the relative permeabilities of the apical and basal surfaces being similar.

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Further Studies On Epoxide Metabolism *In Vitro* By Marine Species

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The results reported here are a continuation of the study we initiated last year on the activities of epoxide metabolizing enzymes in marine species (James, Fouts and Bends, Bull. MDIBL 14, 41, 1975). Epoxides are toxic and potentially carcinogenic organic compounds which may be formed *in vivo* by the action of mixed-function oxidase enzymes on molecules which have aromatic rings or ethylenic linkages in their structure. We have further characterized glutathione (GSH) S-transferase activities towards styrene oxide in liver of the little skate (*Raja erincea*). In addition we have measured the activities of epoxide hydrase and glutathione (GSH) S-tranferase towards styrene oxide in some other vertebrate and invertebrate marine species common to Maine. In some cases, we have compared these values (of styrene oxide metabolism) with the activities of GSH S-transferase towards another alkene oxide, octene oxide.

The methods used for preparation of microsomal and cytosol fractions from homogenates of liver or extra-hepatic organs are as described previously (Bend, Pohl and Fouts, Bull. MDIBL 11, 12, 1972), as are the methods for assay of epoxide hydrase and GSH S-transferase (James, Fouts and Bend, Bull. MDIBL 14, 41, 1975). Except where otherwise stated, the concentration of styrene oxide or octene oxide in the incubation mixture was 1mM and the concentration of GSH S-tranferase activity was 10mM. Assays were routinely carried out at 30°C and the incubation time was 10 minutes for the GSH S-transferase assays and 20 minutes for assay of epoxide hydrase. In experiments where the GSH S-transferase activity was measured in the presence of different concentrations of GSH, the cytosol fraction was dialyzed at 4°C for 24 hours against one change of 0.05M HEPES buffer pH 7.6, to remove any endogenous GSH

Several experiments were carried out using liv skate liver cytosol fraction to determine GSH S-transfe ase activity towards styrene oxide or octene oxide as function of pH in the range 5.7 to 8.3. The results of o experiment are shown in Figure 1. Although the ove variation in measured specific activity (nmoles conjugat • min^{-1} • mg $protein^{-1}$) was not great in the range pH 6 to 8, we usually observed 2 maxima, one between p 7.65 and 7.85. This could be explained by assuming t existence of several forms of GSH S-transferases, w different properties but overlapping substrate specifi (see Pabst, Habig, Jakoby, Biochem. Biophys. Re Comm. 52, 1123, 1973).

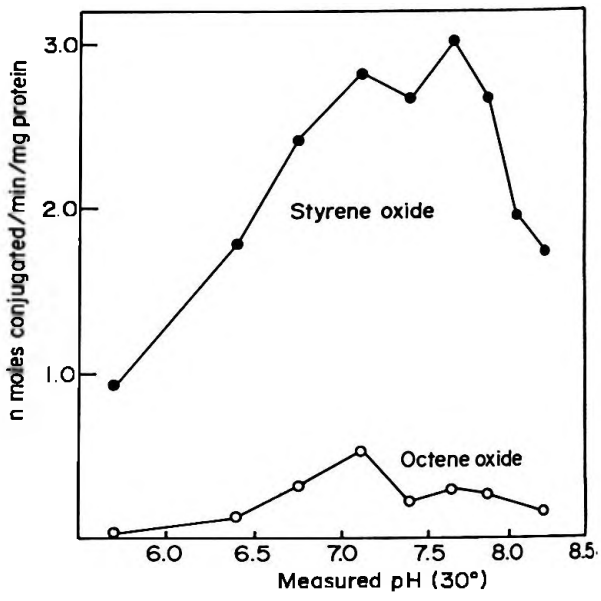
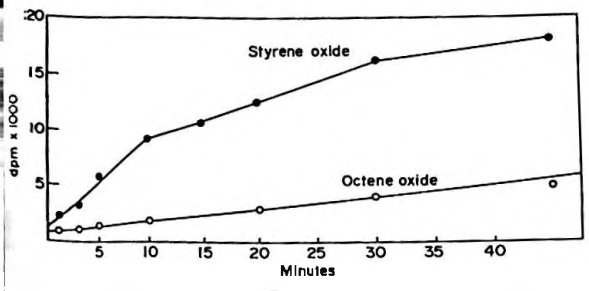


Figure 1 Variation of glutathione S-transferase activity skate hepatic cytosol fraction as a function of pH. The incu tion mixtures contained 1.0 mMolar styrene oxide or 1.0 m lar octene oxide as substrate. All reaction rates were correc for their non-enzymatic component. Results are from a typ experiment that was repeated three times.

We found that the rate of formation of the GS conjugate of styrene oxide increased linearly wi increasing protein concentration up to 4 mg protein skate hepatic cytosol fraction per 1.5 ml incubati mixture, and the rate of formation of the GSH conjuga of octene oxide increased linearly with increasing prote up to 8 mg protein per 1.5 ml incubation volume. Th agrees with previous work in this laboratory where GS S-transferase activity towards 1,2-dichloro-4-nitrobenze in skate hepatic cytosol fraction showed linearity of pro formation up to about 16 mg protein per 3 ml incubati volume (Bend, Fouts, 13, 4, 1973).

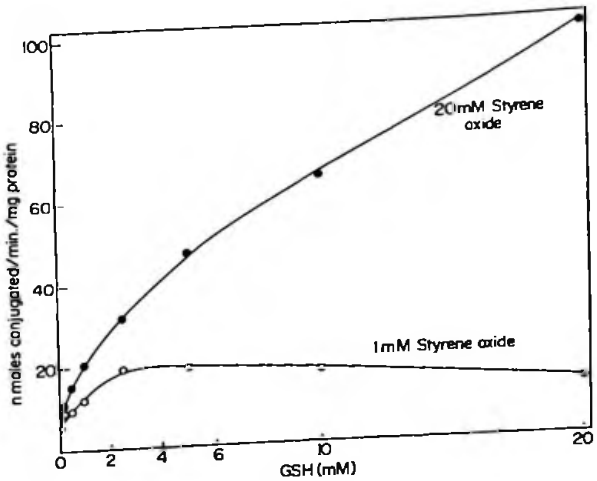
In the presence of 1 to 2 mg of cytosol fracti protein, the rate of styrene oxide conjugation with GS was linear with time up to 10 minutes (Figure 2), while t rate of octene oxide conjugation with GSH was line with time up to 30 minutes of incubation at 30°C.



gure 2 (above) Time course for formation of glutathione njugates in skate hepatic cytosol fractions. Substrate con-ntration (both styrene oxide and octene oxide) was 1.0 Molar. Results are from a single experiment. A repeat experi-ent gave very similar data.

gure 3 (right) Glutathione S-transferase activity of dialyzed ate hepatic cytosol fraction as a function of GSH concentra-on, at 1.0 mMolar and 20.0 mMolar styrene oxide. Specific tivities at 1 mMolar styrene oxide have been multiplied by 0 on the graph, for clarity. Results from a single experiment. epeat experiments at 1.0 mMolar styrene oxide gave similar sults.

For both substances, maximum specific activity of SH S-transferase was observed when the incubation mperature was between 30° and 40°C. The specific ctivites at 12°C, the usual summer water temperature in ne ocean where these fish were caught, were less than



half the specific activities at 30°C. This also agrees with the results shown in the study of GSH S-transferase activity towards 1,2-dichloro-4-nitrobenzene.

Figure 3 shows the variation in specific activity of GSH S-transferase in dialyzed hepatic cytosol fractions of little skate in the presence of varying amounts of GSH, and i mM or 20mM styrene oxide as substrate. Specific activities at the 1 mM styrene oxide concentration are

Table I

Species of Epoxide Metabolizing Activities

Species	Organ	Epoxide Hydrase (Microsomal Fraction)	GSH S-transferase (Cytosol Fraction) Styrene oxide	Octene oxide
		nmoles product formed · min^{-1} · mg $protein^{-1}$		
Rock Crab (*Cancer ittorus*) n=4	Heppatopancreas	1.51±3.1[a]	0.29±0.22[b]	N.D.[b]
	Gill	0.43±0.64[a]	0.83±0.24[b]	0.39±0.51[b]
Lobster (*Homerus americanus*) n=4	Heppatopancreas	21.9±2.4[a]	1.26±0.56[b]	0.08±0.06[b]
	Gill	9.9±5.8[a]	2.27±0.55[b]	0.29±0.06[b]
King of Norway (*Hemitripterus americanus*) n=3	Liver	1.08±0.43[a]	2.57±0.51[b]	3.45±0.96[b]
	Kidney	1.39 (pool)[a]	1.76±0.55[b]	1.20±0.57[b]
Winter Flounder (*Pseudopluronectes americanus*) n=3	Liver	1.99±1.36[a]	4.35±0.93[b]	1.59±0.48[b]
	Kidney	1.80±0.50[a]	4.88±1.35[b]	0.71±0.22[b]
Thorny Skate (*Raja radiata*) n=4	Liver		7.1±2.8[b]	6.18±2.40[b]
Little Skate (*Raja erincea*) n=6	Liver	0.30±0.20[a]	3.07±0.32[b]	0.64±0.10[b]

All specific activities are mean ± S.D. The number of animals tested is given under "Species."

a. Assays were carried out in the presence of 1 mM styrene oxide and incubation time was 20 minutes.

b. Assays were carried out using 1 mM styrene oxide or 1 mM octene oxide and an incubation time of 10 minutes.

multiplied by 10 in this figure for clarity. The kinetics of the reaction do not appear to follow simple Michaelis-Menton theory. Others have found complex kinetics in the GSH S-transferase reactions even in purified enzyme preparations (Pabst, Habig, Jakoby, J. Biol. Chem. 249, 7140, 1974).

The characterization of GSH S-transferase activity alkene oxides was carried out in little skate liver since this was the species used previously to characterize GSH S-transferase activity towards 1,2-dichloro-4-nitrobenzene. We also measured GSH S-transferase activities towards styrene oxide in a number of species as shown in Table I.

In most species, specific activiteis of GSH S-transferase in hepatic or renal cytosol fractions towards octene oxide were much lower than towards styrene oxide. Exceptions were the two teleost species studied, the King of Norway (*Hemitripterus americanus*) and the winter flounder (*Pseudopleuronectes americanus*). Specific activities of both epoxide hydrase and GSH S-transferase towards styrene oxide in liver and kidney of the winter flounder were high, especially microsomal epoxide hydrase. This might indicate that the ability to metabolize epoxides to more polar compounds is intrinsicaly important in the flounder. GSH S-transferase activity towards 1,2-dichloro-4-nitrobenzene in flounder hepatic cytosol fraction was an order of magnitude lower than towards either alkene oxide substrate, whereas in the little skate hepatic cytosol fraction, activity towards styrene oxide was about the same as towards 1,2-dichloro-4-nitrobenzene. This could indicate that skate and flounder have different proportions of the GSH S-transferases which show higher specificity for epoxide substrates.

We were also interested to find high spec activities of epoxide hydrase (about double the activi present in rat liver microsomes) in microsomal prepa tions from hepatopancreas of the lobster (*Homa americanus*) and rock crab [*Cancer ittorus*). Microsom prepared from lobster gill filaments also had high epox hydrase activities. The activities. The activities of G S-epoxide transferases towards styrene oxide or octe oxide in cytosol fractions of hepatopancreas or gill fr the crustacean species were variable and low compa with epoxide hydrase specific activities.

There is one interesting difference between several the marine species studied and rat or guinea pig (Jam Fouts, Bend, Biochem. Pharmacol. In Press, 1975) both mammalian speciea hepatic GSH S-transfer activity (both total and specific) greatly exceeds epo hydrase activity (using 1.0 mM styrene oxide as substr in both assays). In several of the marine spec however, epoxide hydrase activity predominated sugg ing that the microsomal hydrase may be relatively m important in the marine animals for epoxide detoxificat than in guinea pig or rat.

In summary, we may say that all marine species have studied are capable of metabolizing epoxides some extent, both by conjugation with GSH or hydration to the dihydrodiol. The existence in ma species of these pathways for further biotransformat of potential carcinogens, mutagens and cytotoxins co be an important protective mechanism towards envir mental contaminants such as polycyclic aroma hydrocarbons, which are known to be released into t ocean.

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Cytotoxicity Of Dogfish Shark Plasma On Murine Lymphoblasts In Culture

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Adamson (*Proc. Marine Tech. Soc. Foods - Drugs From The Sea 3033, 1972*) in a preliminary report on the effects of Elasmobrance plasma on L1210 cells *in vitro* noted only a 13% inhibition of the growth of L1210 cells in 24 hrs by dogfish plasma. For some time, we have been examining the ability of the serum and plasma of several species to alter the growth of murine lymphoblasts in culture. In the course of these studies, we observed that the plasma of dogfish shark *Squalus acanthias* is cytotoxic to a subline of Leukemia 5178Y cells rendered resitant to L-asparaginase (L5178Y/AR) and to L1210 cells *in vitro*. The present report characterizes this cytotoxicity.

L5178Y/AR and L1210 cells were cultured in Dulbecco-Vogt medium containing 10% of either Fetal Calf Serum (FCS) or shark plasma except where indicated and incubated for 48 hours at 37° in an atmosphere of 95% air and 5% CO_2. Electronic particle sizing of the cells was carried out using a Coulter channelizer and counter.

Table I

Effect of Fetal Calf and Shark Plasma on the Growth of L5178Y/AR *In Vitro*

Dulbecco-Vogt Medium, Supplemented with Fetal Calf Serum or Shark Plasma		Total Cell Count/ml (x 10^6)	
		Initial Count	Count After 48 hrs
Fetal Calf Serum	5%	1.0	3.8
	10%	1.0	4.0
Shark Plasma	5%	1.0	0.9
	10%	1.0	0.86

Legend: Cells were cultured for 48 hours at 37° in an atmosphere of 95 air and 5% CO_2. Trypan blue exclusion counting indicated grea than 90% cells Trypan blue negative in all cultures.

The frequency distribution of DNA content of individu cells was measured by flow microfluorimetry (*Van Dilla e al., Science 163, 1213, 1969*).

L5178Y/AR cells when cultured in FCS exhibit doubling time of about 16 hrs. When shark plasma wa used instead of FCS, multiplication ceases (Table 1). Th mitotic index in the FCS cultures averaged 3.5 percen whereas no mitotic figures were observed in cultures wit shark plasma. Morphologic examination of cells cultur for 48 hrs in shark plasma revealed a striking enlargeme or magalocytosis of the treated cells. This change wa