% of the administered dose and the brain contained and 7% at 4 and 24 hours, respectively. The final agory (Table 1, lower panel) of agents has high t½'s 130 min.) associated with generally higher values in plasma than were for the other two catagories of nts mentioned above. None of the agents in this class V_d's which are suggestive of either moderate tissue trances or slow metabolism the following were drugs nd to have no unusually high T/P ratio (Table 1): nazole, cytoxan, imidazole-4-carboxamide (I-4-C), sine arabinoside (Ara C), and diglycoaldehyde. In this ss of drugs, very high bile to plasma (B/P) ratios were n for cytoxan, Ara C, dibromomannitol (DBM), and lycoaldehyde, while the highest B/P ratios seen in this dy occurred for cytembena (622) and MTX (2200) at 24 rs. The kidney to plasma ratios seen for DBM, zacytidine (5-AC), and Ara C were 1.

Other pharmacokinetic parameters can be measured the excretory pathways, and urinary and biliary asurements are reported as percentages of the total ount excreted compared to the total dose admin-ered. The following compounds were excreted at least % in 24 hours in the urine (values in parantheses are actual amount excreted): puromycin (20), MTX (35), P (21), PIDMS (44), I-4-C (24), cytembena (28), and C (20). An intermediary (7-17%) range of urinary cretion was observed for the following compounds: Act (7), colchicine (9), guanazole (15), cytoxan (12), Ara C 7), DBM (12), and diglycoaldehyde (12). The lowest ounts of urinary excretion were seen for HN_2 (4), ticine (1.4), 5-FU (3), pseudourea (4.4), hycanthone 6) and CDDP (2.9). Only seven compounds were reted in the bile in quanities greater than 1% of the ministered dose: MTX (32), cytembena (16), hycan-one (9.6), puromycin '5.6), cytoxan (3.5), DBM (2), diglycoaldehyde (2). While the total "bookkeeping" adioactivity of most of the agents above is good sidering the number of organs sampled, the possibility excretion via the gills is likely (*Maren, et al., Comp. iochem. Physiol. 26, 853, 1968*) and should be vestigated further.

For 14 of these drugs there are t½'s available for a ammal (usually the human) and the dogfish shark. For TX, 6-MP, colchicine, HN_2 and 5-FU, the t½'s were mparable within a few minutes of each other. Slightly eater differences were seen for the following drugs here dogfish and mammalian t½'s respectively, are ven in parentheses: guanazole (96 vs. 120 min.); Act D .7 vs. 17 min.); cytoxan (68 vs. 20 min.); I-4-C (83 vs. 10 min.); Ara C (93 vs. 25 min); DBM (1.3 vs. 8 hr.); 5-AC (1.5 vs. 3.5 hr); cytembena (1.6 vs. 4 hr.); and CDDP (115 vs. 40 min.). Note that t½ values are higher for the dogfish in four of nine cases, reflecting the expected slower tissue uptake and metabolism. Hence, it would seem that the dogfish is suitable specie upon which to conduct preliminary fate and disposition experiments for xenobiotics.

22 • 1975

Absence Of The "Rehm Effect" In The Gastric Mucosa Of *Squalus acanthius*

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The gastric mucosae of several bony vertebrates respond to depolarization and hyperpolarization by respectively decreasing and increasing the rate of H^+ secretion ("Rehm effect"). In the following, the H^+ secretory rate of the dogfish mucosa proved to be remarkably refractory to a considerable displacement of the transepithelial potential difference in either direction.

Mucosae were mounted as previously described (*Hogben, Science 129: 1224, '59*) in flux chambers at 14°C and bathed by electrolyte solutions (urea-trimethyl-amine-free), whose ionic composition resembles the extracellular fluid of *Squalus*. The serosal solution had 30 mM of HCO_3^- and was gassed by 5% CO_2, 95% O_2, while that at the mucosal surface lacked HCO_3^- and was gassed by 100% O_2. The spontaneous H^+ secretory rate was augmented by 5 carbachol added to the serosalsolution before the first test period. At the end of a 45 minute test period the solution before the first test period. At the end of a 45 minute test period the solution bathing the mucosal surface was removed and titrated automatically to pH 6.85. Paired portions of mucosa were alternately at their spontaneous PD (OmV) and then voltage clamped at 60 mV with one portion being polarized and the other depolarized, for a total of seven 45 minute periods (315').

The results are summarized in Table 1. (The paired differences were corrected for a minor time bias).

Thus, in spite of a substasntial degree of hypo- and hyper- polarization, the H^+ secretory rate was not significantly degreased or increased. In other vertebrates, the H^+ is markedly dependent on the transepithelial PD, prompting the assertion that the H^+ "pump" is "electrogenic". An equally plausible explanation for those vertebrates where the H^+ secretory rate responds to the

Table I

H^+ SECRETION

$\mu Eq.hr^{-1}cm^{-2}$

	Spontaneous potential (~OmV)	Voltage clamped (60mV)	Paired Differences ±95% Conf. Limits
Hypo-polarization (+)	1.35±.21	1.59±.22	0.206±0.382
Hyper-polarization (-)	1.35±.24	1.51±.27	0.108±0.110

(Reference = serosal surface; x±SE, n=6)

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.

23 • 1975

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).

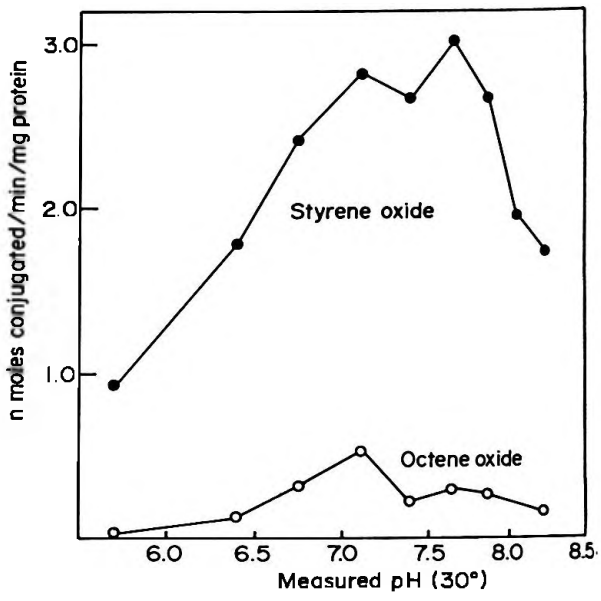

Figure 1 Variation of glutathione S-transferase activity skate hepatic cytosol fraction as a function of pH. The incu tion mixtures contained 1.0 mMolar styrene oxide or 1.0 m lar octene oxide as substrate. All reaction rates were correc for their non-enzymatic component. Results are from a typ experiment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