high density of mitochondrial cristae in the mitochondria is consistent with this notion, as is their intimate relationship to the lipid droplets. Supported by NSF Grant PCM75-14322A02 and NIH Grant IR01 AG0 0961-01.

ROLE OF METABOLISM AND TRANSPORT IN THE EXCRETION OF PHENYLACETIC ACID AND 2,4-DICHLOROPHENOXY ACETIC ACID BY MARINE FISH.

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Phenylacetic acid (PA) has been widely utilized to examine species differences in the enzymatic pathways responsible for detoxification of many chemicals via conjugation with amino acids. Using PA and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) to characterize these pathways in marine fish, James et al. (Bull. MDIBL 13:59-62, 1973, Xenobiotica 7:393-398, 1976) demonstrated that the taurine conjugates (PAT and 2,4-DT) were the major metabolites produced by both winter flounder and dogfish. These compounds were excreted almost exclusively via the urine. However, these and subsequent studies (Pritchard and James, J. Pharmacol. Expt. Therap., in press; James, unpublished) demonstrated an inverse relationship between the extent of metabolism (i.e., taurine conjugation) and the rate of excretion. Since conjugation is generally held to produce more readily excreted metabolites, this was a surprising observation. Therefore, using the winter flounder, *Pseudopleuronectes americanus*, and the spiny dogfish, *Squalus acanthias*, we attempted to answer the following questions: (1) Were parent compound and taurine conjugate transported at substantially different rates? (2) Did differences in renal transport underlie differences in excretion within a single species, e.g., 2,4-D (rapid) vs. PA (slow) in the winter flounder, or between species, e.g., 2,4-D in the flounder (rapid) vs. the dogfish (slow)?

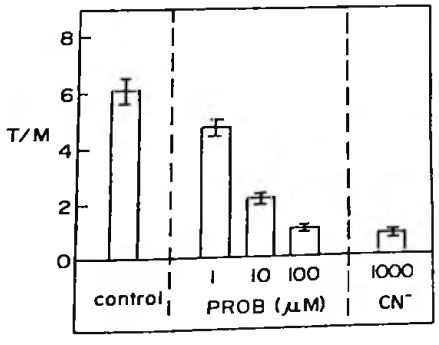
Techniques utilized were the isolated flounder tubule preparation of Forster (Science 108:65-67, 1948) and renal clearance studies in both flounder and dogfish. The details of both flounder preparations have been previously described (Am. J. Physiol. 233:F126-132, 1977). The dogfish clearances were performed as described by Guarino and Anderson (Xenobiotica 7:143, 1976).

Table 1. Concentration dependence of the uptake of phenylacetic acid (PA) and its taurine conjugate (PAT) by isolated flounder tubules.*

[ ] Molar	1	10	100	1000
PA (5)	8.7 ± 1.1	8.0 ± 0.9	5.6 ± 0.7	3.6 ± 0.3
PAT (4)	9.9 ± 1.6	9.8 ± 1.7	8.9 ± 1.3**	5.8 ± 0.5**

* Uptake expressed as the mean tissue-to-medium ratio ± S.E. The figure in parentheses is the number of animals tested. Tubules were incubated for 60 min. at 15°C under 100% O_2.

** $p < 0.05$ versus PA at the same concentration



We began by comparing the transport of PA and PAT *in vitro* and *in vivo*. As shown in Table 1, isolated flounder tubules accumulated both PA and PAT to concentrations much greater than that in the bathing medium. Uptake was concentration dependent. Uptake of both compounds also required metabolic energy and was sensitive to probenecid (Figure 1). Since cyanide (1 mM) reduced the tissue-to-medium ratio (T/M) to essentially 1 for both compounds, significant intracellular binding was absent. Thus, uptake of PA and PAT behaved as if it were mediated by the organic anion transport system and little difference was

Figure 1. Effect of probenecid and cyanide on transport of phenacetyltaurine (PAT) by flounder renal tubules *in vitro*. The teased tubules were incubated with 1 μM ^{14}C - PAT in Forster's saline for 60 min. at 15°C under 100% O_2. Results are expressed as the mean tissue-to-medium ratio (T/M) ± SE (bars) for determinations in five animals. * = $p < 0.05$ vs. the control.

apparent between the handling of PA and PAT. However, chromatographic analysis (under conditions described in Xenobiotica 6:1-13, 1976) of tissue and medium taken at the conclusion of these 60 min. incubations demonstrated that essentially all of the intracellular PA had been converted to PAT by the tubules and that 50% of the label in the medium was also converted to PAT. To focus on the uptake step, paired experiments measured the uptake of ^{14}C-PA or ^{14}C-PAT after shorter incubation times. Again, little difference was seen between the two compounds. PA accumulation was 20 to 30% greater than PAT, values approximating the intracellular conversion of PA to PAT. Mean T/M were 3.5 ± 0.2 for PA vs. 2.8 ± 0.1 for PAT at 5 min and 5.6 ± 0.5 vs. 4.1 ± 0.3 at 10 min (N=4).

In vivo, the clearance of PA or PAT was determined in the flounder after an i.m. dose of 2.5 µmol/kg. Data were normalized to the glomerular filtration rate (GFR) which was measured simultaneously using polyethylene glycol (PEG) as glomerular marker. As shown previously (Am. J. Physiol. 233:F123-132), this dose of PA produced stable plasma PA levels (1.16 ± 0.09 µM with a range from 0.5 to 2.0 µM over 24 clearance periods in 4 animals) during the period of clearance determination (1-6 hours after injection). The mean clearance of PA was 47 times the GFR (range 30-60) clearly indicating net tubular secretion *in vivo*. Probenecid (25 µmol/kg) inhibited tubular secretion nearly 50%. The urine of each of these fish contained ~ 90 to 95% PAT, as previously demonstrated by James and Bend (Xenobiotica 7:393-398, 1976). When PAT was given, clearance values were very similar to those seen following PA.

Considering the above data in conjunction with that previously generated for 2,4-D in the flounder (Bull. MDIBL 17:71-75, 1977), it appears that the slower excretion of PA (and PAT) results from its renal transport not its metabolism. First, PA uptake by flounder tubules *in vitro* is only 25-33% of 2,4-D uptake. At a substrate concentration of 10 µM, the T/M for PA was 9 vs. a T/M of 30-40 for 2,4-D. Similarly, the renal clearance of PA is also much lower (47 times the GFR vs. 250 to 500 times the GFR for 2,4-D) *in vivo*. Finally, the lack of substantive differences between the handling of PA and PAT *in vitro* and *in vivo* also suggests that it is the chemical nature of the parent compound, not its conjugation to taurine, which determines its transport rate and consequently its excretion rate *in vivo*.

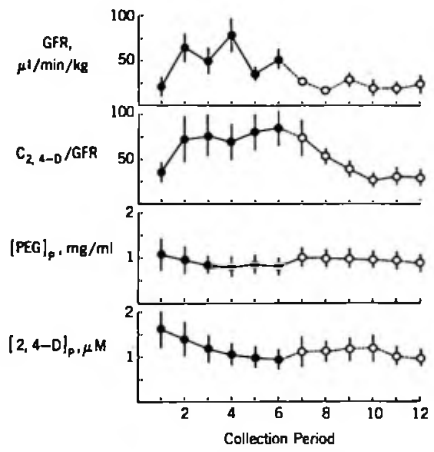


Figure 2. Summary of 2,4-D clearance experiments in the spiny dogfish. Animals were presented with 2.5 µmol/kg ^{14}C-2,4-D and 200 mg/kg 3H-PEG. Bromocresol green (BCG) was presented after collection of 6 control periods. Control measurements were made in 6 animals (O). BCG was given to 3 of these animals (O). Values reported are means ± SE (bars).

The second aspect of the study was a comparison of the renal handling of 2,4-D in the dogfish, where it is extensively metabolized but is excreted more slowly than by the winter flounder. As shown in Figure 2, the clearance of 2,4-D by dogfish kidney averaged about 75 times the GFR after an i.m. dose of 2.5 µmol/kg. The animals did not tolerate a dose of probenecid well, so bromocresol green (BCG), 25 µmol/kg, was used as inhibitor of organic anion transport. After BCG, the GFR fell somewhat in these animals, but the clearance ratio fell even more, reaching stable levels at 30 times the GFR (60% inhibition). Phenol red was much less effective than BCG. Parallel studies in the winter flounder

showed that BCG and phenol red were both effective inhibitors of 2,4-D secretion *in vivo*. However, as shown previously the clearance of 2,4-D by the flounder was high (i.e., 225-450 times the GFR), 3-6 times its clearance in the dogfish. Despite the slower clearance in the dogfish, plasma 2,4-D levels were equal to those in the flounder, suggesting either that absorption from the i.m. site was slower or that a substantial portion of the dose had been sequestered in a depot(s). Since muscle and liver account for 20-40% of the dose during the first 24 hr after 2,4-D injection (Guarino et al., Xenobiotica 7:623-631, 1977), the latter possibility seems likely. Therefore, pending additional studies using 2,4-D taurine, it would appear that the differences between the excretion rate for 2,4-D in the flounder and dogfish may be explained by a combination of lower renal transport capacity in the dogfish and greater availability in the flounder.

Overall, it is clear that the rate of renal tubular transport plays a major role in determining the excretion rate of these foreign organic anions. Secondly, the inverse correlation between extent of taurine conjugation and the rate of excretion does not appear to reflect any causal relationship. The differences between species and between chemicals appear to reflect pharmacokinetic factors, such as storage, and differences in transport of the parent molecules rather than differences between the parent and its conjugate.

HETEROGENEITY OF HEPATIC BENZO(a)PYRENE HYDROXYLASE (ARYL HYDROCARBON HYDROXYLASE) AND 7-ETHOXYRESORUFIN DEETHYLASE ACTIVITIES IN INDIVIDUAL WINTER FLOUNDER, *Pseudopleuronectes americanus*, FROM COASTAL MAINE

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Hepatic microsomal benzo(a)pyrene hydroxylase (AHH) activity is induced in fish that are pretreated with various polycyclic aromatic hydrocarbons, dioxins, polychlorinated biphenyls (PCBs), and polybrominated biphenyls (PBBs). Since all of these compounds have demonstrated or suspected toxicity to a variety of species, including man, the induction of AHH activity in fish has been suggested as a biochemical monitor for pollution of the aquatic environment by selected, toxic chemicals.

Recently, we found that a large percentage of winter flounder (about 85% of the 13 fish studied) had partially induced hepatic microsomal mixed-function oxidase systems and that this induction was identical, or at least very similar, to that caused by the administration of polycyclic hydrocarbons. Our purpose this summer was to perform a similar study on a larger fish population to test for both sex and time-of-year (June to August) effects on the apparent induction phenomenon.

Three enzymatic parameters were measured in the liver of each fish: AHH activity, AHH activity in the presence of 7,8-benzoflavone (BF, 10^{-4} and 5×10^{-4} M) and 7-ethoxyresorufin (7-ERF) deethylase activity. In mammals, and in the little skate, high hepatic AHH activities, the inhibition of these AHH activities by *in vitro* BF, and high 7-ERF activities are associated with the formation of cytochrome P-448 and pretreatment with polycyclic hydrocarbon-like inducing agents.

Winter flounder were collected by drag net and kept in flowing seawater (12-15°). Flounder caught and maintained in this manner appeared healthy. They were held in the tank for at least 2 days and no longer than two weeks before sacrifice. Livers, minus the gallbladders, were removed immediately and placed on ice. They were homogenized in 0.15 M KCl-0.001 M HEPES buffer, pH 7.5, to prepare a 33% w/v homogenate. Hepatic microsomes were obtained, the protein content of whole homogenate and microsomal preparations determined, and AHH activities with whole homogenate (100 µl) or microsomes quantitated as described previously (Pohl, Bend, Guarino and Fouts, Drug Metab. Dispos. 2:545, 1974). Additional AHH incubation mixtures which contained BF (10^{-4} and 5×10^{-4} M) were set up *in vitro*.