SW (Figure 2a; b). In C. arenarius, subsequent net volume loss (6 - 18 h) in control, ablated and sham-operated animals involves extracellular Na but is primarily accompanied by reduction in intracellular Cl and unmeasured

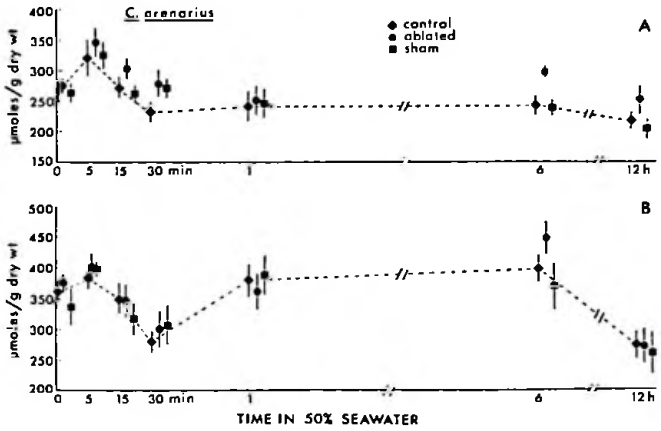

Figure 2.--Ninhydrin positive substances (µmoles/g dry wt) in control, ablated and sham-operated C. arenarius during exposure to 50% seawater. A) amino acids (alanine equivalents); B) imino (proline equivalents).

solute content (Ferraris and Schmidt-Nielsen, 1982). Corresponding with net intracellular volume loss, C. arenarius decreased significantly in imino acid content (Figure 2b) between 6 and 12 h. Decrease in amino acid content was seen simultaneously but was not statistically significant (Figure 2a). As in P. spiralis, there was no consistent, significant effect of decerebration of C. arenarius on amino or imino acid content during exposure to 50% SW (Figure 2a,b). The present study demonstrates that in intertidal Nemertina and Oligochaeta 1) intracellular regulatory volume decrease is accompanied by loss of NPS (amino and imino acids) and 2) neurosecretory and neuroglandular mechanisms have no apparent effect on intracellular RVD vis reduction in NPS. Study supported by NIH awards GM 07047 to Joan D. Ferraris and AM 15972 and AM 15973 to Bodil Schmidt-Nielsen.

THE HEPATIC CYTOSOLIC GLUTATHIONE TRANSFERASES OF MALE LITTLE SKATE: ENZYMES EXHIBITING HIGH REACTIVITY AND STEREOSELECTIVITY WITH POLYCYCLIC ARENE OXIDES

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The involvement of stereoisomerism in xenobiotic metabolism and toxicity has been known for some time. An area in which this is important is carcinogenesis where activation of a chemical species may be dependent upon steric factors, for example with polycyclic aromatic hydrocarbons (PAH). Here there is sequential metabolism of parent PAH to a PAH-oxide to the PAH-diol and finally to the ultimate carcinogenic species, a diol epoxide (Jerina, D.M. et al, in In Vitro Metabolic Activation in Mutagenesis Testing, F.J. deSerres, J.R. Fouts, J.R. Bend, and R.M. Philpot, eds., pp. 159-177, Elsevier, Holland, 1976). It has been shown with the PAH, benzo[α]pyrene (BP), that stereochemical factors play an important role in at least 2 points in this sequence; namely, in the formation of the PAH-oxide (Levin, W. et al, J. Biol. Chem. 255,9067-9074, 1980) and in the enzymic hydrolysis of the PAH-oxide (Armstrong, R.N. et al, J. Biol. Chem. 256, 4726-4733, 1981).

This report is concerned with a family of glutathione transferases (GS-T) isolated from little skate (Raja erinacea) liver which has high catalytic activity with PAH-oxides. Since the reaction of arene oxides with the tripeptide glutathione normally results in detoxication, these enzymes are of considerable interest in biochemical toxicology. Consequently, the major skate enzyme (E-4) was tested for its ability to catalyze the reaction between GSH and several PAH-oxides, including racemic benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide. As detoxication re-

actions often proceed with stereoselectivity, the stereopreference of the various skate glutathione transferases was determined with benzo(a)pyrene 4,5-oxide as substrate.

All chemicals and reagents used were of the highest purity commercially available. All [^{3}H]-PAH-oxides were supplied by Midwest Research Laboratories (Kansas City, Mo.). The source of the enzymes was male little skate hepatic cytosolic fractions. Enzymes E-1, E-2, E-3, E-4 and E-5 were isolated and purified as reported elsewhere (Foureman, G.L and Bend, J.R., MDIBL Bull. 19, 114-118, 1979) as are the specific procedures for the enzyme assays. HPLC conditions and buffers were those reported by Hernandez et al (Hernandez, O.H., et al, BBRC 96, 1494-1502, 1980).

Several polycyclic arene oxides were tested as substrates for the major glutathione transferase isolated from little skate liver, E-4, at identical substrate concentrations (0.01 mM). The data are presented in Table 1. The

TABLE 1

ACTIVITY OF MALE LITTLE SKATE HEPATIC GLUTATHIONE TRANSFERASE E-4 WITH SEVERAL POLYCYCLIC ARENE OXIDES

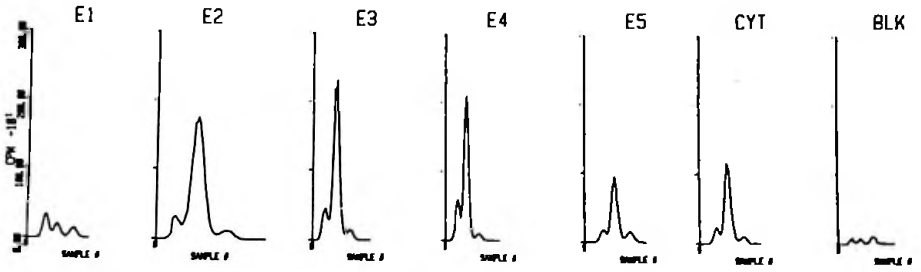
Name	Specific Activity[a]
benzo(a)anthracene 5,6-oxide	46.8 ± 2.1 μmol/min/mg E-4
benzo(a)pyrene 4,5-oxide	14.4 ± 1.9 μmol/min/mg E-4
pyrene 4,5-oxide	13.2 ± 0.4 μmol/min/mg E-4
phenanthrene 9,10-oxide	2.05 ± 0.17 μmol/min/mg E-4
(±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene	0.19 ± 0.03 μmol/min/mg E-4

[a]The incubation conditions were 5 mM GSH, 0.1 M HEPES, pH. 7.6, at 37° and 0.01 mM for the arene oxides. Incubates were extracted with ethyl acetate (3X) and the radioactivity in the aqueous residue quantitated.

highest rate of reactivity observed was with benz[a]anthracene 5,6-oxide (46.8 μmol/min/mg E-4), three times the activity for benzo(a)pyrene 4,5-oxide (BPO). The reactivity of E-4 with the structurally related BPO and pyrene 4,5-oxide was quite similar. However, the specific activity with phenanthrene 9,10-oxide was only one-eighth that for BPO. The lowest rate of enzymatic conjugation observed was with (±)-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene.

Figure 1 shows the HPLC profile of the glutathione adducts of (±)-benzo(a)pyrene 4,5-oxide formed by the little skate hepatic glutathione transferases and cytosol. Each profile consists of 3 peaks; the first 2 peaks are, respectively, the 5-thioether conjugate derived from (+)-4S,5R-BPO and the 4-thioether conjugate from (-)-(4R,5S)-BPO (Hernandez, O.H, et al, BBRC 96, 1494-1502, 1980; Armstrong, R.N. and Jerina, D.M., BBRC 106, 1077-1084, 1981). The third peak is a mixture of the other 2 BPO-GSH conjugate diastereomers. Nonenzymatically, these four diastereomeric conjugates are formed in equal ratios as shown in the profile on the far right (BLK) in Figure 1. Conjugate profiles obtained under identical conditions (i.e., short incubation periods to maximize stereoselectivity) for transferases E-2 through E-5 showed marked prominence of the second peak indicating preferential metabolism at the 4 position of (-)-BPO. E-1 was dissimilar in this respect; it preferred (+)-BPO.

After correction for the non-enzymatic formation of GSH conjugates of BPO in the incubation mixtures, there was no enzymatic formation of the diastereomers in the third HPLC peak with any of the enzymes or in cytosol. That is



to say that GSH conjugates were formed from both (+)- and (-)-enantiomers of BPO but only at epoxide carbon atoms having the R configuration, i.e., C-5 in the case of (+)-4S,5R-BPO (peak 1) and C-4 in the case of (-)-4R,5S-BPO (peak 2). Thus, these enzymes each show enantioselectivity with (+)-BPO and stereospecificity with respect to the R carbon atom of the oxirane ring.

An interesting characteristic of the active site of E-4 is its high reactivity with the K-region polycyclic arena oxides listed in Table 1. The activity with BPO is more than 15-fold that reported for the human transferase μ (Warholm, M et al., BBRC 98, 512-159, 1981) and nearly 150-fold that reported for rat transferase C, the most reactive hepatic transferase with this substrate (Nemoto, N. et al, Nature 255, 512, 1975). Such high enzymatic activities indicate productive transition-state binding of these K-region oxides with E-4.

The stereospecificity demonstrated by E-4 (Figure 1) for an R carbon in the oxirane ring of BPO indicates the active site of this enzyme to be highly ordered, imposing restrictions on this xenobiotic substrate as stringent as those imposed upon endogenous substrates by physiologic enzymes.

That E-4 exhibits reactivity with the diol epoxide substrate is of special significance as this compound is considered to be an ultimate carcinogenic form of benzo(a)pyrene (BP) and has been recently shown to alter DNA structure (Hogan, M., et al, J. Biol. Chem. 256, 4504-4513, 1981). As other studies have demonstrated the formation of appreciable amounts of the precursor, BP-7,8-dihydrodiol, during the metabolism of BP by skate liver microsomes, (Bend et al, in Pesticides and Xenobiotic Metabolism in Aquatic Animals, M.A.Q. Khan, J.J. Lech and J.J. Menn, eds., pp. 297-318, Elsevier, Holland, 1979) and as diol epoxides are apparently poor substrates for epoxide hydrolase (Wood, A. W. et al, J. Biol. Chem. 251, 4882-4890, 1976) the glutathione transferases in this species may be important in the detoxication of this ultimate chemical carcinogen.

BENZO(A)PYRENE METABOLISM IN RECONSTITUTED MONOOXYGENASE SYSTEMS CONTAINING CYTOCHROME P-450 FROM LOBSTER (HOMARUS AMERICANUS) HEPATOPANCREAS FRACTIONS AND NADPH-CYTOCHROME P-450 REDUCTASE FROM PIG LIVER

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A previous study in this laboratory showed that hepatopancreas (HP) of the lobster, Homarus americanus, was a major site of uptake and storage of radioactivity after injection of 1 mg/kg of ^{14}C-benzo(a)pyrene (BaP) into the pericardial sinus (Foureman et al, MDIBL Bull. 18:93, 1978). The BaP-derived radioactivity found in the HP of lobsters sacrificed 3 weeks or 6.5 weeks after BaP administration was primarily (> 98%) hexane-extractable. Analysis of the hexane extracts showed that 97% (3 wk) and 83% (6.5 wk) was unchanged BaP, and that the metabolites present in the extracts were largely phenolic. The non-extractable metabolites (0.5-1.5% of the ^{14}C in HP) were not identified.