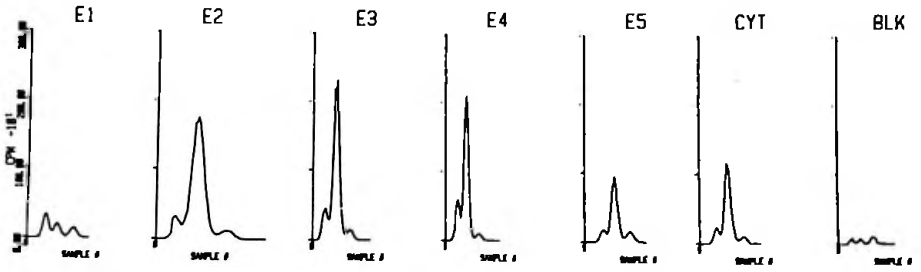

to say that GSH conjugates were formed from both (+)- and (-)-enantiomers of BPO but only at epoxide carbon atoms having the R configuration, i.e., C-5 in the case of (+)-4S,5R-BPO (peak 1) and C-4 in the case of (-)-4R,5S-BPO (peak 2). Thus, these enzymes each show enantioselectivity with (+)-BPO and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

Margaret O. James, Bruce Sherman, Steven A. Fisher and John R. Bend, Department of Medicinal Chemistry, University of Florida, Gainesville, and Laboratory of Pharmacology, NIEHS, Research Triangle Park, N.C.

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.

Because several of the metabolites of BaP are potential carcinogens, we wished to obtain more detailed information of BaP metabolism in lobster HP. Earlier studies in this laboratory and elsewhere had shown that washed microsomes prepared from lobster HP in the usual manner did contain cytochrome P-450, but were very inefficient at metabolizing xenobiotics (Pohl et al, Drug Metab. Disp. 2:545, 1974; Elmamlouk and Gessner, Comp. Biochem. Physiol. 53C:19, 1976). Later studies suggested this might be because (1) NADPH-cytochrome P-450 reductase is solubilized from the endoplasmic reticulum and inactivated during the preparation of microsomes and (2) HP microsomes contain inhibitors of monooxygenation (discussed in Bend et al, Phyletic Approaches to Cancer, C. J. Dawe et al, eds., Japan Sci. Soc. Press Tokyo, 179, 1981). In studies with the spiny lobster, Panulirus argus, we developed a method for preparing a fraction from HP microsomes which contained 2-3 times as much cytochrome P-450 (nmole/mg protein) as microsomes but no inhibitors of monooxygenation (James and Little, Developments in Biochemistry, Vol. 13, 113, Elsevier, NY, 1980). This preparation could be reconstituted with mammalian cytochrome P-450 reductase and was then active in xenobiotic metabolism. In the present study we demonstrate that this method can also be used with HP microsomes from the lobster (Homarus americanus). The HP preparation was used to study the position selective metabolism of BaP and N-demethylation of d-benzphetamine, cytochrome P-450-dependent reactions.

TABLE 1. Properties of the M1 fraction prepared from lobster hepatopancreas microsomes

	Male	Female
Pooled HP wt (g)	122.9	98.1
HP Fraction M1:		
Volume (ml)	10.7	8.7
Protein yield (mg/g HP)	2.6	2.8
Cytochrome P-450 content (nmole/mg protein)	0.55	0.46
NADPH-cytochrome c reductase activity[1] (nmole/min/mg protein)	10.3	5.0
BaP hydroxylase activity[2] (nmole/min/mg protein)	0.010	0.003
Fraction M1 + reductase[3]		
BaP hydroxylase activity[4] (nmole/min/mg protein)	0.168	0.132
Benzphetamine N-demethylase activity (nmole/min/mg protein)	6.1	7.1

[1] Measured at 25°C.

[2] Fluorescence assay, measuring phenolic BaP metabolites, primarily 3-hydroxy BaP. The results shown are from assay at 30°C.

[3] Fraction M1 and cytochrome P-450 reductase from pig liver were mixed in the ratio of 0.1 nmol cytochrome P-450: 500 units of reductase. One unit of reductase catalyzes reduction of 1 nmole cytochrome c per min at 25°C.

[4] Radiochemical assay, measuring all metabolites of BaP.

Separate pools of washed HP microsomes were prepared as described previously (Pohl et al, Drug. Metab. Disp. 2:545, 1974) from 4 male and 4 female lobsters. The lobsters were all in intermoult stage, and weighed about 500 g. Each pool of washed microsomes was resuspended in a buffer containing 0.5% (w/v) sodium cholate, 0.01 M potassium phosphate pH 7.4, 20% glycerol, 0.1 mM EDTA, 0.1 mM dithiothreitol and 0.2 mM phenylmethylsulfonyl fluoride (PMSF). The volume used was equal to the total original weight of HP. The mixture was stirred at 4°C for 2 hr and then recentrifuged at 176,000 g for 60 min. The pale yellow supernatant fraction was discarded and the reddish-brown band

at the bottom of the tube (HP fraction M1) was saved. The protein content, cytochrome P-450 content, BaP hydroxylase activity and NADPH-cytochrome c reductase activity of the M1 fractions are given in Table 1. BaP hydroxylase activities were measured at 20°C and 30°C and were found to be higher at 30°C. Portions of the M1 fractions were mixed as stated in Table 1 with purified cytochrome P-450 reductase which was prepared from pig liver microsomes by the method of Yasukochi and Masters, J. Biol. Chem. 251:5337, 1976. The mixture was assayed for monooxygenase activity with BaP and benzphetamine, and the results are also shown in Table 1.

The individual metabolites of BaP were separated by HPLC and quantitated by scintillation counting of the various fractions. The rates of formation of each metabolite are presented in Table 2. Addition of 7,8-benzoflavone

TABLE 2. Individual metabolites of BaP formed by lobster hepatopancreas M1 fractions mixed with NADPH-cytochrome P-450 reductase from pig liver

	BaP Metabolite Formation[a] (pmole/min/nmole P-450)					% Identified Metabolites	Total Metabolism (nmole/min/nmole P-450)
	DIOLS			QUINONES	PHENOLS		
	9,10-	4,5-	7,8-				
Male, 30°C[b]	7	26	20	86	97	77	0.306
Male, 22°C	5	5	3	40	95	80	0.183
Female, 30°C	6	17	12	69	140	85	0.288

[a] Individual metabolites were separated by the method of Holder et al., Proc. Natl. Acad. Sci. 71:680, 1974, and quantitated as described in James and Little, Chem.-Biol. Interact. 36:229, 1981.

[b] Assay temperature

(ANF) to the incubation tubes caused an increase in the overall rate of metabolism but had little effect on the proportions of each metabolite formed. The enhancement by ANF and the pattern of BaP metabolites found (phenols were the major metabolites) suggest that the lobster cytochrome P-450 is functionally similar to the forms of cytochrome P-450 present in untreated or phenobarbital-induced vertebrates. Addition of TCPO, an epoxide hydrolase inhibitor, to incubation vials abolished formation of BaP-9,10- and 4,5-dihydrodiols and reduced the formation of BaP-7,8-dihydrodiol (data not shown).

This study provides additional evidence that a major reason for the low monooxygenase activities found in vitro in lobster hepatopancreas microsomes is that these microsomes are relatively deficient in NADPH-cytochrome P-450 reductase activity. When mixed with active reductase from vertebrate liver in the absence of HP monooxygenase inhibitors, the lobster cytochrome(s) P-450 was efficient at demethylating benzphetamine, and was also able to metabolize BaP to several metabolites, with phenolic metabolites predominating. However, the overall rate of metabolism of BaP by the reductase-fortified lobster fractions (nmole/min/nmole P-450) was lower than that found with hepatic microsomes from untreated fish or mammals, and substantially lower than rates measured in hepatic microsomes from polycyclic aromatic chemical-induced fish or rats.

The results of this study, together with our earlier studies, suggest that the slow metabolism of BaP observed in vivo is partly due to the relative inefficiency with which the cytochrome(s) P-450 present in lobster HP metabolizes BaP, even under optimal conditions, and may also be due to the low intrinsic cytochrome P-450 reductase: cytochrome P-450 ratio in the lobster hepatopancreas.

BENZO(A)PYRENE HYDROXYLASE AND 7-ETHOXYRESORUFIN O-DEETHYLASE ACTIVITIES IN SEVERAL FISH FROM MAINE EVIDENCE THAT THE HEPATIC MONOOXYGENASE SYSTEM OF ONLY A FEW SPECIES IS INDUCED BY EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBON-TYPE COMPOUNDS IN THE ENVIRONMENT

Steven A. Fisher, Bruce Sherman, Gary L. Foureman and John R. Bend, Laboratory of Pharmacology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina