Dietary fat inhibits the intestinal metabolism of the carcinogen benzo[a]pyrene in fish

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Abstract Following the intestinal absorption of dietary benzo[a]pyrene (BP) by the killifish, this compound becomes incorporated along with dietary triglycerides into membrane-bound fat vacuoles within the intestinal epithelial cell (1985. *J. Lipid Res.* **26:** 428–434). These vacuoles, arising from the smooth endoplasmic reticulum, are important transient structures involved in both the uptake and metabolism of dietary BP and, presumably, other lipophilic toxicants as well. In the present study we used subcellular fractions isolated from the intestines of spot *(Leiostomus xanthurus),* a teleost fish, to study factors that influence the metabolism of BP in a fat vacuole/microsomal system. Triglyceride-solubilized BP is capable of diffusion from fat vacuoles to microsomal enzymes. Increases in the concentration of fat vacuoles decrease the availability of BP to microsomal BP hydroxylase. The effect of fat vacuoles on the activity of BP hydroxylase becomes more pronounced as the concentration of BP in our test system decreases. Addition of cytosolic glutathione transferases to the fat vacuole/microsomal system enhances the activity of BP hydroxylase. Examination of binding of ^{3}H-labeled BP to killifish *(Fundulus heteroclitus)* intestinal cytosolic proteins in vivo indicated that a large fraction of the radioactivity was associated within glutathione transferase. These results suggest that dietary fat inhibits metabolism of low levels of BP in the intestine. A consequence of this would be greater exposure of peripheral tissues to dietary carcinogens.—**Van Veld, P. A., R. D. Vetter, R. F. Lee, and J. S. Patton.** Dietary fat inhibits the intestinal metabolism of the carcinogen benzo[a]pyrene in fish. *J. Lipid Res.* 1987. **28:** 810–817.

Supplementary key words intestine • intracellular fat vacuoles • cytochromes P-450 • glutathione transferases

Polycyclic aromatic hydrocarbons(s) (PAH) are common dietary contaminants of both terrestrial and aquatic ecosystems (1, 2). A potential mode of exposure of PAH and other hydrophobic toxicants is uptake across the gastrointestinal tract. Once absorbed, PAH are subject to a variety of fates including oxidative metabolism by cytochrome P-450-mediated mixed function oxygenase (MFO) systems. In vertebrates, the liver is generally believed to be the major organ involved in MFO activity. However, substantial evidence indicates that the intestine may be an important site of dietary PAH metabolism (3) particularly when the intestinal MFO system has been induced as a result of pre-exposure to these compounds.

During the uptake of dietary benzo[a]pyrene (BP) by the killifish intestine, this PAH becomes incorporated, along with dietary triglyceride into fat vacuoles within the enterocyte (4). Formation of these vacuoles within the smooth endoplasmic reticulum (SER) has been described in mammals (5, 6) and fish (4, 7, 8). The vacuoles are transient structures involved in the processing of dietary fat which eventually leaves the intestine in the form of chylomicrons or, in the case of fish, very low density-sized lipoproteins (7).

Following the absorption of dietary BP, this compound can be extensively metabolized by the intestinal MFO system and removed from the intracellular fat vacuoles (4). However, quantitative measurements of factors affecting the intracellular metabolism of fat-solubilized BP by intestinal MFO enzymes have not previously been reported. This type of information is important because factors inhibiting intestinal MFO activity may result in increased loads of toxicants leaving the intestine en route to other tissues. Therefore, in the present study, we used subcellular fractions from the intestines of fish to measure the influence of fat vacuole concentration, MFO enzyme induction, and cytosolic glutathione transferases on the metabolism of fat-solubilized BP by intestinal microsomal enzymes in vitro. In addition, in vivo studies were conducted to determine whether the disposition of BP may be affected by binding to intestinal cytosolic proteins. We

Abbreviations: BP, benzo[a]pyrene; MFO, mixed function oxygenase; PAH, polycyclic aromatic hydrocarbons; CDNB, 1-chloro-2,4-dinitrobenzene; GSH, reduced glutathione; PC, phosphatidylcholine.

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chose fish as experimental animals because of their availability and ease of handling. Also, fish and mammals share common mechanisms for the intracellular processing of dietary lipids (4, 7–9) and xenobiotics (10–12). Thus, fish are good model organisms for studies of the metabolism of lipophilic toxicants in general.

MATERIALS AND METHODS

Materials

Triolein, Tris-HCl, NADPH, 1-chloro-2,4-dinitrobenzene (CDNB), reduced glutathione (GSH), GSH-agarose, phenylmethyl sulfonyl fluoride (PMSF), phosphatidylcholine (PC), swine skin gelatin, and dithiotreitol (DTT) were purchased from Sigma Chemical Company (St. Louis, MO). Ethylene glycol monomethyl ether (EGME) was purchased from Fisher Scientific Company (Atlanta, GA). Benzo[a]pyrene (BP) was obtained from Aldrich Chemical Company (Milwaukee, WI). [G^{3}H]-benzo[a]pyrene ([^{3}H]BP) (60 Ci/mmol) was purchased from Amersham Corporation (Arlington Heights, IL) and purified by thin-layer chromatography before use. Casamino acids were from Difco Labs (Detroit, MI), bovine serum albumin (BSA) was from United States Biochemical Corporation (Cleveland, OH), tricaine methane sulfonate (TMS) was from Crescent Research Chemicals (Paradise Valley, AZ), and Sephadex G-100 was from Pharmacia Fine Chemicals (Piscataway, NJ). All other reagents and solvents were of analytical grade and purchased from commercial sources.

Fish collection, maintenance, and diet

Spot *(Leiostomus xanthurus)* and salt marsh killifish *(Fundulus heteroclitus)* were collected from the coastal waters of Georgia. Fish were kept in flow-through sea water systems at ambient temperature (20 to 27°C) and fed a laboratory control diet at a rate of approximately 2% body weight per day. The control diet consisted of 400 g of fish meal, 400 g of baby cereal, 100 g of spinach, two chicken eggs, 25 ml of cod liver oil, 4 ml of Polyvisol vitamin drops (Mead Johnson and Co., Evansville, IN), 36 g of gelatin, 700 ml of water, and 300 ml of clam juice. To induce microsomal enzymes, groups of fish were fed the control diet supplemented with BP (16 mg/kg food) dissolved in the cod liver oil. For some experiments, fish were fed [^{3}H]BP in an artificial diet consisting of triolein (9%), phosphatidylcholine (PC) (1%), casamino acids (10%), gelatin (10%), water (70%), and [^{3}H]BP (25 ng/g food) (4).

Enzyme assays

Cytochrome P-450 was assayed by difference spectra (13). BP hydroxylase activity was assayed radiometrically (14). BP hydroxylase reaction mixtures consisted of 50 mM Tris-CHl, pH 7.4, 3.0 mM $MgCl_2$ (buffer A), 0.40 mM NADPH, [^{3}H]BP (various concentrations dissolved in DMSO or fat vacuole carrier), and microsomes (0.5 mg of protein/ml) in a total volume of 1.0 ml. Reactions were initiated by adding NADPH. Blanks consisted of complete reaction mixtures minus NADPH. Incubations, carried out for 10 min at 28°C in a shaking water bath, were terminated by adding 1.0 ml of 0.15 M KOH in 85% DMSO. Samples were extracted four times with 2 ml of hexane, and polar metabolites were quantified by counting the aqueous phase in a liquid scintillation counter. BP hydroxylase reactions were linear over the concentration range of BP and fat vacuoles tested. Glutathione transferase activity was assayed using 1 mM concentrations of CDNB and GSH (15). Blanks consisted of reaction mixtures minus cytosolic protein. One unit of activity is defined as the amount of enzyme that catalyzes the formation of 1 μmol of S-2, 4-dinitrophenyl per min per ml. Protein determinations were performed using BSA as a standard (16).

Lipid determination

Lipids were extracted from intracellular fat vacuoles (17), separated on Chromarods using a solvent system of hexane–ethyl ether–acetic acid 95:5:0.05 and quantified by flame ionization detection with an Iatroscan TH-10 analyzer (18).

Preparation of subcellular fractions

All buffers used in the preparation of subcellular fractions of intestinal cells were kept ice-cold. Fish were anesthetized with tricaine methanesulfonate. Body cavities were opened and the fish were immediately submerged in 1.15% KCl. To prepare microsomes, intestines (including pyloric caecae) of spot were removed and submerged in KCl. The lumen of the intestine was flushed with KCl then flushed with potassium phosphate 100 mM, pH 7.4, containing 1 mM DTT, 1 mM EDTA, 0.1 mM PMSF (in an ethylene glycol monomethyl ether carrier) 20% glycerol, and 1% BSA (buffer B). Intestines were homogenized in four volumes of buffer B using several low speed bursts with a Polytron (Brinkman Instrument), with efforts made to minimize incorporation of air into homogenates. Homogenates were centrifuged twice at 12,000 *g* for 10 min and the pellet and fat pad were discarded after each centrifugation. The 12,000 *g* supernatant was centrifuged at 105,000 *g* for 1 hr, the microsomal pellet was resuspended in potassium phosphate 100 mM, pH 7.4, containing 1 mM DTT, 1 mM EDTA, 0.1 mM PMSF, and 20% glycerol (buffer C), and centrifuged at 105,000 *g* and stored in liquid nitrogen until ready for use. The same general procedure was used to prepare liver microsomes except that a Potter-Elvehjem homogenizer was used.

Cytosols were obtained as the 105,000 *g* supernatant. The same general procedure used to prepare microsomes was followed except buffer C was used for all steps. Cytosolic proteins were fractionated by gel filtration (see below).

Intracellular fat vacuoles were isolated from the intestines of spot 4 hr after they were fed the 9% triolein artificial diet. Initial steps for isolation of fat vacuoles were identical to those described for microsome preparation. The fat layer removed from the tissue homogenate following the initial 12,000 *g* centrifugation provided a crude preparation of intracellular fat vacuoles. This lipid layer was washed with buffer C in a glass tissue homogenizer and then centrifuged (105,000 *g* for 30 min.). After several wash/centrifugation steps, a cream-like preparation was obtained with a relatively constant triglyceride/phospholipid ratio (60:1). Examination of the preparation by light microscopy revealed vacuoles in a size range of 10–100 μm. The vacuoles remained discrete with no visible coalescence.

Artificial fat vacuoles

Artificial fat vacuoles were used to study the effect of fat concentration on the K_m of BP hydroxylase. These vacuoles were prepared with triolein and PC at a mass ratio equal to that of endogenous vacuoles. PC (16.7 mg) dissolved in chloroform–methanol 1:1 was added to a glass test tube and the solvent was evaporated by heating (70°C) under a stream of nitrogen. Triolein (1000 mg) and buffer A (9000 mg) were added to the tube and the mixture was emulsified with a sonic dismembrator (Fisher Scientific) until the size of the artificial vacuoles approximated the size of endogenous fat vacuoles as determined by light microscopy. As with endogenous fat vacuoles, no coalescence of artificial fat vacuoles was observed. Serial dilutions of the emulsion yielded the desired fat vacuole concentrations (1 to 100 mg/ml). [^{3}H]BP in a DMSO carrier was added at concentrations ranging from 14 μM to 56 μM. After 1 hr of equilibration while shaking, fluorescence microscopy indicated that BP was uniformly distributed among the vacuoles.

In vivo binding of BP to cytosolic proteins

The 105,000 *g* supernatant was obtained from killifish intestines at various times after feeding fish a [^{3}H]BP-treated artificial diet (0.5 ng of BP/fish). The supernatants were passed through a Sephadex G-100 column (80 cm × 2.5 cm) that had been equilibrated with buffer C and calibrated with protein standards of known molecular weight. All steps were carried out at 4°C. Fractions were collected and analyzed for radioactivity, glutathione transferase activity, and absorbance at 280 nm (as an indicator of protein).

Isolation of glutathione transferases from fish intestines

The intestines of 20 spot were flushed and homogenized in 50 mM potassium phosphate, pH 7.4, containing 0.1 mM PMSF (buffer D). The 105,000 *g* supernatant obtained from this preparation was passed through a Sephadex G-100 column (80 cm × 2.5 cm) equilibrated with buffer D. Fractions containing glutathione transferase activity were combined and placed onto a column of GSH-Agarose (1 × 5 cm) (19) equilibrated with buffer D. The column was washed with buffer D until protein in the effluent was undetectable. The column was then developed with 50 mM Tris buffer, pH 9.6, containing 5 mM GSH. Fractions containing glutathione transferase activity were combined, dialyzed against buffer A, and concentrated using centrifugal microconcentrators (Amicon Corp.).

RESULTS

Induction of hepatic and intestinal microsomal enzymes

Groups of spot were fed either the laboratory control diet or an identical diet supplemented with BP (16 mg/kg food) for 4 days. The specific content of intestinal cytochrome P-450 enzymes of BP-exposed fish exceeded that of control fish by twofold while the specific content of hepatic cytochromes P-450 appeared to be unaffected by dietary BP treatment **(Table 1).** BP hydroxylase activity associated with the intestinal microsomes of BP-exposed fish exceeded that associated with the intestinal microsomes of control fish by nearly tenfold and approached the level of activity associated with hepatic microsomes (Table 1). Neither cytochrome P-450 nor BP hydroxylase was detected in preparations of fat vacuoles from control and BP-exposed fish.

Availability of fat vacuole-associated BP to BP hydroxylase

Fat vacuoles were isolated from spot intestines 4 hr after they were fed a [^{3}H]BP (25 ng/g) artificial diet (9% triolein). The fat vacuoles obtained were suspended in buffer A (10 mg of triglyceride/ml). Based on the radioactivity present in this preparation, the concentration of BP was estimated to be 192 pM. Following the addition of NADPH, formation of BP metabolites from [^{3}H]BP associated with the fat vacuoles was not detectable over background levels during the 30-min incubation period **(Fig. 1)** indicating negligible BP hydroxylase activity in native fat vacuoles. Addition of intestinal microsomes (0.5 mg of microsomal protein/ml) from BP-induced fish followed by

TABLE 1. Effect of dietary BP on microsomal cytochrome P-450 and BP hydroxylase in intestine and liver of spot

Sample	Treatment[a]	Cytochrome P-450[b] Specific Content	BP Hydroxylase[c]
Intestinal microsomes	Control	0.24 (±0.04)	0.04 (±0.02)
Intestinal microsomes	BP	0.47 (±0.11)[d]	0.34 (±0.10)[d]
Liver microsomes	Control	1.12 (±0.18)	0.31 (±0.24)
Liver microsomes	BP	1.00 (±0.23)	0.49 (±0.19)
Intestinal fat vacuoles	Control	N.D.	N.D.
Intestinal fat vacuoles	BP	N.D.	N.D.

[a]Fish were fed either the laboratory control diet or an identical diet supplemented with BP (16 mg/kg food) at a ratio of 2% body weight per day for 4 days prior to killing.
[b]Nanomoles of P-450/mg microsomal protein; values represent mean ± SE of three groups of five fish.
[c]Nanomoles of BP metabolites · min^{-1} · mg^{-1} microsomal protein; values represent mean ± SE of three groups of five fish.
[d]Significantly different from corresponding control value; $P < 0.01$ (Student's t-test).
[e]N.D., not detected.

NADPH addition to preparations of [^{3}H]BPcontaminated fat vacuoles resulted in conversion of fat vacuole-associated [^{3}H]BP to polar metabolites (Fig. 1) indicating that the [^{3}H]BP associated with fat vacuoles becomes available to microsomal enzymes.

Effect of triglyceride concentration on the metabolism of BP in a fat vacuole/microsomal system

The effect of triglyceride concentration on BP hydroxylase activity in fat vacuole/microsomal systems was determined in dose-response studies in which BP (14 μM to 56 μM) was dissolved in different concentrations of aqueous dispersed artificial fat vacuoles (0 to 100 mg/ml). Michaelis-Menton plots revealed increases in the apparent K_m of BP hydroxylase as fat vacuole concentration increases **(Fig. 2)**. The plots indicate that the magnitude of the effect of fat vacuole additions on BP hydroxylase activity is dependent upon the concentration of BP in the test systems. For example, from the data in the plots we can calculate that in those systems containing fat vacuoles (100 mg/ml) and BP (14 μM) the activity was reduced to 42% of that measured in systems with identical concentrations of BP but without fat vacuoles. For comparison, in those systems containing fat vacuoles (100 mg/ml) and BP (56 μM) the activity of BP hydroxylase was 82% of that measured in systems containing identical concentrations of BP but without fat vacuoles.

Binding of BP to cytosolic proteins in vivo

To determine whether cytosolic proteins may affect the disposition of BP and/or BP metabolites in the intestine, we prepared 105,000 *g* supernatants from the intestines of 20 killifish following 2, 6, or 12 hr of exposure to a dose of [^{3}H]BP (25 ng/g) in the 9% triolein artificial diet. The supernatants were passed through a Sephadex G-100 column. A large percentage of radioactivity was recovered in the void volume of the column **(Fig. 3)** which may represent nonspecific binding to protein-lipid aggregates. Radioactivity recovered in fractions containing glutathione transferase activity increased with time. We were unable to extract the radioactive compound(s) from these fractions with ethyl acetate and we assumed that the radioactivity was associated with BP metabolites covalently bound to glutathione transferase.

Effect of glutathione transferase on BP hydroxylase activity in fat vacuole/microsomal systems

Spot intestine glutathione transferases were partially purified from cytosol by gel filtration followed by affinity chromatography on GSH-Agarose. The activity of glutathione transferases obtained from intestinal cytosol, gel filtration, and affinity chromatography was 0.29, 0.76, and 7.88 units/mg of cytosolic protein, respectively. Fol-

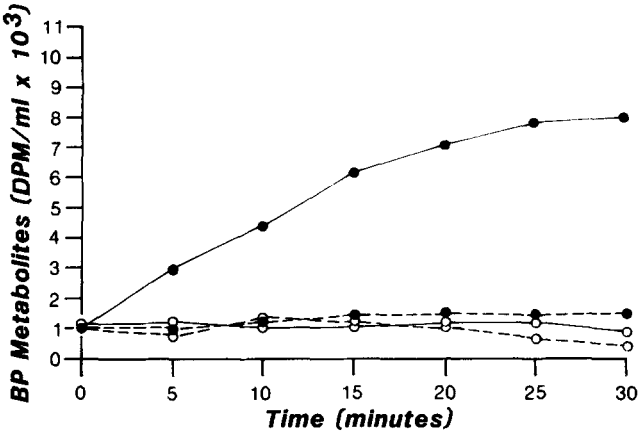

Fig. 1. Production of BP metabolites from fat vacuole-associated [^{3}H]BP by the intestinal microsomes of BP-exposed spot. Reaction mixtures contained fat vacuoles (10 mg of triglyceride/ml) (○—○); fat vacuoles plus NADPH (○--○); fat vacuoles plus microsomes (0.5 mg of protein/ml) (●--●); fat vacuoles, microsomes plus NADPH (●—●). Values represent the average determination from duplicate systems.

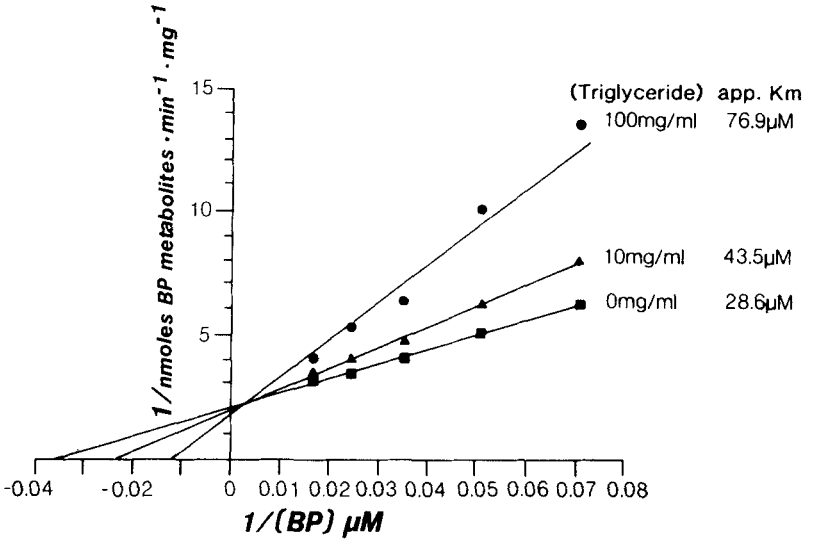

Fig. 2. Double reciprocal plot of BP concentration versus BP hydroxylase activity in artificial fat vacuole/microsomal systems. Artificial fat vacuoles containing [^{3}H]BP were prepared as described in Methods. All systems contained intestinal microsomes (0.5 mg protein/ml) prepared from BP-exposed spot as described in Methods. Values represent the average determination from duplicate systems from which blank (systems without NADPH addition) values have been subtracted.

lowing dialysis and concentration of the glutathione transferase preparation, we tested the effect of this preparation on BP hydroxylase activity in our fat vacuole/microsome system. Test systems contained fat vacuoles (50 mg/ml), microsomal protein (0.5 mg/ml), [^{3}H]BP (28 μM), NADPH (0.40 mM), and identical systems supplemented with the glutathione transferase preparation (0.1–1.0 mg/ml). Conditions of the assay were identical to those described in Methods. BP hydroxylase activity in systems supplemented with 1.0 mg/ml of the glutathione transferase preparation was significantly higher than in systems not supplemented with glutathione transferase **(Table 2)**. Addition of the glutathione transferase preparation at a concentration of 0.1 mg/ml had no significant effect on BP hydroxylase activity.

DISCUSSION

In the present study, we have demonstrated that the intracellular components of intestinal PAH metabolism can be isolated and reconstituted in vitro. The fat vacuoles used in our study represent important structures involved in the absorption and metabolism of lipophilic toxicants by the intestine. During their formation within the enterocyte, the fat vacuoles are believed to acquire a phospholipid coat from the membranes of the SER (5), a major subcellular location of cytochromes P-450. The lipid composition and noncoalescing nature of the endogenous fat vacuoles isolated in our study suggest that these vacuoles are surrounded by phospholipid. However, preparations of natural fat vacuoles contained negligible levels of both cytochromes P-450 and BP hydroxylase activity. Therefore, in our test systems enzymatic activity was entirely

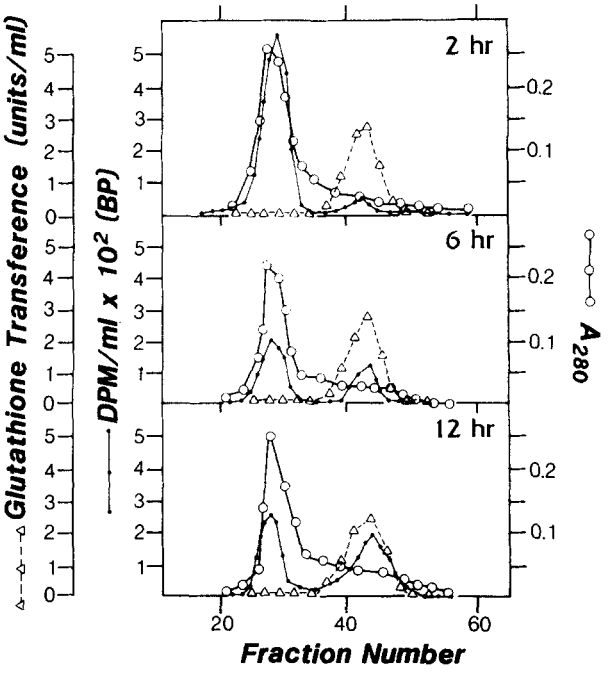

Fig. 3. Binding of BP metabolites to intestinal glutathione transferase in vivo Cytosols were prepared from intestines of 20 killifish at specified times following administration of a [^{3}H]BP diet as described in the text. Cytosols were passed through a gel filtration column as described in text. Values represent single determinations.

TABLE 2. Effect of partially purified glutathione transferase on BP hydroxylase activity in fat vacuole/microsomal systems[a]

Glutathione Transferase (mg/ml)	BP Hydroxylase[b]
0 Control	0.128 ± 0.011
0.1	0.134 ± 0.009
1.0	0.178 ± 0.16[c]

[a]Test systems contained fat vacuoles (50 mg/ml), microsomal protein (0.5 mg/ml), BP (28 μM), NADPH (0.40 mM), and partially purified fish intestinal glutathione transferase a the specified concentrations.

[b]Nanomoles of BP metabolites · min^{-1} · mg^{-1} microsomal protein. Values represent the mean ± SE for three systems from which control values (systems without NADPH addition) have been subtracted.

[c]Significantly different from control value; $P < 0.05$ (Student's t-test).

microsomal. Use of artificial fat vacuoles allows us to expose microsomal enzymes to lipophilic toxicants in physiologically relevant carriers.

In order for fat vacuole-associated BP to become available to microsomal enzymes there must be a transfer of BP from fat vacuoles to microsomes. Our results indicate that transfer of BP occurs between both natural and artificial fat vacuoles and microsomal enzymes. In the cell, transfer of BP from fat vacuoles to the endoplasmic reticulum may occur laterally in a lipid continuum or via simple diffusion of lipophile monomers through the aqueous phase (20, 21). In our study, the concentration of BP available to microsomal enzymes depended on the partitioning of BP between fat vacuoles, microsomes, cytosolic protein, and water. Due to its high lipophilicity (22) BP will reside almost exclusively in lipid structures (4, 23, 24). Increases in the concentration of fat vacuoles in our test system will reduce the amount of BP that partitions into the microsomal fraction. Thus, the increase in the apparent K_m of BP hydroxylase in the presence of fat vacuoles is not due to a decrease in the enzyme's affinity for BP but to a reduction in the amount of substrate available to BP hydroxylase. The reduced availability of substrate results in a decrease in the rate of BP metabolism.

We should point out that the K_m of an enzyme for a substrate refers to the concentration of monomeric substrate in the aqueous phase required to give one-half of the maximum reaction rate. However, values obtained for the apparent K_m of BP hydroxylase in our studies and in those of others (25, 26) far exceeds 10 mM, the reported water solubility of BP (22). Problems in the application of Michaelis-Menton kinetics to microsomal enzyme systems whose substrates are poorly soluble in water have been discussed (23, 27). According to one theory, solubilization of lipophilic hydrocarbons in microsomal membranes may be required for their further metabolism (28). Due to its high lipophilicity, BP could exist at high concentrations in the vicinity of BP hydroxylase, accounting for high K_m values relative to the water solubility of BP. The values obtained for the K_m may not be true constants in the traditional sense but are useful in studies such as ours in which factors affecting the activity of a microsomal enzyme are being studied.

Our results indicate that the influence of fat vacuoles on the activity of BP hydroxylase decreases as the concentration of BP in the test system increases. This observation may help to explain why, in a previous study, varying the concentration of dietary fat had no effect on the amount of BP metabolized by the intestines of rats receiving intraduodenal doses of BP (29). In that study the concentrations of BP used (i.e., 0.2–2 mg of BP per g of triglyceride) may have been high enough such that the amount of BP partitioning into microsomal membranes was sufficient to saturate BP hydroxylase at both doses of fat tested. Residue levels of PAH in food generally lie in the ng/g range (1, 2). At these levels, the concentration of BP available to microsomal proteins would be expected to be influenced by intracellular fat vacuoles, particularly when one considers that, during the uptake of a fatty diet, fat vacuoles may occupy 10–20% of the total volume of the enterocyte (6).

Evidence that glutathione transferase can enhance BP hydroxylase activity has been reported for hepatic microsomal suspensions containing crystalline BP (30). In the present study we have measured enhanced intestinal BP hydroxylase activity in systems containing partially purified intestinal glutathione transferases and natural carriers of lipophilic toxicants. Because the purity of the glutathione transferase preparation in our study was not determined, we do not know the absolute concentration of these enzymes in our test systems. Also, to our knowledge there is no information available on the concentration of glutathione transferase in vertebrate intestinal cytosol. Therefore, comparisons of the concentration of glutathione transferase in our test systems with those encountered in the enterocyte cannot be made. While our results indicate that intestinal glutathione transferase can enhance the activity of BP hydroxylase, extrapolation of these results to physiological conditions must be done with caution.

Within the enterocyte, glutathione transferases may assist in the microsomal oxidation of BP in three complimentary ways. *1*) Glutathione transferases may aid in the transfer of BP between lipid structures (e.g., fat vacuoles and microsomes) by serving as carrier proteins (23, 24). *2*) By covalently binding to BP metabolites (31, 32) or *3*) catalyzing the conjugation of metabolites to the tripeptide GSH (33), glutathione transferases may reduce end product inhibition of BP hydroxylase. Our in vivo binding data suggest that intestinal glutathione transferases covalently bind BP metabolites. This activity may serve not only to reduce end product inhibition of BP hydroxylase but may also reduce the potentially harmful effects resulting from the covalent binding of activated BP metabolites to cellular macromolecules (e.g., DNA).

Binding of native BP to glutathione transferase was not detected in the in vivo binding study. The dissociation constant for glutathione transferase binding to BP $K_d = 10^{-5}$ M) (24) indicates that the protein binds to BP with only a moderate affinity (23). Only by virtue of its high cellular concentration would this protein be expected to have a large effect on the transfer of BP between lipid structures (23). During homogenization of intestinal tissue and preparation of cytosolic fractions, substantial dilution of cytosolic proteins occurs which may account for the nondetectable binding of glutathione transferase to native BP.

Numerous studies with mammals suggest that preexposure of organisms to dietary PAH results in intestinal microsomal MFO systems with high enzymatic activity towards PAH (3). In the present study, exposure of fish to a BP diet for a period of 4 days resulted in BP hydroxylase activity in intestinal microsomes comparable to that in liver microsomes. However, induction of intestinal MFO enzymes apparently can take place over a much shorter time period (3). Thus, intestinal MFO enzymes would be expected to respond to incoming lipophilic toxicants well within the time required for the absorption and processing of dietary fat (4–8).

In conclusion, a considerable amount of research has been directed towards an understanding of the relationship between high dietary fat intake and certain forms of cancer. There is good evidence that dietary fat exerts much of its effect at the promotional phase of chemical carcinogenesis (34, 35). However, due to the high lipophilicity of many carcinogens, it is likely that a high fat diet is also a high carcinogen diet. While it is clear that the intestine is efficient at metabolizing incoming PAH (4,36), factors that influence the efficiency of the intestinal MFO system (e.g., induction, intracellular fat, cytosolic proteins) will also affect the dose and chemical form of PAH that reach peripheral tissues and thus may affect the likelihood of carcinogenesis or toxicity in these tissues.

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