Recently, Denney *et al.* [17] reported that the B form of either human platelet or liver MAO could be selectively precipitated, with retention of enzymatic activity, using a monoclonal antibody to human platelet MAO-B. These latter findings along with those reported herein demonstrate that the two forms of the oxidase can indeed be physically separated by either immunological or simple chromatographic techniques. These findings are also consistent with the hypothesis that the two forms of MAO are, in fact, different proteins; however, the possibility that other factors may account for these differences cannot as yet be ruled out.

As briefly indicated in the introduction, White and Stein [6] have proposed recently that the two oxidases are embedded in the same membrane complex and that this complex in human liver and brain can be disrupted following treatment with phospholipase A2 and/or sodium dodecyl sulfate. Their results demonstrated that the two activities were not clearly separable by electrophoretic or gel filtration techniques. In contrast, the findings presented in this paper indicate that separation of the human brain oxidases occurs under conditions which are relatively mild [18] and would not be expected to be vigorous enough to disrupt the proposed complex. Additionally, the lipid to protein ratio of the enzyme eluting from the ion-exchange column was found to be 0.8 which is very similar to the lipid to protein ratio observed in the outer-mitochondrial membrane [19]. Agreement between the literature value for the native lipid to protein ratio and that found in the partially purified preparation of MAO further supports the hypothesis that treatment with octyglucoside, at the concentrations employed herein, does not disrupt significantly the native lipid–protein interactions.

In summary, these results constitute the first report which demonstrates that the oxidases are separable as catalytically active species by simple chromatographic techniques. These findings do not support the concept that the A and B forms of MAO are held tightly together in a membrane complex which prevents separation of the activities. Furthermore, the inability of previous authors [12, 13] to separate the A and B forms of MAO may be more dependent on the properties of the detergent used to solubilize and chromatograph these proteins than on the state in which these proteins exist within the outer-mitochondrial membrane.

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Regional differences in adrenocortical benzo[*a*]pyrene metabolism in guinea pigs

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The extrahepatic metabolism of xenobiotics has been studied extensively in recent years, in part because of the potential for the production of reactive metabolites, resulting in locally toxic effects. Polycyclic aromatic hydrocarbons, such as benzo[*a*]pyrene (BP), are among the compounds whose adverse effects depend upon activation by microsomal enzymes (see Refs. 1–3). We previously demonstrated high rates of BP metabolism by microsomal preparations from guinea pig adrenal glands [4, 5], and BP is also rapidly metabolized by human fetal adrenals [6, 7]. There is an anatomical zonation of the adrenal cortex [8], and different roles in steroidogenesis have been attributed to each zone. However, relatively little is known about regional differences in the metabolism of xenobiotics within

the adrenal cortex [9, 10]. The studies presented in this communication were done to compare the metabolism of BP by microsomal preparations obtained from the inner (zona reticularis) and outer (zona fasciculata and zona glomerulosa) zones of the guinea pig adrenal cortex.

Adult male English Short Hair guinea pigs, weighing approximately 1000 g, were obtained from the Camm Research Institute (Wayne, NJ) and used in all experiments. Animals were maintained under standardized conditions of light (6:00 a.m.–6:00 p.m.) and temperature (22°) on a diet of Wayne Guinea Pig Diet and water *ad lib.* Animals were killed by decapitation between 8:00 a.m. and 9:00 a.m. Adrenals were quickly removed and placed in cold 1.15% KCl containing 0.05 M Tris–HCl (pH 7.4). Adrenals were bisected longitudinally, and the dark-brown inner zone, consisting primarily of zona reticularis, was gently dissected from the tan outer zone, which was comprised of the zona glomerulosa and zona fasciculata [11]. Tissue from each zone was homogenized, and microsomes were obtained by differential centrifugation [4]. On the basis of steroid Δ^4-hydrogenase activity, which appears to reside solely in the inner zone [11], contamination of the outer zone preparations by inner zone material was approximately 5% or less. Thus, the outer zone enzyme activities presented in this communication are probably not attributable to contamination. In addition, as will be described in a subsequent report, xenobiotic-metabolizing enzymes in the inner and outer zones respond differently to various modifiers of enzyme activity, further suggesting that activity in the outer zone is not the result of inner zone contamination.

BP hydroxylase activity in adrenal microsomes was estimated using two different techniques. The fluorometric assay described by Nebert and Gelboin [12] measures primarily phenolic metabolites, especially 3-hydroxy-BP and 9-hydroxy-BP [13]. Quinine sulfate was calibrated against authentic 3-hydroxy-BP (provided by the chemical repository of the National Cancer Institute) and routinely used as the fluorescence standard. Metabolism of BP was also determined by the isotopic method of DePierre *et al.* [14] as modified by Van Cantfort *et al.* [15]. The latter assay provides an estimate of total BP metabolism. For the isotopic assay, [G-^{3}H]BP was obtained from the Amersham Corp. (Arlington Heights, IL), further purified as described by DePierre *et al.* [14], and diluted with unlabeled BP to a specific activity of 12.5 μCi/μmole. Epoxide hydratase activities in adrenal and liver microsomes were determined using [7-^{3}H]styrene oxide (Amersham Corp.) as the substrate as described by Oesch *et al.* [16] except that the substrate was added in tetrahydrofuran. Samples incubated without microsomes or with heat-inactivated (boiled) microsomes were carried through the entire procedure to correct for any nonenzymatic conversion of styrene oxide to styrene glycol. Cytosolic glutathione-*S*-epoxide transferase activity was also determined using [^{3}H-]styrene oxide as substrate as described by James *et al.* [17]. Analysis of BP metabolism by high pressure liquid chromatography (HPLC) was done essentially as described by Yang *et al.* [18] with modifications previously described [5]. BP and its metabolites were separated with a 30 cm Waters μBondapak C_{18} column (i.d., 3.9 mm). The column was eluted with a concave (curve 8) gradient of 65% methanol in water to 90% methanol over 45 min followed by 15 min at 90% methanol. The solvent flow was 1.0 ml/min, and the eluent was monitored at 254 nm. In addition, when [^{3}H-]BP was used as substrate, 0.5-min fractions were collected, and radioactivity was determined by liquid scintillation counting [5]. Metabolites were quantitated from the amounts of radioactivity under those peaks corresponding to standards provided by the chemical repository of the National Cancer Institute and by establishing standard curves for the u.v. absorbance of known amounts of the authentic metabolites as previously described [5]. Similar results were obtained regardless of whether calculations were based upon radioactivity or u.v. absorbance. Microsomal protein concentrations were determined by the method of Lowry *et al.* [19] using bovine serum albumin as the standard.

Microsomal protein yield was significantly greater in the inner than in the outer adrenocortical zone (Table 1). BP hydroxylase activity, when measured fluorometrically and when expressed per unit tissue weight, was five to six times higher in the inner zone. When activity is expressed per mg of microsomal protein, the differences are smaller but still substantially greater (~ 3-fold) in the inner zone. Since the fluorometric assay measures only phenolic metabolites, it was not surprising that the values obtained with the isotopic assay, which measures total metabolism, were higher in both zones (Table 1). In both inner and outer zones, values with the isotopic assay were approximately 50% greater than those with the fluorometric assay, suggesting a similar ratio of phenolic to total BP metabolites in the two zones. Activity of epoxide hydratase, the enzyme catalyzing the conversion of epoxides to dihydrodiol metabolites, was also far greater in inner than outer zone microsomes. Cytosolic protein concentrations were greater in the inner zone but cytosolic glutathione-*S*-epoxide transferase activity, when expressed per mg protein, was similar in the inner and outer zones. The rates of formation of the various BP metabolites by inner and outer zone microsomes, as determined by HPLC analyses, are indicated in Table 2. The major metabolite produced by both zones was 3-hydroxy-BP, followed by BP-9,10-diol and BP-7,8-diol. The pattern

Table 1. Enzyme activities in inner and outer adrenocortical zones*

	Inner zone	Outer zone
Microsomes		
Protein (mg/g tissue)	49.7 ± 3.8	27.6 ± 2.2†
Benzo[*a*]pyrene hydroxylase (nmoles/min × mg protein)(10^{-1})		
Fluorometric	4.7 ± 0.5	1.6 ± 0.2†
Isotopic	6.6 ± 0.8	2.2 ± 0.3†
Epoxide hydratase (nmoles/min × mg protein)	3.1 ± 0.3	1.1 ± 0.1†
Cytosol		
Protein (mg/g tissue)	78.9 ± 6.8	51.3 ± 4.8†
Glutathione transferase (nmoles/min × mg protein)	41.8 ± 4.6	40.8 ± 4.7

* Values are expressed as means ± S.E. of five to seven experiments.
† $P < 0.05$ (vs corresponding inner zone value).

Table 2. Profile of benzo[a]pyrene (BP) metabolites produced by microsomes obtained from inner and outer adrenocortical zones*

Metabolite	Inner zone (nmoles/min × mg protein)(10^{-2})	Outer zone
3-Hydroxy-BP	51.5 ± 6.0 (62%)†	17.0 ± 1.8 (59%)
9-Hydroxy-BP	5.6 ± 0.6 (7%)	1.8 ± 0.2 (6%)
Quinones	3.0 ± 0.4 (4%)	1.5 ± 0.2 (5%)
BP-7,8-diol	8.9 ± 1.0 (11%)	3.3 ± 0.3 (11%)
BP-4,5-diol	1.6 ± 0.2 (2%)	0.7 ± 0.2 (3%)
BP-9,10-diol	12.7 ± 1.4 (15%)	4.7 ± 0.5 (16%)

* Values are expressed as means ± S.E. of five to seven experiments.

† Values in parentheses indicate percent of total metabolites.

of metabolism was virtually identical in inner and outer zone microsomes, but the rate of production of each metabolite was far greater in the inner zone. The similar pattern of metabolites produced is probably the result of similar cytochrome P-450 isozymes that metabolize BP as well as proportionately similar differences in the activities of BP hydroxylase and epoxide hydratase in the inner and outer zones. The results indicate that, although similar BP metabolites are produced by microsomes from inner and outer adrenocortical zones, the overall rate of metabolism of BP is far greater in the inner zone. Thus, the potential for toxic effects of compounds like BP, which require metabolic activation, may also be greater in the inner zone of the adrenal cortex. Further studies are now under way to examine that hypothesis.

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Glutathione and glutathione *S*-transferases in the urinary bladder of different species

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The glutathione *S*-transferases (EC 2.5.1.18), a family of enzymes with broad and overlapping substrate specificities, catalyze the conjugation of a wide variety of chemically reactive, electrophilic compounds with the nucleophilic tripeptide glutathione. By preventing the interaction of these electrophiles with critical cellular macromolecules, conjugation with glutathione and the resulting formation of water-soluble, glutathione adducts represents a significant step in the detoxication and excretion of many chemical carcinogens and cytotoxicants [1].

Arylamine bladder carcinogens such as 2-naphthylamine are metabolized by hepatic cytochrome P-450 monooxygenases to *N*-hydroxy-2-naphthylamine which is further conjugated with glucuronic acid. The glucuronide then serves as a transport form for the proximate carcinogen which is generated at the slightly acidic pH of the urine