B_1 induced a significant number of liver tumours in fish 9–12 months later. Thus, the risk to fish populations, associated with spawning in contaminated environments, may be due in part to bioactivation of foreign compounds during embryonic development.

In summary, exposure of developing *F. heteroclitus* eggs to PCBs or whole No. 2 fuel oil induced aryl hydrocabon hydroxylase activity in eggs near hatching. PCBs induced AHH activity in both the liver and extrahepatic tissues of similarly exposed yolk sac larvae. AHH activity was present both in hepatic and extrahepatic tissues of control larvae but was undetectable in control eggs. The uninduced activity in yolk sac larval liver was about 50 per cent of that in adult liver. Embryonic metabolism of environmental chemicals could be adaptive during yolk absorption but might also contribute to lesions by production of activated metabolites.

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Comparative effects of mixed function oxidase inhibitors on adrenal and hepatic xenobiotic metabolism in the guinea pig

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In recent years, extra-hepatic xenobiotic metabolism has been studied extensively in a variety of tissues including the lung, kidney, intestine, skin and placenta (see Ref. 1). The oxidative metabolism of polycyclic aromatic hydrocarbons (PAH) such as benzo[*a*]pyrene (BP) by cytochrome P-450-containing enzymes has been of particular interest because of the ubiquitous distribution and potential carcinogenicity, mutagenicity and toxicity of PAH. Extrahepatic metabolism of PAH may represent an important detoxifying mechanism, especially in organs that serve as primary portals of entry to the body, but may also result in the production of highly toxic metabolites [1–3]. The results of numerous investigations indicate that the characteristics of PAH-metabolizing enzymes vary considerably from tissue to tissue. For example, responses to inducing agents, well as to enzyme inhibitors, are highly tissue dependent [1–3]. Such differences in enzyme characteristics may play an important part in determining the relative toxicities of PAH in different organs.

Many foreign compounds, including PAH, are rapidly metabolized by adrenocortical tissue, especially that obtained from human fetuses [4–7] or from guinea pigs [8–

11]. However, relatively little is known about the properties of adrenal drug-metabolizing enzymes. It has been established that the regulation of adrenal and hepatic xenobiotic metabolism operates independently of one another, at least with respect to the influences of age, sex, strain and enzyme inducers [10–17]. The following studies were carried out to further characterize adrenal drug-metabolizing enzymes by comparing the effects of the mixed function oxidase inhibitors, metyrapone, 7, 8-benzoflavone and β-diethylaminoethyl diphenylpropylacetate (SKF 525-A), on adrenal and hepatic metabolism in the guinea pig.

Adult (650–750 g), male English Short Hair guinea pigs were obtained from the Camm Research Institute, Wayne, NJ. Animals were maintained under standardized conditions of lighting (6:00 a.m.–6:00 p.m.) and temperature (22°) on a diet of Wayne Guinea Pig Diet and water *ad lib.* Guinea pigs were decapitated, the adrenals and livers were quickly removed, and microsomes were prepared as described previously [11]. Microsomal pellets were washed once and resuspended at a concentration of 2–3 mg of microsomal protein/ml.

The demethylation of ethylmorphine (EM) was assayed as the amount of formaldehyde [18] formed by adrenal or hepatic microsomes, as described previously [11]. Benzo[*a*]pyrene hydroxylation was determined using the fluorometric assay of Nebert and Gelboin [19]. Quinine sulfate was calibrated against authentic 3-OH-benzo[*a*]pyrene and routinely used as the fluorescence standard. Steroid 21-hydroxylase activity was measured as the rate of conversion of 11β-hydroxyprogesterone to corticosterone [11]. Microsomal protein concentrations were determined by the method of Lowry *et al.* [20] using bovine serum albumin as the standard.

Metyrapone was a far more potent inhibitor of EM demethylation than of BP hydroxylation in both adrenal and hepatic microsomes (Table 1). EM metabolism was inhibited to a greater extent in liver than in adrenal microsomes but the effects of metyrapone on BP hydroxylation were similar in both adrenal and liver microsomes. As expected, SKF 525-A produced a concentration-dependent inhibition of EM metabolism. Concentrations as low as 50 μM significantly decreased enzyme activity. The effects of SKF 525-A on adrenal and hepatic EM demethylase activity were virtually identical. In contrast, the actions of SKF 525-A on BP metabolism were tissue dependent. Hepatic BP hydroxylase activity was uniformly decreased by SKF 525-A. The concentration-dependent inhibition of hepatic BP metabolism by SKF 525-A was similar to the inhibition of hepatic EM demethylation. However, the nature of the effect of SKF 525-A on adrenal BP hydroxylation was dependent upon the amount of drug employed. Concentrations of SKF 525-A between 50 and 100 μM increased enzyme activity (Table 1). Higher concentrations of SKF 525-A inhibited BP metabolism. SKF 525-A also decreased adrenal microsomal steroid 21-hydroxylase activity but to a lesser extent than EM metabolism was decreased. Benzoflavone (BF) was a more potent inhibitor of hepatic than of adrenal BP hydroxylation. No evidence of enhancement of enzyme activity was obtained at any of the BF concentrations studied in either tissue. BF had little or no effect on EM demethylation in adrenal or hepatic microsomes or on adrenal 21-hydroxylation.

The results demonstrate some differences and some similarities in the characteristics of adrenal and hepatic mixed function oxidases in guinea pigs. Inhibition of BP metabolism by metyrapone was not tissue dependent. Metyrapone has been shown to be a more potent inhibitor of BP metabolism in microsomes obtained from control rats than in those from 3-methylcholanthrene-induced animals [21]. Our results, therefore, suggest that the adrenal and hepatic enzymes in the guinea pig do not differ with regard to their degree of 'induction'. We demonstrated previously that metyrapone also inhibited adrenal microsomal steroid 21-hydroxylation [22]. Thus, metyrapone appears to be a relatively non-specific inhibitor of adrenal mixed function oxidases in the guinea pig.

In contrast to the actions of metyrapone, BF has been found to selectively inhibit BP metabolism in microsomes from rats treated with 3-methylcholanthrene and to increase activity in microsomes from control rats [23]. However, like metyrapone, BF fails to differentiate adrenal and hepatic BP metabolism in the guinea pig. BP hydroxylase

Table 1. Effects of metyrapone, 7,8-benzoflavone and SKF 525-A on adrenal and hepatic benzo[*a*]pyrene (BP) hydroxylase and ethylmorphine (EM) demethylase activities*

Inhibitor (M)	Microsomal enzyme activities (% of control) EM demethylase Adrenal	EM demethylase Liver	BP hydroxylase Adrenal	BP hydroxylase Liver
Metyrapone				
0	100	100	100	100
2×10^{-4}	75 ± 3†	58 ± 3†	92 ± 3†	94 ± 4
5×10^{-4}	67 ± 3†	46 ± 4†	86 ± 4†	90 ± 3†
1×10^{-3}	60 ± 4†	39 ± 3†	74 ± 3†	79 ± 4†
2×10^{-3}	47 ± 3†	30 ± 3†	66 ± 4†	73 ± 4†
Benzoflavone				
0	100	100	100	100
5×10^{-6}	98 ± 5	97 ± 4	98 ± 3	92 ± 5
1×10^{-5}	101 ± 4	98 ± 4	93 ± 5	84 ± 6†
5×10^{-5}	99 ± 3	99 ± 5	84 ± 6†	68 ± 4†
1×10^{-4}	94 ± 5	95 ± 5	68 ± 6†	56 ± 5†
SKF 525-A				
0	100	100	100	100
5×10^{-5}	91 ± 3†	90 ± 3†	126 ± 4†	86 ± 3†
1×10^{-4}	77 ± 4†	76 ± 4†	118 ± 3†	67 ± 3†
2×10^{-4}	60 ± 4†	64 ± 4†	84 ± 2†	54 ± 3†
5×10^{-4}	48 ± 3†	52 ± 4†	49 ± 3†	41 ± 5†

* Values are expressed as mean per cent of control ± S.E.; there were six to eight determinations per value.

† $P < 0.05$ (vs corresponding control value).

in guinea pig adrenal and hepatic microsomes responds to BF like the 'induced' enzyme in rat liver. It is possible that the enzyme in 'control' guinea pigs is at least partially 'induced' as a result of dietary or other environmental factors. Similar inhibition of BP metabolism by BF was also found in the rat adrenal [17] but not in the fetal human adrenal gland [24]. The absence of BF effects on adrenal EM metabolism or on 21-hydroxylation suggests that different forms of cytochrome P-450 may be involved in those reactions than in BP metabolism. We suggested previously that adrenal drug and steroid metabolism may be catalyzed by different species of cytochrome P-450 [10, 11], but the actions of BF suggest that multiple cytochrome P-450 moieties may be involved in adrenal metabolism of different xenobiotics.

Although SKF 525-A produced similar decreases in adrenal and hepatic EM metabolism, its effects on BP hydroxylase activity clearly indicate differences in the adrenal and hepatic enzymes. SKF 525-A has been widely used as an inhibitor of mixed function oxidase reactions but, to our knowledge, has never before found to enhance enzyme activity. However, low concentrations (50–100 μM) of SKF 525-A increased adrenal BP metabolism, whereas larger amounts produced the expected inhibition. These effects of SKF 525-A on adrenal BP metabolism in the guinea pig contrast with the lack of effect reported in the fetal human adrenal [24] and in the rat adrenal [16]. However, in each of the latter studies, only a single concentration of SKF 525-A was tested and effective concentrations may have been overlooked. It is also possible that the actions of SKF 525-A on adrenal BP metabolism are species-specific. The divergent effects of SKF 525-A on adrenal BP and EM metabolism further suggest that different cytochrome P-450 moieties catalyze each reaction.

The mechanism responsible for the SKF 525-A-mediated increase in adrenal BP metabolism remains to be resolved. Since the drug has no apparent effect on microsomal epoxide hydrase activity [25], changes in the pattern of BP metabolism are not likely to account for increases in the production of fluorescent metabolites (phenols). In addition, we have found recently that, even in the absence of SKF-525-A, guinea pig adrenal microsomes convert BP almost exclusively to phenols [26]. The biphasic effect of SKF 525-A on metabolism may indicate the involvement of more than one mono-oxygenase in adrenal BP hydroxylation. Alternatively, SKF 525-A may exert a biphasic effect on a single enzyme. Further investigation is now needed to consider these as well as other possible mechanisms.

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