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METABOLISM OF POLYCYCLIC AROMATIC HYDROCARBONS IN THE RAT OVARY

COMPARISON WITH METABOLISM IN ADRENAL AND LIVER TISSUES

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Abstract—The activities of polycyclic aromatic hydrocarbon metabolizing enzymes and their regulation in the rat ovary were investigated and compared to those in the rat adrenal and liver. 7,12-Dimethylbenz[*a*]anthracene and benz[*a*]pyrene, both potent carcinogens, were converted by ovarian microsomes to hydrophilic products by the same cytochrome P-450 dependent monooxygenase with an apparent K_m of about 0.05–0.1 μM. These data indicate that the ovarian AHH has a 20- and 200-fold higher affinity for polycyclic aromatic hydrocarbons than the adrenal and liver, respectively. 3-Methylcholanthrene (MC) and 7,12-dimethylbenz[*a*]anthracene apparently induce ovarian AHH but in an irreproducible manner, suggesting a possible regulation by endogenous factors. Among various inhibitors tested, ellipticine, SU-9055 and α-napthoflavone were the most efficient; steroids, e.g. cholesterol and estradiol, were less efficient inhibitors. A comparison of the adrenal and liver with respect to metabolite patterns for DMBA and BP reveals that there are striking qualitative as well as quantitative similarities between the adrenal and ovarian systems, whereas the liver appears to be different. Also, glutathione-*S*-transferase activity is inducible by MC in the liver but not in the adrenal or ovary. DT-diaphorase is induced by MC in all three organs but only by DMBA in the liver and ovary. In contrast, epoxide hydrolase is not induced by MC or DMBA in either of these organs. These results suggest that the ovarian and adrenal systems involved in bioactivation and detoxification of xenobiotics are closely related whereas those of the liver are different.

The gonads are receiving increasing attention as important sites of detoxification of drugs and xenobiotics (for a review see Ref. 1). Since it is generally accepted that most carcinogens also act as mutagens and that these substances after bioactivation cause a decreased capacity for reproduction [2–4], as well as other toxic effects [5], it appears essential to gain further insight into gonadal detoxification. Thus, it has been shown that the ovary and testis contain cytochrome P-450 dependent monooxygenase(s) [6, 7], glutathione-*S*-transferase(s) [8, 9] and epoxide hydrolase [7, 10]. To some extent these activities are controlled by the pituitary through gonadotropic hormones [7, 11] (see also Ref. 9). Both ovarian [6] and testicular [4, 6, 12] metabolism of benzo[*a*]pyrene (BP) has been reported to be inducible by MC or TCDD, well-known inducers of BP metabolism in the liver and several other organs [13, 14].

Toxic effects of polycyclic hydrocarbons on reproduction have been observed in both rats [15] and mice [3]. In the former case spermatogenesis is affected whereas the oocytes seem to be the main target in the the latter case. The reasons for the discrepancy between different species [16] and strains of the same species [3] are presently not known. It has been suggested, however, that reproductive functions of both the testis [4] and ovary [17] in humans are affected by various xenobiotics and carcinogens, resulting in cancer, infertility, early menopause, teratogenic effects and possible silent genetic defects.

The present investigation aims at a further characterization of the polycyclic hydrocarbon metabolizing system of the rat ovary using the carcinogens DMBA and BP as substrates, with special reference to metabolite patterns, inducibility and activities of glutatione-*S*-transferase, DT-diaphorase and epox-

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† Abbreviations: MC, 3-methylcholanthrene; *o,p'*-DDD, 2,2-bis(4-chlorophenyl,2-chlorophenyl)-1,1-dichloroethane; SKF, β-diethyl-aminoethyl-diphenylpropyl-acetate; CDNB, 1-chloro-2,4-dinitrobenzene; SU-9055, 3-(1,2,3,4-tetrahydro-1-oxo-2-naphtyl)pyridine; ellipticin, 5,11-dimethyl-(6H)-pyrido-(4-3B)carbazole; AHH, aryl hydrocarbon hydroxylase; DMBA, 7,12-dimethylbenz[*a*]anthracene; 12-OHM-7-MBA, 12-hydroxymethyl-7-methylbenz[*a*]anthracene; 7-OHM-12-MBA, 7-hydroxymethyl-12-methylbenz[*a*]anthracene; 7,12-diOHMBA, 7,12-dihydroxymethylbenz[*a*]anthracene; DMBA-5,6-diol, 5,6-dihydro-5,6-dihydroxy-7,12-dimethylbenz[*a*]anthracene; BP, benz[*a*]pyrene; BP-9,10-diol, 9,10-dihydro-9,10-dihydroxybenz[*a*]pyrene; BP-4,5-diol, 4,5-dihydro-4,5-dihydroxybenz[*a*]pyrene; BP-7,8-diol, 7,8-dihydro-7,8-dihydroxybenz[*a*]pyrene; BP-6,12-diol, 6,12-dihydro-6,12-dihydroxybenz[*a*]pyrene; 9-OH-BP, 9-hydroxybenz[*a*]pyrene; 3-OH-BP, 3-hydroxybenz[*a*]pyrene.

ide hydrolase. The results indicate that the ovarian AHH activity produces metabolite patterns that are similar to those obtained with adrenals rather than with the liver and is induced by MC in an irreproducible manner. Also, the activity and inducibility of ovarian glutathione-*S*-transferase, and DT-diaphorase and epoxide hydrolase activities resemble those of the adrenal enzymes.

MATERIALS AND METHODS

Ovaries, adrenals and livers were removed from five female Sprague–Dawley rats (> 180 g) after decapitation, and fractionated immediately. The microsomal fractions obtained from pooled organs were prepared essentially as described by Mattison and Thorgeirsson [6], Ogle [18] and Ernster *et al.* [19], respectively. Frozen organs or subcellular preparations were also used since these showed unchanged monooxygenase activities. Incubations for estimating NADPH-dependent DMBA and BP metabolism either by HPLC analysis or by the distribution method were carried out as described previously [20]. Soluble glutathione-*S*-transferase activity was measured with CDNB according to Habig *et al.* [21]. Soluble DT-diaphorase was assayed as described previously [22]. Epoxide hydrolase activity was estimated with [^{3}H]styrene-7,8-oxide as substrate and [^{3}H]styrene-7,8-diol formed was determined as described by Seidegård *et al.* [23]. Induction of microsomal monooxygenases, glutathione-*S*-transferases and DT-diaphorases was performed by administering MC or DMBA (80 and 50 mg/kg, respectively), dissolved in corn oil, i.p., 48 hr prior to sacrifice. [^{14}C]DMBA (97.4 mCi/mmole) and [^{14}C]BP (21.7 mCi/mmole) were obtained from NEN Chemicals (D-6072 Dreireich, West Germany): [^{3}H]BP (17 Ci/mmole) was obtained from Amersham (U.K.). SU-9055 was a gift from Ciba-Geigy (Basel, Switzerland) and ellipticine was a gift from the National Cancer Institute (NIH, Bethesda, MD). Reference metabolites of BP and DMBA were gifts from Dr J. W. DePierre (University of Stockholm, Sweden) and Dr Jan-Åke Gustafsson (Department of Medical Nutrition, Karolinska Institute, Stockholm, Sweden), and Dr P. L. Grover (Chester Beatty Research Institute, London, U.K.), respectively.

Table 1. Subcellular distribution of ovarian AHH activity*

Fraction	Substrate	Sp. act.† (pmoles/min·mg protein)
Mitochondria	DMBA	0.1 ± 0.1 (4)
Mitochondria	BP	1.5 ± 0.03 (4)
Microsomes	DMBA	4.0 ± 0.01 (4)
Microsomes	BP	7.4 ± 0.6 (4)
Soluble	DMBA	< 0.0 (4)
Soluble	BP	1.0 ± 0.3 (4)

* Rat ovaries were fractionated and activities estimated as described in Materials and Methods. Additions were 5 μM DMBA or BP.

† Mean ± S.D.; numbers within parentheses denote number of experiments.

RESULTS

As indicated in Table 1 the metabolism of DMBA and BP, catalyzed by the rat ovary, showed a similar subcellular distribution. Ninety per cent of the measurable activity was confined to the microsomal fraction with little or no activity in the mitochondria and soluble fraction. Taking the routinely observed microsomal contamination of mitochondria into account (cf. Ref. 24), allows us to conclude that ovarian AHH activity is localized exclusively in the

Table 2. Effect of inhibitors on ovarian AHH activity*

Conditions	Concentration of inhibitor (μM)	Sp. act.† (pmoles/min·mg protein)	Inhibition (%)
Microsomes	—	1.50 ± 0.27 (2)	—
+ carbon monoxide	—	0.25 (2)	83
+ DMBA	5	0.77 ± 0.03 (2)	49
+ SU-9055	100	0.14 (1)	91
+ ellipticine	10	0.04 ± 0.01 (2)	97
+ α-naphthoflavone	100	0.06 ± 0.02 (2)	96
+ β-naphthoflavone	100	0.90 ± 0.07 (2)	40
+ estradiol	100	1.15 ± 0.11 (2)	23
+ progesterone	100	1.36 ± 0.24 (2)	9
+ cholesterol	100	0.74 ± 0.05 (2)	51
+ testosterone	100	1.34 ± 0.25 (2)	11
+ spironolactone	100	1.22 (1)	19
+ SKF	100	0.99 ± 0.13 (2)	34
+ metyrapone	100	0.81 ± 0.20 (2)	46
+ ethylmorphine	100	1.55 ± 0.13 (2)	0
+ *o,p'*-DDD	100	0.79 (1)	47
+ biphenyl‡	100	1.79 (1)	0

* The AHH assay was carried out as described in Materials and Methods. The concentration of BP was 5 μM; carbon monoxide was added continuously during the assay as a carbon monoxide–oxygen mixture (90:10).

† Mean ± S.D.; numbers within parentheses denote number of experiments.

‡ The biphenyl used was 3,4,5,2′,4′,6′-hexachlorobiphenyl.

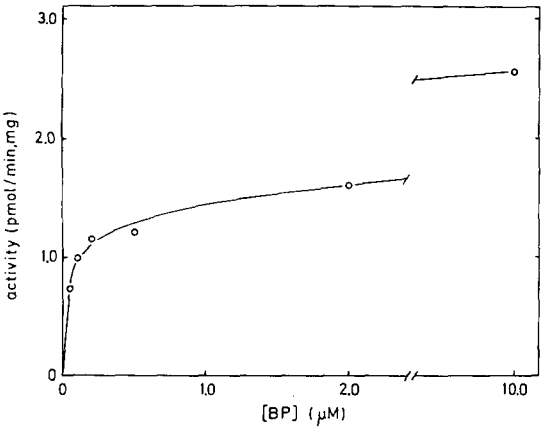

Fig. 1. Concentration dependence for BP metabolism catalyzed by microsomes from the rat ovary. Assay of hydroxylase activity was carried out as described in Materials and Methods. Protein concentration was 0.5 mg/ml.

endoplasmic reticulum. With both BP and DMBA as substrates ovarian AHH activity was NADPH-dependent (not shown, cf. Materials and Methods) and inhibited by carbon monoxide, SU-9055, ellipticine and α-naphthoflavone (Table 2); *o,p'*-DDD, metyrapone, SKF and β-naphthoflavone were less inhibitory. Various inhibitory steroids include cholesterol and estradiol but these were generally less potent. Biphenyl, ethylmorphine and progesterone had little, if any, effect (Table 2). DMBA inhibited BP metabolism and vice versa (not shown), suggesting the involvement of a common cytochrome P-450. The assay of AHH activity in the presence of different concentrations of BP gave an apparent K_m of approximately 0.05 μM (Fig. 1). However, even though a marked tendency for saturation was noted up to 0.5 μM BP, the activity was still continuously but slowly increasing up to 10 μM (Fig. 1). This tendency was maintained up to 100 μM (not shown). The approximately 50% inhibition obtained at equimolar concentrations of DMBA and BP (Table 2), using BP as the labeled substrate, suggests that the K_m value for DMBA is about the same as that for BP.

Ovarian AHH activity has been reported to be induced by MC [6]. As shown in Table 3 this has not been possible to reproduce, although some induction, between three- and four-fold, was observed occasionally with MC and DMBA. Attempts to increase the extent of induction by pre-treatment with 320 mg MC/kg instead of 80 mg MC/kg (cf. Materials and Methods) were not successful (not shown). In fact, in some cases pretreatment with MC resulted in decreased rather than increased activities. The activities in both control and induced rats varied markedly and were between 0.5 and 16 pmoles/min·mg protein, irrespective of the substrate used (not shown). Moreover, the control values for the rats that showed induction were considerably lower than those that did not show induction, which is indicated by the large variability in the control activity (Table 3). In agreement with previous reports [25–27], rat adrenal AHH activity, which was about 200 pmoles/min·mg, was not inducible by MC or DMBA but was suppressed by these agents (Table 3). In contrast, under the same conditions and in the same rat, liver metabolism of BP, which was about 45 pmoles/min·mg in the control rats, was consistently induced about 30-fold by MC pretreatment; metabolism of DMBA was induced 20- and 2-fold by MC and DMBA pretreatment, respectively (Table 3). These findings indicate that the inducibility and maximal activity of ovarian AHH is variable in this strain of rat and possibly regulated by endogenous factors (cf. Discussion).

The metabolite patterns for both BP and DMBA conversions catalyzed by ovarian, adrenal and liver microsomes revealed significant diffferences. With BP as substrate the metabolite patterns in control

Table 3. Effect of pretreatment with MC and DMBA on AHH in the rat ovary, adrenal and liver*

Organ	Substrate	Pretreatment	Sp. act.† (pmoles/min·mg protein)
Ovary	BP	—	6.1 ± 9.2 (8)
Adrenal	BP	—	263.2 ± 15.8 (8)
Liver	BP	—	44.1 ± 14.5 (8)
Ovary	BP	MC	13.8 ± 3.8 (8)
Adrenal	BP	MC	207.5 ± 19.0 (8)
Liver	BP	MC	1290.6 ± 290.5 (8)
Ovary	DMBA	—	4.3 ± 2.1 (9)
Adrenal	DMBA	—	202.9 ± 28.0 (9)
Liver	DMBA	—	64.1 ± 2.7 (9)
Ovary	DMBA	MC	10.9 ± 3.1 (9)
Adrenal	DMBA	MC	149.7 ± 21.1 (9)
Liver	DMBA	MC	1167.2 ± 139.1 (9)
Ovary	DMBA	DMBA	10.2 (1)
Adrenal	DMBA	DMBA	191.0 ± 9.2 (2)
Liver	DMBA	DMBA	143.4 ± 8.5 (2)

* Estimations of AHH and pretreatment with MC and DMBA were carried out as described in Materials and Methods.

† Mean ± S.D.; numbers within parentheses denote number of experiments.

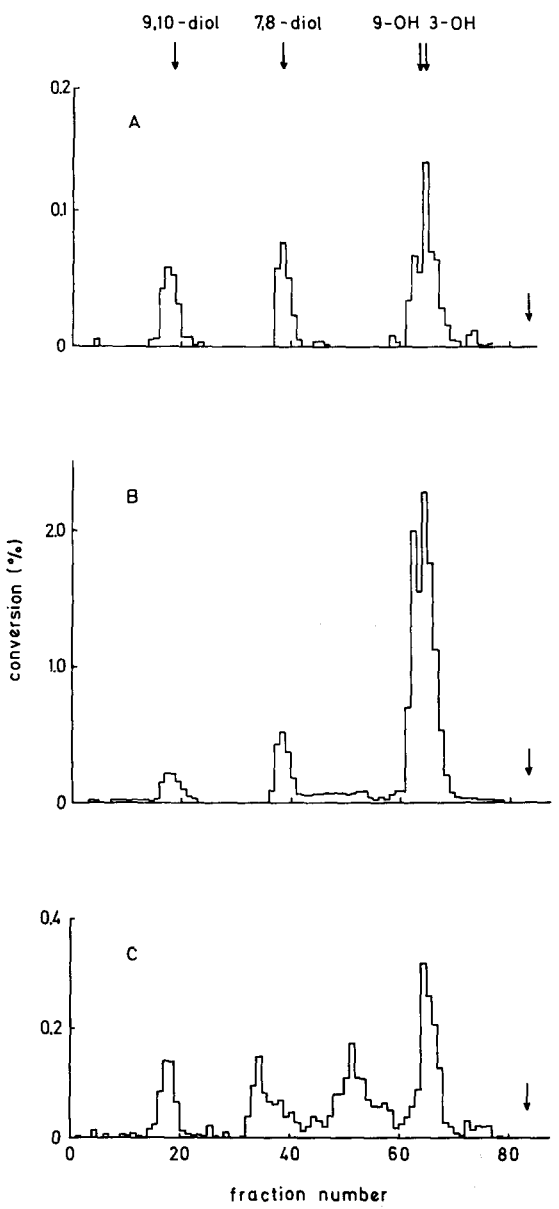

Fig. 2. Metabolism of BP catalyzed by untreated rat ovary (A), adrenal (B) and liver (C) microsomes. The concentration of the substrate was 50 μM. Protein concentration was 0.5 mg/ml. The arrow indicates where BP was eluted. 9,10-diol, BP-9,10-diol; 4,5-diol, BP-4,5-diol; 7,8-diol, BP-7,8-diol; 6,12-diol, BP-6,12-diol; 9-OH, 9-OH-BP; 3-OH, 3-OH-BP.

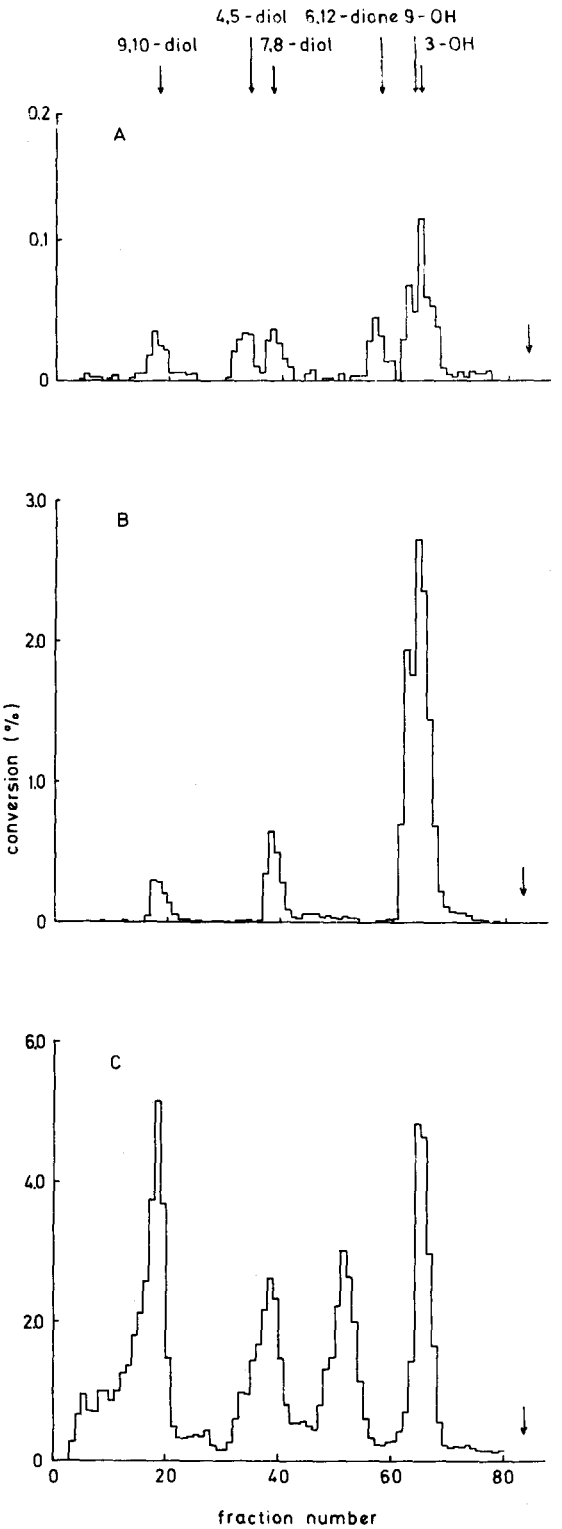

Fig. 3. Metabolism of BP catalyzed by MC-pretreated rat ovary (A), adrenal (B) and liver (C) microsomes. MC pretreatment was carried out as described in Materials and Methods; conditions were as in Fig. 2.

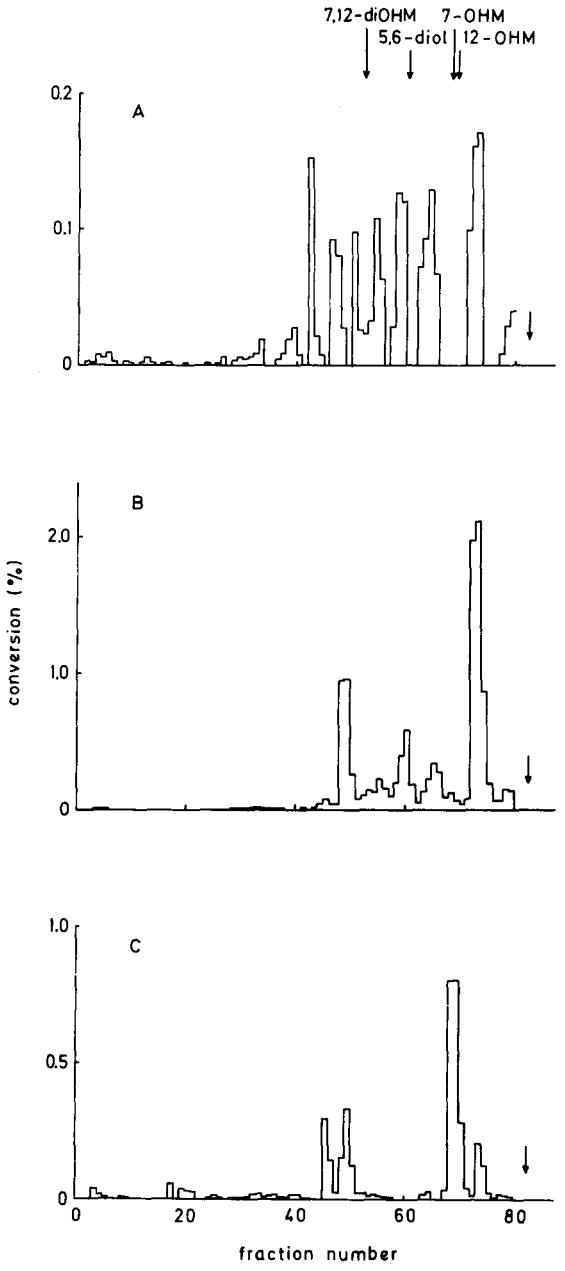

Fig. 4. Metabolism of DMBA catalyzed by non-treated rat ovary (A), adrenal (B) and liver (C) microsomes. Conditions were as in Fig. 2. The arrow indicates where DMBA was eluted. 7,12-diOHM, 7,12-diOHMBA; 5,6-diol, DMBA-5,6-diol; 7-OHM, 7-OHM-12-MBA; 12-OHM. 12-OHM-7-MBA.

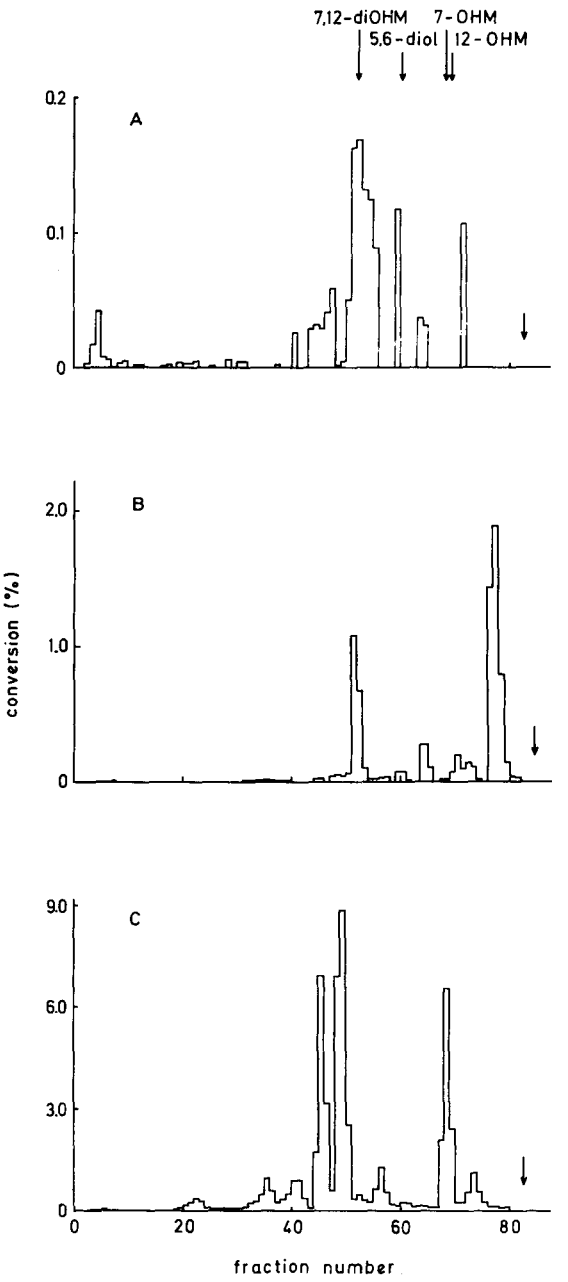

Fig. 5. Metabolism of DMBA catalyzed by MC-pretreated rat ovary (A), adrenal (B) and liver (C) microsomes. Conditions were as in Fig. 2.

ovary and adrenal were qualitatively nearly identical: in both cases 9,10-diol, 7,8-diol, 3-OH and 9-OH metabolites were formed, the main difference being an enhanced relative formation of the 3-OH and 9-OH derivatives in the adrenal (Fig. 2A and B). In the liver, the pattern was different with respect to the content of quinones, which were essentially lacking in the ovarian and adrenal incubations (Fig. 2C). As compared to the control ovary (Fig. 2A) the MC-pretreated ovary differed markedly in that 4,5-diol and 6,12-dione appeared as new products. In the MC-pretreated adrenal (Fig. 3B) the patterns were unchanged. The induced liver showed the well-established general increase in all BP metabolites and the appearance of new hydrophilic products (Fig. 3C).

Exchange of BP for DMBA as substrate resulted in metabolite patterns that were somewhat more

Table 4. Effect of pretreatment with MC and DMBA on glutathione-*S*-transferase and DT-diaphorase activities in the rat ovary, adrenal and liver*

Organ	Activity	Pretreatment	Sp. act.† (nmoles/min·mg protein)
Ovary	GSH-S-T	—	334.6 ± 36.4 (4)
Adrenal	GSH-S-T	—	467.0 ± 46.9 (4)
Liver	GSH-S-T	—	880.8 ± 360.2 (4)
Ovary	GSH-S-T	MC	333.6 ± 52.6 (4)
Adrenal	GSH-S-T	MC	456.1 ± 138.2 (4)
Liver	GSH-S-T	MC	2730.0 ± 905.3 (4)
Ovary	GSH-S-T	DMBA	249.8 (1)
Adrenal	GSH-S-T	DMBA	392.9 (1)
Liver	GSH-S-T	DMBA	708.8 (1)
Ovary	DT	—	173.9 ± 52.6 (5)
Adrenal	DT	—	232.3 ± 25.5 (4)
Liver	DT	—	1230.0 (1)
Ovary	DT	MC	783.7 ± 197.5 (6)
Adrenal	DT	MC	360.5 ± 87.3 (5)
Liver	DT	MC	8270.0 (1)
Ovary	DT	DMBA	338.7 (1)
Adrenal	DT	DMBA	202.8 (1)
Liver	DT	DMBA	2350.0 (1)

* Estimations of glutathione-*S*-trasnferase (GSH-S-T) and DT-diaphorase (DT) activities and pretreatment with MC and DMBA were carried out as described in Materials and Methods.

† Mean ± S.D.; numbers within parentheses denote number of experiments.

complicated to interpret, partly because all metabolites could not be identified with the available reference compounds. However, in this case similarities were also seen between ovarian and adrenal metabolite patterns. In the control ovary (Fig. 4A) two minor metabolites were tentatively identified as the 3-OH and 8,9-diol derivatives, respectively, whereas the relative contribution of these metabolites was much higher in the control adrenal (Fig. 4B). Pretreatment with MC suppressed the formation of the unidentified minor metabolites in the adrenal but had otherwise no or little effect on the major metabolites (Fig. 5B). In the ovary, pretreatment with MC resulted in a dramatic change in the distribution of the metabolites (Fig. 5A), producing one major metabolite identified as 7,12-diOHMBA. In contrast to the ovary and adrenal, which did not produce substantial amounts of the 7-OHM-12-MBA, this derivative was one of three major metabolites in both the control (Fig. 4C) and MC-pretreated liver (Fig. 5C). It is surprising to note that induction of the liver affected all metabolites to the same relative extent (cf. Refs 28 and 29). Thus the formation of 7-OHM-12-MBA was induced about 18 times compared to about 20 times overall induction.

The effect of pretreatment with MC and DMBA on glutathione-*S*-transferase, DT-diaphorase and epoxide hydrolase activities was also investigated. As shown in Table 4 the glutathione-*S*-transferase activities in the control ovary, adrenal and liver were between 300–900 nmoles/min·mg, the highest activity being in the liver. Attempts to induce this activity by MC or DMBA was successful only with MC in the liver, which gave a double activity. DT-

Table 5. Effect of pretreatment with MC and DMBA on epoxide hydrolase activities in the rat ovary, adrenal and liver*

Organ	Pretreatment	Sp. act.† (nmoles styrene-diol formed/min·mg protein)
Ovary	—	0.79
Adrenal	—	0.28
Liver	—	7.10
Ovary	MC	1.12
Adrenal	MC	0.15
Liver	MC	7.66
Ovary	DMBA	0.80
Adrenal	DMBA	0.23
Liver	DMBA	5.61

* Estimations of epoxide hydrolase activities and pretreatment with MC and DMBA were carried out as described in Materials and Methods.

† Mean of duplicate experiments.

diaphorase activities were comparable in the control ovary and adrenal, i.e. about 200 nmoles/min·mg, but was six-fold higher in the control liver (Table 4). Induction of DT-diaphorase in the ovary and adrenal by DMBA or MC gave two- to five-fold increased activities; liver DT-diaphorase activity was doubled by DMBA and increased seven-fold by MC (Table 4). Epoxide hydrolase, assayed with styrene-7,8-oxide as substrate, showed an activity of about 0.8 nmoles/min·mg in the ovary and 0.3 nmoles/min·mg in the adrenal; the corresponding liver activity was 10-fold higher than that of the ovary (Table 5). None of these organs revealed any significant induction of epoxide hydrolase activity after pretreatment with MC or DMBA; in fact, in some instances a decreased activity was observed.

DISCUSSION

The present data show that the rat ovary actively converts DMBA and BP to more hydrophilic products in a carbon monoxide sensitive and NADPH-dependent manner, indicating that both hydrocarbons are metabolized by a cytochrome P-450 dependent monooxygenase. BP metabolism is inhibited by DMBA, which suggests that the same cytochrome P-450 is involved in the conversion of the two hydrocarbons, as well as by other established cytochrome P-450 (P-448) inhibitors, e.g. α-naphthoflavone, ellipticine and SU-9055. An interesting feature of the ovarian AHH is its low K_m value for its substrate which is 0.05–0.1 μM. The corresponding value for adrenal AHH has been estimated to be below 3 μM with DMBA as substrate and below 1 μm with BP as substrate [20]. A considerably higher value, about 20 μM, has been reported for liver AHH [30, 31]. In this context it should be pointed out that K_m values of membrane-bound enzymes for hydrophobic substrates tend to increase with the protein concentration, and hence the membrane lipid concentration in the assay (cf. Ref. 32). However, in the present investigation all assays were carried out in the presence of the same protein concentration. The high affinity of ovarian and adrenal AHH for its substrates also indicates that, under physiological conditions where exposure to polycyclic aromatic hydrocarbons most likely occurs at nonsaturating concentrations, K_m and V_{max} values are equally important parameters. Even though the liver may also have a small amount of high-affinity AHH, which may be masked by large amounts of low-affinity AHH, the present results suggest that the adrenal and ovary may be substantially more potent in bioactivation of xenobiotics than is indicated by their maximal AHH activities. Thus, these organs may be more sensitive *in vivo* to carcinogens of the aromatic hydrocarbon type than, for example, the uninduced liver. The possibility that a similar high affinity of AHH may be involved in chemical carcinogenesis in the human ovary is being investigated.

Analysis of the metabolite pattern for adrenal, ovarian and liver metabolism of BP and DMBA indicates, on the one hand, similarities between the ovary and adrenal, and on the other, differences between the ovary–adrenal and liver. Qualitatively, the BP metabolite patterns found with the control ovary and adrenal are almost identical whereas that of the MC-pretreated ovary resembles the control and induced livers. With DMBA as substrate the main differences are that the ovary and adrenal essentially do not produce 7-OHM-12-MBA, a metabolite generated in large amounts by both the control and induced livers and which has been proposed to be highly toxic [5, 33]. Thus, the "methyl-hydroxylase" suggested to be responsible for the formation of 7-OHM-12-MBA in liver [29] has a much lower activity in the adrenal and ovary. These data agree with previously published reports on the ovarian metabolism of BP in control and induced Sprague–Dawley rats [16]. Quantitatively, however, the present results do not reveal a consistent overall induction as has been reported earlier [6, 16], but rather appearances of new products. This variability may be due to an endogeneous regulation of the AHH, possibly mediated by the pituitary (cf. Refs. 7, 9, 11).

Metabolism of DMBA in adrenals from untreated male Sprague–Dawley rats has been studied previously in this laboratory [20, 34]. The present data indicate that, with respect to DMBA metabolism, there are no major sex differences in this strain of the rat, except that the rate of the overall adrenal metabolism appears to be about two-fold higher in the female rat. In the liver, DMBA metabolism involves the formation of 7-OHM-12-MBA, a compound which has been claimed to be responsible for DMBA-induced adrenal necrosis [33] as well as other toxic effects [5]. Earlier reports have indicated that the generation of this metabolite is only slightly inducible or even suppressed after MC treatment [26, 28]. In the present communication it is shown that MC induces all liver metabolites of DMBA approximately to the same extent (cf. Ref. 32).

Glutathione-*S*-transferase [35] and DT-diaphorase [36] are believed to have an important role in conjugation reactions and reduction of quinones, respectively. Whereas the transferases in the liver are induced by MC (cf. Ref. 35), those in the adrenal and ovary are not. DT-diaphorase is inducible in the liver [37] as well as in both the ovary and adrenal. The fact that DMBA-induced necrosis is prevented by inducers of DT-diaphorase [5] may therefore be due to induction of adrenal DT-diaphorase activity. Epoxide hydrolase does not appear to be inducible at all by MC or DMBA.

The steady-state level of an epoxide intermediate of a particular carcinogen is probably one of several parameters that is related to the potency of this carcinogen [13, 38]. In the Sprague–Dawley rat the ratio of epoxide-generating to epoxide-metabolizing systems, i.e. the ratio of AHH to epoxide hydrolase plus conjugating activities, seems to be approximately 5 times higher in the adrenal as compared to the liver in control animals. Provided that a similar relation holds for the Mendel–Osborn rat strain, this may explain the fact that, as compared to the liver, the rate of spontaneous tumor formation in the adrenal of that particular strain is very high [39]. In the ovary, the ratio of AHH to epoxide hydrolase plus conjugating activities is of the same order of magnitude as that of the liver. Nevertheless, the

incidence of ovary cancer is higher as compared to liver cancer in the Mendel–Osborn rat strain [39]. The previously discussed high affinity of the adrenal and ovary for polycyclic aromatic hydrocarbons may contribute to this discrepancy. In adult humans the situation is quite the opposite, i.e. adrenal tumors are rare compared to ovary and liver tumors [40, 41], which correlates well with the very low and high activity of AHH and epoxide hydrolase, respectively, in the adrenal.* The properties of the human ovary with regard to these activities are presently being investigated.

In conclusion, there are several lines of evidence to indicate that the enzymes involved in detoxification reactions in the ovary and adrenal are similar with respect to their properties and regulation. This relationship may be due to the possibility that these reactions in both the adrenal and ovary, in contrast to the liver, are related to steroidogenesis and/or inactivation of steroids rather than detoxification of xenobiotics.

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* D. Papadopoulos, E. Hallberg and J. Rydström, in preparation.

REFERENCES

1. W. L. Heinrich and M. R. Juchau, in *Extrahepatic Metabolism of Drugs and Other Foreign Compounds*, (Ed. T. E. Gram), p. 319. S. P. Medical and Scientific Books, New York (1980).
2. D. W. Nebert, S. S. Thogeirsson and G. H. Lambert, *Envir. Hlth Perspect.* **18**, 35 (1976).
3. D. R. Mattison and S. S. Thorgeirsson, *Cancer Res.* **39**, 3471 (1979).
4. I. P. Lee and R. L. Dixon, *Envir. Hlth Perspect.* **24**, 117 (1978).
5. C. B. Huggins, *Experimental Leukemia and Mammary Cancer. Induction, Prevention and Cure.* University of Chicago Press, Chicago (1979).
6. D. R. Mattison and S. S. Thorgeirsson, *Cancer Res.* **38**, 1368 (1978).
7. I. P. Lee, K. Suzuki, H. Mukhtar and J. R. Bend, *Cancer Res.* **40**, 2486 (1980).
8. H. Mukhtar, I. P. Lee, G. L. Foureman and J. R. Bend, *Chem. biol. Interact.* **22**, 153 (1978).
9. H. Mukhtar, R. M. Philpot and J. R. Bend, *Biochem. biophys. Res. Commun.* **81**, 89 (1978).
10. F. Oesch, H. Glatt and H. Schmassman, *Biochem. Pharmac.* **26**, 603 (1977).
11. T. M. Guenthner, D. W. Nebert and R. H. Menard, *Molec. Pharmac.* **15**, 719 (1979).
12. A. Aitio and M. G. Parkki, *Toxic. appl. Pharmac.* **44**, 107 (1978).
13. J. W. DePierre and L. Ernster, *Biochim. biophys. Acta* **473**, 149 (1978).
14. A. Poland and A. Kende, *Fedn. Proc.* **33**, 2404 (1976).
15. E. V. Jensen, E. Ford and C. B. Huggins, *Proc. natn. Acad. Sci. U.S.A.* **50**, 454 (1964).
16. D. R. Mattison, D. M. West and R. H. Menard, *Biochem. Pharmac.* **28**, 2101 (1979).
17. D. R. Mattison and S. S. Thorgeirsson, *Lancet* **1**, 187 (1978).
18. T. F. Ogle, *J. Steroid Biochem.* **8**, 1033 (1977).
19. L. Ernster, P. Siekevitz and G. E. Palade, *J. Cell Biol.* **15**, 541 (1962).
20. J. Montelius, D. Papadopoulos, M. Bengtsson and J. Rydström, *Cancer Res.* **42**, 1479 (1982).
21. W. H. Habig, M. J. Pabst and W. B. Jacoby, *J. biol. Chem.* **249**, 7130 (1974).
22. C. Lind and B. Höjeberg, *Archs Biochem. Biophys.* **207**, 217 (1981).
23. J. Seidegård, J. W. DePierre, M. S. Moron, K. A. M. Johannesen and L. Ernster, *Cancer Res.* **37**, 1075 (1977).
24. G. L. Sottocasa, in *Biochemical Analysis of Membranes* (Ed. A. H. Maddy), p. 55. John Wiley, New York (1976).
25. T. L. Dao and R. M. Varela, *Cancer Res.* **26**, 1015 (1966).
26. P. Sims, *Biochem. Pharmac.* **19**, 2261 (1970).
27. N. G. Zampaglione and G. J. Mannering, *J. Pharmac. exp. Ther.* **185**, 676 (1973).
28. P. Sims, *Biochem. Pharmac.* **19**, 795 (1970).
29. S. K. Yang and W. V. Dower, *Proc. natn. Acad. Sci. U.S.A.* **72**, 2601 (1975).
30. A. P. Alvares, G. R. Shilling and R. Kuntzman, *Biochem. biophys. Res. Commun.* **30**, 588 (1968).
31. J. W. DePierre, M. S. Moron, K. A. M. Johannesen and L. Ernster, *Analyt. Biochem.* **63**, 470 (1974).
32. A. Y. H. Lu, D. M. Jerina and W. Levin, *J. biol. Chem.* **252**, 3715 (1977).
33. D. N. Weathly, A. G. Hamilton, A. T. Currie, E. Boyland and P. Sims, *Nature, Lond.* **211**, 1311 (1966).
34. J. Rydström, J. Montelius, D. Papadopoulos, E. Hallberg, L. Helfer and M. Bengtsson, in *Biochemistry, Biophysics and Regulation of Cytochrome P-450* (Eds. J.-Å. Gustafsson, J. Carlstedt-Duke, A. Mode and J. Rafter), p. 491. Elsevier/North-Holland, Amsterdam (1980).
35. D. M. Jerina and J. R. Bend, in *Biological Reactive Intermediates: Formation, Toxicity and Inactivation* (Eds. D. Jollow, J. J. Kocsis, R. Snyder and H. Vainio), p. 207. Plenum, New York (1976).
36. C. Lind, H. Vadi and L. Ernster, *Archs Biochem. Biophys.* **190**, 97 (1978).
37. C. Huggins and R. Fukunishi, *J. exp. Med.* **119**, 923 (1964).
38. M. K. Buening, P. G. Wislocki, W. Levin, H. Yagi, D. R. Thakker, H. Akagi, M. Koreeda, D. M. Jerina and A. H. Conney, *Proc. natn. Acad. Sci. U.S.A.* **75**, 5358 (1978).
39. *NIH Rodents.* NIH Publication No 81-606 (1981), US Department of Health and Human Services.
40. O. Bodansky (Ed.), *Biochemistry of Human Cancer.* Academic Press, New York (1975).
41. *Cancer Incidence in Sweden* 1974. National Board of Health and Welfare, Cancer Registry, Stockholm (1980).