selected model of inflammatory reaction. This difference could be explained, according to Vane [1-3], either by a variation in the sensitivity of the prostaglandin synthetases with respect to the anti-inflammatory drugs or by the altered role of prostaglandins in the determination of the various inflammatory responses. In the latter case, the action of the anti-inflammatory agents could be attributed to other mechanisms.

Institut Léon Fredericq. J. DAMAS
Physiologie humaine, normale et pathologique,
and
Laboratoire de Radiobiologie. C. DEBY
32, *blvd de la Constitution,*
University of Liège,
4000-*Liège.*
Belgium

REFERENCES

1. J. R. Vane, *Nature, New Biol.* **231**, 232 (1971).
2. S. H. Ferreira and J. R. Vane, in *The Prostaglandins* (Ed. P. W. Ramwell), Vol. II, p. 1. Plenum Press, New York (1974).
3. S. H. Ferreira and J. R. Vane, *A. Rev. Pharmac.* **14**, 57 (1974).
4. C. Deby, G. Barac and Z. M. Bacq, *Archs int. Pharmacodyn.* **208**, 363 (1974).
5. E. J. Ånggård and C. Larsson, *Acta physiol. scand., Suppl.* **376**, abstr. 15 (1973).
6. C. Larsson and E. J. Ånggård, *J. Pharm. Pharmac.* **25**, 635 (1973).
7. C. Deby, M. Descamps, F. Binon and Z. M. Bacq, *Biochem. Pharmac.* **24**, 1089 (1975).
8. M. Colot, M. Vandamme, M. Dirks, J. Beersaerts and R. Charlier, *Arch. int. Pharmacodyn.* **208**, 328 (1974).
9. M. Colot, M. Vandamme, M. Dirks, J. Beersaerts, G. Deridder and R. Charlier, *Thérapie* **28**, 775 (1973).
10. S. H. Ferreira and B. B. Vargaftig, *Br. J. Pharmac.* **50**, 543 (1974).
11. C. Deby, J. Damas and Z. M. Bacq, *Bull. Acad. R. Méd. Belg.*, in press (1975).
12. G. Van Den Berg, T. Bultsma and W. Th. Nauta, *Biochem. Pharmac.* **24**, 1115 (1975).

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Effect of benzo(a)pyrene and chlorpromazine on aryl hydrocarbon hydroxylase activity from rat tissues

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Aryl hydrocarbon hydroxylase (AHH), an inducible mixed-function oxidase system, is found in animal tissues, essentially those at the portals of entry to the body [1–12]. This enzyme system metabolizes polycyclic hydrocarbons and related compounds [11, 13, 14]. As polycyclic hydrocarbons are found in the environment, the metabolism of such compounds has been the object of many investigations [3, 13, 15–19]. The biotransformation process renders these compounds more water-soluble, and hence more excretable, but during this process of some polycyclic hydrocarbons, toxic intermediates may be formed [20, 21]. A polycyclic hydrocarbon, benzo(a)pyrene, has been implicated through an AHH formed intermediated, as one of the precarcinogens found in cigarette smoke and thereby a contributor to the rapidly increasing lung cancer in man [15, 16] and perhaps to carcinogenesis in other tissues. Genetic and physical factors as well as xenobiotics from drugs, dietary and environmental sources have also been shown to increase or decrease the activity of AHH [19, 22–26].

From recent studies discussed by Gillette, Mitchell and Brodie [24, 26] it appears that mixed-function oxidases of extrahepatic tissues may also be of importance in the development of serious drug toxicities through activated intermediates. Thus there is a need to examine the distribution, levels, induction response and related contribution to total body xenobiotic metabolism, of these enzyme systems.

This communication reports on the distribution and relative levels of AHH in nine rat tissues, with data on the induction response to two xenobiotics, BP and CP,* to which man may become exposed.

The materials were obtained from known commercial sources as indicated in a previous paper [23]. Dr. Harry V. Gelboin of NIH U.S.A. generously donated the 3-OH-benzo(a)pyrene.

Animals. Male rats weighing between 150–200 grams or female rats in a 250–300 gram weight range (on their 15th day of pregnancy) were obtained from the Sprague–Dawley Laboratory in Madison, Wisconsin. Housing was in overhanging steel cages (20 × 11 × 8 in.) over Sanicel as bedding and in well-ventilated rooms at 76° ± 1°F with a 12-hr light cycle. Rat chow (Ralston Purina Co. in St. Louis, Mo.) was fed *ad lib.* The experiment was started after a 5-day acclimation period.

AHH Assay. In vitro drug metabolism was assayed by a microsomal hydroxylase found in various animal species and different tissues that catalyzes the ring hydroxylation of benzo(a)pyrene yielding a mixture of hydroxylated products, the major one being 3-OH-benzo(a)pyrene.

All rats were guillotined and exsanguinated between 9:00 a.m. and 12:00 noon and tissues were used immediately after washing in cold saline. Tissues were removed and prepared in the cold as follows:

Lungs from male rats were scraped with a clean single-edged razor blade to remove connective tissues of the lobar bronchi, their major branches, and the accompanying branches of the pulmonary veins and articles. Lymph nodes from male rats were removed from the mesentery and stripped of fascia. Two rats were pooled for each assay. The submaxillary and sublingual salivary glands from two male rats were pooled for each assay. One kidney from each male rat was removed and minced for assaying. With prostate tissue, two rats were pooled for each determination. Portions of the mammary glands were removed and scraped (as done with lung) to remove connective tissue. The livers of newborn male rats from separate litters were minced into 1-cm pieces and pooled. All tissues were homogenized as previously described [23].

After removing and preparing the tissues the AHH activity was assayed using modifications of the methods of Wattenberg and Kuntzman [1, 27] as previously de-

* Abbreviations used: BP = 3,4-benzo(a)pyrene. CP = chlorpromazine.

Table 1. Benzo(a)pyrene effect on AHH in rat tissues

Group	Tissue	Tissue AHH units* Control	Tissue AHH units* Benzo(a)pyrene treated	Fold increase of mean	Significance P value less than
Adult male rats	Lymph node	0.85 ± 0.62 (5)	9.40 ± 1.91 (5)	11.1	0.001
	Lung	7.12 ± 0.90 (6)	73.33 ± 25.02 (2)	10.3	0.05
	Prostate	2.56 ± 1.25 (5)	13.24 ± 1.57 (5)	5.2	0.001
	Kidney	51.77 ± 4.03 (6)	192.01 ± 38.82 (6)	3.7	0.01
	Salivary glands	1.43 ± 0.22 (6)	3.91 ± 0.53 (6)	2.7	0.01
	Liver	857.56 ± 82.06 (6)	1955 ± 185.73 (6)	2.3	0.001
	Spleen	4.45 ± 0.43 (6)	8.90 ± 1.19 (6)	2.0	0.001
Adult female rats	Mammary gland (lactating)	1.25 ± 0.89 (5)	11.39 ± 1.60 (5)	9.1	0.001
Newborn male rats	Liver	350.45 ± 33.21 (5)	552.72 ± 61.81 (5)	1.6	0.05

Benzo(a)pyrene in 5% tween 80 and 0.9% saline was dosed i.p. to adult male rats at 25 mg/kg 1 day prior to enzyme assay. Controls were treated with Tween–saline vehicle only. Adult female rats were dosed similarly but three times, 2–4 days *post partum* every 72 hr. The lactating mammary glands of the mothers and the livers of the suckling male young were assayed on the 10th day post partum. The number of experiments is reported in brackets. Each experiment was performed with tissue from 1 adult or 3–6 newborn rats.

* One AHH unit = 1.0 pg of 3-OH-benzo(a)pyrene formed/mg wet wt tissue/min. Results are given as means ± S.E.M.

scribed [23] except that the incubation medium contained 215 μmole KCl, 10 μmole $MgCl_2$, 3·5 μmole NADPH, 3·9 μmole NADH, 0.5 ml 12,000 *g* tissue supernatant and then sufficient 0.1 M K_2HPO_4–KH_2PO_4 buffer pH 7.4 to a total volume of 3.0 ml. The reaction was then initiated by the addition of 100 μg of benzo(a)pyrene in 0.1 ml acetone. After incubation for 15 min at 37°, separation and estimation of the products was performed on an Amico–Bowman Spectrophotofluorometer as previously described [23].

The importance of AHH in extra-hepatic tissues as a protective device for metabolizing polycyclic hydrocarbons at the portals of entry to the body has been the subject of a number of communications [1–3, 13, 15–19]. AHH has previously been reported in intestine, lung, kidney, skin, placenta, testes and adrenals. In light of the importance of AHH mediated metabolism, in this laboratory, other portals of entry or exit to the body were investigated.

The data in Table 1 indicate that from rats on a Purina Chow diet, there is an endogenous AHH activity in the lymph node, prostate, salivary glands and lactating mammary gland in addition to those tissues previously reported. Although the endogenous levels of AHH are quite low in the extrahepatic tissues when compared to liver, after induction with PB, the fold increase is equal to or greater than that for liver. The importance of this induction is that these tissues function as portals of entry or exit in the body in metabolism of xenobioties. It is noteworthy however that liver AHH and to a lesser extent kidney and lung AHH provide the primary means of metabolizing polycylic hydrocarbons in both BP induced and non-induced animals. Liver tissue has approximately

Table 2. Chlorpromazine effect on AHH in rat tissues

Group	Tissue	Tissue AHH units* Control	Tissue AHH units* Chlorpromazine treated	Fold increase of mean	Significance P value less than
Adult male rats	Lung	12.46 ± 1.86 (6)	50.20 ± 11.68 (6)	4.0	0.01
	Kidney	54.38 ± 10.39 (6)	113.43 ± 7.57 (6)	2.1	0.001
	Liver	798.90 ± 60.27 (6)	964.92 ± 75.74 (6)	1.2	0.05
	Salivary glands	1.92 ± 0.52 (6)	2.85 ± 0.71 (6)	1.5	N.S.
	Prostate	5.13 ± 1.28 (5)	5.55 ± 1.98 (5)	1.1	N.S.
	Spleen	4.27 ± 0.48 (6)	4.10 ± 0.70 (6)	1.0	N.S.
	Lymph node	3.14 ± 0.62 (5)	2.35 ± 0.51 (5)	0.7	N.S.
Adult female rats	Mammary gland (lactating)	2.67 ± 1.60 (5)	4.70 ± 1.17 (5)	1.8	N.S.
Newborn male rats	Liver	356.46 ± 55.25 (5)	392.51 ± 91.08 (5)	1.1	N.S.

Chlorpromazine in 0.9% saline was dosed i.p. to adult male rats at 20 mg/kg 1 day prior to enzyme assay. Controls were treated with saline vehicle only. Adult female rats were dosed similarily but three times, 2–4 *post partum* every 72 hr. The lactating mammary glands and the livers of the suckling male young were assayed on the 10th day. The number of experiments is reported in brackets. Each experiment was performed with tissue from 1 adult or 3–6 newborn rats.

* One AHH unit = 1.0 pg of 3-OH-benzo(a)pyrene formed/mg/wet wt tissue/min. Results are given as means ± S.E.M.

120-fold more AHH activity than lung tissue and approximately 17-fold more AHH activity than kidney tissue under normal conditions i.e., without BP induction but on a Purina Chow diet. Upon treatment of rats with BP however, the induced liver AHH activity becomes only 27-fold greater than lung and only 10-fold greater than kidney AHH activity. Hence, upon induction kidney and lung tissues become quantitatively more significant in the metabolism of the total body intake of hydrocarbons. Of importance also is that lymph node, prostate, mammary glands and salivary glands can be induced up to levels approaching that of 'normal' lung.

The data in Table 2 indicates that CP, a commonly used drug, given in one low dose can induce significantly lung and kidney as well as liver. As with the BP induced animals, the liver again plays the most significant role in metabolizing polycyclic hydrocarbons but the kidney and lung become quantitatively more important in metabolizing these hydrocarbons after induction with CP [28].

Lactating female rats were given PB or CP in three doses, 72 hr apart to determine if the mammary glands could be induced. BP induces the mammary gland AHH activity 9.1-fold in this experiment but even so, in preliminary studies some BP (or metabolites) appear to be secreted and to induce the newborn rat livers. A previous report [29] with methylcholanthrene dosing indicated that this might be the case, for tumors were found in the young, however, no determinations of AHH levels were made. The increase in the mammary gland AHH activity is of interest in that apparently there was not full protection of the young even by this induction. This suggests that in lactating women who smoke heavily, BP might possibly be secreted into the milk. A parallel case can be made from the reports that the placenta, from smoking pregnant women [13, 30], has induced levels of AHH. One wonders how much BP gets through to the fetus even though this portal of entry the placenta has been induced and what this does to fetal steroid levels [31].

The results reported above indicate that AHH is an enzyme system which can be measured and induced in many rat tissues. This AHH activity in these various tissues is of importance not only in protective tissues of entry into the body, but also in light of the focusing knowledge on carcinogenic intermediates of certain polycyclic hydrocarbons and toxic intermediates of certain drugs [15–17, 19–21]. This paper expands the number of tissues which may form such intermediates. Of importance also is that the significant metabolism of polycyclic hydrocarbons occurs primarily via the liver, but upon induction with either BP or CP, kidney and lung tissues apparently become much more significant in metabolizing these hydrocarbons to which the animal has or may become exposed.

Department of Pharmacology, Hahnemann Medical College and Hospital, Philadelphia, Pennsylvania 19102, U.S.A.

EDWARD I. CIACCIO
HERMAN DE VERA*

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REFERENCES

1. L. W. Wattenberg, L. J. Leong and P. J. Strand, *Cancer Res.* **22,** 1120 (1962).
2. H. V. Gelboin and N. R. Blackburn, *Cancer Res.* **24,** 356 (1964).
3. L. W. Wattenberg and L. J. Leong, *Cancer Res.* **25,** 365 (1965).
4. R. M. Welch, V. E. Harrison and A. H. Conney, *Science, N.Y.* **160,** 541 (1968).
5. D. W. Nebert and H. V. Gelboin, *Archs Biochem. Biophys.* **134,** 76 (1969).
6. E. Schlede, R. Kuntzman, S. Haber and A. H. Conney, *Cancer Res.* **30,** 2893 (1970).
7. F. J. Weibel, J. C. Lentz, L. Diamond and H. V. Gelboin, *Archs Biochem. Biophys.* **144,** 78 (1971).
8. L. W. Wattenberg, *Cancer Res.* **28,** 99 (1971).
9. G. Kellermann, C. R. Shaw and M. L. Kellermann, *New. Engl. J. Med.* **289,** 934 (1973).
10. T. S. Tamulski, C. E. Morreal and T. L. Dao, *Cancer Res.* **33,** 3117 (1973).
11. B. G. Lake, R. Hopkins, J. Chakrabory, J. W. Bridges and D. V. Parke, *Drug Metab. Disp.* **1,** 342 (1973).
12. L. Wheeler and A. Strother, *Drug Metab. Disp.* **2,** 533 (1974).
13. A. H. Conney, R. Welch, R. Kuntzman, R. Chang, M. Jacobson, A. D. Monro-Faure, A. D. Peck, A. W. Bye, A. Poland, P. J. Pappers, M. Finster and J. A. Wolff, *Ann. N.Y. Acad. Sci.* **179,** 155 (1971).
14. L. Diamond, R. McFall, J. Miller and H. V. Gelboin, *Cancer Res.* **32,** 731 (1972).
15. C. Heidelberger, *Fedn Proc.* **32,** 2154 (1973).
16. J. A. Miller and E. C. Miller, *J. natn. Cancer Inst.* **47,** V. (1971).
17. J. A. Miller, in *'Toxicants Occuring Naturally in Foods'* (Ed. by F. M. Strong). Nat. Acad. Sci., Washington, D.C. (1974).
18. T. Maugh II. *Science, N.Y.* **183,** 940 (1974).
19. A. H. Conney and J. J. Burns, *Science, N.Y.* **178,** 576 (1972).
20. P. L. Grover, A. Hewer and P. Sims, *Biochem. Pharmac.* **23,** 323 (1974).
21. P. L. Grover, *Biochem. Pharmac.* **23,** 333 (1974).
22. D. P. Sanvordeker and H. J. Lambert, *Drug Metab. Rev.* **3,** 201 (1974).
23. R. P. Carlson and E. I. Ciaccio, *Biochem. Pharmac.* in press.
24. J. R. Gillette, J. R. Mitchell and B. B. Brodie, *A. Rev. Pharmac.* **14,** 271 (1974).
25. J. R. Gillette, *Biochem. Pharmac.* **23,** 2785 (1974).
26. J. R. Gillette, *Biochem. Pharmac.* **23,** 2927 (1974).
27. R. Kuntzman, L. C. Mark, L. Brand, M. Jacobson, W. Levin and A. H. Conney, *J. Pharmac. exp. Ther.* **152,** 151 (1966).
28. K. C. Aurori and E. S. Vesell, *Drug Metab. Disp.* **2,** 566 (1974).
29. H. Shay, S. Weinhouse, M. Gruenstein and B. Freedman, *Cancer* **3,** 891 (1960).
30. M. R. Juchau and P. K. Zachariah, *Biochem. Pharmac.* **24,** 227 (1975).
31. T. R. Tephly and G. J. Mannering, *Molec. Pharmac.* **4,** 10 (1968).