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KINETICS, ACTIVATION, AND INDUCTION OF AORTIC MONO-OXYGENASES— BIOTRANSFORMATION OF BENZO[A]PYRENE*,†

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Abstract—Aortic aryl hydrocarbon hydroxylase (EC 1.14.14.2), a cytochrome P-450-dependent mono-oxygenase complex potentially important in the etiology of atherosclerosis, was detected previously in aortic homogenates of humans, monkeys and rabbits [M. R. Juchau, J. A. Bond and E. P. Benditt, *Proc. natn. Acad. Sci. U.S.A.* **73**, 3723 (1976)]. The present study more fully characterizes the mono-oxygenase activity in aortas of treated and untreated New Zealand White rabbits. A 2-fold activation was obtained with NADH (7×10^{-4} M) at saturating concentrations of NADPH. Addition of heme (9 μM) increased the enzymatic activity 2- to 4-fold during a 15-min incubation and over 25-fold during a 2-hr incubation. The results suggest the presence of relatively high concentrations of apoprotein in the aortic tissues. Kinetic studies in the presence of heme yielded an apparent K_m of 1×10^{-4} M and V_{max} of 15.24 pmoles/mg of protein/min with respect to NADPH. A sigmoidal curve was obtained with varying benzo[a]pyrene concentrations (0.5 to 80 μM), suggesting the possibility of allosterism. Aroclor 1254, 3-methylcholanthrene and 5,6-benzoflavone acted to induce the cytochrome P-450-dependent mono-oxygenase, while phenobarbital and pregnenolone 16α-carbonitrile demonstrated little, if any, induction capacity. Analyses of metabolites formed in induced aortas with high-pressure liquid chromatography revealed the formation (in each case) of primarily the phenolic metabolites of benzo[a]pyrene.

Focal proliferation of smooth muscle cells in the intima of the aorta is a necessary requirement for the genesis and development of atherosclerosis in man and experimental animals [1–4]. Benditt and Benditt [5] have provided evidence that human atherosclerotic plaques are monoclonal in origin. Their data indicated that the focal lesions of large atherosclerotic plaques in black females were composed of cells that produce only one of the isoenzymes of glucose 6-phosphate dehydrogenase (G-6-P-D), while the surrounding nonplaque tissue of the intima was a mosaic of cells containing different isoenzymes of G-6-P-D. It has also been found that uterine leiomyomas, which are benign smooth muscle tumors, and certain pre-neoplastic lesions are derived from single precursor cells [6]. These data imply that the smooth muscle cells of the aorta may undergo some type of cellular alteration, presumably a mutation, to cause a subsequent formation of intimal lesions.

Therefore, it seems possible that environmental procarcinogens (or promutagens) could traverse the walls of the aorta, be metabolically activated, and initiate atherosclerotic lesions by means of a somatic cell mutation. Recent evidence in our laboratory has demonstrated that the walls of the aorta in humans, monkeys and rabbits possess a mixed-function oxygenase system, aryl hydrocarbon hydroxylase (EC 1.14.14.2) (AHH), that has been heavily implicated in the conversion of promutagens to proximate or ultimate mutagens [7]. In addition, treatment of chickens with two known promutagens, benzo[a]pyrene (BP) and 7,12-dimethylbenz[a]anthracene (DMBA), gave rise to increases in both the number of plaques formed in the aorta and in the rate of plaque development, without a concomitant rise in blood levels of cholesterol [8]. Treatment of mice with 3-methylcholanthrene (3-MC) [9] also appeared to produce an increased incidence of sclerotic lesions. The importance of these findings lends itself to support of the hypothesis that promutagens can act to initiate atherosclerotic lesions.

If the hypothesis is to be tested critically, it is first necessary to demonstrate the presence of mono-oxygenase activity in the aortas of various species. As alluded to earlier, this has been done [7]. An obvious extension of this finding is a more extensive characterization of the aortic enzyme activity such that one can more fully understand the "behavior" of this aortic enzyme complex. The following report focuses on the analysis of the biotransformation of promutagens in the aorta as determined by three separate methods [radiometric, fluorometric and high-pressure liquid chromatography [h.p.l.c.]). The kinetics of the reaction were investigated and, in addition, we studied the effects of a number of activators added *in vitro*. The effects of several inducing agents on aortic mono-oxygenase activity were also compared.

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MATERIALS AND METHODS

Tissues. Thoracic and abdominal aortas of New Zealand White rabbits were obtained from Totem Farms/Lab Associates, Kirkland, WA. Less than 2 hr elapsed between the time of death and delivery of aortas to the laboratory, during which time the tissues were maintained at 0–4°. (No loss of activity could be detected during the 2-hr period.) While on ice, individual aortas were stripped of nearly all excess fatty tissue. Aortas then were pooled, weighed, and homogenized with 4 vol. of cold potassium phosphate buffer (0.1 M, pH 7.35). Due to the fibrous and elastic nature of the tissue, homogenization was carried out with a Polytron PT10 homogenizer for 60 sec at a setting of 6. The procedure was performed for two 30-sec intervals at 4°. After each interval, aortas were allowed to cool for 60 sec. Homogenates were centrifuged at 10,000 *g* (International model B 20) for 20 min at 4°. The pellets were discarded and the supernatant fluid was collected for either immediate use or storage (−80°) for subsequent use. Protein values were determined by the method of Lowry *et al.* [10] using bovine serum albumin as a standard.

Chemicals. [7,10-^{14}C]-BP [sp. act.: 25.3 mCi/mmole, 98 per cent pure by thin-layer chromatography (T.L.C.) on silica gel in hexane fraction] was obtained from Amersham/Searle, Arlington Heights, IL. Hematin was obtained from CalBiochem, Los Angeles, CA; 5,6-benzoflavone from Aldrich Chemical Co., Milwaukee, WI; Aroclor 1254 from Monsanto Co., St. Louis, MO; 3-methylcholanthrene from Mann Research Lab., New York, NY; benzo[a]pyrene from Eastman Kodak Co., Rochester, NY; and Aquasol and Omnifluor from New England Nuclear, Boston, MA. NADPH, NADH, bovine serum albumin (BSA), glucose 6-phosphate dehydrogenase and glucose 6-phosphate (G-6-P) were obtained from Sigma Chemical Co., St. Louis, MO. Pregnenolone 16α-carbonitrile (PCN) was a gift from G. D. Searle & Co., Chicago, IL. All other reagents and chemicals utilized were of the highest purity commercially available.

Enzyme assays and analyses. Three separate methods were utilized in the analyses of benzo[a]pyrene metabolism. A standard fluorometric assay was employed for the detection of very low levels of enzyme activity. A radiometric assay was utilized for cases in which heme was added as an activator of the system since heme interferes with the standard fluorometric assay. The radiometric assay provides a method for the estimation of rates of formation of all phenolic metabolites. High-pressure liquid chromatography was utilized in order to assess the formation of various phenols, quinones and dihydrodiols.

Analysis of the rates of formation of the 3-hydroxy-metabolite of BP (BP-3-ol) from aortic and liver homogenates was performed with the highly sensitive fluorometric assay [11], modified slightly [12]. Aortic whole homogenates, equivalent to 6–7 mg protein, were incubated with vigorous shaking in a Dubnoff incubator at 37.5° for 3 hr under 100% oxygen tension. Incubation flasks contained BP (9.98×10^{-5} M, final concentration), G-6-P (2.50×10^{-3} M, final concentration), 2 units of G-6-P-D, NADPH (1.15×10^{-3} M, final concentration), and NADH (0–1.41×10^{-3} M), BSA (0–1.0 mg/ml) $MgCl_2$ (0–0.2 M), or nicotinamide (0–2.25×10^{-3} M), and sufficient potassium phosphate buffer (0.1 M, pH 7.35) to bring the total volume to 2 ml. Control flasks were incubated in the absence of NADPH and samples were run in duplicate. Liver 10,000 *g* supernatant fraction, equivalent to 1.5 to 2.5 mg protein, was incubated under the same conditions except that typical incubation flasks contained BP (2.0×10^{-4} M, final concentration), G-6-P (2.5×10^{-3} M, final concentration) and NADPH (1.31×10^{-3} M, final concentration) brought to a 2-ml total volume with potassium phosphate buffer (0.1 M, pH 7.35). Control flasks for the livers consisted of BP blanks run in triplicate. All reactions were started by the addition of BP.

Analyses of the rates of formation of phenolic metabolites of BP from aortic homogenates were performed with a radiometric assay utilizing [7,10-^{14}C]-BP as substrate [13] and slight modifications as described below. The selection of the radiometric assay for BP hydroxylation was based on earlier observations by Brown and Kupfer [13] that the presence of heme in the incubation mixture yielded non-reproducible results when the fluorometric assay was utilized. Furthermore, these investigators noted that heme quenches the fluorescence of the BP-3-ol metabolite of BP. Because of the positive results obtained in the studies involving heme as an activator, this assay was the one most frequently employed in our study.

Aortic 10,000 *g* supernatant fraction equivalent to 3–5 mg protein was incubated with vigorous shaking in a Dubnoff incubator at 37.5° for 15 min or 2 hr (as specified below) under 100 per cent oxygen tension. Typical incubation flasks contained: [7,10-^{14}C]-BP (100,000 dis./min), BP (40 nmoles), NADPH (1.1×10^{-3} M, final concentration), NADH (7.0×10^{-4} M, final concentration), heme (0 to 9.0 μM, final concentration), and sufficient potassium phosphate buffer (0.1 M, pH 7.35) to bring the total volume to 1.0 ml. Additions of BSA (0.5 mg/ml) and $MgCl_2$ (5×10^{-3} M, final concentration) in the presence and absence of heme were also studied with both a 15-min and a 2-hr incubation period. Sample blanks consisted of heat-inactivated (100°, 5 min) tissue fractions. All samples were run in triplicate and reactions were started by the addition of BP. Phenolic metabolites were extracted into 1 N NaOH [13], neutralized with HCl, and samples were counted in 10 ml Aquasol on a Beckman 9000 series liquid scintillation counter with a counting efficiency consistently greater than 85 per cent.

Kinetic studies with respect to BP and NADPH. The radiometric assay was utilized in this portion of the investigation. Because of the inherently low mono-oxygenase activity in the aorta [7], it was necessary to use very high specific activities of [7,10-^{14}C]-BP. Varying concentrations of BP (0.5 to 80 μM, 10^5–10^7 dis./min) or NADPH (0–3.0×10^{-3} M) were added to standard incubation flasks. When studying enzyme kinetics with respect to BP, the ratio of the concentration of radiolabeled BP to the molar concentration of total BP in incubation vessels was held constant in order to avoid further dilution of the labeled substrate.

Induction studies. Thirty New Zealand White male rabbits (Totem Farms/Lab Associates, Kirkland, WA) approximately 8 weeks of age and weighing 1.7 to 2.0 kg were placed into six groups of five rabbits each. Animals were housed at the animal facility (Animal Medicine) at the University of Washington, placed on a 12-hr light/dark cycle and fed commercial grade rabbit chow (Wayne Lab-blox for rabbits) and water *ad lib.* After a 1.5 day acclimatization period, animals were treated as follows: group 1, corn oil (1 ml/kg); group 2, 3-MC (40 mg/kg) in corn oil; group 3, Aroclor 1254 (500 mg/kg) in corn oil; group 4, phenobarbital (20 mg/kg) in 0.9% saline; group 5, PCN (80 mg/kg) in H_2O plus 1 drop of Tween 80; and group 6, 5,6-benzoflavone (20 mg/kg) in corn oil. All animals were injected i.p. once daily for 5 days, killed on day 7, and their aortas excised and prepared as discussed above. Rabbits treated with the Aroclor 1254 regimen were killed early (day 5) due to the generally debilitated appearance of the animals. In spite of this, the magnitude of induction with Aroclor 1254 was high and in accord with that observed in tissues of healthy animals in other laboratories.

Radiometric assays for the induction studies were performed on both the aortas and livers from the different treatment groups. Because of the small quantity of supernatant fluid obtained from individual aortas, it was necessary to pool all the aortas from the respective groups. The effects of the various inducing agents on enzymatic activity in aortas were studied in the presence and absence of heme. Optimal concentrations obtained in the kinetic studies of BP (80.0 μM, 222,000 dis./min) and NADPH (1.2×10^{-3} M) were utilized for these assays. Incubation times were 1 hr for aortic preparations and 15 min for hepatic preparations.

High-pressure liquid chromatography analyses. Acetone–hexane (2:8) extracts containing BP metabolites from triplicate incubation flasks containing aortic tissue preparations were pooled for each of the six different treatments as discussed above. The pooled extracts were evaporated to dryness under gentle nitrogen flow in the dark. The metabolites were redissolved in 4.0 ml methanol, which then was evaporated to near dryness and reconstituted to 1.0 ml in methanol. Eight μl of the methanolic solution was injected into a high-pressure liquid chromatograph (Micromeritics model 7000-011) for analyses. The metabolites were separated with a 2.1 mm × 8.0 cm Vydac Reverse Phase (the Separations Group, Hesperia, CA) precolumn connected to a 4.1 mm × 25 cm Partisil ODS microparticulate column (Whatman, 10 μm) operated at ambient temperature with a constant flow rate of 1.0 ml/min and a 30–100% methanol–water gradient. Individual fractions were collected at 24-sec intervals directly into scintillation-counting vials. Samples were collected for 30 min. Scintillation fluors (12 g Omnifluor in 1 liter of Triton X-100 and 2 liters of toluene) were added and the samples were counted in a Beckman 9000 series liquid scintillation counter with a counting efficiency consistently greater than 85 per cent. Sufficient counts were accumulated to provide for 95 per cent confidence intervals. No significant radioactivity (<1 per cent) remained in the aqueous phase. Retention times of the radioactive metabolites were compared with the standard BP compounds listed in Table 1.

Table 1. High-pressure liquid chromatography retention times for standard compounds (BP and synthesized metabolites) utilized in the described experiments

Reference compound	Retention time (min)	Fraction No.*
BP	30.0	75
BP-3-ol	26.8	67
BP-9-ol	25.6	64
BP-6,12-quinone	23.2	58
BP-3,6-quinone	22.0	55
BP-4,5-quinone	21.6	54
BP-1,6-quinone	20.0	50
BP-4,5-epoxide	19.2	48
BP-*trans*-7,8-diol	18.0	45
BP-*cis*-4,5-diol	17.2	43
BP-*trans*-4,5-diol	17.2	43
BP-*trans*-9,10-diol	12.4	31

* Fraction in which the peak concentration appeared for each reference standard.

RESULTS

Results from the determination of optimal conditions that yielded maximal mono-oxygenase activity are presented in Table 2. In the fluorometric assay, additions of NADH, $MgCl_2$ and BSA increased AHH activity in a concentration-dependent fashion with maximal activity seen at 7.15×10^{-4} M, 2.35×10^{-3} M, and 0.5 mg/ml respectively. Heme activation in the radiometric assay was also seen to be concentration-dependent with maximal activity at 6–9 μM. Addition of nicotinamide had very little

Table 2. Per cent activation of aortic AHH with different modifiers.*

Incubation time	modifier	Conc	per cent of control
3 hr	NADH	0	100
		3.5×10^{-4} M	150
		7.1×10^{-4} M	208
		1.4×10^{-3} M	175
3 hr	Mg^{2+}	0	100
		2.3×10^{-3} M	162
		5.0×10^{-3} M	127
		1.0×10^{-2} M	138
		2.0×10^{-2} M	53
3 hr	BSA	0	100
		0.125 mg/ml	126
		0.5 mg/ml	184
		1.0 mg/ml	109
		2.0 mg/ml	131
15 min	Heme	0	100
		1 μM	104
		2 μM	109
		3 μM	133
		4 μM	159
		6 μM	184
		9 μM	179

* All data were derived from fluorometric analyses except that from heme (radiometric). Controls = 100 per cent (see Materals and Methods).

Table 3. Per cent activation of aortic AHH with combinations of modifiers using the radiometric assay

Time (min)	Modifier	Per cent of control*
15	None	100
15	BSA + Mg^{2+}	142
15	Heme	371
15	BSA, Mg^{2+}, Heme	190
120	None	100
120	BSA + Mg^{2+}	100
120	Heme	2751
120	BSA, Mg^{2+}, Heme	2367

* Control = 100 per cent (see Materals and Methods).

effect on enzymatic activity, and in fact was seen to be inhibitory at a concentration of 6.25×10^{-4} M. The results with BSA correspond well with those of other investigators [14] who also found enhanced hydroxylation of BP, but with 1.0 mg/ml of BSA. In the radiometric assay, the optimal concentrations of BSA and $MgCl_2$ were seen to have no effects on enzymatic activity at a 2-hr incubation, but about a 40 per cent activation for a 15 min incubation (Table 3). These same concentrations of BSA and $MgCl_2$ were also slightly inhibitory in the presence of heme (Table 3). Heme (1–9 μM) increased enzymatic hydroxylase activity about 2.5-fold for a 15 min incubation period, but over 25-fold for a 2-hr incubation (Table 3). Additions of fresh heme to heat-inactivated tissue preparations or additions of heme to incubation flasks containing no tissue protein were made in order to examine the possibility that the increased activities observed were due to a non-enzymatic reaction catalyzed by hematin; no activity was observed in either case.

Earlier work [7] indicated that, in both induced and uninduced rabbit aortas, the formation of products using BP as substrate was linear for extended time periods. Times of incubation utilized in this study were within the period of linearity. Kinetic studies with varying concentrations of BP

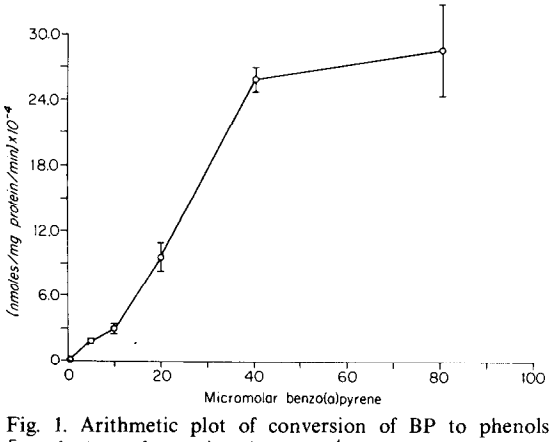

Fig. 1. Arithmetic plot of conversion of BP to phenols [nmoles/mg of protein/min $\times 10^{-4}$ vs BP concentration (μM)]. Each point represents samples run in triplicate. Vertical bars represent the mean $\pm$ S. D. The final protein value was 2 mg/ml and incubation time was 15 min.

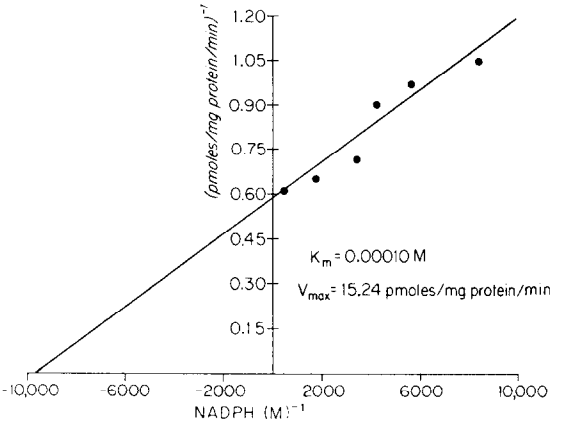

Fig. 2. Double reciprocal plot of conversion of BP to phenols [nmoles/mg of protein/min vs NADPH concentration (M)]. Each point represents samples run in triplicate. The final protein value was 2 mg/ml and incubation time was 15 min.

(0–80 μM) produced a sigmoid curve (Fig. 1). Double-reciprocal and Scatchard plots of these data were non-linear. The apparent K_m and V_{max} could be estimated, however, from the arithmetic curve. An apparent K_m of 2.7×10^{-5} M and a V_{max} of 2.86 pmoles/mg of protein/min were estimated (at suboptimal NADPH concentrations). This experiment was performed twice, with similar kinetics observed in each experiment. Because the sigmoid curve suggested the possibility of cooperativity, a Hill coefficient was determined in order to provide an estimate of the number of subunits involved. Hill plots of these data, however, consistently yielded a Hill coefficient of n = 1, suggesting the absence of any apparent cooperativity. Kinetic analysis with respect to varying concentrations of NADPH yielded the more classical Michaelis–Menten kinetics. Lineweaver–Burk plots of the data yielded an apparent K_m of 1.36×10^{-4} M and a V_{max} of 13.17 pmoles/mg of protein/min. Replication of this experiment yielded an apparent K_m of 1.04×10^{-4} M and a V_{max} of 15.24 pmoles/mg of protein/min (Fig. 2). Accurate K_m and V_{max} values were not

Table 4. Formation of phenolic products from BP in 10,000 g fractions of rabbit livers and aortas incubated in the presence or absence of heme*

Treatment	Aorta incubated in absence of heme	Aorta incubated in presence of heme	Liver incubated in absence of heme
Corn oil	0.42 ± 0.18	9.75 ± 1.59	19.00 ± 0.30
Aroclor 1254	1.95 ± 0.06	14.85 ± 1.56	11.64 ± 1.65
3-MC	1.20 ± 0.12	11.82 ± 0.66	29.16 ± 1.80
Phenobarbital	0.45 ± 0.09	11.16 ± 1.86	30.90 ± 0.32
PCN	0.48 ± 0.12	14.28 ± 1.08	20.05 ± 1.21
5.6-Benzoflavone	0.66 ± 0.12	10.35 ± 0.75	20.76 ± 1.58

* Each value reported represents the mean specific activity (pmoles/mg of protein/min) ± S. D. of triplicate determinations (duplicate for livers) obtained with pooled samples of five rabbits. Heme concentration: 9 μM. Aortic preparations were incubated for 1 hr, hepatic preparations for 15 min. For details of incubation, see Materials and Methods.

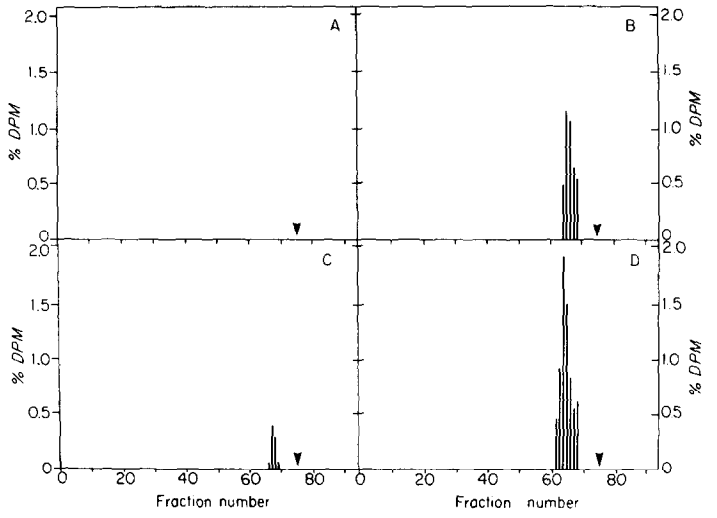

Fig. 3. High-pressure liquid chromatographic profiles of [^{14}C]-BP metabolites formed in incubation flasks containing 10,000 *g* fractions of (A) control aortas incubated without heme, (B) control aortas incubated with heme, (C) aortas from 5,6-benzoflavone-pretreated rabbits incubated without heme, and (D) aortas from 5.6-benzoflavone-pretreated rabbits incubated with heme. Final protein concentrations in reaction flasks were: A and B, 1.8 mg/ml; C and D, 2.4 mg/ml. Arrows represent the peak fraction that co-chromatographed with benzo[a]pyrene.

ascertainable in the absence of heme due to the extremely low enzymtic activity. However, the specific activities presented represent V_{max} since 2-fold increases in BP or NADPH did not result in increased activity (data not shown).

Results from the induction study are presented in Table 4. Most evident from the table is the fact that Aroclor 1254, 3-MC and 5,6-benzoflavone acted to induce the cytochrome P-450-dependent aortic monooxygenase. On the other hand, phenobarbital and PCN demonstrated little, if any, induction capacity in these aortas. Data from livers are presented for comparative purposes. Interestingly, when aortas were incubated in the presence of heme, the induction previously seen with Aroclor 1254, 3-MC and 5,6-benzoflavone was somewhat masked, and specific activities were increased markedly in both treated as well as control groups. For example, the 5-fold induction observed with Aroclor 1254-pretreated aortas incubated with heme was seen to be only about 1.5-fold when incubated with heme. The 3-fold induction observed with 3-MC-pretreated aortas incubated without heme was only 1.2-fold when incubated with heme. Similar trends are seen with the other treatments.

The results from the investigation of metabolic

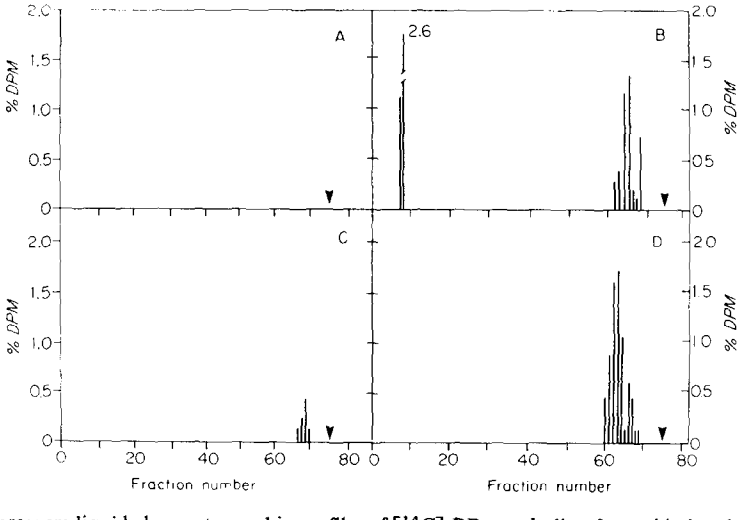

Fig. 4. High-pressure liquid chromatographic profiles of [^{14}C]-BP metabolites formed in incubation flasks containing 10,000 *g* fractions of (A) aortas from polychlorinated biphenyl-pretreated rabbits incubated without heme, (B) aortas from polychlorinated biphenyl-pretreated rabbits incubated with heme, (C) aortas from 3-MC-pretreated rabbits incubated without heme, and (D) aortas from 3-MC-pretreated rabbits incubated with heme. Final protein concentrations in reaction flasks were: A and B, 1.9 mg/ml; C and D, 2 mg/ml. Arrows represent the peak fraction that cochromatographed with benzo[a]pyrene.

profiles with h.p.l.c. are presented in Figs. 3 and 4. With the exception of Fig. 4B (Aroclor 1254 pretreatment), all the metabolites appeared in fractions that cochromatographed with the BP-3-ol and the 9-hydroxy metabolite of BP (BP-9-ol). This does not, however, suggest that only these metabolic products were formed in aortic metabolism of BP. Indeed, given the low mono-oxygenase activity in rabbit aortas, it should be expected that only those metabolic products that are typically produced in higher concentrations would be observed in h.p.l.c. profiles. No detectable metabolites cochromatographed with BP-9-ol when analysis was performed on incubations without the additions of heme. No phenolic products were detected in incubations containing aortic tissues from phenobarbital or PCN-pretreated rabbits if heme was omitted from incubation flasks. Additions of heme resulted in h.p.l.c. profiles that were very similar to those exhibited with other treatments. Therefore, other profiles are not shown.

DISCUSSION

Cytochrome P-450-dependent mono-oxygenase has been found in aortas of humans, monkeys and rabbits [7]. If one accepts the hypothesis that the focal proliferation of smooth muscle cells in the intima of the aorta is due to a somatic cell mutation [5], then it becomes of paramount importance to investigate more fully this enzyme complex which is strongly implicated in the conversion of promutagens to ultimate mutagens and carcinogens. The results from the present study have contributed to a greater understanding of this enzyme complex.

The results presented in this study are consistent with those seen in research on other extra-hepatic tissues. Induction and activation are known to increase the specific activities of various hepatic and extra-hepatic mono-oxygenases. Presently, we have shown that the enzyme complex is capable of activation by NADH, $MgCl_2$, BSA and heme. This activation has allowed for a better characterization of the aortic mono-oxygenase but, in addition, has elevated the levels of enzymatic activity such that accurate assessments of metabolic profiles and other characteristics could be made.

Activation of aortic mono-oxygenase activity by NADH suggests that the second electron transfer to the ternary complex (oxycytochrome P-450) may be mediated via cytochrome b_5 [15] or that NADH (via cytochrome b_5) provides an electron reservoir to prevent lipid peroxidative destruction of P-450 during partial uncoupling when substrate and molecular oxygen are both bound to the P-450 enzyme active-site [16].

The magnitude of heme activation revealed an aspect of the enzyme complex that appeared to set it apart from hepatic mono-oxygenases. Further work will be necessary to determine whether this phenomenon in unique to the aorta, although preliminary studies in our laboratory with the lung, testes and kidney indicate much less activation in those tissues. A 20–40 per cent activation of BP hydroxylase by heme has been reported for hepatic tissues [13]. The work reported in this paper, however, reveals more than a 25-fold activation by heme after a 2-hr incubation. This suggests the presence in the aortic homogenates of a relatively large pool of free apocytochrome P-450. Previous investigators have postulated the existence of a free apocytochrome P-450 in mammalian livers [17–19]. The marked increase in specific activities observed when aortas were incubated with heme seemed to indicate that increased heme incorporation into the apoprotein masked the effect of the inducers. These data might be explained by imagining that the inducers stimulate the incorporation of heme into the apoprotein. Studies in hepatic systems [20] would tend to contradict this viewpoint.

Treatment of rabbits with various inducing agents confirmed our earlier report [7] of the inducibility of mono-oxygenase by 3-MC (about 2.5-fold). More interesting was the nearly 5-fold induction seen with Aroclor 1254 (PCB).* The pervasiveness with which our environment is exposed to these inducing agents (especially PAH and PCB) is well documented. Induction of this system implies increased formation of reactive intermediates, some of which are toxic and some of which form covalent bonds with DNA *in vitro* [21, 22]. Thus, the increased bioconversion of PAH to highly cytotoxic phenolic metabolites in arterial wall cells could result in cell death and/or damage with a consequent cell proliferation. On the other hand, the bioconversion of these drugs to epoxides or diol-epoxides that react with DNA could produce mutations that also lead to increased proliferation.

BP metabolites have been demonstrated to be both cytotoxic and mutagenic in mammalian cells [23] and bacteria [24]. The cytochrome P-450-dependent mono-oxygenase is functional in the conversion of polycyclic aromatic hydrocarbons to arene oxides, phenols, quinones, and in conjunction with other enzymes (epoxide hydratase and glutathione *S*-epoxide transferase) to dihydrodiols and water soluble conjugates [25–28]. Utilization of the h.p.l.c. analyses has enabled us to more critically evaluate the overall aortic metabolism of PAH; the data indicated that the major metabolities of BP were phenols. In only one instance (polychlorinated biphenyl-treated aorta) was any other metabolite detected. Known standards did not coelute with this peak. It could represent, however, a contaminant or an as yet unidentified metabolite, presumably a diol. Interestingly, when tissues were incubated with heme, the BP-9-ol metabolite was increased markedly.

The possibility that highly reactive intermediates formed in other tissues (i.e. liver) could accumulate in the body fluids to such a degree to cause mutations in the aorta seems somewhat unlikely, especially in light of the fact that most reactive chemicals have such short half-lives [29]. It would seem more plausible that procarcinogens and promutagens could be transported via plasma low density lipoproteins to the aorta [30] and bioactivated by tissue mono-oxygenases. This investigation has helped to characterize the "active" role of the aorta in its interactions with procarcinogens and promutagens and the possible importance of these interactions in the etiology of atherosclerosis.

* PCB, polychlorinated biphenyls; and PAH, polycyclic aromatic hydrocarbons.

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