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2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin Induction of Aryl Hydrocarbon Hydroxylase in Female Rat Liver. Evidence for *De Novo* Synthesis of Cytochrome P-448

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SUMMARY

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The effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on heme synthesis and the synthesis of cytochrome P-448, as reflected in aryl hydrocarbon hydroxylase activity, were determined in female rat liver. TCDD treatment did not increase the activity of δ-aminolevulinic acid synthetase, the rate limiting enzyme in heme biosynthesis. In contrast, a 23-fold increase in the level of cytochrome P-448-mediated aryl hydrocarbon hydroxylase was observed 24 hours after TCDD treatment. Actinomycin D and cycloheximide, but not $CoCl_2$, completely prevented the induction of new cytochrome P-448 and increased aryl hydrocarbon hydroxylase activity by TCDD. *In vitro* reconstitution of cytochrome P-450 and increases in enzymatically active aryl hydrocarbon hydroxylase levels were achieved by adding hemin to whole liver homogenates from rats given TCDD (a hemoprotein inducer) and $CoCl_2$ (a heme depleter). These results suggest that TCDD induces *de novo* protein synthesis of apocytochrome P-448, which combines with heme to yield new cytochrome P-448 and a concomitant increase in aryl hydrocarbon hydroxylase activity. Therefore it is concluded that TCDD selectively induces the formation of cytochrome P-448 leading to increased aryl hydrocarbon hydroxylase activity, and that protein synthesis, rather than heme synthesis, is the rate-limiting event in this process.

INTRODUCTION

2,3,7,8 - Tetrachlorodibenzo - *p* - dioxin[3] (TCDD), an unwanted contaminant formed in the commercial synthesis of the herbicide 2,4,5-trichlorophenoxy acetic acid (1), is among the most potent teratogens (2–4) and acneogens known (5), and has an LD_{50} of 45 μg/kg in female rats (6). Administration of TCDD to rats results in the proliferation of both smooth and rough endoplastic reticulum (7) as well as increases in the activities of several hepatic enzymes including aryl hydrocarbon hydroxylase (benzo[α]pyrene monooxygenase, EC 1.14.14.2) (8, 9), biphenyl 2-hydroxylase (9, 10) and uridine diphosphate glucuronyltransferase (8, 11). Hepatic cytochrome P-448[4], a particular form of cytochrome P-

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[3] The abbreviations used are TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; AHH, aryl hydrocarbon hydroxylase; ALA, δ-aminolevulinic acid.

[4] In this paper cytochromes P-450 and P-448 are used to denote the hepatic microsomal CO-binding pigments found in control and TCDD pretreated rats

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450, is increased by prior treatment with some polycylic hydrocarbons (e.g., 3-methylcholanthrene, benz[a]anthracene, β-naphthoflavone, or benzo[α]pyrene) or TCDD. At least four (12, 13) and probably six different forms of cytochrome P-450 have been identified (14, 15); these hemoproteins are referred to collectively as cytochrome P-450. Further elucidation of the mechanism of the effects of chemical agents on the microsomal mixed function oxidase enzyme system may permit a fuller understanding of the individual components of this system. In particular, TCDD may be useful in assessing the specific role of cytochrome P-448 in toxification and detoxification reactions of clinically used drugs and environmental toxicants such as polycyclic hydrocarbons (16, 17) in mammals.

In the present studies the mechanism of TCDD-induced increases in microsomal cytochrome P-448 and AHH activity in female rat liver was investigated. Female rats were chosen because AHH activity is lower than in male rats (8) and because of the interest in TCDD-induced teratogenesis (2–4). The magnitude and specificity of TCDD-induced AHH activity was compared to the cytochrome P-450-dependent aminopyrine N-demethylation *in vitro*, and the role of heme or protein synthesis in the regulation of TCDD-mediated cytochrome P-448 induction was determined, using drugs and other agents which specifically alter these processes in mammalian cells. Finally, the pharmacologic and toxicologic implications of the effects of TCDD on specific components of the microsomal mixed function oxidase system in mammalian liver are discussed.

MATERIALS AND METHODS

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin was synthesized in the Environmental Chemistry and Biology Branch of NIEHS. D,L-Ethionine, actinomycin D, cycloheximide, benzo[α]pyrene, isocitric dehydrogenase (Type I), L-δ-phosphatidylcholine, L-δ-phosphatidylethanolamine, isocitric acid, succinyl-CoA synthetase (succinic thiokinase) (EC 6.2.1.4), adenosine 5-triphosphate, guanosine 5-triphosphate and coenzyme A (free acid) were obtained from Sigma Chemical Company. Cobaltous chloride ($CoCl_2$) was purchased from K&K Laboratories. [G-^{3}H] Benzo[α]pyrene was obtained from Amersham Searle. Hemin chloride was purchased from Calbiochem. Other chemicals were of reagent grade and were obtained from standard commercial sources.

Treatment of animals. Female Sprague-Dawley rats (CD strain) (200–250 g), obtained from Charles River Laboratories, were housed singly in hanging wire cages with food and water ad libitum. TCDD in corn oil was administered to rats by intraperitoneal injection or by gavage. Anhydrous $CoCl_2$ was dissolved in distilled water and administered by subcutaneous injection at a dose of 60 mg/kg. Cycloheximide and actinomycin D were dissolved in 50% ethanol and administered intraperitoneally. D,L-Ethionine was suspended in corn oil and given in a dose of 500 mg/kg *per os*.

Preparation of tissues. All animals were killed by decapitation. Livers were excised, weighed and homogenized with six strokes of a Potter-Elvehjem homogenizer fitted with a Teflon pestle. Homogenization occurred at 4° in 7 volumes of 0.25 M sucrose containing 0.05 M Tris-HCl buffer, pH 7.5, (for mitochondria) or in 7 volumes of 1.15% KCl containing 0.02 M Tris-HCl, pH 7.5 (for microsomes). The microsomal data in Table 1 were obtained from experiments using livers which were homogenized in 0.25 M sucrose containing 0.05 M Tris-HCl, pH 7.5. Homogenates were centrifuged for 10 min at 600 × *g*, and the mitochondria were sedimented from the resulting supernatant solution by centrifugation at 9,000 × *g* for 15 min. Mitochondria were resuspended in 0.25 M sucrose buffer containing 0.05 M Tris-HCl buffer, pH 7.5, and recentrifuged at 9,000 × *g* for 15 min; the final mitochondrial pellet was suspended in 0.05 M Tris-HCl buffer, pH 7.5, so that each milliliter of suspension contained about 15 mg/ml mitochondrial protein. The microsomal fraction was prepared from the 9,000 × *g* su-

respectively. When reduced and complexed with carbon monoxide, both cytochrome P-450 and P-448 exhibit a Soret maximum in the 450 nm region and can not be easily spectrally separated. Cytochrome P-448 is also called cytochrome P_1-450.

TABLE 1

TCDD-induced increases in cytochrome P-450 and hemoprotein mediated enzyme activity in female rat liver

Female rats were given either corn oil or TCDD (2 μg/kg, *per os*). After 1, 3, 7 or 28 days, the animals were sacrificed and the hepatic δ-aminolevulinic acid synthetase, cytochrome P-450, aminopyrine N-demethylase and aryl hydrocarbon hydroxylase were determined, as described in MATERIALS AND METHODS. All data are expressed as the mean ± standard error of the mean (n = 4 or more). Statistical comparisons are made between corn oil and TCDD treatment at each of the four time periods.

	δ-Aminolevulinic acid synthetase	Cytochrome P-450	Aminopyrine-N-demethylase	Aryl hydrocarbon hydroxylase
	(nmoles ALA/mg protein/hr)	*(nmoles/mg protein)*	*(nmoles formaldehyde/mg protein/min)*	*(nmoles polar products/mg protein/hr)*
Day 1				
Corn oil	1.00 ± 0.13	0.79 ± .06	4.67 ± .27	5.0 ± .43
TCDD	1.01 ± 0.14	1.10 ± .17	5.18 ± .48	113.0 ± 6.4[c]
Day 3				
Corn oil	0.88 ± 0.05	0.82 ± .09	5.36 ± .43	4.2 ± .46
TCDD	0.93 ± 0.11	1.22 ± .12[b]	6.43 ± .77	155.0 ± 8.2[c]
Day 7				
Corn oil	0.68 ± 0.14	0.96 ± .07	5.39 ± .34	4.3 ± .37
TCDD	0.79 ± 0.15	1.56 ± .19[b]	6.39 ± .17	195.0 ± 5.1[c]
Day 28				
Corn oil	0.74 ± 0.09	0.89 ± .07	5.12 ± .35	3.5 ± .50
TCDD	0.92 ± 0.07	1.17 ± .09[a]	5.36 ± .56	45.0 ± 10.0[c]

[a] $p < 0.05$.
[b] $p < 0.01$.
[c] $p < 0.001$.

pernatant solution by centrifugation at 105,000 × *g* for 60 min. The microsomes were resuspended in 30 ml of 0.05 M Tris-HCl buffer, pH 7.5, containing 0.15 M KCl and recentrifuged at 105,000 × *g* for 60 min. The final pellet was gently resuspended in 0.05 Tris-HCl buffer, pH 7.5, using a Potter-Elvehjem homogenizer with a Teflon pestle so that the final suspension contained approximately 15 mg/ml microsomal protein. Protein concentrations were determined by the method of Lowry *et al.* (18) using bovine serum albumin (fraction V) as a standard.

Enzyme assays. ALA synthetase activity was determined as previously described by Woods (19) with the modification that EDTA and mercaptoethanol were omitted from the incubation media. Cytochrome P-450 was determined on an ACTA III spectrophotometer by carbon monoxide difference spectroscopy following reduction with dithionite. An extinction coefficient of 91 mM^{-1} cm^{-1} was assumed for the difference in absorption between the Soret maximum in the 450 nm region and 490 nm (20). This measurement of cytochrome P-450 determines the sum of all related cytochromes by using a single composite extinction coefficient. Formaldehyde produced by the demethylation of aminopyrine was determined by the Nash reaction (21). Aryl hydrocarbon hydroxylase activity was measured by the radiometric assay of DePierre *et al.* (22), wherein all polar metabolites benzo[α]pyrene are determined. Student's *t*-test was used to determine levels of statistical significance.

In vitro reconstitution of hepatic cytochrome P-450 and increased aryl hydrocarbon hydroxylase activity. After treatment with the indicated chemicals, rats were sacrificed and their livers perfused *in situ* with cold isotonic KCl to remove hemoglobin. Livers were homogenized to produce a 50% suspension in 0.1 M phosphate buffer, pH 7.4. Ten milliliters of whole liver homogenate was incubated for 20 min at 37° with 1 mM phosphatidylcholine and 0.25 mM phosphatidylethanolamine[5],

[5] Subsequent work of Meier and Meyer (23) and

either with or without 40 μM hemin. After microsomes were prepared as described above, the microsomal cytochrome P-450 content and AHH activity were determined in the microsomal suspensions from liver homogenates incubated either with or without hemin. Holocytochromes, formed by reconstitution of apocytochromes with hemin *in vitro,* were determined by measurements of their characteristic absorption peak in the 450 nm region, and cytochrome P-448 was determined by its functional enzyme activity (AHH)[6].

RESULTS

Time course of TCDD-induced alterations in cytochrome P-450 content and AHH activity. As an initial approach to understanding the mechanism of action of TCDD with regard to its effects on heme biosynthesis and microsomal mixed function oxidase activity in mammalian liver, time course studies were performed. Results of these studies are shown in Table 1. One, three and seven days after the administration of either TCDD or corn oil to rats, the liver weights per 100 g of body weight were significantly elevated 9.4, 21 and 13% in the TCDD-treated rats. Twenty-eight days after the administration of either TCDD or corn oil the liver weight per 100 g of body weight was only 7.2% higher (not statistically significant) in the TCDD-treated rats. Microsomal protein per gram of liver was 9.5, 7.3, 20 and 9.6% higher in rats given TCDD 1, 3, 7 and 28 days before sacrifice when compared to rats given corn oil at the same times. Only the 20% increase in microsomal protein per gram of liver observed in TCDD-treated rats on day 7 was statistically significant. TCDD did not alter heme biosynthetic pathway enzymes in female rat liver. No change was observed in the activity of ALA synthetase, a finding similar to results previously reported (25). TCDD also had no effect on the activity of hepatic ALA dehydratase, heme synthetase, and uroporphyrinogen I synthetase (unpublished results). Total cytochrome P-450 levels were 39, 49, 63 and 32% higher than corn oil treated controls 1, 3, 7 and 28 days after TCDD administration; however, the activity of aminopyrine-N-demethylase, a cytochrome P-450-mediated enzyme, was not significantly elevated after TCDD treatment. In contrast, the activity of aryl hydrocarbon hydroxylase, a cytochrome P-448-mediated enzyme (26–29), was increased 23, 37, 45 and 13 fold at 1, 3, 7 and 28 days after administration of TCDD. Thus cytochrome P-450 and aryl hydrocarbon hydroxylase activity were increased by TCDD administration, reaching a peak seven days after TCDD administration. Cytochrome P-450 levels and aryl hydrocarbon hydroxylase activity 28 days after TCDD administration were higher than corn oil treated control values but much lower than the peak values of day 7. A hypsochromic shift of 1 nm occurred in the maximum of the Soret absorption peak in the difference spectra of microsomes of rats given 2 μg/kg TCDD *per os* three days before sacrifice. A 2 nm shift occurred at the dose of 20 μg/kg TCDD.

Time course studies of hepatic microsomal cytochrome P-450 and AHH were also performed within the first 24 hours after i.p. administration of TCDD. Levels of hepatic microsomal cytochrome P-450 rose from 0.92 to 1.21 and 1.20 nmoles/mg protein 18 and 24 hours after TCDD administration. Time course effects of TCDD on AHH levels are shown in Fig. 1. After an initial four hour lag period, AHH activity was increased from 5.0 to 9.8, 49 and 122 nmoles/mg protein/hour at 6, 12 and 24 hours after 2 μg/kg TCDD was administered.

Effects of $CoCl_2$ and protein synthesis inhibitors on TCDD induced increases in cytochrome P-450 and aryl hydrocarbon hydroxylase. The results of the time course studies suggest that TCDD acts to specifically increase the synthesis of cytochrome

Bond *et al.* (24) have shown that phosphatidylcholine and phosphatidylethanolamine are not necessary for *in vitro* reconstitution of cytochrome P-450 and increased aryl hydrocarbon hydroxylase activity.

[6] Hepatic microsomal mixed function oxidases are a membrane bound multicomponent enzyme system dependent on microsomal lipid, NADPH cytochrome C reductase and hemoprotein (26, 27). The enzymatic specificity is known to reside in the hemoprotein (cytochrome P-450 or cytochrome P-448) part of this multicomponent system (26–28).

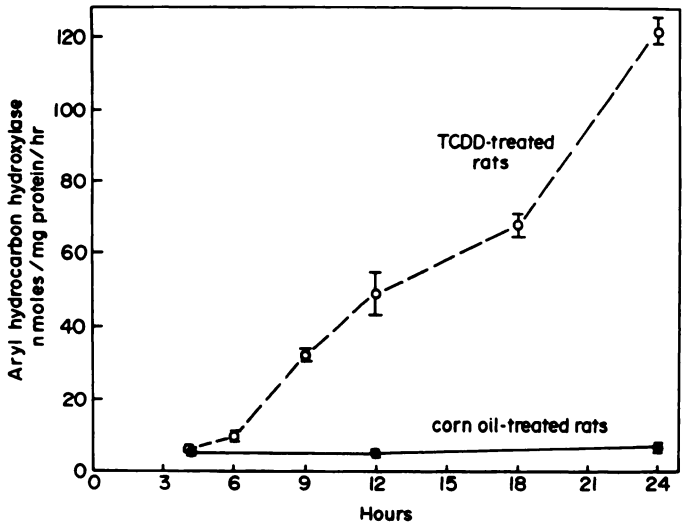

FIG. 1. *Time course of TCDD induction of aryl hydrocarbon hydroxylase in female rat liver*

TCDD was administered intraperitoneally to female rats at zero time. Hepatic microsomal aryl hydrocarbon hydroxylase activity was determined 4, 6, 9, 12, 18 and 24 hours after treatment. Vertical lines indicate the standard error of the mean from determinations of enzyme activity of four or more rats.

P-448-mediated mixed function oxidase activity independent of an effect on heme biosynthesis. To further substantiate this hypothesis, the effects of drugs which specifically inhibit the processes of heme and protein synthesis in mammalian liver were investigated in control and TCDD-treated rats.

Cycloheximide, an inhibitor of cytoribosomal protein synthesis in mammalian cells (30), was initially selected as a protein synthesis inhibitor. $CoCl_2$ was utilized to deplete hepatic heme levels (31, 32); this agent is known to inhibit heme biosynthesis (33, 34) and increase heme catabolism (31). Results of these experiments are shown in Table 2. When either $CoCl_2$ or cycloheximide were given singly to rats the anticipated decreases in both cytochrome P-450 and AHH were observed. During the 24 hours immediately following treatment with TCDD and either cycloheximide or $CoCl_2$, the total cytochrome P-450 content was decreased by about one-third in comparison with that of rats treated with TCDD alone. Microsomal AHH activity was also decreased by about one-third in livers of rats treated with both TCDD and $CoCl_2$ when compared to values obtained with TCDD alone. In contrast, concurrent administration of cycloheximide and TCDD reduced the hepatic microsomal AHH activity by almost two-thirds when compared to the maximal values obtained with TCDD. In the presence of $CoCl_2$, TCDD increases cytochrome P-450; cycloheximide prevents the TCDD-induced increase in cytochrome P-450. These results suggest that a substantially greater effect results from inhibition of protein synthesis, as compared with depletion of heme levels, on the TCDD-mediated induction of cytochrome P-448 and AHH. They indicate further that the principal effect of TCDD in this regard may be mediated through a selective increase in apocytochrome P-448 synthesis.

To further investigate the importance of *de novo* synthesis of apocytochrome P-448, the effects of various agents known to inhibit protein synthesis by different mechanisms were examined with respect to their effects on TCDD-induced increases in aryl hydrocarbon hydroxylase activity. These results are shown in Fig. 2. Actinomycin D is known to act at the transcriptional level to inhibit protein synthesis (35). Ethionine has also been used as a protein synthesis

TABLE 2

Effect of $CoCl_2$ and cycloheximide on TCDD-induced increases in cytochrome P-450 and aryl hydrocarbon hydroxylases

Rats were sacrificed twenty-four hours after the indicated treatment and cytochrome P-450 and aryl hydrocarbon hydroxylase activities were determined in hepatic microsomes as described in MATERIALS AND METHODS. All data are mean ± standard error (n).

Treatment			Cytochrome P-450	Aryl hydrocarbon hydroxylase
Compounds	Dose	Route		
			(nmoles/mg protein)	*(nmoles/mg protein/hour)*
Corn Oil	1 ml/kg	po	1.02 ± 0.03 (6)	8.0 ± 0.55 (7)
Cycloheximide	2 mg/kg	ip	0.72 ± 0.04 (4)[a]	4.5 ± 0.73 (4)[b]
$CoCl_2$	60 mg/kg	sc	0.54 ± 0.03 (5)[a]	4.9 ± 0.60 (6)[b]
TCDD	2 μg/kg	po	1.22 ± 0.03 (6)[a]	143. ± 9.9 (5)[a]
TCDD plus	2 μg/kg	po		
Cycloheximide	2 mg/kg	ip	0.80 ± 0.04 (4)[a, c]	51. ± 11.1 (4)[a, c]
TCDD plus	2 μg/kg	po		
$CoCl_2$	60 mg/kg	sc	0.78 ± 0.03 (5)[a, c]	102. ± 9.9 (5)[a, d]

[a] $p < .001$ vs. corn oil.
[b] $p < .01$ vs. corn oil.
[c] $p < .001$ vs. TCDD.
[d] $p < .01$ vs. TCDD.

inhibitor (36). In these studies the twelve hour time point (Fig. 1) was selected to examine the effects of ethionine, cycloheximide and actinomycin D on TCDD-induced increases in AHH. TCDD increased AHH activity from 4.9 to 49 nmole/mg protein/hour. Both actinomycin D and cycloheximide completely prevented TCDD induction of AHH. Although ethionine (500 mg/kg, p.o.) was not as effective as actinomycin D or cycloheximide in preventing TCDD-induced protein synthesis, it nevertheless reduced the increase in AHH activity expected from TCDD administration by 86%.

In vitro reconstitution of total hepatic microsomal cytochrome P-450 and increased AHH activity. To further test the hypothesis that TCDD selectively increases the synthesis of apocytochrome P-448, *in vitro* reconstitution of microsomal cytochrome P-450 was performed. Results of these experiments are shown in Fig. 3. When whole liver homogenates from control rats were incubated with hemin *in vitro,* cytochrome P-450 content of hepatic microsomes increased by 25 pmoles/mg protein as compared to incubation without hemin. Levels of reconstituted cytochrome P-450 from rats treated with both $CoCl_2$ and TCDD were 151 pmoles/mg protein, a 600% increase. Similarily, *in vitro* incubation of liver homogenates with hemin increased AHH activity by 2.1 and 9.4 nmoles products/mg protein/hr in control and in $CoCl_2$ plus TCDD-treated rats, respectively. Thus a 450% greater increase in AHH activity occurred in liver homogenates from rats treated with both TCDD and $CoCl_2$ as compared to liver homogenates from corn oil-treated rats. Neither TCDD nor $CoCl_2$ treatment alone increased *in vitro* reconstitution of cytochrome P-450 or increased AHH activity. Cycloheximide, either alone or in combination with TCDD, resulted in no significant increase in cytochrome P-450 or AHH activity when compared to corn oil-treated controls.

DISCUSSION

These studies indicate that TCDD induces the synthesis of apocytochrome P-448, which then combines with heme to form new cytochrome P-448, resulting in increased AHH activity. TCDD did not induce or otherwise alter ALA synthetase, the rate-limiting enzyme of heme biosynthesis (37). This finding agrees with previous work from this laboratory demonstrating that TCDD in dosages as high as 25 μg/kg has no effect on ALA synthetase in rats (25). Similar results were obtained

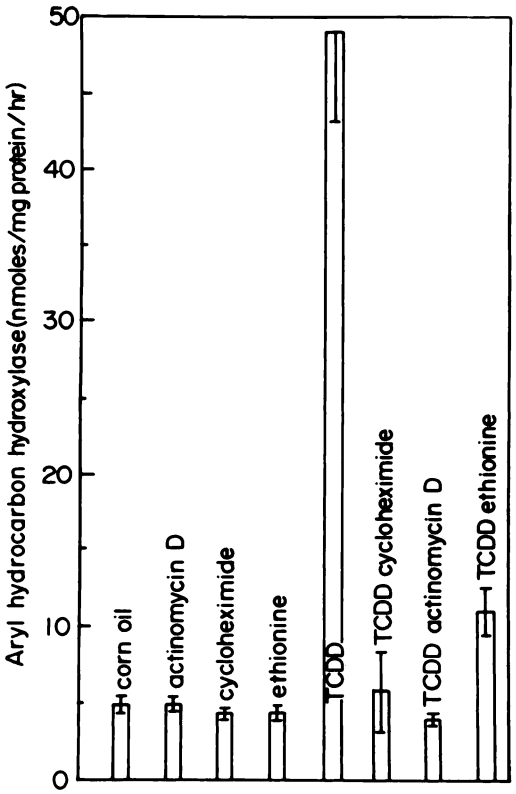

FIG. 2. *Effect of three protein synthesis inhibitors on TCDD induction of aryl hydrocarbon hydroxylase activity*

Actinomycin D (2 mg/kg, ip), cycloheximide (5 mg/kg, ip), ethionine (500 mg/kg, po), TCDD (2 μg/kg, ip), or corn oil (1 ml/kg, ip) was administered to female rats 12 hours prior to sacrifice. Aryl hydrocarbon hydroxylase activity was determined in hepatic microsomes from treated female rats. Vertical lines indicate the standard error of the mean from determinations of enzyme activity of four or more rats.

with mice and guinea pigs (38). These results suggest that, despite possible increased heme requirements to form greater levels of cytochrome P-448 after TCDD treatment, the available hepatic heme pool is sufficient to provide the additional required heme (39, 40) without altering the feedback regulation by heme of ALA synthetase (41). Although phenobarbital increases ALA synthetase, cytochrome P-450 and 3-methyl-4-monomethyl aminobenzene N-demethylase (39), acute administration of phenylbutazone is known to increase cytochrome P-450 and o-nitroanisole demethylase activity without increasing ALA synthetase activity (42). Secobarbital and allobarbital treatment increase ALA synthetase activity in rats by 40 and 240%, respectively (43). Hepatic cytochrome P-450 content and the activities of some hepatic hemoprotein mediated enzyme activities were increased three days after administration of these two compounds to rats (43). TCDD does not increase ALA synthetase activity even after 28 days of exposure to TCDD. The halflife of [^{14}C]TCDD in female rats has been determined to be 30 days (44).

As previously noted by Lucier *et al.* (8), aminopyrine-N-demethylase activity is not increased by TCDD, a finding that strongly suggests that total cytochrome P-450 levels are not induced by this agent. In contrast, TCDD increased the activity of the cytochrome P-448-dependent enzyme aryl hydrocarbon hydroxylase (26–29) by 45-fold in this study. The fact that hepatic AHH activity was substantially increased in TCDD-treated animals, whereas no increase was observed in levels of aminopyrine-N-demethylase, strongly supports the contention that TCDD specifically induces the cytochrome P-448 form of microsomal hemoprotein rather than the other hemoproteins of the cytochrome P-450 class. Both cycloheximide and $CoCl_2$ prevent hepatic microsomal AHH levels from reaching the maximal values observed in TCDD pretreated rats. When cycloheximide (2 mg/kg) was administered to rats, incorporation of [^{3}H]leucine into rat liver microsomal proteins was greatly inhibited two hours after administration, but incorporation was more than fully restored after 24 hours (45). The short duration of action of cycloheximide at low dose levels may explain why this agent is not totally effective in reducing TCDD-induced increases in AHH activity over long time periods (24 hr).

$CoCl_2$ is known to decrease hepatic microsomal cytochrome P-450 (46, 47), ethylmorphine N-demethylase activity (46) and AHH activity (47). Although $CoCl_2$ partially reduced the total microsomal cytochrome P-450 levels and AHH activity in TCDD-treated rats in the present study, it was not as effective as were protein synthe-

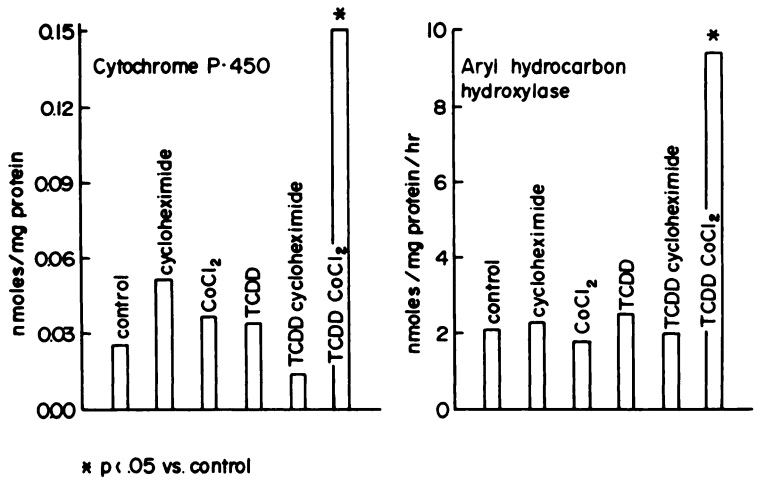

FIG. 3. *In vitro reconstitution of total hepatic microsomal cytochrome P-450 and increases in aryl hydrocarbon hydroxylase activity*

Whole liver homogenates from rats which received the indicated treatment were incubated for 20 minutes at 37°C with 1 mM phosphatidylcholine and 0.25 mM phosphatidylethanolamine either with or without 40 μM hemin. Following incubation, microsomes were prepared, and the difference in cytochrome P-450 content or aryl hydrocarbon hydroxylase activity resulting from *in vitro* incubation with hemin was determined. Values are expressed as nmoles of additional cytochrome P-450 content or aryl hydrocarbon hydroxylase activity resulting from reconstitution of free apocytochrome(s) with hemin.

sis inhibitors in preventing TCDD-induced increases in AHH activity. These findings suggest that $CoCl_2$-induced depletion of intracellular heme does not prevent synthesis of apocytochrome P-448 and that protein synthesis, rather than heme synthesis, may be the primary and rate-limiting event in the synthesis of cytochrome P-448. It is recognized that $CoCl_2$ is less efficient in reducing hepatic heme levels (31, 32) than cycloheximide is in reducing protein synthesis (45). However, the hypothesis that apocytochrome P-448 is the limiting factor in the formation of new cytochrome P-448 is consistent with the observation of Correia and Meyer (48), who demonstrated *in vitro* reconstitution of microsomal cytochrome P-450 with hemin in liver homogenates of $CoCl_2$-treated rats given phenobarbital, 3-methyl-cholanthrene, or pregnenolone 16α-carbonitrile. The functional activity of the reconstituted hemoproteins was also demonstrated in their investigation by increased ethylmorphine N-demethylase activity in $CoCl_2$ and phenobarbital pretreated rats. *De novo* protein synthesis of apocytochrome P-448, as suggested by the present studies, is also consistent with the work of Hook *et al.* (9), which showed that actinomycin D reduces TCDD-induced AHH in male rats, and with the work of Nebert and Gielen (49), which demonstrated that actinomycin D and cycloheximide inhibited phenobarbital or benz[a]anthracene-induced AHH activity in fetal rat hepatocyte cultures. Niwa *et al.* (50) showed inhibition of TCDD-induced AHH activity by cycloheximide and actinomycin D in cell culture. The polycyclic hydrocarbon β-napthoflavone has been shown to induce cytochrome P-448 via *de novo* protein synthesis (12). TCDD and 3-methylcholanthrene are known to act similarly in the induction of AHH (51). Different staining patterns in SDS-polyacrylamide gels of rat hepatic microsomal proteins and partially purified hemoproteins have been observed after 3-methylcholanthrene pretreatment (52, 53). The fact that the 3-methylcholanthrene-induced increases in AHH activity, cytochrome P-450 levels, and the ratio of 455 to 430 nm peaks in the ethyl isocyanide difference spectra are antagonized by both ethionine and actinomycin D (54) offers further support of a selective effect of TCDD on apocytochrome P-448

synthesis, as suggested by the current studies. In conclusion, evidence has been presented that TCDD acts via *de novo* protein synthesis of apocytochrome P-448 to form new cytochrome P-448 and to increase hepatic microsomal aryl hydrocarbon hydroxylase activity. TCDD is not only a toxic environmental contaminant but also a chemical agent which may specifically alter the cytochrome P-448 metabolism of both endogenous steroid hormones (10) and exogenous polycyclic hydrocarbon carcinogens. In this regard TCDD has been useful as a pharmacologic tool in further elucidating the specific role of cytochrome P-448-mediated enzyme activities.

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