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# Differential Induction of Two 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin-Inducible Forms of Cytochrome P-450 in Extrahepatic versus Hepatic Tissues

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SUMMARY

The present study examines the induction of two isozymes of cytochrome P-450, P-448$_{HCB}$ and P-448$_{MC}$, by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in hepatic and a number of extrahepatic tissues of male rats. These isozymes were quantitated by radioimmunoassay (RIA). TCDD induces both forms of cytochrome P-448 markedly in liver. In extrahepatic tissues, TCDD induces cytochrome P-448$_{MC}$ but not cytochrome P-448$_{HCB}$. Induction of cytochrome P-448$_{MC}$ is greatest in liver > kidney > lung > intestine > spleen > testes > brain (no significantly increased). The results in liver, kidney, and lung were confirmed by a technique that depends on both electrophoretic mobility and immunological characteristics (sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by transfer to nitrocellulose paper and immunostaining of the protein). Cytochrome P-448$_{HCB}$ is a minor constitutive form in livers of control male rats (5% of the total cytochrome P-450). In contrast, cytochrome P-448$_{MC}$ is below the level of detection in control livers using the immunostaining technique (<0.6% by RIA). These results indicate that induction of cytochrome P-450 isozymes by TCDD is tissue-dependent in the rat. The response of extrahepatic tissues to TCDD is more limited than that of liver.

## INTRODUCTION

Compounds such as 3-MC,[1] BNF, TCDD, and certain polychlorinated biphenyls such as HCB appear to interact with a common hepatic cytosolic receptor (1) to subsequently induce isozymes of cytochrome P-450 which are characterized by CO-reduced complexes with absorption maxima at 448 nm (2, 3). These forms are sometimes termed cytochrome(s) P-448. 3-MC and TCDD have been shown to induce at least two forms of hepatic cytochrome P-448 in the rabbit and mouse (4, 5). Recently, we purified two forms of hepatic cytochrome P-448 from the rat (6) and demonstrated that both forms are induced markedly by 3-MC and HCB (7). These isozymes differ in their substrate specificities, immunological properties, peptide maps, and molecular weights on SDS-polyacrylamide gel electrophoresis. For purposes of this paper, the form of cytochrome P-448 with a monomeric molecular weight of 52,000 isolated from HCB-treated rats is termed cytochrome P-448$_{HCB}$, and the 55,000 molecular weight form isolated from 3-MC-treated rats is termed cytochrome P-448$_{MC}$.[2]

Although TCDD induces two forms of cytochrome P-448 (Form 4 and Form 6) in the liver of rabbits (5), Liem *et al.* (9) demonstrated that TCDD induces only one form in extrahepatic tissue (Form 6). These observations suggest that induction of the two forms of cytochrome P-448 is tissue-specific in this species. However, Dees *et al.* (10) recently suggested that both Form 4 and Form 6 are induced in extraheptic tissues of the rabbit, since they observed an increase in immunostaining of extraheptic tissues using antibodies to both forms. 3-MC and TCDD have also been shown to induce monooxygenases in a number of extrahepatic tissues in the rat (11–13). Studies by Guengerich and co-workers (13) indicated that cytochrome P-448$_{MC}$ (termed BNF-B$_2$ by these workers) is induced in both kidney and lung. Earlier studies from this laboratory suggested that this cytochrome might also be induced in testes, with some evidence for induction in the small intestine (12).

[1] The abbreviations used are: 3-MC, 3-methylcholanthrene; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; HCB, 3,4,5,3′,4′,5′-hexachlorobiphenyl; SDS, sodium dodecyl sulfate; RIA, radioimmunoassay; IgG, immunoglobulin; IA, immunoabsorbent; AHH, aryl hydrocarbon hydroxylase; BNF, β-naphthoflavone; anti-MC, antiserum to cytochrome P-448$_{MC}$; anti-HCB, antiserum to cytochrome P-448$_{HCB}$; PB, phenobarbital.

[2] Cytochrome P-448$_{MC}$ isolated from 3-MC-treated rats is presumably identical with cytochrome P-450c (3). P-448$_{HCB}$ is also induced by isosafrole (7) and is probably identical with cytochrome P-450*d* (8). Cytochrome P-450$_{PB}$ is the major cytochrome isolated from PB-treated rats.

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In the present study, rats were treated with TCDD (the most potent of the 3-MC type inducers), and two immunochemical techniques were utilized to determine whether the pattern of induction of the two isozymes of cytochrome P-448 in extrahepatic tissues differs from that observed in liver. The two isozymes were quantitated using a double-labeled RIA which detects as little as 0.06 pmole of cytochrome P-$448_{MC}$ or P-$448_{HCB}$ (7). Second, we utilized a technique described by Guengerich and coworkers (13) for transfer of cytochromes from SDS gels to nitrocellulose paper, coupled with immunostaining of the P-450 on nitrocellulose. The latter technique permits more specific identification of cytochromes by a combination of monomeric molecular weight and immunological reactivity. Our results indicate that TCDD induces two isozymes of cytochrome P-448 in liver (P-$448_{MC}$ and P-$448_{HCB}$), but only one form is induced in extrapheptic tissues (P-$448_{MC}$).

## METHODS

*Chemicals.* Goat anti-rabbit IgG and horseradish peroxidase-rabbit anti-horseradish peroxidase complex were purchased from Cappel Laboratories (Cochranville, Pa.). 3,3′-Diaminobenzidine tetrahydrochloride was purchased from Sigma Chemical Company (St. Louis, Mo.). Nitrocellulose sheets (0.45 μm) were purchased from Bio-Rad Laboratories (Richmond, Calif.).

*Treatment of animals.* Male Sprague-Dawley rats (60–80 g) were dosed orally with TCDD (25 μg/kg) in corn oil/acetone (10:1) and killed 3 days later. Tissues were excised, and hepatic microsomes were prepared by differential centrifugation as described earlier. For liver, microsomes from three individual animals were prepared. For extrahepatic tissues, microsomes from three animals were pooled; three such pools were prepared for each tissue.

*Cytochrome and antibody preparations.* Cytochromes P-$448_{HCB}$, P-$448_{MC}$, and P-$450_{PB}$ (the major cytochrome from PB-induced livers) were purified to apparent homogeneity as described elsewhere (6). Both cytochromes P-$448_{MC}$ and P-$448_{HCB}$ contained only a single band on SDS-polyacrylamide gel electrophoresis even when amounts as high as 10 μg of protein per well were electrophoresed. The specific contents were 18.8 nmoles/mg of protein for cytochrome P-$448_{HCB}$, 13.6 nmoles/mg for cytochrome P-$448_{MC}$, and 10 nmoles/mg for cytochrome P-$450_{PB}$.

Antiserum to cytochrome P-$448_{HCB}$ (anti-HCB) was raised in rabbits and treated with an IA containing partially purified cytochrome P-$448_{MC}$ bound covalently to Sepharose gel as described previously (7). Similarly, antiserum to cytochrome P-$448_{MC}$ (anti-MC) was treated with an IA containing covalently bound cytochromes P-$448_{HCB}$ and P-$450_{PB}$.

*RIA.* P-$448_{MC}$ and P-$448_{HCB}$ were radioiodinated by New England Nuclear Corporation (Boston, Mass.), using the "Bolton-Hunter reagent" as described previously (7). After separation of unreacted Bolton-Hunter reagent from $^{125}$I-P-450 on a Sephadex G-25 column, more than 95% of the radioactivity was found to be associated with the protein and less than 5% with unreacted Bolton-Hunter reagent as determined by instant thin-layer chromatography (14). The specific activity was approximately 40 μCi/μg. Approximately 97% of the radioactivity of the radiolabeled P-$448_{MC}$ was trichloroacetic acid-precipitable, and at least 78% of the radioactivity could be precipitated with anti-MC. Approximately 89% of the radioactivity of P-$448_{HCB}$ was trichloroacetic acid-precipitable, and at least 73% could be precipitated with anti-HCB.

Each microsomal preparation from liver, kidney, and lung was solubilized at a concentration of 1.5 mg of protein per milliliter, using 0.6% cholate as described previously (7). Each of the three microsomal pools from intestine, spleen, brain, and testes was solubilized with 1.2% cholate at a microsomal concentration of 4 mg of protein per milliliter. The RIA for cytochromes P-$448_{HCB}$ and P-$448_{MC}$ was performed on each of the solubilized preparations as described earlier (7). Standard curves were prepared daily, using purified cytochromes P-$448_{HCB}$ and P-$448_{MC}$. Tissues were analyzed in duplicate at two or three concentrations.

*Other methods.* Hepatic cytochrome P-450 content was determined from the CO-reduced difference spectrum, using an extinction coefficient of 91 $mM^{-1}$ $cm^{-1}$ (15). Cytochrome P-450 in extraheptic samples was determined by the dithionite difference spectrum of CO-saturated microsomes, using an extinction coefficient of 100 $mM^{-1}$ $cm^{-1}$ for the dithionite-CO difference spectrum (16). AHH activity was measured at 37° by estimation of fluorescent metabolites as previously described (17). Protein was determined by the method of Lowry *et al.* (18), using bovine serum albumin as a standard.

*Electrophoresis and immunostaining.* SDS-polyacrylamide gel electrophoresis was performed as described by Laemmli (19). Gels were stained with Coomassie blue when large amounts of proteins were applied to the gels (2 μg) or with an ultrasensitive silver stain when small amounts of proteins were used (20). Proteins were transferred from the gels to nitrocellulose sheets (blotted) as described by Towbin *et al.* (21), using a current setting of 150 mamp for 16 hr. The nitrocellulose sheets were subsequently immunostained as described by Towbin *et al.* (21) and modified by Domin *et al.* (22), as will be described more completely elsewhere. Briefly stated, the nitrocellulose sheets were treated with bovine serum albumin (3%) to saturate nonspecific binding, incubated with rabbit anti-P-$448_{MC}$ or anti-P-$448_{HCB}$ (diluted 1:1000), incubated with goat anti-rabbit γ-globulin (2.5%), and then treated with horseradish peroxidase-rabbit anti-horseradish peroxidase complex. Finally, they were stained for 30 min at room temperature with a freshly prepared solution of 3,3′-diaminobenzidine (38 mg/100 ml) and 0.007% $H_2O_2$ in buffer (10 mM Tris containing 0.9% NaCl). They were then rinsed twice for 30 sec and soaked for an additional 5 min in buffer and dried.

## RESULTS

*Induction of AHH, Cytochrome P-450 content, and Specific isozymes.* As shown in Table 1, TCDD increased AHH activity in liver (13-fold), kidney (135-fold), lung (22-fold), intestine (106-fold), and spleen (48-fold), and produced slight increases in brain (7.5-fold) and testes (2-fold). These were accompanied by increases in total cytochrome P-450 in liver (2-fold), kidney (5-fold), lung, and intestine as measured spectrally.

Figure 1 shows a standard curve for determination of cytochromes P-$448_{HCB}$ and P-$448_{MC}$ by RIA. Inhibition of binding is linear when plotted versus the logarithm of the concentration of isozyme between 0.06 and 5 pmoles/assay for both cytochromes. The standard curves showed approximately equal sensitivity for the two cytochromes. The RIA for cytochromes P-$448_{MC}$ and P-$448_{HCB}$ had less than 1% cross-reactivity toward the heterologous antigens with the IA-absorbed antisera used in the present study (7). Figure 1 also shows curves for inhibition of binding of radioiodinated P-$448_{MC}$ and P-$448_{HCB}$ by solubilized cytochrome P-450 from liver microsomes at various dilutions. The percentage inhibition is plotted versus the amount of total cytochrome P-450 assayed. Similarly, Fig. 2 shows curves for inhibition of binding of $^{125}$I-P-$448_{MC}$ by solubilized kidney microsomes for TCDD-treated rats. In all three cases, the slope of the curves generated was similar to that of the standard curve.

Measurements of isozyme content (Table 1) indicated that TCDD increased cytochrome P-$448_{MC}$ dramatically in liver (109-fold), kidney (73-fold), lung (15-fold), intes-

TABLE 1

*Differential induction of cytochrome P-450 isozymes in hepatic versus extrahepatic tissues*

Values represent means of three determinations ± standard error. Male rats weighing 60–80 g were dosed orally with TCDD (25 μg/kg) in corn oil/acetone (10:1) or vehicle alone and killed 3 days later.

| Tissue | AHH | | Cytochrome P-450 | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | TCDD | Total | | P-448$_{MC}$[a] | | P-448$_{HCB}$[a] | |
| | | | Control | TCDD | Control | TCDD | Control | TCDD |
| | *pmoles/mg protein/min* | | *pmoles/mg protein* | | *pmoles/mg protein (%)* | | *pmoles/mg protein (%)* | |
| Liver | 607 ± 80 | 7590 ± 780[b] (13-fold) | 860 ± 90 | 1740 ± 210[b] (2-fold) | 4.8 ± 2.8 (0.6%) | 523 ± 60[b] (30%) | 46.4 ± 7 (5%) | 268 ± 12[b] (16%) |
| Kidney | 12 ± 1 | 1619 ± 134[b] (135-fold) | 50 ± 7 | 238 ± 76 (4.8-fold) | 0.9 ± 0.3 (2%) | 66 ± 15[b] (31%) | 1.6 ± 0.5 (3%) | 4.9 ± 1.3 (2%) |
| Lung | 25 ± 5 | 542 ± 22[b] (22-fold) | 62 ± 12 | 119 ± 11[b] | 2.6 ± 0.6 (2%) | 39 ± 6[b] (36%) | 2.1 ± 0.3 | 3.8 ± 1.3 (3%) |
| Intestine | 0.9 ± 0.2 | 96 ± 19[b] (106-fold) | ND[c] | 26 ± 1[b] | 0.23 ± 0.05 | 19 ± 3[b] (73%) | 1.0 ± 0.1 | 0.7 ± 0.04 |
| Spleen | 0.4 ± 0.1 | 19 ± 8 (48-fold) | ND | ND | 0.33 ± 0.06 | 8.4 ± 1.3[b] | 0.23 ± 0.03 | 0.7 ± 0.1[b] |
| Testes | 3.7 ± 0.1 | 8.1 ± 15 (2.1-fold) | 55 ± 15 | 50 ± 9 | 0.05 ± 0.01 (0.1%) | 2.5 ± 0.3[b] (5%) | 0.08 ± 0.003 | 0.15 ± 0.05 |
| Brain | 0.41 ± 0.02 | 3.0 ± 1.7 (7.5-fold) | ND | ND | 0.12 ± 0.02 | 0.13 ± 0.04 | 0.19 ± 0.04 | 0.15 ± 0.02 |

[a] Isozyme content was determined by RIA.
[b] Significantly greater than controls ($p < 0.05$), Student's $t$-test.
[c] ND = not detected.

tine (83-fold), and spleen (25-fold). TCDD produced a slight increase in testicular P-448$_{MC}$. Cytochrome P-448$_{MC}$ was increased from <0.6% of the total cytochrome P-450 in liver microsomes of control rats to 30% of the total in liver microsomes from TCDD-treated rats. The cytochrome P-448$_{MC}$ content of kidney and lung increased from <2–3% of the total P-450 (control rats) to 31% and 36% of the total in TCDD-induced rats. Cytochrome P-448$_{MC}$ accounted for 73% of the total cytochrome P-450 in intestinal microsomes from TCDD-induced rats.

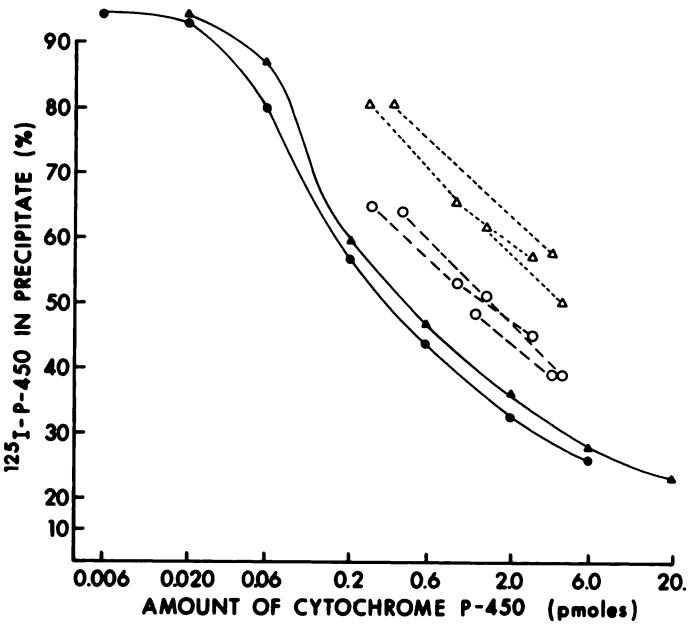


FIG. 1. *Standard curves for determination of cytochromes P-448$_{MC}$ and P-448$_{HCB}$ in the RIA*

Standard curves for purified cytochromes: P-448$_{MC}$ (●) and P-448$_{HCB}$ (▲). *Dashed lines* represent inhibition of binding of $^{125}$I-P-448$_{MC}$ (○) or $^{125}$I-P-448$_{HCB}$ (△) by solubilized liver microsomes from TCDD-treated rats. Each line represents different dilutions of a sample from an individual animal. Data are plotted versus *total* cytochrome P-450 in the sample.

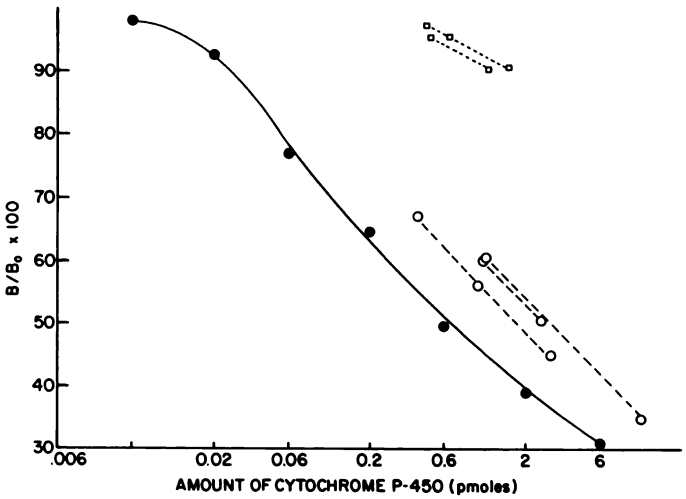


FIG. 2. *Curves showing the inhibition of binding of P-448$_{MC}$ by different dilutions of solubilized kidney microsomes*

The *solid line* represents a standard curve for determination of P-448$_{MC}$. The *dashed lines* represent different dilutions from solubilized kidney microsomes from TCDD-treated (○) or control (□) rats. Each line represents different dilutions of solubilized microsomes from an individual animal.

In contrast, TCDD induced cytochrome P-448$_{HCB}$ in liver, but not in extrahepatic tissues. In liver, TCDD increased cytochrome P-448$_{HCB}$ from 5% of the total P-450 in control liver to 16% in TCDD-induced livers. However, P-448$_{HCB}$ accounted for only 3–4% of the total cytochrome P-450 in treated kidney and lung microsomes

from either control or TCDD-induced rats. A statistically significant increase in P-448$_{HCB}$ in spleen was so slight it was considered to be of doubtful significance.

*Electrophoresis and immunostaining.* When SDS-polyacrylamide gel electrophoresis was performed on liver microsomes and the gels were stained for protein with Coomassie blue or silver stain, TCDD increased protein-staining bands with molecular weights identical with those of purified cytochromes P-448$_{HCB}$ and P-448$_{MC}$ (data not shown). Increases could not be detected in extrahepatic tissues by this method.

When the proteins were transferred electrophoretically from SDS-polyacrylamide gels to nitrocellulose sheets and stained using the immunochemical staining procedures described under Methods, transfer from the gel to the paper was complete, as indicated by the disappearance of protein-staining bands from the gel after the transfer using the ultrasensitive silver stain. The lower limit of sensitivity for the immunostaining procedure was approximately 0.5 pmole for cytochrome P-448$_{MC}$ and 0.125 pmole for cytochrome P-448$_{HCB}$ (Fig. 3). Cross-reactivity was observed between anti-HCB and P-448$_{MC}$ before purification on immunoabsorbents (data not shown). Although we did not observe cross-reactivity between anti-MC and P-448$_{HCB}$ in our earlier study (6), we have subsequently noted cross-reactivity between other lots of anti-MC (from different rabbits) and P-448$_{HCB}$. Therefore, anti-MC was routinely passed over a P-448$_{HCB}$ immunoabsorbent as a precautionary measure. Anti-MC is also routinely treated with a P-450$_{PB}$ immunoabsorbent in our laboratory. After immunoabsorption, no cross-reactivity (<5%) was detected in the immunostaining procedure between anti-HCB and P-448$_{MC}$ or P-448$_{PB}$ or between anti-MC and P-448$_{HCB}$ or P-450$_{PB}$ (Fig. 3).

Using this procedure, cytochrome P-448$_{MC}$ was not detected in liver or extrahepatic microsomes from control rats, as indicated by the absence of any staining in the 55,000 molecular weight region (Fig. 3A and B). In contrast, cytochrome P-448$_{MC}$ was readily detected in liver, kidney, lung, and intestinal microsomes from TCDD-induced rats. A trace of cytochrome P-448$_{MC}$ was also detected in splenic microsomes from TCDD-treated rats; however, this cytochrome could not be detected in testes by this method (Fig. 3B). Cytochrome P-448$_{MC}$ could not be detected in liver microsomes from control rats when the amount of total cytochrome P-450 per well was increased to 100 pmoles (116 $\mu$g of protein) (data not shown). This suggests that less than 0.5% of the cytochrome P-450 in microsomes from control male rat liver is cytochrome P-448$_{MC}$. Some cross-reactivity of anti-MC to additional proteins with molecular weights differing from that of P-448$_{MC}$ was observed at very high concentrations of liver microsomes and in intestinal microsomes (Fig. 3A and B). However, cytochrome P-448$_{MC}$ could be identified in TCDD-treated microsomes by its molecular weight as well as by staining with anti-MC. The small band with high molecular weight in the pure P-448$_{MC}$ preparation is probably an aggregate of the protein.

In contrast to cytochrome P-448$_{MC}$, cytochrome P-448$_{HCB}$ was easily detected in control liver microsomes (Fig. 3C). TCDD produced an increase in the amount of this protein in liver microsomes. No other bands were detected in liver or extrahepatic microsomes, indicating the specificity of the antibody. Cytochrome P-448$_{HCB}$ was not detectd in kidney microsomes from control or TCDD-induced rats, even when the total cytochrome P-450 per well was as high as 7.2 pmoles (200 $\mu$g of protein per well). Since the sensitivity of the assay was at least 0.125 pmole/well, the amount of cytochrome P-448$_{HCB}$ in TCDD-treated kidneys was less than 1.7% of the total cytochrome P-450. Nor was cytochrome P-450$_{HCB}$ detected in microsomes from lungs, intestine, testes, or spleen from control or TCDD-induced rats (Fig. 3D). In contrast, cytochrome PP-448$_{MC}$ was readily detected in kidney microsomes from TCDD-treated rats when the total P-450 was only 0.25 pmole per well.

## DISCUSSION

The present study shows that induction of P-450 isozymes by TCDD is different in hepatic and extrahepatic tissues. TCDD induces cytochromes P-448$_{MC}$ and P-448$_{HCB}$ in liver, as has been noted previously in our laboratory, but induces only cytochrome P-448$_{MC}$ in extrahepatic tissues. These results were demonstrated quantitatively by an RIA procedure which detects as little as 0.06 pmole of either cytochrome. These results were confirmed by immunostaining blots of SDS-polyacrylamide gels of liver, kidney, and lung microsomes from control and TCDD-induced rats. The second procedure, although less sensitive than the RIA, is more specific than the RIA, since it depends on both antibody specificity and electrophoretic mobility of the antigen. No indication of any increase in cytochrome P-448$_{HCB}$ was observed in kidney or lung microsomes from TCDD-treated rats, although cytochrome P-448$_{MC}$ could be detected easily in these tissues by this procedure.

These results are similar to those of Liem *et al.* (9), who demonstrated that TCDD induced Form 6 but not Form 4 in rabbit lungs and kidneys whereas it induced both forms in rabbit liver. Our results in the rat contrast with those of Dees *et al.* (10), who reported that TCDD increased immunofluorescent staining of slices of kidney and lung of rabbits using antibody to Form 4. The immunohistological procedure may be more sensitive than either the RIA or immunostaining of western blots of gels. However, immunohistological staining and the RIA procedure have a disadvantage inasmuch as any cross-reactivity of the antibodies for other cytochromes or other proteins will be detected as immunostaining in the tissue or as an overestimation of the isozyme in the RIA. We previously noted that anti-MC cross-reacted slightly with P-450$_{PB}$ (7). Moreover, anti-MC and anti-HCB have some cross-reactivity before immunoabsorption. Reik *et al.* (23) have shown that P-450*c* and P-450*d* (presumably identical to P-448$_{MC}$ and P-448$_{HCB}$, respectively), although not immunochemically identical, do share some immunochemical determinants. Therefore, procedures which rely only on immunochemical reactivity should be interpreted with some caution. The blotting procedure identifies the protein on the basis of its mo-

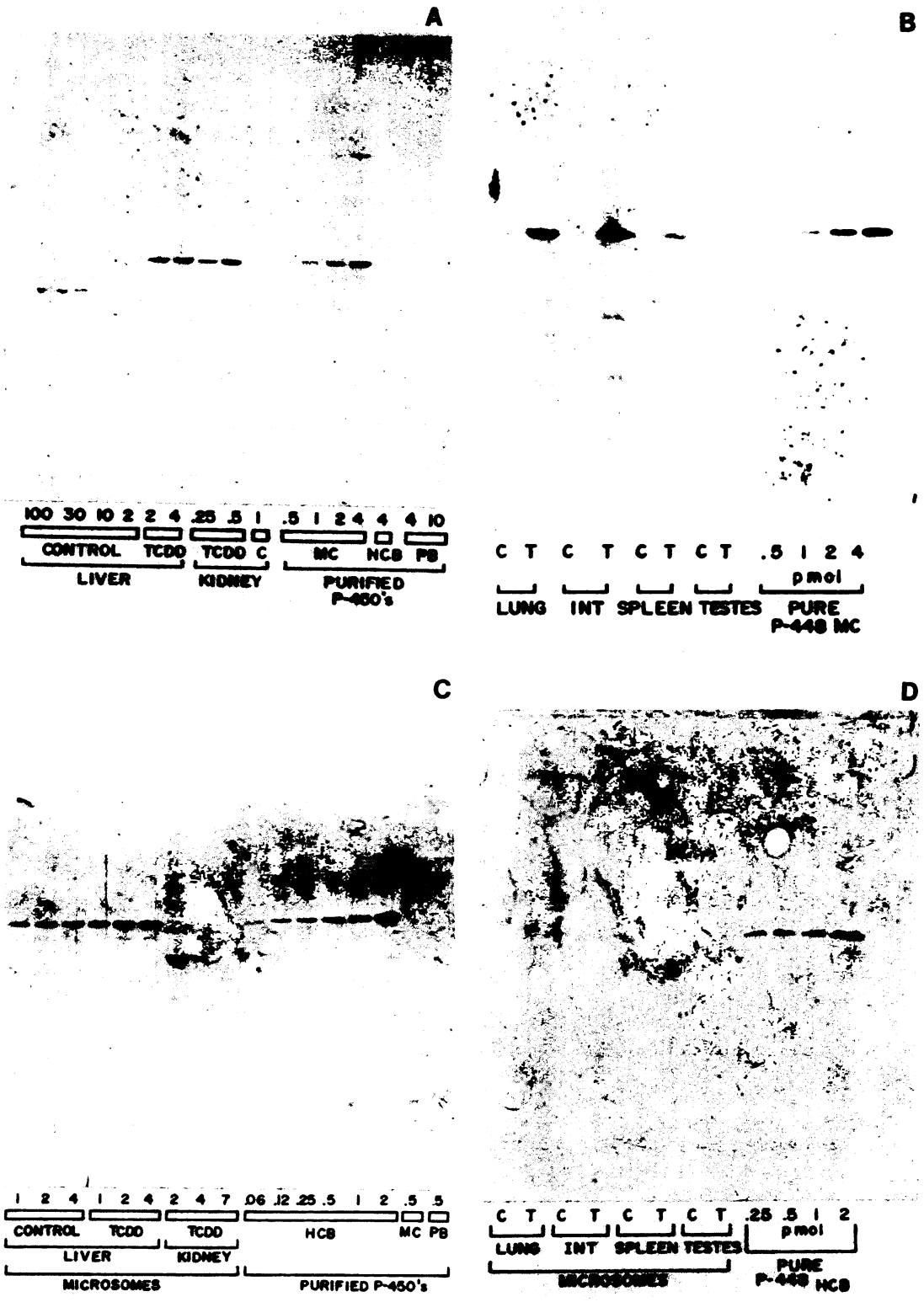


FIG. 3. *Identification of cytochromes P-448$_{MC}$ and P-448$_{HCB}$ in control and TCDD-induced rat liver and kidney microsomes by SDS-polyacrylamide gel electrophoresis followed by transfer of the proteins to nitrocellulose and immunostaining of the proteins*

SDS polyacrylamide gel electrophoresis was carried out in 0.5 × 10 cm lanes according to the method of Laemmli (19). Samples were transferred to nitrocellulose sheets which were subsequently treated with anti-P-448$_{MC}$ (A and B) or anti-P-448$_{HCB}$ (C and D) and stained as described under Methods. Liver and extrahepatic microsomes from control or TCDD-treated rats were electrophoresed in the indicated lane. Purified cytochromes were included in each gel as indicated: *HCB*, P-448$_{HCB}$; *MC*, P-448$_{MC}$; *PB*, P-450$_{PB}$. The numbers below each lane represent the amount of total P-450 electrophoresed in that lane (picomoles per well). For B and D, the amounts of total P-450 or microsomal protein per well were as follows: lung, 1 pmole; intestine (*INT*), 0.2 mg of protein; spleen, 0.1 mg of protein; testes, 0.25 mg of protein.

lecular weight as well as its immunochemical reactivity. It should be noted that proteins are denatured during the transfer to the nitrocellulose sheets. Only the portion of the antibody which recognizes the denatured antigens will produce immunostaining. Therefore, this procedure does not prove that the original antiserum is completely specific. Moreover, this procedure will not distinguish between immunochemically similar proteins with identical molecular weights. However, it is obviously more specific than procedures which rely only on an immunochemical reaction. In the present study, the blotting experiments provide additional evidence that P-$448_{HCB}$ is present in control liver and that P-$448_{MC}$ is present in extrahepatic tissues from TCDD-induced rats.

Earlier studies by Guengerich and Mason (12) first demonstrated the presence of cytochrome P-$448_{MC}$ (P-450 BNF-$B_2$) in 3-MC-induced liver, kidney, and lung on the basis of Ouchterlony immunodiffusion studies. These studies suggested that testes, small intestine, colon, and stomach might also contain cytochrome P-$448_{MC}$. Guengerich *et al.* (13) later demonstrated the presence of P-450 BNF-$B_2$ in liver, kidney, and lung of BNF-treated rats using SDS-polyacrylamide gel electrophoresis combined with immunochemical staining. Our RIA results show that TCDD induced P-$448_{MC}$ in kidney, lung, intestine, spleen, and testes, but that cytochrome P-$448_{HCB}$ was not induced in these tissues. Reconstitution studies with cytochromes P-$448_{MC}$ and P-$448_{HCB}$ have shown that AHH activity in 3-MC-induced microsomes appears to be associated with P-$448_{MC}$ but not P-$448_{HCB}$ (6, 8). The results of the RIA for P-$448_{MC}$ paralleled increases in AHH activity in these tissues. TCDD also appeared to produce a small increase in AHH activity in brain. This increase was not detected by the RIA. It is uncertain whether this is because P-$448_{MC}$ was not increased in brain or because the amounts of this cytochrome are at the limits of detection by the RIA.

The induction of P-$448_{MC}$ and P-$448_{HCB}$ in rat liver by TCDD, 3-MC, HCB, and isosafrole has now been reported in a number of laboratories (7, 24–27). The present study reports lower values for cytochromes P-$448_{MC}$ and P-$448_{HCB}$ in livers of TCDD-treated rats (30% and 16% of the total P-450, respectively) than those recently reported by Guengerich *et al.* (24, 25) in the livers of BNF-treated rats. These authors reported that $\beta$NF-B (presumably P-$448_{MC}$) and $\beta$NF/ISF-G (presumably P-$448_{HCB}$) accounted for 83% and 33% of the total P-450. Since quantitation of a number of P-450 isozymes accounted for 183% of the spectrally determined P-450, Guengerich *et al.* suggested that a large proportion of the cytochrome P-450 must be present as apocytochrome. However, there is to date no conclusive evidence for the presence of large amounts of apocytochrome in liver microsomes. Our RIA results are consistent with our spectral determinations. Moreover, we have found that the results of RIA determinations on liver microsomes from 3-MC-, HCB-, and Aroclor 1254-treated rats agreed with the results of radial immunodiffusion studies on the same samples in our laboratory. We previously reported that P-$448_{MC}$ and P-$448_{HCB}$ represent 50% and 30% of the total cytochrome P-450 in liver microsomes from 3-MC-induced male rats and 28% and 45% of the total cytochrome P-450 in liver microsomes from HCB-treated rats (7). Therefore, the proportion of isozymes varies somewhat with the particular inducer. Differences between laboratories may also reflect differences in antibody specificity or may reflect differences between strains of animals.

The present study indicates that cytochrome P-$448_{HCB}$ is a minor constitutive form in the liver, accounting for approximately 5% of the total cytochrome P-450 in control microsomes from male Sprague-Dawley rats. An earlier study from our laboratory indicated that cytochrome P-$448_{HCB}$ constituted less than 2% of the total cytochrome P-450 in control liver microsomes (7). However, the sensitivity of the RIA procedure for P-$448_{HCB}$ was improved in the present study, subsequent to synthesis of a second batch of $^{125}$I-P-$448_{HCB}$, presumably because less denaturation of the cytochrome occurred during iodination. Our present results are similar to values recently reported by Parkinson *et al.* (26) and Thomas *et al.* (27) for P-450*d* (5–7% of the total cytochrome P-450) in livers of Long-Evans rats. However, the values obtained by both the radial immunodiffusion (26, 27) and the RIA procedures depend only on immunoreactivity. The results of the immunostaining procedure provide more substantial evidence that cytochrome P-$448_{HCB}$ is in fact a constitutive form.

In contrast, the immunostaining procedure did not detect cytochrome P-$448_{MC}$ in livers of control male rats (<0.5% of total cytochrome P-450). The results of the RIA procedure also suggested that the concentration of P-$448_{MC}$ was <0.6% of total cytochrome P-450. These values are somewhat lower than the results of radial immunodiffusion studies which indicate that cytochrome P-$448_{MC}$ (P-450*c*) may account for as much as 3% of the total P-450 in control male rat livers (26). However, results as low as 1% have been reported from the same laboratory (27). Moreover, the immunostaining procedure is considerably more specific than either the RIA or the radial immunodiffusion procedures. It is possible that the relative amounts of isozymes may vary in different strains of laboratory rats. However, an alternative explanation for the differences is the relative specificity of the procedures. In our animals, cytochrome P-$448_{HCB}$ is definitely a constitutive form (approximately 5% of the total P-450), whereas cytochrome P-$448_{MC}$ cannot be detected (<0.5% of the total P-450). These results do not rule out the probability that lower amounts of this isozyme do occur in control microsomes at concentrations below the limits of sensitivity of the present procedure.

In summary, our results show that, although two forms of cytochrome P-450 (P-$448_{HCB}$ and P-$448_{MC}$) are induced by TCDD in liver, only one of these forms (P-$448_{MC}$) is induced in extrahepatic tissues. Cytochrome P-$448_{HCB}$ has also been shown to be a minor constitutive form (approximately 5% of the total P-450). Since cytochrome P-$448_{HCB}$ metabolizes aflatoxin and arylamines such as 2-aminofluorene and Trp-P-2 (a pyrolysis product of the amino acid tryptophan) to mutagens (28), this cytochrome may be an important constitutive form in the activation of these precarcinogens. The differential

induction of the two isozymes in hepatic and extrahepatic tissues may be important in the specificity of certain carcinogens for particular organs.

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