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SHORT COMMUNICATIONS

Differential Induction by 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin of Multiple Forms of Rabbit Microsomal Cytochrome *P*-450: Evidence for Tissue Specificity

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SUMMARY

LIEM, H., U. MULLER-EBERHARD AND E. F. JOHNSON. Differential induction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin of multiple forms of rabbit microsomal cytochrome *P*-450: Evidence for tissue specificity. *Mol. Pharmacol.* **18:** 565–570 (1980).

Four forms of cytochrome *P*-450, forms 2, 3, 4, and 6, are presently being isolated and characterized in this laboratory. Induction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin of specific forms of microsomal cytochrome P-450 was studied in adult rabbit kidney and lung. The induction of a single prominent cytochrome *P*-450 form was observed; this cytochrome was identified as form 6. The assignment of the inducible pulmonary and renal cytochrome as form 6 was established by its mobility on sodium dodecyl sulfate–polyacrylamide gel electrophoresis, its peptide map, and its specific antigenicity. In contrast, previous findings have shown that form 4 is the major cytochrome *P*-450 induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in adult rabbit liver. Thus, the specific induction of cytochrome *P*-450 forms 4 and 6 is tissue dependent.

INTRODUCTION

Compounds such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD),[1] 3-methylcholanthrene (MC), and β-naphthoflavone (BNF) are potent inducers of hepatic cytochrome *P*-450. In general, the administration of these compounds to experimental animals leads to the induction of forms of microsomal cytochrome *P*-450 characterized by an absorption maximum of the reduced CO–hemoprotein complex which occurs at a shorter wavelength (448 nm) than that observed for constitutive cytochrome *P*-450 (450 nm). The resulting change in cytochrome *P*-450 composition is associated with an increase in the activity of a limited number of monooxygenases which include benzo[*a*]pyrene hydroxylase, 7-ethoxyresorufin *O*-deethylase, and 2-acetylaminofluorene *N*-hydroxylase (1).

It is now recognized that more than one form of cytochrome *P*-450 is induced in the liver by the above compounds. Two inducible forms of cytochrome *P*-450, forms 4[2] and 6, have been purified in our laboratory from the livers of TCDD-treated adult rabbits (2–5). The two forms can be distinguished by several criteria which include their mobility on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), their peptide maps, and their unique antigenic properties. In addition, the two forms display different substrate specificities. Whereas form 4 is more active than form 6 in the catalysis of 2-acetylaminofluorene *N*-hydroxylation (6), form 6 catalyzes the hydroxylation of benzo[*a*]pyrene at a much higher rate than form 4 (4). Both forms metabolize 7-ethoxyresorufin *O*-deethylation at similar rates (7).

When TCDD is administered to the mother rabbit prior to delivery, form 6 is the principal form of cytochrome *P*-450 in neonate liver (5). On the other hand, form 4 is the predominant cytochrome *P*-450 in hepatic microsomes following the pretreatment of adult rabbits with TCDD (3). Atlas *et al.* (8, 9) have noted that MC induces benzo[*a*]pyrene hydroxylase activity but not 2-acetylaminofluorene *N*-hydroxylase in rabbit kidney and in the lung of neonates. These observations suggest that the induction of forms 4 and 6 may be tissue specific as well as age dependent. To test this hypothesis, we examined the effect of TCDD on the occurrence of forms 4 and 6 in adult kidney and lung microsomes, using SDS-PAGE, peptide mapping, and immunological detection. By these experimental criteria, we have established that TCDD induces only form 6 and not form 4 in the kidney and lung of adult rabbits.

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* Dedicated to Prof. Fritz Hartman, Hannover, Germany, on the occasion of his 60th birthday.

[1] Abbreviations used: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; MC, 3-methylcholanthrene; BNF, β-naphthoflavone; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

[2] Forms 2 and 4 are designated LM_2 and LM_4 by Haugen *et al.* (19).

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EXPERIMENTAL PROCEDURES

Male New Zealand white rabbits weighing 3–4 kg were employed. Treatment of the animals with TCDD and isolation of the microsomes have been described (2). The procedure for isolation of microsomes from kidney and lung was modified in that tissues were first minced with scissors and then homogenized with a Potter–Elvehjem homogenizer.

Protein concentration was measured by a modification (10) of the procedure of Lowry *et al.* (11). Bovine serum albumin served as the standard. Cytochrome *P*-450 content was determined from the reduced CO difference spectrum using an extinction coefficient of 91 mM^{-1} cm^{-1} (12). Activity of cytochrome *P*-450 reductase was assessed spectrophotometrically using cytochrome c as an electron acceptor (2).

Benzo[*a*]pyrene hydroxylase activity was determined as described by Nebert and Gelboin (13) and 7-ethoxyresorufin *O*-deethylase activity was determined by the procedure of Burke and Mayer (14) with minor modifications (7).

Polyacrylamide gel electrophoresis was performed in the presence of sodium dodecyl sulfate as described by Laemmli (15). Densitometric measurements were determined using stained gels and a Gilford gel scanning apparatus.

The procedure for peptide mapping was that of Cleveland *et al.* (16). The isolation and recovery of polypeptides from polyacrylamide gels were accomplished as described elsewhere (17). Kidney or lung microsomes (50 μg) were loaded on each track (8.0 × 100 × 3.0 mm) of a polyacrylamide gel. The band corresponding to form 6 was excised from the gel, and the slices from 10 tracks were combined for electrophoretic elution (17). A total of 3–5 μg of protein was recovered from 10 tracks (500 μg of microsomal protein). Based on the specific contents of the microsomes, ca. 3 and 5% of the kidney and lung microsomal proteins are forms of cytochrome *P*-450. Assuming that form 6 accounts for between 20 and 80% of the cytochrome, the expected yield of protein would be 3–12 and 5–20 μg for kidney and lung, respectively.

The preparation and characterization of antibodies against forms 4 and 6 have been described, as have the immunological methods used in this study (2, 5).

TCDD was a gift from Dow Chemical Co. and 3-hydroxybenzo[*a*]pyrene was kindly provided by Dr. Harry Gelboin of the National Cancer Institute. All reagents for SDS-PAGE were purchased from Bio-Rad Laboratories. Coomassie brilliant blue, NADPH (tetrasodium salt), cytochrome *c* (horse heart type III), crystalline bovine serum albumin (fatty acid free), phosphorylase *a*, papain, sodium cholate, and sodium deoxycholate were purchased from Sigma Chemical Co. Benzo[*a*]pyrene (gold label) was obtained from Aldrich Chemical Co., and resorufin and 7-ethoxyresorufin were from Pierce Chemical Co. All other chemicals were of the highest commercially available grade.

RESULTS

As shown in Table 1, treatment of the rabbit with TCDD causes an increase in total microsomal cytochrome *P*-450 content in the kidney but not in the lung. Despite the apparent lack of induction of cytochrome *P*-450 in the lung, benzo[*a*]pyrene hydroxylase and 7-ethoxyresorufin *O*-deethylase activities are greater in lung microsomes from TCDD-treated rabbits relative to those from control animals. The 7-ethoxyresorufin *O*-deethylase activity increases in both kidney and lung microsomes from almost undetectable levels to 20% of the activity previously found (7) in the liver microsomes of TCDD-treated adults. The activity of benzo[*a*]pyrene hydroxylase increases 10 times or more above control values in the kidney and 3 times or more in the lung microsomes. This enhancement in monooxygenase activities is not accompanied by an increase in NADPH cytochrome *c* reductase activity in either organ. The observed increase in enzymatic activities suggests that form 6 is induced by TCDD in these organs.

Since the four forms of cytochrome *P*-450 isolated in this laboratory can be distinguished by SDS-PAGE (18), microsomes from kidney and lung were examined by SDS-PAGE to identify which of these forms may have been induced by TCDD. In the kidney as well as in the lung, a component migrating with the same relative mobility as purified form 6 appears to be induced by TCDD (Fig. 1). On the other hand, an increase in the concentration of a component with the same relative mobility as form 4 was not observed following TCDD treatment. Form 2, the major phenobarbital-inducible cytochrome (19), and forms 4 and 6, the two forms inducible by TCDD in adult rabbit liver (2–5), are shown for comparison. Densitometric scans (Fig. 2) of kidney and lung microsomes from control and TCDD-treated animals as well as control microsomes supplemented with

TABLE 1

Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on renal and pulmonary cytochrome P-450 and related enzymes in rabbits

Assays were conducted as described in Experimental Procedures. Values represent means ± SE ($N \geq 4$).

	Kidney		Lung	
	Control	TCDD	Control	TCDD
Cytochrome *P*-450[a]	0.18 ± 0.1	0.57 ± 0.06	0.94 ± 0.15	0.90 ± 0.17
Cytochrome *P*-450 reductase[b]	0.021 ± 0.002	0.020 ± 0.004	0.294 ± 0.045	0.201 ± 0.040
7-Ethoxyresorufin[c]	<10	440 ± 50	<10	410 ± 70
Benzo[*a*]pyrene[c] hydroxylase	<3	29 ± 1	<3	9 ± 1

[a] nmol/mg protein.
[b] μmol cytochrome *c* reduced/min/mg protein.
[c] pmol product formed/min/mg protein.

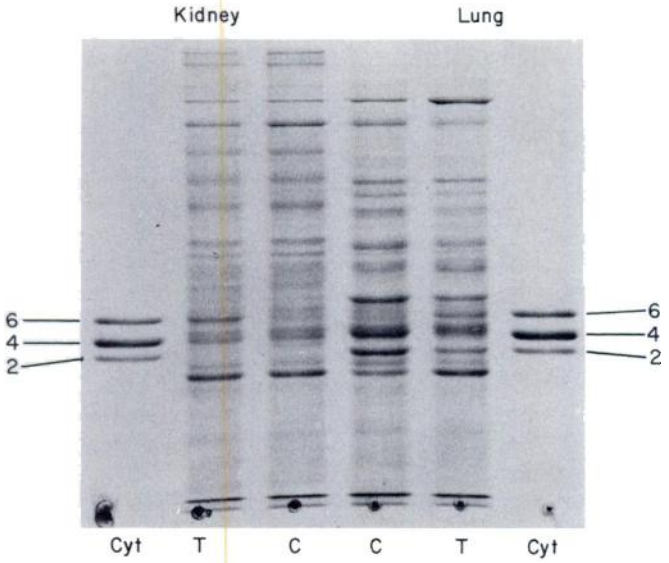

FIG. 1. *Electrophoretic comparison of microsomes of kidney and lung (30 μg) from TCDD-treated (T) versus untreated adult animals (C)*

The outside tracks contain three isolated forms of cytochrome *P*-450, forms 2, 4, and 6 (2 μg each). In both tissues, the treatment of the animal effects an increase in the amount of a protein which has the same mobility as form 6. Details of the electrophoretic procedure are provided in the text. Migration occurred from the top of the gel toward the bottom.

purified form 6 show the relative migration of the induced protein to be similar to that of form 6 purified from hepatic microsomes. It can be seen in Figs. 1 and 2 that the induction of a microsomal protein with a mobility equivalent to that of form 6 in the lung is accompanied by a decrease in the amount of another microsomal protein with a mobility equivalent to that of purified form 2. One of two forms of cytochrome *P*-450 highly purified from control rabbit lung was shown to be identical to form 2 purified from liver by several criteria (20, 21).

The identity of the form of cytochrome P-450 induced by TCDD in kidney and lung microsomes of rabbit as form 6 is further substantiated by peptide mapping. Johnson *et al.* (17) have shown that forms 2, 4, and 6 can be identified by peptide "fingerprints" specific for each cytochrome form when they are subjected to partial digestion with *S. aureus* V_8 protease, α-chymotrypsin, or papain and the resulting peptides are visualized on SDS-PAGE. As shown in Fig. 3, the peptide maps of the induced protein isolated from kidney and lung microsomes of TCDD-treated rabbits closely resembles that of purified form 6. Although some differences in the intensities of the bands are observed, a similar size distribution of the peptides is observed in each case.

Immunological characterization of kidney and lung microsomes of TCDD-treated rabbits also indicates that form 6 is present. On Ouchterlony diffusion plates (Fig. 4), solubilized microsomes from kidney and lung isolated from TCDD-treated rabbits are shown to react against the antibody raised to purified form 6. Only one precipitin line is observed. In addition, a line of complete immunological identity is seen between purified form 6 and the precipitin lines of lung and kidney microsomes. However, antibodies raised to purified form 4 do not appear to react with these microsomes.

DISCUSSION

Our studies show that the induction of multiple forms of microsomal cytochrome *P*-450 in adult rabbits following TCDD treatment varies from organ to organ. Whereas form 4 is the major form of cytochrome *P*-450 induced by this agent in the liver of adult rabbits, form 6 is induced in the kidney and lung. The identity of the TCDD-inducible cytochrome in kidney and lung microsomes as form 6 was suggested by the similar mobilities of the electrophoretic species induced by TCDD and form 6 purified from liver microsomes when analyzed by SDS-PAGE. This identification was corroborated by peptide mapping. When subjected to limited proteolysis, peptide "fingerprints" of both the TCDD-induced pul-

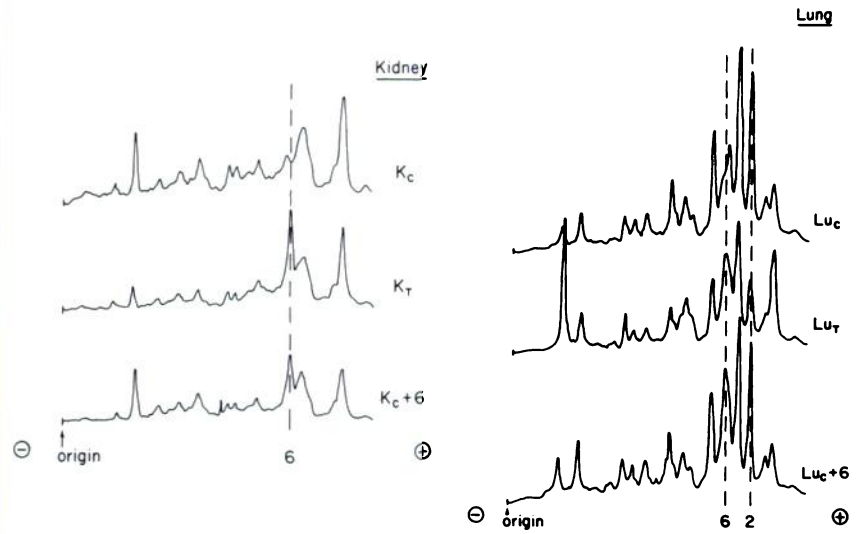

FIG. 2. *Densitometric tracings of kidney and lung microsomes after SDS–polyacrylamide gel electrophoresis*

For both kidney (K) and lung (Lu) (30 μg), the tracings depict microsomes from an untreated animal (K_C or Lu_C), microsomes from a TCDD-treated animal (K_T or Lu_T), and microsomes from an untreated animal to which purified form 6 (2 μg) was added (K_C + 6 or Lu_C + 6).

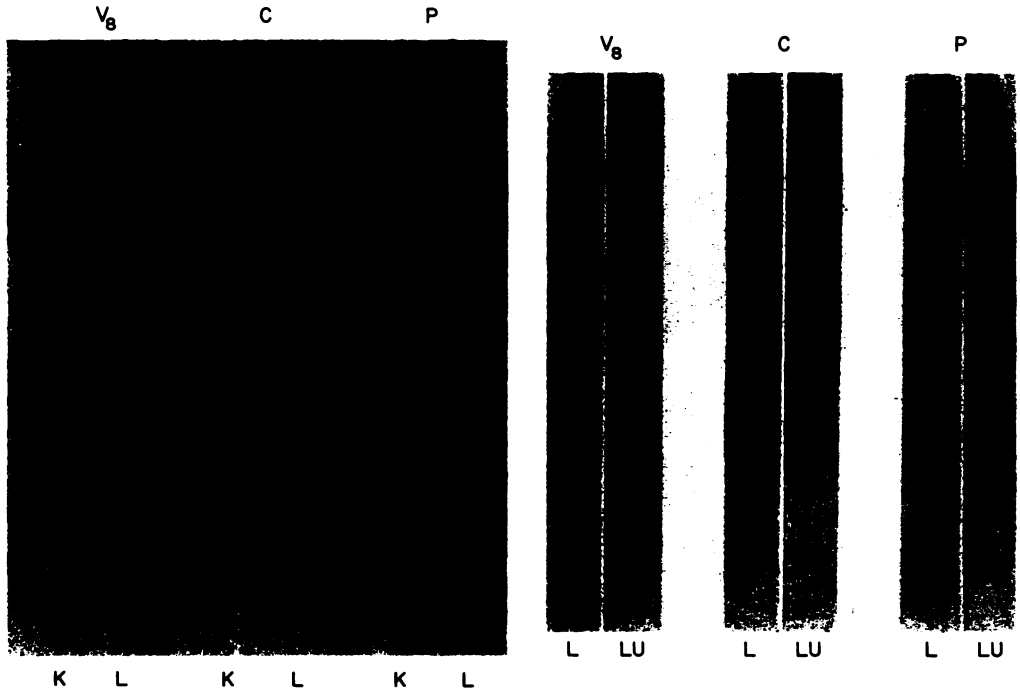

FIG. 3. *Peptide mapping*

The peptides generated by limited proteolysis of purified cytochrome *P*-450 form 6 (10 μg) purified from liver microsomes (L) and the inducible proteins isolated from lung (Lu) or kidney (K) (3–5 μg) microsomes from TCDD-treated rabbits were analyzed by SDS-PAGE. The procedure used here is described by Johnson *et al.* (17). The samples were incubated with either *S. aureus* V_8 protease (V_8), α-chymotrypsin (C), or papain (P) at 37°C for 30 min.

monary and renal microsomal proteins recovered from polyacrylamide gels closely resembled that of purified hepatic form 6. Although this method might not detect small differences in amino acid composition of the peptides, the fact that the inducible protein cannot be distinguished from purified form 6 using three different proteolytic enzymes strongly indicates that the TCDD-inducible cytochrome *P*-450 species in kidney and lung is

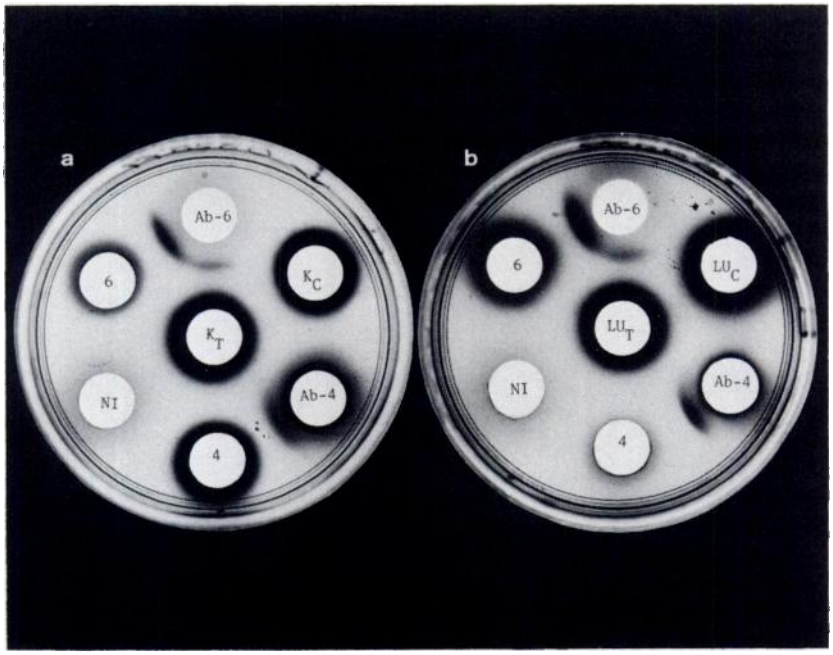

FIG. 4. *Ouchterlony double diffusion studies*

The wells in plate *a* contained the following: Ab-6, antibody directed against form 6; 6, purified form 6 (3.2 μM); NI, nonimmune serum; 4, purified form 4 (16.9 μM); Ab-4, antibody directed aganst form 4; K_C, kidney microsomes from untreated adult rabbits; and K_T, kidney microsomes from TCDD-treated adult rabbits. The wells in plate *b* contained the following: Ab-6, antibody directed against form 6; 6, purified form 6 (14.9 μM); NI, nonimmune serum; 4, purified form 4 (4.2 μM); Ab-4, antibody directed against form 4; Lu_C, lung microsomes from untreated adult rabbits; and Lu_T, lung microsomes from TCDD-treated adult rabbits.

identical to form 6. This assignment was confirmed by immunological studies; antibody raised against hepatic form 6 reacts with kidney and lung microsomes only after TCDD pretreatment of the rabbits. Precipitin lines of complete identity are formed between purified form 6 and kidney microsomes as well as between form 6 and lung microsomes. In contrast, the induction of form 4 by TCDD could not be detected by either SDS-PAGE or immunological means. The lack of an immunological reaction to antibodies against form 4 does not indicate the absence of form 4, as this form could be present in amounts below detectability. Nevertheless, we can conclude that form 4, the major form of cytochrome *P*-450 which is induced by TCDD in adult rabbit liver, is not induced in kidney and lung microsomes. The exclusive induction of form 6 by TCDD in these two tissues is therefore similar to the inductive response of the livers of newborn rabbits to TCDD (5).

For a more direct identification of the extrahepatic forms of cytochrome *P*-450, isolation of the cytochromes from these organs would be desirable, since a direct comparison of the functional, physicochemical, and immunological characteristics of the cytochromes could then be made. At present, few extrahepatic forms of cytochrome *P*-450 have been purified to homogeneity. Two major constitutive forms of cytochrome *P*-450 have been highly purified from lung microsomes of rabbits (20–23). One of these, designated cytochrome *P*-450 I (20) or fraction A (23), has been shown to be the same cytochrome as form 2 (the major hepatic PB-inducible cytochrome *P*-450) by its molecular weight, substrate specificity, and immunological properties (20, 21, 23) as well as by peptide mapping (22). The other one, designated cytochrome *P*-450 II, is immunochemically distinct from forms 2 and 4. On the other hand, Guengerich found in his study that fraction A could be distinguished from form 2 by their apparent isoelectric points (23). These differences, however, could be due to artifacts in the method of isoelectric focusing (24). To our knowledge these are the only forms of cytochrome *P*-450 which have been isolated from rabbit extrahepatic tissues and which have been extensively compared with those isolated from the liver.

In the present study, the induction of form 6 is accompanied by an increase in total cytochrome *P*-450 content in the kidney but not in the lung. The induction of form 6 in the pulmonary microsomes appears to be compensated for by the decrease in another microsomal protein, which on SDS-PAGE has the mobility of purified form 2. This phenomenon, an increased synthesis of one form of cytochrome *P*-450 occurring at the expense of the synthesis of another, has been encountered by others (9, 25) and could explain the absence of an increase in the total cytochrome *P*-450 content in the lung as observed here.

Since the cellular composition of the kidney and lung is heterogeneous, it can be assumed that the induction of cytochrome *P*-450 form 6 may occur only in certain cell types. For instance, Boyd (26) has indicated that Clara (pulmonary nonciliated bronchiolar) cells have a much higher cytochrome *P*-450-dependent monooxygenase activity than other cell types in the lung, an organ estimated to contain over 40 different specific cell types. Another illustration of tissue-cell specificity of induction was recently observed by Zenser *et al.* (27). These authors reported that the cortex of the kidney is more sensitive to MC induction than the medulla. Thus, the induction of cytochrome *P*-450 form 6 in the kidney and lung may also be specific to an as yet unidentified subpopulation of cells.

The induction of form 6 in the microsomes of kidney and lung of adult animals treated with TCDD correlates well with the induction of aryl hydrocarbon hydroxylase activity in these organs as it does in the neonate liver (5). It has been known for some time that the cytochrome *P*-450 monooxygenases play a central role in both the activation and the detoxification of carcinogens such as benzo[*a*]pyrene and 2-acetylaminofluorene. These two pathways (activation and detoxification) can be catalyzed by different forms of cytochrome *P*-450 (6). Consequently, the occurrence and amounts of different forms of cytochrome *P*-450 in tissues/organs will be a determinant of the balance between the activation and detoxification of substrates. Induction of form 6 in neonatal liver and in adult kidney and lung obviously affects this balance and may have a profound effect on the susceptibility of these tissues to carcinogenesis.

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REFERENCES

1. Thorgeirsson, S. S., and D. W. Nebert. The *Ah* locus and the metabolism of chemical carcinogenesis and other foreign compounds. *Advan. Cancer Res.* **25**: 149–193 (1977).
2. Johnson, E. F., and U. Muller-Eberhard. Resolution of two forms of cytochrome P-450 from liver microsomes of rabbits treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Biol. Chem.* **252**: 2839–2845 (1977).
3. Johnson, E. F., and U. Muller-Eberhard. Purification of the major cytochrome P-450 of liver microsomes from rabbits treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Biochem. Biophys. Res. Commun.* **76**: 652–659 (1977).
4. Johnson, E. F., and U. Muller-Eberhard. Multiple forms of cytochrome P-450: Resolution and purification of rabbit liver aryl hydrocarbon hydroxylase. *Biochem. Biophys. Res. Commun.* **76**: 644–651 (1977).
5. Norman, R. L., E. F. Johnson and U. Muller-Eberhard. Identification of the major cytochrome P-450 form transplacentally induced in neonatal rabbits by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Biol. Chem.* **253**: 8640–8647 (1978).
6. Johnson, E. F., D. S. Levitt, U. Muller-Eberhard and S. S. Thorgeirsson. Evidence that multiple forms of cytochrome P-450 catalyze divergent pathways of 2-acetylaminofluorene metabolism. *Cancer Res.* (in press).
7. Johnson, E. F., G. E. Schwab and U. Muller-Eberhard. Multiple forms of cytochrome P-450: Catalytic differences exhibited by two homogeneous forms of rabbit cytochrome P-450. *Mol. Pharmacol.* **15**: 708–718 (1979).
8. Atlas, S. A., S. S. Thorgeirsson, A. R. Boobis, K. Kumaki and D. W. Nebert. Differential induction of murine *Ah* locus-associated monooxygenase activities in rabbit liver and kidney. *Biochem. Pharmacol.* **24**: 2111–2116 (1975).
9. Atlas, S. A., A. R. Boobis, J. S. Felton, S. S. Thorgeirsson and D. W. Nebert. Ontogenetic expression of polycyclic aromatic compound-inducible monooxygenase activities and forms of cytochrome P-450 in rabbit. Evidence for temporal control and organ specificity of two genetic regulatory systems. *J. Biol. Chem.* **252**: 4712–4721 (1977).
10. Bensadoun, A., and D. Weinstein. Assay of proteins in the presence of interfering materials. *Anal. Biochem.* **70**: 241–250 (1976).
11. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275 (1951).
12. Omura, T., and R. Sato. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.* **239**: 2370–2378 (1964).
13. Nebert, D. W., and H. V. Gelboin. Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture. *J. Biol. Chem.* **243**: 6242–6249 (1968).
14. Burke, M. D., and R. T. Mayer. Ethoxyresorufin: Direct fluorimetric assay of

a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. *Drug Metab. Disp.* **2:** 583–588 (1974).
15. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature* **227:** 680–685 (1970).
16. Cleveland, D. W., S. G. Fischer, M. W. Kirschner and U. K. Laemmli. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. *J. Biol. Chem.* **252:** 1102–1106 (1977).
17. Johnson, E. F., M. C. Zounes and U. Muller-Eberhard. Characterization of three forms of rabbit microsomal cytochrome P-450 by peptide mapping utilizing limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. *Arch. Biochem. Biophys.* **192:** 282–301 (1979).
18. Johnson, E. F. Isolation and characterization of a constitutive form of rabbit liver microsomal cytochrome P-450. *J. Biol. Chem.* **255:** 304–309 (1980).
19. Haugen, D. A., T. H. van der Hoeven and M. J. Coon. Purified liver microsomal cytochrome P-450: Separation and characterization of multiple forms. *J. Biol. Chem.* **250:** 3567–3570 (1975).
20. Wolf, C. R., M. M. Szutowski, L. M. Ball and R. M. Philpot. The rabbit pulmonary monooxygenase system: Characteristics and activities of two forms of pulmonary cytochrome P-450. *Chem. Biol. Interact.* **21:** 29–43 (1978).
21. Serabjit-Singh, C. J., C. R. Wolf and R. M. Philpot. The rabbit pulmonary monooxygenase system. Immunochemical and biochemical characterization of enzyme components. *J. Biol. Chem.* **254:** 9901–9907 (1979).
22. Slaughter, S. R., C. R. Wolf, T. P. Marcinizyn and R. M. Philpot. Characterization of purified forms of rabbit pulmonary cytochrome P-450 and comparison with the hepatic cytochrome P-450 induced by phenobarbital, in *Microsomes, Drug Oxidations, and Chemical Carcinogenesis* (M. J. Coon *et al.*, eds.), 4th International Symposium on Microsomes and Drug Oxidations, Ann Arbor, Michigan, 1979. Academic Press, New York, 171–174 (1979).
23. Guengerich, F. P. Preparation and properties of highly purified cytochrome P-450 and NADPH-cytochrome P-450 reductase from pulmonary microsomes of untreated rabbits. *Mol. Pharmacol.* **13:** 911–923 (1977).
24. Guengerich, F. P. Artifacts in isoelectric focusing of the microsomal enzymes cytochrome P-450 and NADPH-cytochrome P-450 reductase. *Biochim. Biophys. Acta* **577:** 132–141 (1979).
25. Haugen, D. A., M. J. Coon and D. W. Nebert. Induction of multiple forms of mouse liver cytochrome P-450. Evidence for genetically controlled *de novo* protein synthesis in response to treatment with β-naphthoflavone or phenobarbital. *J. Biol. Chem.* **251:** 1817–1827 (1976).
26. Boyd, M. R. Evidence for the Clara cell as a site of cytochrome P-450-dependent mixed-function oxidase activity in lung. *Nature* **269:** 713–715 (1977).
27. Zenser, T. V., M. B. Mattammal and B. B. Davis. Differential distribution of the mixed-function oxidase activities in rabbit kidney. *J. Pharmacol. Exp. Ther.* **207:** 719–725 (1978).

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