0031-6997/80/3104-0277$02.00/0
PHARMACOLOGICAL REVIEWS

Vol. 31, No. 4
Printed in U.S.A.

Multiplicity of Mammalian Microsomal Cytochromes P-450

ANTHONY Y. H. LU AND SUSAN B. WEST

Department of Animal Drug Metabolism and Radiochemistry, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey

I. Historical Background

THE EXISTENCE of more than one liver microsomal drug-metabolizing enzyme in different animal species was first postulated more than 20 years ago by Axelrod based on a study of the liver N-demethylation of several narcotics (5). His observation that the rabbit demethylated *l*-methadone and meperidine faster than the rat, while the rat system metabolized morphine more rapidly than the rabbit suggested that the responsible enzymes in rabbit and rat liver are different. An early study by Conney et al. (16) suggested not only that the drug-metabolizing enzymes of various species differ but that the liver of a single species contains several enzymes that

catalyze the same reaction. They found that the administration of benzo(a)pyrene to rats markedly increased the microsomal metabolism of substrates such as benzo(a)pyrene, acetanilide, zoxazolamine, and 3-methyl-4-monomethylaminoazobenzene yet decreased the metabolism of meperidine and benadryl and had no effect on the metabolism of chlorpromazine.

Since these initial observations, studies on the nature of the drug-metabolizing enzyme system have focused primarily on the liver microsomal hemeprotein, cytochrome P-450, for several important reasons. In 1965, Cooper and coworkers (21, 120) established that cytochrome P-450 is the terminal oxidase of the liver microsomal drug-metabolizing enzyme system. Subsequently, cytochrome P-450 was shown to be the substrate-binding site of the hydroxylation system (51, 135). The question than asked was whether a single cytochrome P-450 could account for the broad substrate specificity, the induction properties, and the spectral characteristics of the microsomal drug-metabolizing system or were there, in fact, many different forms of cytochrome P-450. The data accumulated over the past 15 years from studies with microsomes as well as with purified cytochrome P-450 isozymes overwhelmingly support the concept that there are multiple forms of cytochrome P-450. In this paper, we shall review the evidence for the existence of multiple forms of microsomal cytochrome P-450 and their role in the metabolism of drugs, mutagens, and carcinogens. Since much of the relevant evidence from studies with microsomes has been extensively reviewed elsewhere by others (76, 103, 104, 111, 114, 142, 150), we will limit our discussion for the most part to studies with purified cytochromes P-450. In addition to this article, several other reviews on the purification and multiplicity of microsomal cytochromes P-450 have also appeared recently (19, 20, 37, 58, 79, 84, 90, 101).

II. Multiplicity of Cytochromes P-450: Microsomal Studies

A. *Induction Studies*

The use of different inducers to manipulate the biochemical and biophysical properties of the microsomal hydroxylation system has played a major role in establishing the existence of multiple forms of cytochrome P-450. In a series of important studies, liver microsomes prepared from phenobarbital (PB)-treated rats were shown to differ with respect to substrate specificity and the absorption maxima of their reduced CO and ethylisocyanide difference spectra from liver microsomes prepared from 3-methylcholanthrene (3-MC)-treated rats (2, 32, 76, 103, 138–140). The biochemical and physical changes observed after 3-MC treatment were postulated to be due to the induced synthesis of a new hemeprotein, designated "cytochrome P-448" (2) or "cytochrome P_1-450" (138). Other studies showed that the induction of cytochrome P-448 could be blocked by inhibitors of protein and nucleic acid synthesis such as ethionine, puromycin, and actinomycin D (76). Moreover, the change in CO difference spectrum and ethylisocyanide difference spectrum after 3-MC treatment could not be duplicated by the addition of 3-MC to rat liver microsomes (3). PB treatment of rats elevated microsomal cytochrome P-450 levels but the hemeprotein synthesized had the same absorption maximum in its reduced CO difference spectrum as microsomal cytochrome P-450 from untreated animals. When optimum inducing doses of PB and 3-MC were administered simultaneously to rats, the induction was additive, i.e. the level of cytochrome P-450 was elevated to the sum of the hemeprotein concentrations obtained when PB and 3-MC were given separately (8). These results support the hypothesis that the syntheses of cytochrome P-450 and P-448 are under separate genetic control. Other inducers such as pregnenolone 16α-carbonitrile (PCN) were also found to differ from PB and 3-MC and were presumed to induce the formation of a different species of cytochrome P-450 (98, 132).

B. *In Vitro Inhibition Studies*

A variety of different compounds selectively affect the microsomal metabolism of various substrates. The effect of a compound is dependent on the substrate, type of reaction, and source of microsomes. For example, Wiebel et al. (162) observed that 7,8-benzoflavone inhibited the hydroxylation of benzo(a)pyrene by liver microsomes from 3-MC-treated rats but had no effect on benzo(a)pyrene metabolism in microsomes from PB-treated rats. Hydroxylation of benzo(a)pyrene by liver microsomes from untreated rats was stimulated. In contrast, ellipticine inhibited benzo(a)pyrene hydroxylation in all three types of microsomes (78). Metyrapone and SKF-525A, on the other hand, inhibit the metabolism of a variety of substrates in microsomes from PB-treated rats but have little effect on metabolism by microsomes from 3-MC-treated rats.

Testosterone can be hydroxylated by liver microsomes at several different positions. Chlorthion strongly inhibits the hydroxylation of testosterone by liver microsomes from untreated rats at the 16α-position, while hydroxylation at the 6β- and 7α-positions are only minimally affected (17). Carbon monoxide also differentially inhibits the hydroxylation of testosterone at these three positions (17). These results provide indirect support for the concept of multiple forms of cytochrome P-450 with different specificities.

C. *Kinetic Studies*

The metabolism of many drugs and carcinogens by liver microsomes appears to follow Michaelis-Menten kinetics, and kinetic data from these studies can be fitted to a linear plot by using Lineweaver-Burk analysis or other similar types of plots. However, the kinetic data from compounds such as aminopyrine (126), 7-ethoxycoumarin (151), and aniline (154) cannot be fitted to a

single linear plot and instead appear multiphasic, a result consistent with the interpretation that more than one cytochrome P-450 is involved in catalysis of a compound.

D. Developmental Studies

Liver microsomal hydroxylation of testosterone at the 6β-, 7α-, and 16α-positions exhibit different age-dependent patterns (17). The rate of 16α-hydroxylation of testosterone is low in newborn rats; it remains low until 4 weeks of age and then increases markedly. The rate of 6β-hydroxylation increases up to 2 weeks of age, plateaus until the age of 7 weeks, and then increases. The rate of 7α-hydroxylation in the newborn is faster than that of either 6β- or 16α-hydroxylation; it continues to increase until 4 weeks of age and then decreases with age. These results suggest that these three pathways are under separate genetic control and are catalyzed by different species of cytochrome P-450. The developmental patterns of many other hydroxylase activities (e.g. 2- and 4-hydroxylation of biphenyl) have also been shown to differ from one another.

E. Genetic Studies

In an extensive series of studies with genetically inbred mice, Nebert and associates (112, 114) have shown that the murine Ah locus regulates the polycyclic aromatic hydrocarbon-dependent induction of numerous drug-metabolizing enzyme activities in the liver as well as other tissues. Their results suggest that a number of structural genes are involved in the induction of cytochrome P-448 and that as many as 15 distinct inducible forms of cytochrome "P-450" can be regulated by the Ah locus and receptor (M. Lang and D. W. Nebert, personal communication).

III. Multiplicity of Cytochromes P-450: Purification Studies

A. Solubilization, Resolution, and Reconstitution of Microsomal Hydroxylation System

Although studies with microsomes strongly suggest that cytochrome P-450 and cytochrome P-448 represent two different proteins, the argument has been made that cytochrome P-448 could result from the binding of the inducer or its metabolites to the native cytochrome P-450 or conformational change of cytochrome P-450, rather than represent the synthesis of a new hemeprotein. Thus, definitive proof for the existence of multiple forms had to await the isolation and purification of different forms of cytochrome P-450.

In 1968, the liver microsomal hydroxylation system was solubilized with detergent and resolved by column chromatography into three components that, when recombined, catalyzed the metabolism of fatty acids (85). The three components were identified as cytochrome P-450, NADPH-cytochrome *c* reductase (also known as NADPH-cytochrome P-450 reductase), and phosphatidylcholine (87, 143). The same reconstituted hydroxylation system was soon found to catalyze the biotransformation of steroids, drugs, carcinogens, and a variety of other foreign compounds (86, 88, 89, 93, 99, 100). The successful resolution and reconstitution of the microsomal drug-metabolizing enzyme system thus opened the door and paved the way for the eventual purification of cytochrome P-450.

B. Which Component Controls Substrate Specificity?

The reconstituted system has been used to demonstrate that the substrate specificity of the hydroxylation system is determined primarily by cytochrome P-450 (88, 89, 93). NADPH-cytochrome *c* reductase and microsomal lipids isolated from a variety of different microsomal sources were generally interchangeable. Thus, it is primarily the cytochrome P-450 fraction that is responsible for the difference in substrate specificity between the liver microsomes of control, PB-, and 3-MC-treated rats (83, 89, 94), control and ethanol-treated rats (153), male and female rats (9), starved and normal rats (7), control and 3-MC-treated hamsters (82), and inducible and non-inducible mouse strains (113). Although recent studies have shown that the metabolism of some substrates by certain forms of cytochrome P-450 can be affected by the presence of cytochrome b_5 (49, 53, 54, 91, 95, 144) and membranes composed of different lipids (10, 54, 55), cytochrome P-450 is by far the most important factor in determining substrate specificity.

C. Criteria for Multiple Forms

In recent years, cytochrome P-450 has been purified from a variety of sources and characterized. In general, the following criteria have been used to demonstrate that various purified cytochromes P-450 are distinct proteins. While the results of a single approach are subject to alternative interpretations, the evidence taken as a whole overwhelmingly supports the existence of multiple forms of microsomal cytochrome P-450.

1. Polyacrylamide Gel Electrophoresis in the Presence of Sodium Dodecyl Sulfate (SDS). Because of its hydrophobic nature, mammalian cytochrome P-450 is usually isolated as a high molecular weight aggregate with an apparent molecular weight between 300,000 and 500,000 (18, 38). The extent of aggregation of the purified preparation can be influenced by factors such as detergent, lipid, and ionic strength. It is thus of little value to use gel filtration or analytical ultracentrifugation to compare the apparent molecular weights of different cytochrome P-450 preparations. On the other hand, SDS-gel electrophoresis has proved to be an extremely useful tool in the characterization of cytochrome P-450 since it can be used not only to evaluate the purity of an enzyme preparation but also to determine under denaturing conditions the subunit molecular weight of different preparations. Since SDS-gel electrophoresis can normally resolve proteins whose subunit molecular weight differ by

as little as 1,000 daltons, it can be used as a convenient and sensitive technique for distinguishing multiple forms of cytochrome P-450.

Several early studies with SDS-gel electrophoresis suggested that there are multiple forms of cytochrome P-450 with different subunit molecular weights in liver microsomes. Alvares and Siekevitz (4) reported in 1973 that the SDS-gel electrophoretic pattern of partially purified cytochrome P-450 (from PB-treated rats) differed considerably in the 47,000 to 52,000 molecular weight region from the pattern observed with partially purified cytochrome P-448 (from 3-MC-treated rats). Welton and Aust (157) found that microsomes could be resolved on SDS gels in the 50,000 molecular weight region into three major protein-staining bands. The intensity of the 49,000 molecular weight band was increased after PB-treatment whereas the intensity of the 53,000 molecular weight band was increased after 3-MC-treatment. These protein bands were presumed to contain cytochrome P-450 because of positive staining with benzidine, which tests for heme-dependent peroxidase activity.

In recent years, various species of microsomal cytochrome P-450 with subunit molecular weights between 45,000 to 60,000 have been purified. These purified cytochrome P-450 preparations comigrate on SDS gels with microsomal protein-staining bands that have been identified as cytochrome P-450 based on heme-staining, developmental, and induction patterns. Thus, solubilization and purification have not artificially created protein species not normally present in microsomal membranes. In addition, the differences in electrophoretic mobility of various cytochrome P-450 species cannot be simply attributed to the different carbohydrate content of the molecules since rabbit P-450LM$_2$ and P-450LM$_4$ both contain one glucosamine and two mannose residues per subunit yet differ in subunit molecular weight by about 6,000 daltons (44). Rabbit P-450LM$_4$ has also been found by amino acid analysis to have about 60 more residues than P-450LM$_2$ (44). Therefore, it can be concluded that the differences in electrophoretic mobility of various forms of cytochrome P-450 on SDS gels are most likely due to real differences in subunit molecular weight.

As previously noted (37, 58, 79), the electrophoretic mobility of cytochrome P-450 samples can be considerably affected by the choice of gel system, standards, buffers, heating procedure, SDS concentration, and sample size. Thus, different subunit molecular weights have been reported for a given cytochrome P-450 preparation by different laboratories as well as by the same laboratory (see tables 1 to 6). Therefore, a comparison of the subunit molecular weight of different forms determined by different research groups has been unsatisfactory and confusing. Despite these discrepancies, it should be pointed out that on the same gel the relative order of electrophoretic mobilities of these preparations is quite reproducible.

If a standard SDS-gel electrophoresis system were to be run under strictly controlled conditions, it might be possible to make a direct comparison between different purified forms from several laboratories. The system described by Laemmli (77) has been widely used and appears to be one of the most sensitive to small molecular weight differences. One way to resolve apparent discrepancies in reported molecular weights would be for a single laboratory to run SDS gels under strictly controlled conditions on all the different purified cytochrome P-450 preparations.

2. *Spectral Properties.* Differences in the reduced CO, ethylisocyanide, octylamine, cyanide, and substrate difference spectra observed within microsomal preparations, as well as with partially purified cytochrome P-450 preparations, have led to the suggestion that these different spectral properties are due to the presence of different forms of cytochrome P-450 (2, 15, 57, 138). These early observations have been largely confirmed by studies wth purified enzymes. The absorption maximum of the reduced CO complexes of various cytochrome P-450 preparations ranges from 447 to 452 nm (tables 1 to 6) while the 455 to 430 ratio at pH 7.4 of the reduced ethylisocyanide complexes varies from 0.3 to 2.0 (48, 97). Spectral parameters determined in microsomal suspensions appear to represent a composite value of all the different forms of cytochrome P-450 in the particular microsomal preparation.

While one laboratory, with instruments currently available, can reproducibly determine the absorption maximum of the reduced CO complex of a purified cytochrome P-450 preparation within ± 0.5 nm, the same measurement on the same protein by different laboratories has yielded values that differ significantly from one another. The variation in the reported absorption maximum for the same protein has been as large as ± 1 nm. Thus, a report that two different cytochrome P-450 preparations have different reduced CO maxima is not sufficient evidence that they are different hemeproteins. On the other hand, if two preparations have identical CO maxima, it does not necessarily mean that they are the same protein. For example, the CO maxima of the major 3-MC-inducible form of cytochrome P-450 purified from rats and rabbits are identical (447 nm), yet these two enzymes have been shown to be different proteins based on their catalytic activities, immunological properties, and subunit molecular weights (72).

Most purified microsomal cytochromes P-450 appear to be in a low-spin state. However, purified rabbit LM$_4$ exists predominantly in a high-spin state (42, 44, 73). It is unlikely that the spin state of a cytochrome P-450 preparation can be used to distinguish multiple forms of cytochrome P-450 since the spin state of rabbit LM$_4$ can be easily affected by detergents, protein concentration, and other factors (44, 79). On the other hand, it should be noted that the absorption maximum of the reduced CO complex of cytochrome P-450 cannot be altered under similar conditions (79).

3. *Catalytic Activity.* The various purified cytochrome

P-450 preparations possess different but overlapping substrate specificities when reconstituted in a nonmembranous system (33, 45, 48, 49, 63, 80, 97, 115, 128, 134) as well as in various liposomal systems (11, 54, 55). This property has been used as an important criterion for demonstrating the existence of multiple forms of cytochrome P-450. The difference in specificity among forms is dependent on the substrate. For example, various cytochrome P-450 isozymes catalyze the N-demethylation of benzphetamine or the hydroxylation of benzo(a)pyrene at rates that can differ by a factor of a hundred (33, 45, 60, 97, 134). In contrast, the rates at which a variety of different forms of cytochrome P-450 hydroxylate aniline are quite similar (29, 45, 86, 134).

The rates at which various cytochrome P-450 isozymes catalyze a reaction not only differ markedly, but many also exhibit positional selectivity and stereoselectivity in the metabolism of such compounds as benzo(a)pyrene (23, 24, 92, 145, 163), testosterone (45, 48, 134), and warfarin (26, 69). Compounds such as SKF-525A and 7,8-benzoflavone selectively affect the microsomal metabolism of various substrates. Their effect on a particular reaction depended on which purified cytochrome P-450 isozyme was used as the catalyst (63, 73, 137, 156). The apparent K_m of a substrate also depended on the cytochrome isozyme used as the terminal oxidase and can vary significantly from one isozyme to another. For example, the K_m for O-deethylation of 7-ethoxycoumarin is 27 μM for PB rat fraction B, 322 μM for PB rat fraction D, and 36 μM for 3-MC rat fraction B (36).

4. Immunological Properties. Antibodies prepared against various purified cytochrome P-450 preparations generally show high specificity and thus provide another means for distinguishing multiple forms of cytochrome P-450. For example, antibodies against rabbit forms 2, 4, and 6 did not crossreact with each other when tested by Ouchterlony double-diffusion analysis (22, 62) although testing by radioimmunoassay revealed a slight crossreactivity between forms 2 and 4 (22). Similarly, antibodies produced against rat cytochrome P-450$_a$, P-450$_b$, and P-450$_c$ (134), or B fractions of PB- and 3-MC-treated rats (36) were also highly specific. Antibodies against various rat cytochrome P-450 preparations either did not crossreact or crossreacted very poorly with cytochromes P-450 purified from rabbits, mice, and humans (79, 147, 148, 155).

Antibodies against various forms of cytochrome P-450 inhibit cytochrome P-450-catalyzed reactions in microsomes as well as in reconstituted cytochrome P-450 systems (22, 66, 105, 136, 146–148, 158). The inhibition patterns observed are dependent on the source of microsomes, the substrate, and the type of reaction.

5. Peptide Mapping. Another valuable criterion for demonstrating multiple forms of cytochrome P-450 is a comparison of the peptide fragments of different enzyme preparations after limited proteolysis. With the procedure developed by Cleveland et al. (14), peptide fragments generated by limited proteolysis can be separated by SDS-gel electrophoresis and the peptide maps of different enzyme preparations can be compared. Thus, while the peptide maps generated from several different NADPH-cytochrome P-450 reductase preparations (purified from rat liver, rabbit liver, and rabbit lung) are quite similar (35), the peptide maps obtained from major forms of rat and rabbit cytochrome P-450 are very different (35, 64, 134). These results suggest that the primary structure of these cytochrome P-450 species is distinct and that the differences between individual proteins are not due to small alterations of the polypeptide chain. Because of its simplicity and sensitivity, peptide mapping should prove useful for evaluating structural similarities or dissimilarities of cytochrome P-450 species isolated in the future.

6. Amino Acid Sequence. The ultimate proof of a different primary structure for different species of cytochrome P-450 will be a comparison of the total amino acid sequence of each isozyme. Because of its large subunit molecular weight and hydrophobic nature, no purified cytochrome P-450 has yet been totally sequenced. However, four purified cytochromes P-450 (rabbit LM$_2$, rat P-450$_a$, rat P-450$_b$, and rat P-450$_c$) have been partially sequenced (12, 43). The sequences of the first 19 amino acids on the N-terminal end of the three forms from rats are quite different while rabbit LM$_2$ and rat P-450$_b$, the major forms induced by phenobarbital, show partial homology. All four contain a high percentage of hydrophobic amino acids. The N- and C-terminal residues are, respectively, methionine and arginine for rabbit LM$_2$, methionine and methionine for rat P-450$_a$, glutamic acid and serine for rat P-450$_b$, and isoleucine and leucine for rat P-450$_c$ (12, 43). Along with results from peptide mapping (35, 64, 134), distinct end-terminal sequences provide further support for the multiplicity of cytochromes P-450 and suggest that these cytochromes P-450 are separate gene products rather than posttranslational modifications of a common precursor.

D. Nomenclature

The various purified cytochrome P-450 preparations that have been reported in the literature up to March 1980 are listed in tables 1 to 6. Partially purified preparations that either have a low specific content (less than 10 nmol per mg of protein) or are known to contain more than one form of cytochrome P-450 have not been included. A glance at these tables should convince the reader that the nomenclature presently used by various laboratories to designate different forms of cytochrome P-450 is neither uniform nor based on a common rationale. It is undoubtedly confusing to the uninitiated reader. Yet, selection of a suitable nomenclature for the multiple forms of cytochrome P-450 has been hindered by the complexity of this family of isozymes as well as by the large number of newly discovered cytochromes P-450. Thus far, a suitable nomenclature that succinctly

and accurately describes the properties and functions of cytochrome P-450 has not been agreed upon by investigators in this field. Historically, terms such as cytochromes P-450 and P-448 (or P_1-450), which refer to the absorption maximum of the reduced CO complex of the cytochrome, have been used to denote the cytochrome present in liver microsomes of untreated or PB-treated animals vs. 3-MC-treated animals (2, 138). This nomenclature is clearly outdated. The absorption maxima of the reduced CO complex of the major induced forms of cytochrome P-450 are not all at 450 or 448 nm and, in addition, different forms of cytochrome P-450 sometimes have an identical absorption maximum (see tables 1 to 6). More recent systems of nomenclature based on induction by a specific inducer or order of elution from a chromatography column have not proved to be more definitive or useful for comparing the forms isolated in one laboratory with those isolated by another.

The IUPAC-IUB Commission on Biochemical Nomenclature recommended that "multiple forms of an enzyme should be distinguished on the basis of electrophoretic mobility, with the number 1 being assigned to that form having the highest mobility toward the anode" (116). This system has been adopted by Haugen et al. (45) for identifying multiple forms of rabbit liver cytochrome P-450 with the modification that the tissue of origin is designated as LM for "liver microsomal." Recently, Johnson et al. (58, 63) have also adopted a similar system for the various forms of cytochrome P-450 from 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-treated rabbits. However, this system of nomenclature has not yet been adopted for the multiple forms of cytochrome P-450 isolated from other species or other tissues. Although some may feel that it would be premature to adopt it for all cytochrome P-450 systems, the case can be made that even an imperfect system is better than further delay. The confusing variety of names being used to refer to the forms of cytochrome P-450 isolated from rat liver microsomes is a good example of a family of isozymes critically in need of a uniform system of nomenclature. As a first step, a specific gel system run under strictly controlled conditions would have to be chosen with the understanding that the present technology for identifying gel bands as cytochrome P-450 is not completely reliable. If additional cytochrome P-450 isozymes are isolated from a particular tissue or species at some future time, the system would probably need to be revised. Unless a common reference point is agreed upon soon, the isolation of additional forms of cytochrome P-450 can only increase the present state of confusion.

E. Multiple Forms of Cytochrome P-450 in Rabbit Microsomes

The various forms of cytochrome P-450 that have been purified from the liver and lung microsomes of rabbits are listed in tables 1 and 2, respectively. As noted in the tables, several of the forms purified in one laboratory are probably identical to forms isolated by other laboratories. On the basis of several criteria, at least five different forms of rabbit cytochrome P-450 have been purified from either lung or liver microsomes and the properties of each will be briefly discussed below.

Form 1. This cytochrome P-450 species, which has been highly purified by Johnson and Muller-Eberhard (60) from the liver microsomes of TCDD-treated rabbits, was reported to have a subunit molecular weight of 48,000; its reduced CO complex has an absorption maximum of 450.5 nm. Although it catalyzes the metabolism of benzphetamine, benzo(a)pyrene, 7-ethoxyresorufin, and acetanilide, activities are generally low. Antibodies produced against forms 2, 4, and 6 do not react with form 1 as assayed by Ouchterlony double-diffusion analysis (58, 62). It has also been distinguished from other forms by peptide mapping (64). A similar preparation of lower specific content termed LM_1 has been obtained by Guengerich from the liver microsomes of untreated rabbits (33), but it has not been determined whether these two preparations are the same form.

Form 2. Form 2 is the major species of cytochrome P-450 in liver microsomes of PB-treated rabbits and it has been well-characterized by many laboratories (22, 33, 44, 52, 63). It has been called LM_2 (43, 44), P-450_1 (42, 49, 50), 70 mM phosphate fraction (40), and P-450 (52, 67). Reports of the subunit molecular weight range from 48,500 to 50,000; the absorption maximum of the reduced CO complex is 451 nm. The N- and C-terminal residues are methionine and arginine, respectively (44). It also contains one residue of glucosamine and two residues of mannose per subunit (44). Form 2 is most active in catalyzing the metabolism of substrates such as benzphetamine, cyclohexane, biphenyl, warfarin, and parathion, and it is rather inactive in metabolizing polycyclic aromatic hydrocarbons.

Philpot and coworkers (136, 164) have isolated from the lung microsomes of untreated rabbits a form of cytochrome P-450, designated cytochrome P-450I, whose subunit molecular weight, immunological properties, and catalytic activity are indistinguishable from rabbit liver form 2. Guengerich (34) has also isolated from the lung microsomes of untreated rabbits a similar cytochrome P-450 termed fraction A that is immunologically indistinguishable from liver form 2. It has not been determined whether cytochrome P-450I and fraction A are identical.

Form 3. This cytochrome P-450 species has been purified from liver microsomes of untreated as well as PB- or TCDD-treated rabbits (59) but is apparently not induced by these two inducers. It has a reported subunit molecular weight of 51,000 and an absorption maximum in its reduced CO difference spectrum of 450 nm. When compared to rabbit forms 2, 4, and 6, form 3 is the most active of the four cytochromes in catalyzing N-demethylation of aminopyrine. It is rather inactive in the metabolism of benzphetamine, ethoxyresorufin, and benzo(a)pyrene. Form 3 can also be distinguished from forms 2,

TABLE 1

*Different forms of cytochrome P-450 purified from rabbit liver microsomes**

Preparation	Treatment of Animals	CO Maximum	Subunit Molecular Weight	Comments	Reference
Form 1	TCDD	450.5	48,000	Originally designated form a	60
LM_2	PB	451	48,700	Major PB-inducible form	44, 45
Form 2	PB	451	48,500	Identical to LM_2	63
LM_2	PB	451	49,000	Identical to LM_2 above	33
$P\text{-}450_1$	PB	451	49,000	Probably identical to LM_2	42, 49, 50, 52
70 mM phosphate fraction, hydroxylapatite column	PB	451	49,000	Probably identical to LM_2	40
P-450	PB	Not available	49,700	Probably identical to LM_2	67
Form 3	None	450	51,000	Constitutive form	59
LM_{3b}	None	451	52,000	Identical to form 3	75
LM_3	PB	449	51,000		55
LM_4	BNF	448	55,300	Major BNF-inducible form	44, 45
Form 4	TCDD	447	54,500	Originally designated form c, major TCDD-inducible form in adult liver, identical to LM_4	61
P-448	3-MC	447	51,000	Identical to LM_4	72
LM_{4b}	BNF	447	53,000	Probably identical to LM_4	33
$P\text{-}448_1$	3-MC	448	54,000	Probably identical to LM_4	41, 42, 49, 50
0.3 M phosphate fraction, hydroxylapatite column	PB	447	53,000	Probably identical to LM_4	40
P-448	None	448	53,000	Probably identical to LM_4	46
Form 6	TCDD	448	57,500	Originally designated form b, preferentially induced by TCDD in newborn rabbit liver.	60, 117
P-450	None	449	52,000		108
Form B	None	448	51,500		128

* The abbreviations used are: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PB, phenobarbital; BNF, β-napthaflavone; 3-MC, 3-methylcholanthrene.

TABLE 2

*Different forms of cytochrome P-450 purified from rabbit lung microsomes**

Preparation	Treatment of Animals	CO Maximum	Subunit Molecular Weight	Comments	Reference
1. P-450I	None	452	51,000 or 52,000	Identical to the major PB-inducible form from rabbit liver microsomes, i.e. LM_2 or form 2.	136, 164
2. P-450II	None	450	53,000 or 58,000		141, 164
3. Fraction A	None	451.5	49,000		34

* The abbreviation used is: PB, phenobarbital.

4, and 6 by its subunit molecular weight, peptide map, and immunological properties.

A constitutive form of cytochrome P-450, termed LM_{3b}, has been purified by Koop and Coon (75) from liver microsomes of untreated rabbits. It is reported to have a subunit molecular weight of 52,000 and the absorption maximum of its reduced CO complex is 451 nm. Exchange of enzyme preparations between the two laboratories has confirmed that form 3 purified by Johnson (59) is identical to LM_{3b}.[1]

Another form has been isolated by Ingelman-Sundberg from the liver microsomes of PB-treated rabbits and is referred to as "LM_3." It has a subunit molecular weight of 51,000 and a CO maximum of 449 nm. It appears to be identical to form 3*c* recently isolated by Coon and coworkers.[1]

Form 4. Form 4 is the major liver microsomal form of cytochrome P-450 induced in adult rabbits by 3-MC, TCDD, and β-napthoflavone (BNF). It has been well-characterized by a number of laboratories (22–24, 33, 41, 42, 44, 49, 50, 63, 72). It has been called LM_4 (44, 45), LM_{4b} (33), P-448 (72), $P\text{-}448_1$ (42, 49, 50), and 0.3M phosphate fraction (40). The reported subunit molecular weight of form 4 ranges from 51,000 to 55,300. It has an absorption maximum in its reduced CO difference spectrum of 447 to 448 nm. This protein contains one residue

[1] Presented at the International Symposium on Biochemistry, Biophysics, and Regulation of Cytochrome P-450, June 15–18, 1980, Stockholm, Sweden.

of glucosamine, one residue of glucose, and two residues of mannose per subunit (44). The C-terminal amino acid is lysine; the N-terminal residue is probably blocked (44). Form 4 has been isolated predominantly in the high-spin state, but it can be converted to the low-spin state by a number of reagents (44). Form 4 isolated from BNF- or TCDD-treated rabbits (44, 61) is most likely identical to form 4 isolated from PB-treated or untreated rabbits (44). Unlike the major form of cytochrome P-450 isolated from the liver microsomes of 3-MC-treated rats, rabbit form 4 is rather inactive in catalyzing the metabolism of polycyclic aromatic hydrocarbons. It catalyzes the metabolism of acetanilide, biphenyl, ethoxyresorufin, and benzo(a)pyrene 7,8-diol, but is a rather inactive catalyst toward most other cytochrome P-450 substrates.

Guengerich (34) has partially purified a cytochrome P-450, designated LM_{4a}, from the liver microsomes of BNF-treated rabbits. It has the same subunit molecular weight and absorption maximum in its reduced CO difference spectrum as LM_{4b} but catalyzes the metabolism of benzo(a)pyrene at a somewhat faster rate.

Form 6. Studies by Johnson and coworkers (60, 117) have shown that form 6 is induced in both adult and neonate rabbit liver by TCDD. It has a subunit molecular weight of 57,500; the absorption maximum of its reduced CO complex is at 448 nm. Form 6 catalyzes the metabolism of ethoxyresorufin and is approximately 100-fold more active than forms 2, 3, and 4 in metabolizing polycyclic aromatic hydrocarbons such as benzo(a)pyrene (59). It can be distinguished from forms 2, 3, and 4 by a number of criteria. It has not been determined whether LM_7 isolated by Guengerich from untreated rabbits (33) is a distinct form of cytochrome P-450 or is related to form 6.

Other Partially Purified Rabbit Cytochrome P-450 Fractions. Other cytochrome P-450 fractions have been isolated from rabbit liver microsomes. Their relationship to the preceding five forms has not been determined. The following have been isolated from untreated rabbits: a) a species of cytochrome P-450 that has a high affinity for cytochrome b_5 and requires cytochrome b_5 and NADH-cytochrome b_5 reductase in addition to NADPH-cytochrome P-450 reductase to catalyze the NADPH-dependent O-demethylation of *p*-nitroanisole (108), and b) fractions A and B purified by Philpot and Arinc (128). Cytochrome P-450 species isolated from PB-treated rabbits include a) a mixture of two cytochromes P-450 designated $LM_{1,7}$ (45), b) LM_{xx} (33), and c) P-450III (40).

The laboratories of Philpot and coworkers (136, 164) and Guengerich (34) have each isolated two forms of cytochrome P-450 from the lung microsomes of untreated rabbits. They were termed cytochrome P-450I and cytochrome P-450II and fractions A and B, respectively. Cytochrome P-450I and fraction A have already been discussed above. Whether P-450II and fraction B are related to each other or to other forms of cytochrome P-450 present in rabbit liver microsomes is unknown.

F. Multiple Forms of Cytochrome P-450 in Rat Liver Microsomes

The various forms of cytochrome P-450 that have been purified from the liver microsomes of rats and some of their properties are listed in table 3. As was noted with the rabbit, several of the forms purified in one laboratory are probably identical to forms isolated by other laboratories. However, due to the lack of a uniform nomenclature or structural information, the assignment of identity in table 3 for some of the cytochrome P-450 species should be considered tentative. By using criteria such as subunit molecular weight, absorption maximum of the reduced CO complex, immunological and catalytic properties, and partial amino acid sequence, from five to seven forms of cytochrome P-450 are present in rat liver microsomes. Those forms for which the most definitive evidence is available will be briefly discussed first.

1. Major PB-Inducible Form. This form is the major species of cytochrome P-450 in liver microsomes of PB-treated rats (33, 134, 161) and is also induced by polychlorinated biphenyls such as Aroclor 1254 (134). The following preparations are most likely this species: $P\text{-}450_b$ (134), fraction B isolated from PB-treated rats (33), fraction C (161), peak I*b* (25), and P-450 (66, 106). Reports of subunit molecular weight range from 49,000 to 53,000; the absorption maximum of its reduced CO difference spectrum ranges from 450 to 451 nm. The N- and C-terminal amino acids are glutamic acid and serine, respectively (12). This form of cytochrome P-450 catalyzes the metabolism of such substrates as benzphetamine, N,N-dimethylaniline, parathion, and N,N-dimethylphentermine (33, 66, 109, 110, 134, 161). It is immunologically distinct from the major PB-inducible form in rabbit liver microsomes, form 2 (66, 148).

2. Major 3-MC-Inducible Form. This form is the major species of cytochrome P-450 induced by 3-MC and is also induced by BNF and polychlorinated biphenyls such as Aroclor 1254 and 3,4,5,3′,4′-pentachlorobiphenyl. The following preparations are most likely this species: $P\text{-}450_c$ (134), fraction B isolated from 3-MC-treated rats (33), peak II (25), P-448 (106, 133), and H-II (121). Reports of subunit molecular weight of this cytochrome P-450 range from 53,500 to 56,000; its reduced-CO complex has an absorption maximum of 447 to 448 nm. The N- and C-terminal residues are isoleucine and leucine (12). It is one of the most efficient of the cytochromes P-450 in catalyzing the metabolism of a variety of polycyclic aromatic hydrocarbons as well as 7-ethoxycoumarin, 7-ethoxyresorufin, and zoxazolamine. This form is immunologically distinct from the major 3-MC-inducible form in rabbit liver microsomes, form 4 (72, 148).

3. PCN-Inducible Form. This species of cytochrome P-450, although relatively unstable, has recently been purified from rats treated with PCN by Elshourbagy and Guzelian (25). It has a subunit molecular weight of 51,000 and an absorption maximum in its reduced CO difference

TABLE 3
*Different forms of cytochrome P-450 purified from rat liver microsomes**

Preparation	Treatment of Animals	CO Maximum	Subunit Molecular Weight	Comments	Reference
P-450$_b$	Aroclor 1254 PB	450	52,000	Major PB-inducible form	134
Fraction C	PB	450	52,000	Identical to P-450$_b$	161
Fraction B	PB	449.6	53,000	Major PB-inducible form; probably identical to P-450$_b$, although the end terminals of these two preparations have been found to be different	33, 36
Peak I$_b$, phosphocellulose column	PB	450.7	50,000	Probably identical to P-450$_b$	25
P-450	PB	451	50,000	Probably identical to P-450$_b$	106
P-450	PB	Not available	48,500	Probably identical to P-450$_b$	66
P-450$_c$	Aroclor 1254 3-MC	447	56,000	Major 3-MC-inducible form	134
Fraction B	3-MC	447.6	56,000	Major 3-MC-inducible form; probably identical to P-450$_c$, although the end terminals of these two preparations have been found to be different.	33, 36
Peak II, DE-52 column	3-MC	448	54,000	Probably identical to P-450$_c$	25
P-448	3-MC	448	56,500	Probably identical to P-450$_c$	106
H-II fraction	3,4,5,3′,4′-Pentachlorobiphenyl	447	53,500	Probably identical to P-450$_c$	121
Peak III, phosphocellulose column	PCN	450	51,000	Major PCN-inducible form	25
P-450$_a$	Aroclor 1254 PB 3-MC	452	48,000		134
Fraction D	PB	449.2	54,000		33, 36
50 mM phosphate fraction, CM-Sephadex	Cholestyramine	Not available	50,000		40
80 mM phosphate fraction, CM-Sephadex	Cholestyramine	Not available	50,000		40
Fraction A	None	450	52,200		1
Fraction B	None	450.8	52,400		1

* The abbreviations used are: PB, phenobarbital; 3-MC, 3-methylcholanthrene; PCN, pregnenolone 16α-carbonitrile.

spectrum of 450 nm. It catalyzes the N-demethylation of ethylmorphine and aminopyrine but at a slower rate than would be expected from studies with liver microsomes from PCN-treated rats. On the basis of several criteria, it is a different isozyme than either the major PB- or 3-MC-inducible forms.

4. P-450$_a$. This species of cytochrome P-450 has been purified from PB-, 3-MC-, and Aroclor-treated rats by Ryan et al. (134). It has a subunit molecular weight of 48,000 and the absorption maximum of its reduced CO complex is at 452 nm. It contains methionine as both its N- and C-terminal amino acid and a partial sequence of the first 19 amino acids on its N-terminal end differs considerably from the sequences of either the PB(P-450$_b$)- or 3-MC(P-450$_c$)-inducible forms (12). Cytochrome P-450$_a$ preferentially catalyzes the hydroxylation of testosterone at the 7α-position but is a rather inactive catalyst for the metabolism of other substrates (134).

5. P-450$_d$. This species of cytochrome P-450 has been purified from rats treated with isosafrole by Levin and coworkers.[1] It has a subunit molecular weight of 52,000, identical to P-450$_b$, and its reduced CO complex has an absorption maximum of 447 nm, indistinguishable from P-450$_c$. Cytochrome P-450$_d$ was isolated as an isosafrole metabolite-cytochrome complex and has poor catalytic activity toward a number of substrates either before or after removal of the bound isosafrole metabolite. P-450$_d$ shows partial identity with P-450$_c$ when analyzed by Ouchterlony double-diffusion techniques but does not crossreact with antibodies prepared against cytochromes P-450$_a$ or P-450$_b$. Peptide maps of proteolytic digests of cytochrome P-450$_d$ indicate that its primary structure differs from P-450$_a$, P-450$_b$, and P-450$_c$.

6. Other Rat Cytochrome P-450 Fractions. Other cytochrome P-450 fractions have been isolated from liver microsomes of untreated, PB-, and cholestyramine-treated rats. Several have been highly purified but their relationship to the preceding four forms has not been determined.

A. Two forms of cytochrome P-450, fraction A and fraction B, have been purified from the liver microsomes of untreated rats by Agosin et al. (1). The subunit molec-

ular weight of both forms is 52,000 and the absorption maximum of the reduced CO complex is 450 nm for fraction A and 450.8 nm for fraction B. Neither of the forms is very active in catalyzing the metabolism of substrates such as benzphetamine, *p*-chloro-N-methylaniline, or 7-ethoxyresorufin, but both enzymes actively catalyze the metabolism of juvenile hormone and its analogue, fraction B being the better catalyst.

B. Gibson and Schenkman (30) have also partially purified two forms of cytochrome P-450 from untreated rats. The reduced CO complex absorption maximum is at 449.5 nm for P-450I, and 448.5 nm for P-450II. The subunit molecular weight is 52,000 for P-450II and is undetermined for P-450I because of its low specific content. The relationship between P-450I and P-450II and fractions A and B obtained by Agosin et al. (1) is not known.

C. Besides the major PB-inducible form of cytochrome P-450 and P-450$_a$, several other species of cytochrome P-450 have been isolated from the liver microsomes of PB-treated rats by Guengerich (33). Referred to as fraction A, fraction C, and fraction D, it is not known whether any of these fractions correspond to P-450$_a$.

D. Recently, Hansson and Wikvall (40) have isolated two forms of cytochrome P-450 referred to as "50 mM phosphate fraction and 80 mM phosphate fraction" from cholestyramine-treated rats. It is not known whether these forms of cytochrome P-450 are related to PCN-inducible P-450 (25), P-450$_a$ (134), fractions A, C, or D (33, 36), or fractions I and II (30).

G. Multiple Forms of Cytochrome P-450 in Mouse Liver Microsomes

Although inbred strains of mice have been used to study the genetic mechanisms of control of cytochrome P-450 induction, less interest has been shown in isolating and purifying multiple forms of the mouse isozymes than has been the case for the cytochrome P-450 isozymes from rat or rabbit. The four forms of liver microsomal cytochrome P-450 from the mouse that have been purified to date are listed in table 4. Undoubtedly, more forms of mouse cytochrome P-450 will be purified in the future since preliminary information from partially purified mouse preparations suggests their presence (48).

Two forms of cytochrome P-450 have been highly purified from the liver microsomes of PB-treated B6D2F$_1$/J mice by Huang et al. (48). The reported subunit molecular weight of A_2 is 50,000 and for C_2, 56,000. The absorption maximum of their reduced CO complexes is at 451 nm for A_2 and at 450 nm for C_2. Two other species have been partially purified; the absorption maximum of the reduced CO complex of A_1 is 450 nm and for C_1, 449 nm. All four fractions can be clearly distinguished by their substrate specificities. Interestingly, the C_1 fraction crossreacts quite well with antibody produced against the major PB-inducible form from rat liver microsomes, while A_1, A_2, and C_2 crossreact very poorly, which suggests that most of the forms of cytochrome P-450 from PB-treated mice differ from those isolated from PB-treated rats.

Negishi and Nebert (115) have purified two forms of cytochrome P-450, referred to as P-448 and P$_1$-450, from 3-MC-treated C57BL/6N mice. Although the subunit molecular weights of the two forms are indistinguishable, the absorption maximum of their reduced CO complexes is at 449.3 nm for P$_1$-450 and 448 nm for P-448. These two forms were distinguished from each other by peptide mapping, immunological properties, and their abilities to catalyze the metabolism of benzo(a)pyrene and acetanilide.

H. Multiple Forms of Cytochrome P-450 in Pig Microsomes

Two cytochromes P-450 have recently been purified from pig tissues by Masters et al. (105). As shown in table 5, one form of cytochrome P-450 has been purified from kidney microsomes of untreated pig and has a subunit molecular weight of 56,000. The other form, purified from liver microsomes of PB-treated pig, has a subunit molecular weight of 52,000. Although the absorption maximum of the reduced CO complexes of both forms is at 450 nm, they can be distinguished by their immunological properties. Both forms of cytochrome P-450 are active in the ω- and ω-1 hydroxylation of lauric acid.

I. Multiple Forms of Cytochrome P-450 in Human Liver Microsomes

Although the presence of multiple forms of cytochrome P-450 in experimental animals has been convincingly established, evidence for multiple forms of human cytochrome P-450 has only recently begun to appear. Earlier studies with human adult and fetal liver as well as human placenta (70, 71, 127) have provided indirect evidence consistent with the presence of multiple forms of human

TABLE 4

*Different forms of cytochrome P-450 purified from mouse liver microsomes**

Preparation	Treatment of Animals	CO Maximum	Subunit Molecular Weight	Reference
A_2 from B6D2F$_1$/J mice	PB	451	50,000	48
C_2 from B6D2F$_1$/J mice	PB	450	56,000	48
P$_1$-450 from B-6 mice	3-MC	449.3	55,000	115
P-448 from B-6 mice	3-MC	448	55,000	115

* The abbreviations used are: PB, phenobarbital; 3-MC, 3-methylcholanthrene.

TABLE 5

*Different forms of cytochrome P-450 purified from pig microsomes**

Preparation	Treatment of Animals	CO Maximum	Subunit Molecular Weight	Reference
P-450 from pig kidney microsomes	None	450	56,000	105
P-450 from pig liver microsomes	PB	450	52,000	105

* The abbreviation used is: PB, phenobarbital.

cytochrome P-450. For example, Kapitulnik et al. (71) found that in human autopsy livers benzo(a)pyrene hydroxylase activity correlated well with zoxazolamine, coumarin, and hexobarbital hydroxylase activities, but poorly with 7-ethoxycoumarin deethylase and antipyrine hydroxylase activities. Metabolism of 7-ethoxycoumarin by human liver also did not correlate well with metabolism of antipyrine. Addition of 7,8-benzoflavone to human liver homogenates or microsomes increased the rate of benzo(a)pyrene and zoxazolamine hydroxylation but had little or no effect on the rates of metabolism of 7-ethoxycoumarin, coumarin, and hexobarbital (70). There is a marked individuality in the degree of stimulation by 7,8-benzoflavone on the metabolism of various compounds by liver samples from different individuals. These results suggest that human liver contains multiple forms of cytochrome P-450 but that the relative proportion of each form varies among individuals.

Several laboratories have recently reported on the purification of cytochrome P-450 from human liver. Kamataki and coworkers (68, 74) partially purfied cytochrome P-450 from human fetal and adult livers. The absorption maximum of the reduced CO complex was at 449 nm for the fetal liver cytochrome and 451 to 452 nm for the adult liver cytochrome. The catalytic activity of these preparations was generally low. Beaune et al. (6) have used column chromatography to separate human liver cytochrome P-450 into several fractions. Fraction A has a subunit molecular weight of about 45,000 and the absorption maximum of its reduced CO complex is at 450 nm. Not much information is available on the other cytochrome P-450 fractions. Bosterling and Trudell (11) have obtained three cytochrome P-450 fractions from human liver microsomes. One of the fractions (HA-2) has been highly purified and has a subunit molecular weight of 52,500. Magnetic circular dichroism studies suggest that the heme environment of human HA-2 differs from that of rat cytochrome P-450.

More recently, human liver microsomal cytochrome P-450 has been purified to homogeneity by Wang et al. (155). As noted in table 6, the cytochrome P-450 isolated from different individuals has significantly different subunit molecular weights. Human cytochrome P-450 and rat cytochrome P-450 are clearly different proteins as indicated by their lack of crossreactivity as assayed by Ouchterlony double-diffusion analysis. However, human cytochromes P-450 are immunologically more similar to the major PB-inducible form of rat cytochrome P-450 than to the major 3-MC-inducible form as judged by complement fixation or inhibition of catalytic activity by antibodies (155). Complement fixation studies have also indicated that the cytochromes P-450 isolated from the liver of different individuals are not identical.

TABLE 6

Different forms of cytochrome P-450 purified from human liver microsomes

Preparation	CO Maximum	Subunit Molecular Weight	Reference
Patients 6 and 8	450	53,000	155
Patient 3	450	55,500	155
HA-2 fraction	Not available	52,500	11
LH-20 fraction	451–452	Not available	68

Thus, it is clear that human cytochrome P-450 is also a family of isozymes that are distinct from the cytochromes P-450 isolated from experimental animals. The information available thus far does not permit an estimate of the number of multiple forms of cytochrome P-450 in human tissues. A fair assumption is that there may be a large number of forms, in view of the genetic and environmental differences among and between populations. However, a definitive answer must await the purification of cytochrome P-450 from other human samples.

IV. Partial Separation of Various Forms of Cytochrome P-450 within the Microsomal Membrane

Over the years, the argument has been made that the isolation and purification of multiple forms of cytochrome P-450 from various species and tissues is an artifact and is the result of the use of detergents and the disruption of membranes. The case for multiplicity could perhaps be made stronger if various forms of cytochrome P-450 could be separated (even partially) into different membrane fractions without the use of detergents or other disruptions of the microsomal membrane. Indeed, such studies have been successfully performed and the results are consistent with purification studies.

Glaumann (31) separated microsomes into rough, smooth I, and smooth II subfractions with a cation-containing sucrose gradient. If the microsomes were from 3-MC-treated rats, the absorption maximum of the reduced-CO complexes was at 448 nm for rough and smooth I fractions and at 450 nm for smooth II fractions. Similarly, Mailman et al. (102) have separated the microsomes from untreated rats and mice into several smooth and rough subfractions with a two-step discontinuous sucrose gradient. These submicrosomal fractions exhibited different spectral characteristics including differences in the absorption maximum of their reduced CO difference spectra. Thus, these results are consistent with purification studies and suggest that different forms of cytochrome P-450 may be located in different parts of the endoplasmic reticulum.

V. Identification and Quantification of Various Forms of Cytochrome P-450 in Microsomal Preparations

Since it is clear that the types and proportions of the various forms of microsomal cytochrome P-450 in a tissue will determine the metabolic profile of a compound and affect its biological and toxicological properties, efforts

have been made to show either that a particular form of cytochrome P-450 is present or to quantify various forms of cytochrome P-450 in microsomal preparations. Some of the approaches currently being employed are discussed briefly below.

A. Estimation by Chemical Probes

Some compounds, including certain substrates, inhibitors, and other ligands, react selectively with various forms of microsomal cytochrome P-450 and useful information can sometimes be derived by studying the selective interactions between microsomal cytochrome P-450 and these compounds. For example, enantiomeric selectivity and perturbation of product ratios are observed in the metabolism of warfarin and are dependent on the form of cytochrome P-450 used as the catalyst (129, 130). Attempts are being made to use the results from metabolism of warfarin as a means of estimating the forms of cytochrome P-450 present in a preparation (131). Metyrapone, 7,8-benzoflavone, and tetrahydrofuran selectively inhibit the O-deethylation of 7-ethoxycoumarin (152) and have been used to probe the relative distribution of the different forms of cytochrome P-450 in various microsomal preparations.

Compounds such as piperonyl butoxide, safrole, amphetamine, and its derivatives are capable of forming product adducts with cytochrome P-450 (28, 47). Other compounds, like metyrapone, can selectively bind to different forms of cytochrome P-450 with different affinities. Based on the combined results from these two types of studies, the estimation of several classes of cytochrome P-450 in liver microsomes has been attempted (159, 160).

One advantage of this type of analysis is that it is relatively simple to perform. However, it is limited by its inherent selectivity. Only a rough estimate can be made concerning the composition of microsomal cytochrome P-450 if the probe used is not truly selective for a particular form of cytochrome P-450. Other inherent problems associated with this method include difficulties in demonstrating saturation and estimating extinction coefficients. In addition, it is often very difficult to correlate the forms of cytochrome P-450 differentiated by this technique with various purified forms of cytochrome P-450.

B. SDS-Gel Electrophoresis

The electrophoretic pattern on SDS gels of the protein-staining bands from a microsomal preparation has been used as a simple and convenient approach to estimate the forms of cytochrome P-450 present in different sources of microsomes. The assignment of a band in the 50,000 molecular weight region as cytochrome P-450 has been based on heme-staining and/or its relative intensity upon treatment of the animal with an inducer. This method is limited by the finite resolution of protein bands and the reliability of heme-staining. One must be cautious in equating the change in the intensity of a particular band to a change in concentration of a particular form of cytochrome P-450. Microsomes contain many other proteins whose subunit molecular weights are between 45,000 and 60,000 and, thus, a single band is unlikely to be a single protein. In addition, some of these proteins are also inducible (e.g. epoxide hydrase and UDP-glucuronyltransferase). Identification of a heme-containing protein band in microsomes is not always straightforward and in SDS gels is complicated by heme exchange and nonspecific binding of heme to nonheme proteins.

In recent years, the results obtained from SDS gel electrophoresis of microsomal preparations have sometimes been overinterpreted. It is becoming more common to find a protein band on SDS-gel being equated to single species of cytochrome P-450, and changes in the intensity of protein bands between 45,000 to 60,000 after treatment with a compound are being used to quantify the various species of cytochrome P-450 in microsomes. It is even possible to find reports in which all the protein-staining bands on a densitometer tracing in the molecular weight range of 45,000 to 60,000 are labeled as apocytochromes P-450.

SDS-gel electrophoresis, coupled with other techniques, can undoubtedly be used to show that qualitative changes in cytochrome P-450 occur upon treatment of animals with different compounds. However, it should not be used to quantify various species of cytochrome P-450 in a microsomal sample for the following reasons: a) cytochrome P-450 accounts for only 5% to 15% of the total liver microsomal protein in microsomes from untreated or PB-treated rats. Thus, protein bands whose subunit molecular weights are between 45,000 to 60,000 should not all be labeled as apocytochrome P-450. In addition, in microsomes prepared from other tissues, cytochrome P-450 may be a minor protein species. b) From 5 to 10 or more polypeptides can be resolved on SDS-gels in the molecular weight range of 45,000 to 60,000 depending on the conditions and gel system chosen. Densitometer tracings usually cannot resolve all of these bands. c) Some proteins that have already been identified, and perhaps others that have not yet been isolated and characterized, are also induced by the various treatments used and have or could have subunit molecular weights that fall between 45,000 to 60,000.

C. Peptide Mapping

On the other hand, Johnson et al. (64) have used SDS gels in conjunction with limited proteolysis to show that a particular form of cytochrome P-450 is present in rabbit liver microsomes. Microsomal proteins were first resolved by SDS-gel electrophoresis and the protein band in question was then subjected to partial proteolysis. The resulting peptide fragments were reanalyzed by SDS-gel electrophoresis and compared to the peptide fragments obtained from the partial proteolysis of the purified form of this species of cytochrome P-450. By use of this tech-

nique, Norman et al. (117) have shown that form 6 is the single cytochrome P-450 species induced transplacentally in the liver microsomes of newborn rabbits by TCDD. This method can be used to monitor the occurrence and induction of a specific form of cytochrome P-450 only if that species is the major component in the microsomal protein band being subjected to proteolysis.

D. Column Chromatography

Warner and Neims (156) have shown that rat liver microsomal cytochrome P-450 from untreated rats can be solubilized and resolved into four peaks by anion-exchange chromatography with an overall recovery of greater than 75%. Pretreatment of animals with BNF or PB changes the distribution of cytochrome P-450 in the different peaks.

Because of its good recovery, this method can be used to show that qualitative changes in the cytochrome P-450 population occur in animals after various in vivo treatments. However, this method is not suitable for quantification since each peak may contain more than one form of cytochrome P-450. In addition, chromatography of microsomal protein after various animal pretreatments may yield corresponding peaks that do not contain the same form of cytochrome P-450.

E. Immunological Analysis

The purification of various forms of cytochrome P-450 and the subsequent ability to prepare specific antibodies against these forms provide perhaps one of the best potential methods for quantifying various forms of cytochrome P-450 in microsomes. By using a radial immunodiffusion technique, Thomas et al. (146) have determined that the major PB-inducible form of cytochrome P-450 (PB-P-450 or P-450$_b$) accounts for 62% to 76% of the total microsomal cytochrome P-450 in the liver microsomes of PB-treated rats. Less than 10% of the total microsomal cytochrome P-450 in these PB-microsomes appears to be the 3-MC-inducible form (cytochrome P-448 or P-450$_c$). The 3-MC-inducible form, on the other hand, represents 83% to 96% of the total microsomal cytochrome P-450 in the liver microsomes of 3-MC-treated rats. Microsomal preparations from untreated rats contain less than 10% of either form of cytochrome P-450. In a similar study Omura et al. (119) reported that liver microsomes from PB- or 3-MC-treated rats contain approximately 50% of the total microsomal cytochrome P-450 as the PB-inducible or 3-MC-inducible form of cytochrome P-450. The reason for these different estimates for the amount of the major forms of cytochrome P-450 in liver microsomes from induced rats is unclear. However, based on other types of analysis such as column chromatography (156), the liver microsomes from 3-MC-treated rats most probably contain much less than 83% to 96% of the total microsomal cytochrome P-450 as the 3-MC-inducible form. When more forms of cytochrome P-450 are purified and specific antibodies are prepared, this method promises to be quite useful for identification and quantification of the various forms of cytochrome P-450 in microsomes prepared from different sources.

F. Affinity Chromatography

If all the cytochrome P-450 species in a given set of microsomes could be purified collectively in high yield, SDS-gel electrophoresis of the purified sample might yield a gel pattern that could be used for the quantification of various species of cytochrome P-450. Affinity chromatography is a potentially useful tool for accomplishing this objective since a single step could yield a purified enzyme preparation in high yield.

Some progress has been made recently in developing affinity columns for the purification of microsomal cytochrome P-450. With tryptamine or 2,4-dichloro-6-phenylphenoxyethylamine as a ligand, highly purified cytrochrome P-450 can be obtained directly from detergent-solubilized microsomes with a yield of between 70% to 80% (96). SDS-gel electrophoresis of the highly purified cytochrome P-450 yields three to four protein-staining bands, which presumably are all cytochrome P-450 apoproteins. It is not yet known whether all the different forms of cytochrome P-450 in a particular microsomal preparation have been isolated by this procedure. Nevertheless, this procedure could be very useful for the quantification of most of the cytochrome P-450 species in a microsomal preparation. Two implicit assumptions in this technique are that 1) each species of cytochrome P-450 has a different subunit molecular weight and 2) each can be separated from the others by SDS-gel electrophoresis.

VI. How Many Forms of Microsomal Cytochrome P-450?

The evidence available thus far overwhelmingly supports the existence of multiple forms of cytochrome P-450 in microsomes prepared from liver and other tissues. However, it will probably be some time before the actual number of different forms of cytochrome P-450 in these tissues is determined with certainty. The difficulties in making such an assessment are due to a number of factors: a) The lack of a uniform system of nomenclature as well as different methods of purification make it difficult to compare different preparations from different laboratories. b) SDS-gel electrophoresis may not be able to resolve cytochrome P-450 species whose subunit molecular weights differ by less than 500 to 1,000 daltons. Thus, it is not known whether a seemingly electrophoretically homogeneous preparation contains more than one species. The eventual determination of amino acid sequences would help to resolve this area of uncertainty. c) Up to the present time, most efforts at purification have concentrated on the major inducible cytochrome P-450 species, and very few of the minor species or uninduced species have been studied. In addition, little work has been done on microsomal cytochrome P-450 species

in tissues other than liver, lung, and kidney.

Despite these uncertainties, it is generally agreed that there is a large but finite number of cytochrome P-450 species in mammalian microsomes. Looking back at tables 1 to 6 and the discussion that accompanied them, a conservative estimate of the minimum number of forms in various types of microsomes would be: 1) five to six forms of cytochrome P-450 in rabbit liver and lung microsomes taken collectively, 2) five to seven forms of cytochrome P-450 in rat liver microsomes, 3) four to six forms in mouse liver microsomes, 4) two forms in pig liver microsomes, and 5) an undetermined number in human liver microsomes. Since the forms isolated from different species are most likely different, the minimum total number of cytochrome P-450 species described to date is more than 20. It is quite likely that this number will easily double or triple when the cytochromes P-450 from other tissues and other species are carefully examined.

In contrast to the idea that there exists a large but finite number of cytochrome P-450 species, Nebert (111) has postulated the existence of possibly hundreds or thousands of discrete forms of cytochrome P-450 that would vary slightly in order to be able to metabolize the thousands of substrates of widely differing chemical structues efficiently. Nebert (111) argues that, like the immunoglobulins, the cytochrome P-450 polypeptides may have both variable and constant regions. Immunochemical studies and amino acid sequencing techniques may not be sensitive enough to determine whether a purified cytochrome P-450 contains only one form or many forms with only very minor differences.

To judge from the peptide maps generated from several inducible species of purified cytochrome P-450 (25, 35, 64, 134) it is clear that the primary structures of these P-450s are fairly different. In addition, the subunit molecular weights of some of these species differ by as much as 10,000. Thus, the available data are not consistent with inducible forms having only minor modifications in primary structure. Certainly, amino acid sequencing of various forms of cytochrome P-450 could determine how similar in primary structure they are and whether there exist variable and constant regions. The existence of many forms of cytochrome P-450 in a single preparation will be more difficult to rule out since many of the techniques available are not sensitive enough to detect a single change of amino acid or a very minor alteration in the polypeptide sequence.

VII. Some Unanswered Questions

Despite the overwhelming evidence supporting the idea of multiple forms of cytochrome P-450 in microsomes, this concept has not received universal acceptance due to several as yet unaccounted-for observations. For example, Fischer and Spencer (27) found that when protein synthesis in young female rats is blocked by the administration of actinomycin D or puromycin, there is a decrease in the P-450 content of the microsomes but treatment with benzanthracene still leads to the formation of cytochrome P-448. Furthermore, these investigators found that the absorption maximum of the reduced CO difference spectrum can be shifted from 450 to 448 nm by treating the liver microsomes from immature female rats with benzanthracene. On the other hand, this shift in absorption maximum does not occur if microsomes from adult female rats are used. Kahl et al. (65) have also found that induction of cytochrome P-448 in rats by 3-MC still occurs even when protein synthesis and the increase in cytochrome level are blocked by treatment of the rat with cycloheximide. These results are in contrast to the findings of Alvares et al. (3, 76) that induction of cytochrome P-448 requires protein synthesis and cannot be duplicated in vitro. These seemingly contradictory results can be interpreted in several ways: 1) The formation of cytochrome P-448 is not due to true enzyme induction but perhaps to an alteration in membrane structure (i.e. cytochromes P-450 and P-448 are interconvertible). 2) The formation of cytochrome P-448 occurs by a modification of the preexisting cytochrome in a posttranslational step that is not blocked by cycloheximide. 3) Alternatively, protein synthesis may have been only partially blocked in these experiments, allowing the synthesis of some cytochrome P-448 but decreasing total cytochrome P-450 content.

Parke and coworkers (107, 123, 125) have observed that when animals are treated with carcinogens, the increase in biphenyl 2-hydroxylase activity is biphasic, i.e. an initial stimulatory phase followed later by enzyme induction. Parke (122–124) argued that since the initial stimulation occurs within 15 min of treatment with the carcinogen, and since biphenyl 2-hydroxylation is preferentially catalyzed by cytochrome P-448, the stimulation may represent a conformational change of preexisting cytochrome P-450 to P-448.

While the results of the studies just cited are subject to alternative interpretations, they are nevertheless inconsistent with the rich literature supporting the existence of multiple forms of cytochrome P-450. These studies should perhaps be repeated before definitive conclusions are reached.

VIII. Regiospecificity of Multiple Forms of Cytochrome P-450 in Drug, Mutagen, and Carcinogen Metabolism

Even though various forms of cytochrome P-450 have broad and overlapping substrate specificities, they often exhibit remarkable regioselectivity and stereoselectivity. For example, rat cytochrome P-450$_a$, P-450$_b$, and P-450$_c$ preferentially hydroxylate testosterone at the 7α-, 16α-, and 6β-positions, respectively (134). Cytochromes P-450 isolated from mice and rabbits also preferentially hydroxylate testosterone at specific positions (45, 48). The metabolism of warfarin by liver microsomal cytochrome P-450 provides another excellent example of the regio-

selectivity and stereoselectivity of various forms of cytochrome P-450. Fasco et al. (26) have shown that rabbit LM_4 metabolizes warfarin only when warfarin is in the R-configuration and the coumarin side of the warfarin molecule is oriented toward the active site of the enzyme. Rabbit LM_2, on the other hand, is able to metabolize warfarin in either its R- or S-configuration but forms different products depending on the configuration of warfarin. When warfarin is in the S-configuration, rabbit LM_2 acts on the coumarin side of the molecule, but when warfarin is in the R-configuration, LM_2 acts on the phenyl ring. The regioselectivity and stereoselectivity toward warfarin of the major 3-MC-inducible form of liver microsomal cytochrome P-450 from 3-MC-treated rats are very similar to those of rabbit LM_4 but the rat enzyme is 40-fold more active than rabbit LM_4 (69). The major PB-inducible form of cytochrome P-450 from PB-treated rats and rabbit LM_2 have similar regioselectivities but their stereoselectivities are opposite in orientation (69).

The differing abilities of various forms of cytochrome P-450 to convert various chemicals such as benzo(a)pyrene, 2-aminoanthracene, and tryptophan pyrolysis products to metabolites that are mutagenic in the Ames test has been reported (56, 81, 118). For example, cytochrome P-448 isolated from 3-MC-treated rats is one of the most active enzymes that catalyze benzo(a)pyrene-induced mutagenesis whereas other forms of cytochrome P-450 are rather inactive, a result that is consistent with metabolism studies (81). In addition to differences in overall metabolic rates, various forms of cytochrome P-450 also exhibit positional selectivity and stereoselectivity in the oxygenation of benzo(a)pyrene and (−)*trans*-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene (23, 24, 92, 145, 163). For example, when benzo(a)pyrene is used as the substrate, the ratio of 3-hydroxybenzo(a)pyrene to 9-hydroxybenzo(a)pyrene formation ranges from 1 to 20 depending on the form of cytochrome P-450 catalyzing the reaction (92). The stereoselective conversion of (−)*trans*-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene to (±) 7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (diol epoxide 1) and (±)7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (diol epoxide 2) is also dependent on the particular cytochrome P-450 species used (24, 145, 163). Thus, cytochrome P-448 from 3-MC-treated rats and rabbit LM_4 preferentially convert (−)*trans*-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene to diol epoxide 2 whereas the rabbit LM_7 fraction preferentially metabolizes this compound to diol epoxide 1. Rabbit LM_2 gives somewhat more diol epoxide 2 than diol epoxide 1. Since the (+) and (−) enantiomers of diol epoxides 1 and 2 have different mutagenic and carcinogenic activities in different bioassay systems (13, 165), the relative amount of each enantiomer produced by different forms of cytochrome P-450 could well be an important factor in determining the susceptibility of different tissues, species, and strains to the carcinogenic action of benzo(a)pyrene.

IX. Conclusion

The multiplicity of microsomal cytochrome P-450 has been reviewed. Available evidence indicates that cytochrome P-450 exists in multiple forms, and differences in substrate specificity, positional specificity, and stereospecificity of the various forms of cytochrome P-450 play an important role in regulating the balance between activation and inactivation of a given chemical. Thus, differences in the composition and proportion of the various forms of cytochrome P-450 in a tissue are important factors to consider when evaluating drug and carcinogen metabolism and their related toxicities.

Acknowledgments. We are grateful to numerous investigators for providing us with reprints and preprints. We also wish to thank Mrs. G. Mancinelli for her assistance in the preparation of this manuscript.

REFERENCES

1. Agosin, M., Morello, A., White, R., Repetto, Y., and Pedemonte, J.: Multiple forms of noninduced rat liver cytochrome P-450. Metabolism of 1-(4′-ethylphenoxy)-3,7-dimethyl-6,7-epoxy-trans-2-octene by reconstituted preparations. J. Biol. Chem. **254:** 9915–9920, 1979.
2. Alvares, A. P., Schilling, G., Levin, W., and Kuntzman, R.: Studies on the induction of CO-binding pigments in liver microsomes by phenobarbital and 3-methylcholanthrene. Biochem. Biophys. Res. Commun. **29:** 521–526, 1967.
3. Alvares, A. P., Schilling, G., Levin, W., and Kuntzman, R.: Inability of substrates to alter the carbon monoxide and isocyanide difference spectra of microsomal hemeprotein. J. Pharmacol. Exp. Ther. **176:** 1–10, 1971.
4. Alvares, A. P., and Siekevitz, P.: Gel electrophoresis of partially-purified cytochrome P-450 from liver microsomes of variously-treated rats. Biochem. Biophys. Res. Commun. **54:** 923–929, 1973.
5. Axelrod, J.: The enzymatic N-demethylation of narcotic drugs. J. Pharmacol. Exp. Ther. **117:** 322–330, 1956.
6. Beaune, P., Dansette, P., Flinois, J. P., Columelli, S., Mansuy, D., and Leroux, J. P.: Partial purification of human liver cytochrome P-450. Biochem. Biophys. Res. Commun. **88:** 826–832, 1979.
7. Bernhardsson, C., Bjorkhem, I., Danielsson, H., and Wikvall, K.: 12α-Hydroxylation of 7α-hydroxy-4-cholesten-3-one by a reconstituted system from rat liver microsomes. Biochem. Biophys. Res. Commun. **54:** 1030–1038, 1973.
8. Bidelman, K., and G. J. Mannering: Induction of drug metabolism. V. Independent formation of cytochrome P-450 and P_1-450 in rats treated with phenobarbital and 3-methylcholanthrene simultaneously. Mol. Pharmacol. **6:** 697–701, 1970.
9. Bjorkhem, I., Danielsson, H., and Wikvall, K.: Hydroxylation of bile acids by reconstituted systems from rat liver microsomes. J. Biol. Chem. **249:** 6439–6445, 1974.
10. Bosterling, B., Stier, A., Hildebrandt, A. G., Dawson, J. H., and Trudell, J. R.: Reconstitution of cytochrome P-450 and cytochrome P-450 reductase into phosphatidylcholine-phosphatidylethanolamine bilayers: Characterization of structure and metabolic activity. Mol Pharmacol. **16:** 332–342, 1979.
11. Bosterling, B., and Trudell, J. R.: Purification of human and rat cytochromes P-450 and P-450 reductase and their reconstitution into phospholipid vesicles. *In* Microsomes, Drug Oxidations and Chemical Carcinogenesis, ed. by M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. R. Gillette, and P. J. O'Brien, pp. 115–118, Academic Press, New York, 1980.
12. Botelho, L. H., Ryan, D. E., and Levin, W.: Amino acid compositions and partial amino acid sequences of three highly purified forms of liver microsomal cytochrome P-450 from rats treated with polychlorinated biphenyls, phenobarbital, or 3-methylcholanthrene. J. Biol. Chem. **254:** 5635–5640, 1979.
13. Buening, M. K., Wislocki, P. G., Levin, W., Yagi, H., Thakker, D. R., Akagi, H., Koreeda, M., Jerina, D. M., and Conney, A. H.: Tumorigenicity of the optical enantiomers of the diastereomeric benzo(a)pyrene 7,8-diol-9,10-epoxides in newborn mice: Exceptional activity of (+)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene. Proc. Nat. Acad. Sci. U.S.A. **75:** 5358–5361, 1978.
14. Cleveland, D. W., Fischer, S. G., Kirschner, M. W., and Laemmli, U. K.: Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J. Biol. Chem. **252:** 1102–1106, 1977.
15. Comai, K., and Gaylor, J. L.: Existence and separation of three forms of cytochrome P-450 from rat liver microsomes. J. Biol. Chem. **248:** 4947–4955, 1973.
16. Conney, A. H., Gillette, J. R., Inscoe, J. K., Trams, E. R., and Posner,

H. S.: Induced synthesis of liver microsomal enzymes which metabolise foreign compounds. Science **130:** 1478–1479, 1959.

17. CONNEY, A. H., LEVIN, W., JACOBSON, M., KUNTZMAN, R., COOPER, D. Y., AND ROSENTHAL, O.: Specificity in the regulation of the 6β-, 7α- and 16α-hydroxylation of testosterone by rat liver microsomes. *In* Microsomes and Drug Oxidations, ed. by J. R. Gillette, A. H. Conney, G. J. Cosmides, R. W. Estabrook, J. R. Fouts, and G. J. Mannering, pp. 279–295, Academic Press, New York, 1969.
18. COON, M. J., HAUGEN, D. A., GUENGERICH, F. P., VERMILION, J. L., AND DEAN, W. L.: Liver microsomal membranes: Reconstitution of the hydroxylation system containing cytochrome P-450. *In* The Structural Basis of Membrane Function, ed. by Y. Hatefi and L. Diavadi-Ohaniance, pp. 409–427, Academic Press, New York, 1976.
19. COON, M. J., AND VATSIS, K. P.: Biochemical studies on chemical carcinogenesis: Role of multiple forms of liver microsomal cytochrome P-450 in the metabolism of benzo(a)pyrene and other foreign compounds. *In* Polycyclic Hydrocarbons and Cancer, vol. 1, ed. by H. V. Gelboin and P. O. P. Ts'o, pp. 335–360, Academic Press, New York, 1978.
20. COON, M. J., VERMILION, J. L., VATSIS, K. P., FRENCH, J. S., DEAN, W. L., AND HAUGEN, D. A.: Biochemical studies on drug metabolism: Isolation of multiple forms of liver microsomal cytochrome P-450. *In* Concepts in Drug Metabolism, ed. by D. M. Jerina, pp. 46–71, American Chemical Society Symposium Series, Washington, D.C., 1977.
21. COOPER, D. Y., LEVIN, S., NARASHIMHULU, S., ROSENTHAL, O., AND ESTABROOK, R. W.: Photochemical action spectrum of the terminal oxidase of mixed-function oxidase systems. Science **147:** 400–402, 1965.
22. DEAN, W. L., AND COON, M. J.: Immunochemical studies on two electrophoretically homogeneous forms of rabbit liver microsomal cytochrome P-450: P-450$_{LM2}$ and P-450$_{LM4}$. J. Biol. Chem. **242:** 3255–3261, 1977.
23. DEUTSCH, J., LEUTZ, J. C., YANG, S. K., GELBOIN, H. V., CHIANG, Y. L., VATSIS, K. P., AND COON, M. J.: Regio- and stereoselectivity of various forms of purified cytochrome P-450 in the metabolism of benzo(a)pyrene and (−)trans-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene as shown by product formation and binding to DNA. Proc. Nat. Acad. Sci. U.S.A. **75:** 3123–3127, 1978.
24. DEUTSCH, J., VATSIS, K. P., COON, M. J., LEUTZ, J. C., AND GELBOIN, H. V.: Catalytic activity and stereoselectivity of purified forms of rabbit liver microsomal cytochrome P-450 in the oxygenation of the (−) and (+) enantiomers of *trans*-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene. Mol. Pharmacol. **16:** 1011–1018, 1979.
25. ELSHOURBAGY, N. A., AND GUZELIAN, P. S.: Separation, purification and characterization of a novel form of hepatic cytochrome P-450 from rats treated with pregnenolone-16α-carbonitrile. J. Biol. Chem. **255:** 1279–1285, 1980.
26. FASCO, M. J., VATSIS, K. P., KAMINSKY, L. S., AND COON, M. J.: Regioselective and stereoselective hydroxylation of R and S warfarin by different forms of purified cytochrome P-450 from rabbit liver. J. Biol. Chem. **253:** 7813–7820, 1978.
27. FISCHER, P. W. F., AND SPENCER, T.: Spectral changes in liver microsomal cytochrome P-450 from benzanthracene-treated normal and partially hepatectomized rats. Chem.-Biol. Interact. **8:** 253–259, 1974.
28. FRANKLIN, M. R.: Inhibition of mixed-function oxidations by substrates forming reduced cytochrome P-450 metabolic-intermediate complexes. Pharmacol. Ther. A **2:** 227–245, 1977.
29. FUJITA, T., AND MANNERING, G. J.: Electron transport components in hepatic microsomes: Solubilization, resolution and recombination to reconstitute aniline hydroxylase activity. J. Biol. Chem. **248:** 8150–8156, 1973.
30. GIBSON, G. G., AND SCHENKMAN, J. B.: Purification and properties of cytochrome P-450 obtained from liver microsomes of untreated rats by lauric acid affinity chromatography. J. Biol. Chem. **253:** 5957–5963, 1978.
31. GLAUMANN, H.: Chemical and enzymatic composition of microsomal subfractions from rat liver after treatment with phenobarbital and 3-methylcholanthrene. Chem.-Biol. Interact. **2:** 369–380, 1970.
32. GNOSSPELIUS, Y., THOR, H., AND ORRENIUS, S.: A comparative study on the effects of phenobarbital and 3,4-benzpyrene on the hydroxylating enzyme system of rat-liver microsomes. Chem.-Biol. Interact. **1:** 125–137, 1969/1970.
33. GUENGERICH, F. P.: Separation and purification of multiple forms of microsomal cytochrome P-450: Activities of different forms of cytochrome P-450 towards several compounds of environmental interest. J. Biol. Chem. **252:** 3970–3979, 1977.
34. GUENGERICH, F. P.: Preparation and properties of highly-purified cytochrome P-450 and reductase from pulmonary microsomes of untreated rabbits. Mol. Pharmacol. **13:** 911–923, 1977.
35. GUENGERICH, F. P.: Comparison of highly-purified microsomal cytochromes P-450 and NADPH-cytochrome P-450 reductases by peptide mapping. Biochem. Biophys. Res. Commun. **82:** 820–827, 1978.
36. GUENGERICH, F. P.: Separation and purification of multiple forms of microsomal cytochromes P-450: Partial characterization of three apparently homogeneous cytochromes P-450 isolated from liver microsomes of phenobarbital- and 3-methylcholanthrene-treated rats. J. Biol. Chem. **253:** 7931–7939, 1978.
37. GUENGERICH, F. P.: Isolation and purification of cytochrome P-450 and the existence of multiple forms. Pharmacol. Ther. A **6:** 99–121, 1979.
38. GUENGERICH, F. P., AND HOLLADAY, L. A.: Hydrodynamic characterization of highly purified and functionally active liver microsomal cytochrome P-450. Biochemistry **18:** 5442–5449, 1979.
39. GUENGERICH, F. P., AND MASON, P. S.: Immunological comparison of hepatic and extrahepatic cytochromes P-450. Mol. Pharmacol. **15:** 154–164, 1979.
40. HANSSON, R., AND WIKVALL, K.: Hydroxylation in biosynthesis and metabolism of bile acids. Catalytic properties of different forms of cytochrome P-450. J. Biol. Chem. **255:** 1643–1649, 1980.
41. HASHIMOTO, C., AND IMAI, Y.: Purification of a substrate complex of cytochrome P-450 from liver microsomes of 3-methylcholanthrene-treated rabbits. Biochem. Biophys. Res. Commun. **68:** 821–827, 1976.
42. HASHIMOTO-YUTSUDO, C., IMAI, Y., AND SATO, R.: Multiple forms of cytochrome P-450 purified from liver microsomes of phenobarbital- and 3-methylcholanthrene-pretreated rabbits. II. Spectral Properties. J. Biochem. **88:** 505–516, 1980.
43. HAUGEN, D. A., ARMES, L. G., YASUNOBU, K. T., AND COON, M. J.: Amino-terminal sequence of phenobarbital-inducible cytochrome P-450 from rabbit liver microsomes: Similarity to hydrophobic amino-terminal segments of preproteins. Biochem. Biophys. Res. Commun. **77:** 967–973, 1977.
44. HAUGEN, D. A., AND COON, M. J.: Properties of electrophoretically homogeneous phenobarbital-inducible and β-naphthoflavone-inducible forms of liver microsomal cytochrome P-450. J. Biol. Chem. **251:** 7929–7939, 1976.
45. HAUGEN, D. A., VAN DER HOEVEN, T. A., AND COON, M. J.: Purified liver microsomal cytochrome P-450: Separation and characterization of multiple forms. J. Biol. Chem. **250:** 3567–3570, 1975.
46. HLAVICA, P., AND HULSMANN, S.: Studies on the mechanism of hepatic microsomal N-oxide formation. N-oxidation of N,N-dimethylaniline by a reconstituted rabbit liver microsomal cytochrome P-448 enzyme system. Biochem. J. **182:** 109–116, 1979.
47. HODGSON, E., AND PHILPOT, R. M.: Interaction of methylenedioxyphenyl-(1,3-benzodioxole) compounds with enzymes and their effects on mammals. Drug Metab. Rev. **3:** 231–301, 1974.
48. HUANG, M. T., WEST, S. B., AND LU, A. Y. H.: Separation, purification and properties of multiple forms of cytochrome P-450 from the liver microsomes of phenobarbital-treated mice. J. Biol. Chem. **251:** 4659–4665, 1976.
49. IMAI, Y.: Reconstituted O-dealkylase systems containing various forms of liver microsomal cytochrome P-450. J. Biochem. **86:** 1697–1707, 1979.
50. IMAI, Y., HASHIMOTO-YUTSUDO, C., SATAKE, H., GIRARDIN, A., AND SATO, R.: Multiple forms of cytochrome P-450 purified from liver microsomes of phenobarbital and 3-methylcholanthrene pretreated rabbits. I. Resolution, purification and molecular properties. J. Biochem. **88:** 489–503, 1980.
51. IMAI, Y., AND SATO, R.: Substrate interaction with hydroxylase system in liver microsomes. Biochem. Biophys. Res. Commun. **22:** 620–626, 1966.
52. IMAI, Y., AND SATO, R.: A gel-electrophoretically homogeneous preparation of cytochrome P-450 from liver microsomes of phenobarbital pretreated rabbits. Biochem. Biophys. Res. Commun. **60:** 8–14, 1974.
53. IMAI, Y., AND SATO, R.: The roles of cytochrome b_5 in a reconstituted N-demethylase system containing cytochrome P-450. Biochem. Biophys. Res. Commun. **75:** 420–426, 1977.
54. INGELMAN-SUNDBERG, M., GLAUMANN, H., AND JOHANSSON, I.: Properties of purified forms of rabbit liver microsomal cytochrome P-450 and NADPH-cytochrome P-450 reductase in reconstituted phospholipid vesicles. *In* Microsomes, Drug Oxidations and Chemical Carcinogenesis, ed. by M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. R. Gillette and P. J. O'Brien, pp. 513–516, Academic Press, New York, 1980.
55. INGELMAN-SUNDBERG, M., JOHANSSON, I., AND HANSSON, A.: Catalytic properties of the liver microsomal hydroxylase system in reconstituted phospholipid vesicles. Acta Biol. Med. Ger. **38:** 379–388, 1979.
56. ISHII, K., ANDO, M., KAMATAKI, T., KATO, R., AND NAGAO, M.: Metabolic activation of mutagenic tryptophan pyrolysis products by a purified cytochrome P-450-dependent monooxygenase system. Cancer Lett., in press.
57. JEFCOATE, C. R. E., GAYLOR, J. L., AND CALABRESE, R. L.: Ligand interactions with cytochrome P-450. I. Binding of primary amines. Biochemistry **8:** 3455–3463, 1969.
58. JOHNSON E. F.: Multiple forms of cytochrome P-450: Criteria and significance. *In* Reviews in Biochemistry Toxicology, vol. I, ed. by E. Hodgson, J. R. Bend, and R. M. Philpot, pp. 1–26, Elsevier/North-Holland, New York, 1979.
59. JOHNSON, E. F.: Isolation and characterization of a constitutive form of rabbit liver microsomal cytochrome P-450. J. Biol. Chem. **255:** 304–309, 1980.
60. JOHNSON, E. F., AND MULLER-EBERHARD, U.: Multiple forms of cytochrome P-450: Resolution and purification of rabbit liver aryl hydrocarbon hydroxylase. Biochem. Biophys. Res. Commun. **76:** 644–651, 1977.
61. JOHNSON, E. F., AND MULLER-EBERHARD, U.: 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.
62. JOHNSON, E. F., AND MULLER-EBERHARD, U.: 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.
63. JOHNSON, E. F., SCHWAB, G. E., AND MULLER-EBERHARD, U.: Multiple forms of cytochrome P-450: Catalytic differences exhibited by two homogeneous forms of rabbit cytochrome P-450. Mol. Pharmacol. **15:** 708–718,

1979.
64. JOHNSON, E. F., ZOUNES, M. C., AND MULLER-EBERHARD, U.: 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:** 282–289, 1979.
65. KAHL, G. F., ZIMMER, B., GALINSKY, T., JONEN, H. G., AND KAHL, R.: Induction of cytochrome P-448 by 3-methylcholanthrene in the rat during inhibition of protein synthesis *in vivo*. *In* Microsomes and Drug Oxidations, ed. by V. Ullrich, I. Roots, A. Hildebrandt, R. W. Estabrook, and A. H. Conney, pp. 551–558, Pergamon Press, Oxford, 1977.
66. KAMATAKI, T., BELCHER, D. H., AND NEAL, R. A.: Studies of the metabolism of parathion and benzphetamine using an apparently homogeneous preparation of rat liver cytochrome P-450: Effect of a cytochrome P-450 antibody preparation. Mol. Pharmacol. **12:** 921–932, 1976.
67. KAMATAKI, T., LIN, M. C. M. L., BELCHER, D. H., AND NEAL, R. A.: Studies on the metabolism of parathion with an apparently homogeneous preparation of rabbit liver cytochrome P-450. Drug Metab. Dispos. **4:** 180–189, 1976.
68. KAMATAKI, T., SUGIURA, M., YAMAZOE, Y., AND KATO, R.: Purification and properties of cytochrome P-450 and NADPH-cytochrome c reductase from human liver microsomes. Biochem. Pharmacol. **28:** 1993–2000, 1979.
69. KAMINSKY, L. S., FASCO, M. J., AND GUENGERICH, F. P.: Comparison of different forms of purified cytochrome P-450 from rat liver by immunological inhibition of regio- and stereoselective metabolism of warfarin. J. Biol. Chem. **255:** 85–91, 1980.
70. KAPITULNIK, J., POPPERS, P. J., BUENING, M. K., FORTNER, J. G., AND CONNEY, A. H.: Activation of monooxygenases in human liver by 7,8-benzoflavone. Clin. Pharmacol. Ther. **22:** 475–485, 1977.
71. KAPITULNIK, J., POPPERS, P. J., AND CONNEY, A. H. Comparative metabolism of benzo[a]pyrene and drugs in human liver. Clin. Pharmacol. Ther. **21:** 166–176, 1977.
72. KAWALEK, J. C., LEVIN, W., RYAN, D., THOMAS, P. E., AND LU, A. Y. H.: Purification of liver microsomal cytochrome P-448 from 3-methylcholanthrene-treated rabbits. Mol. Pharmacol. **11:** 874–878, 1975.
73. KAWALEK, J. C., AND LU, A. Y. H.: Reconstituted liver microsomal enzyme system that hydroxylates drugs, other foreign compounds and endogenous substrates. VIII. Different catalytic activities of rabbit and rat cytochromes P-448. Mol. Pharmacol. **11:** 201–210, 1975.
74. KITADA, M., AND KAMATAKI, T.: Partial purification and properties of cytochrome P-450 from homogenates of human fetal livers. Biochem. Pharmacol. **28:** 793–797, 1979.
75. KOOP, D. R., AND COON, M. J.: Purification and properties of P-450 LM_{3b}, a constitutive form of cytochrome P-450, from rabbit liver microsomes. Biochem. Biophys. Res. Commun. **91:** 1075–1081, 1979.
76. KUNTZMAN, R., LEVIN, W., SCHILLING, G., AND ALVARES, A.: The effects of 3-methylcholanthrene and phenobarbital on liver microsomal hemoprotein and on the hydroxylation of benzpyrene. *In* Microsomes and Drug Oxidations, ed. by J. R. Gillette, A. H. Conney, G. J. Cosmides, R. W. Estabrook, J. R. Fouts, and G. J. Mannering, pp. 349–363, Academic Press, New York, 1969.
77. LAEMMLI, U. K.: Cleavage of structural proteins during the assembly of the head of bacteriophase T_4. Nature (London) **227:** 680–685, 1970.
78. LESCA, P., LECOINTE, P., PAOLETTI, C., AND MANSUY, D.: Ellipticines as potent inhibitors of drug metabolism. Protective effect against chemical mutagenesis and carcinogenesis. Biochimie (Paris) **60:** 1011–1018, 1978.
79. LEVIN, W.: Purification of liver microsomal cytochrome P-450: Hopes and promises. *In* Microsomes and Drug Oxidation, ed. by V. Ullrich, I. Roots, A. Hildebrandt, R. W. Estabrook, and A. H. Conney, pp. 735–747, Pergamon Press, New York, 1977.
80. LEVIN, W., RYAN, D., HUANG, M. T., KAWALEK, J., THOMAS, P. E., WEST, S. B., AND LU, A. Y. H.: Characterization of multiple forms of highly purified cytochrome P-450 from the liver microsomes of rats, mice and rabbits. *In* Microsomes and Drug Oxidations, ed. by V. Ullrich, I. Roots, A. Hildebrandt, R. W. Estabrook, and A. H. Conney, pp. 185–191, Pergamon Press, New York, 1977.
81. LEVIN, W., WOOD, A. W., LU, A. Y. H., RYAN, D., WEST, S., CONNEY, A. H., THAKKER, D. R., YAGI, H., AND JERINA, D. M.: Role of purified cytochrome P-448 and epoxide hydrase in the activation and detoxification of benzo(a)pyrene. *In* Concepts in Drug Metabolism, ed. by D. M. Jerina, pp. 99–126, American Chemical Society Symposium Series, Washington, D.C., 1977.
82. LOTLIKAR, P. D., LUHA, L., AND ZALESKI, K.: Reconstituted hamster liver microsomal enzyme system for N-hydroxylation of the carcinogen 2-acetylaminofluorene. Biochem. Biophys. Res. Commun. **59:** 1349–1355, 1974.
83. LOTLIKAR, P. D., AND ZALESKI, K.: Ring- and N-hydroxylation of 2-acetaminofluorene by rat liver reconstituted cytochrome P-450 enzyme system. Biochem. J. **150:** 561–564, 1975.
84. LU, A. Y. H.: Liver microsomal drug-metabolizing enzyme system: Functional components and their properties. Fed. Proc. **35:** 2460–2463, 1976.
85. LU, A. Y. H., AND COON, M. J.: Role of hemeprotein P-450 in fatty acid ω-hydroxylation in a soluble enzyme system from liver microsomes. J. Biol. Chem. **243:** 1331–1332, 1968.
86. LU, A. Y. H., JACOBSON, M., LEVIN, W., WEST, S. B., AND KUNTZMAN, R.: Reconstituted liver microsomal enzyme system that hydroxylates drugs, other foreign compounds and endogenous substrates. IV. Hydroxylation of aniline. Arch. Biochem. Biophys. **153:** 294–297, 1972.
87. LU, A. Y. H., JUNK, K. W., AND COON, M. J.: Resolution of the cytochrome P-450 containing ω-hydroxylation system of liver microsomes into three components. J. Biol. Chem. **244:** 3714–3721, 1969.
88. LU, A. Y. H., KUNTZMAN, R., WEST, S., AND CONNEY, A. H.: Reconstituted liver microsomal enzyme system that hydroxylates drugs, other foreign compounds and endogenous substrates. I. Determination of substrate specificity by the cytochrome P-450 and P-448 fractions. Biochem. Biophys. Res. Commun. **42:** 1200–1206, 1971.
89. LU, A. Y. H., KUNTZMAN, R., WEST, S., JACOBSON, M., AND CONNEY, A. H.: Reconstituted liver microsomal enzyme system that hydroxylates drugs, other foreign compounds and endogenous substrates. II. Role of the cytochrome P-450 and P-448 fractions in drug and steroid hydroxylations. J. Biol. Chem. **247:** 1727–1734, 1972.
90. LU, A. Y. H., AND LEVIN, W.: The resolution and reconstitution of the liver microsomal hydroxylation system. Biochim. Biophys. Acta **344:** 205–240, 1974.
91. LU, A. Y. H., LEVIN, W., SELANDER, H., AND JERINA, D. M.: Liver microsomal electron transport systems. III. The involvement of cytochrome b_5 in the NADPH-supported cytochrome P-450-dependent hydroxylation of chlorobenzene. Biochem. Biophys. Res. Commun. **61:** 1348–1355, 1974.
92. LU, A. Y. H., LEVIN, W., VORE, M., CONNEY, A. H., THAKKER, D. R., HOLDER, G., AND JERINA, D. M.: Metabolism of benzo(a)pyrene by purified liver microsomal cytochrome P-448 and epoxide hydrase. *In* Carcinogenesis, vol. 1, Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism and Carcinogenesis, ed. by R. I. Freudenthal and P. W. Jones, pp. 115–126. Raven Press, New York, 1976.
93. LU, A. Y. H., LEVIN, W., WEST, S., JACOBSON, M., RYAN, D., KUNTZMAN, R., AND CONNEY, A. H.: The role of cytochrome P-450 and P-448 in drug and steroid hydroxylations. Ann. N.Y. Acad. Sci. **212:** 156–174, 1973.
94. LU, A. Y. H., LEVIN, W., WEST, S. B., JACOBSON, M., RYAN, D., KUNTZMAN, R., AND CONNEY, A. H.: Reconstituted liver microsomal enzyme system that hydroxylates drugs, other foreign compounds, and endogenous substrates. VI. Different substrate specificities of the cytochrome P-450 fractions from control and phenobarbital-treated rats. J. Biol. Chem. **248:** 456–460, 1973.
95. LU, A. Y. H., LEVIN, W., WEST, S. B., VORE, M., RYAN, D., KUNTZMAN, R., AND CONNEY, A. H.: Role of cytochrome b_5 in NADPH- and NADH-dependent hydroxylation by the reconstituted cytochrome P-450 or P-448-containing system. *In* Cytochromes P-450 and b_5: Structure, Function and Interaction, ed. by D. Y. Cooper, O. Rosenthal, R. Snyder, and C. Witmer, pp. 447–466, Plenum Press, New York, 1975.
96. LU, A. Y. H., MORIN, J. W., AND WARNER, M.: Purification of liver microsomal cytochrome P-450 by affinity chromatography. Symposium on Biochemistry, Biophysics and Regulation of Cytochrome P-450, Stockholm, June 15–19, 1980.
97. LU, A. Y. H., RYAN, D., KAWALEK, J., THOMAS, P., WEST, S. B., HUANG, M. T., AND LEVIN, W.: Multiplicity of liver microsomal cytochrome P-450: Separation, purification and characterization. Biochem. Soc. Trans. **4:** 169–172, 1976.
98. LU, A. Y. H., SOMOGYI, A., WEST, S., KUNTZMAN, R., AND CONNEY, A. H.: Pregnenolone-16 α-carbonitrile: A new type of inducer of drug-metabolizing enzymes. Arch. Biochem. Biophys. **152:** 457–462, 1972.
99. LU, A. Y. H., STROBEL, H. W., AND COON, M. J.: Hydroxylation of benzphetamine and other drugs by a solubilized form of cytochrome P-450 from liver microsomes: Lipid requirement for drug demethylation. Biochem. Biophys. Res. Commun. **36:** 545–551, 1969.
100. LU, A. Y. H., STROBEL, H. W., AND COON, M. J.: Properties of a solubilized form of the cytochrome P-450-containing mixed-function oxidase of liver microsomes. Mol. Pharmacol. **6:** 213–220, 1970.
101. LU, A. Y. H., AND WEST, S. B.: Reconstituted mammalian mixed-function oxidases: Requirements, specificities and other properties. Pharmacol. Ther. A **2:** 337–358, 1978.
102. MAILMAN, R. B., TATE, L. G., MUSE, K. E., COONS, L. B., AND HODGSON, E.: The occurrence of multiple forms of cytochrome P-450 in hepatic microsomes from untreated rats and mice. Chem.-Biol. Interact. **10:** 215–228, 1975.
103. MANNERING, G. J.: Properties of cytochrome P-450 as affected by environmental factors: Qualitative changes due to administration of polycyclic hydrocarbons. Metabolism **20:** 228–245, 1971.
104. MANNERING, G. J., SLADEK, N. E., PARLI, C. J., AND SHORMAN, D. W.: Formation of a new P-450 hemoprotein after treatment of rats with polycyclic hydrocarbons. In Microsomes and Drug Oxidations, ed. by J. R. Gillette, A. H. Conney, G. J. Cosmides, R. W. Estabrook, J. R. Fouts, and G. J. Mannering, pp. 303–327, Academic Press, New York, 1969.
105. MASTERS, B. S. S., YASUKOCHI, Y., OKITA, R. T., PARKHILL, L. K., TANIGUCHI, H., AND DEES, J. H.: Laurate hydroxylation and drug metabolism in pig liver and kidney microsomes and in reconstituted systems from pig liver and kidney. *In* Microsomes, Drug Oxidations and Chemical Carcinogenesis, ed. by M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. R. Gillette, and P. J. O'Brien, pp. 709–720, Academic Press, New York, 1980.
106. MASUDA-MIKAWA, R., FUJII-KURIYAMA, Y., NEGISHI, M., AND TASHIRO, Y.: Purification and partial characterization of hepatic microsomal cytochrome P-450s from phenobarbital and 3-methylcholanthrene-treated

rats. J. Biochem. 86: 1383–1394, 1979.
107. McPherson, F. J., Bridges, J. W., and Parke, D. V.: The effects of benzopyrene and safrole on biphenyl 2-hydroxylation and other drug-metabolizing enzymes. Biochem. J. 154: 773–780, 1976.
108. Miki, N., Sugiyama, T., and Yamano, T.: Purification and some properties of cytochrome P-450 with high affinity for cytochrome b_5. *In* Microsomes, Drug Oxidations and Chemical Carcinogenesis, ed. by M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. R. Gillette, and P. J. O'Brien, pp. 27–34, New York, 1980.
109. Miwa, G. T., West, S. B., and Lu, A. Y. H.: Studies on the rate-limiting enzyme component in the microsomal monooxygenase system: Incorporation of purified NADPH-cytochrome c reductase and cytochrome P-450 into rat liver microsomes. J. Biol. Chem. 253: 1921–1929, 1978.
110. Miwa, G. T., Zweig, J. S., Walsh, J. S. A., and Lu, A. Y. H.: Kinetic isotope effects in cytochrome P-450-catalyzed oxidation reactions. I. Substrate-cytochrome P-450 interactions. *In* Microsomes, Drug Oxidations and Chemical Carcinogenesis, ed. by M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. R. Gillette, and P. J. O'Brien, pp. 363–366, Academic Press, New York, 1980.
111. Nebert, D. W.: Genetic aspects of enzyme induction by drugs and chemical carcinogens. *In* The Induction of Drug Metabolism, ed. by R. W. Estabrook and E. Lindenlaub, pp. 419–452, F. K. Schattauer Verlag, Stuttgart, New York, 1979.
112. Nebert, D. W., and Gielen, J. E.: Genetic regulation of aryl hydrocarbon hydroxylase induction in the mouse. Fed. Proc. 31: 1315–1325, 1972.
113. Nebert, D. W., Heidema, J. K., Strobel, H. W., and Coon, M. J.: Genetic expression of aryl hydrocarbon hydroxylase induction. Genetic specificity resides in the fraction containing cytochromes P-448 and P-450. J. Biol. Chem. 248: 7631–7636, 1973.
114. Nebert, D. W., and Jensen, N. M.: The Ah locus: Genetic regulation of the metabolism of carcinogens, drugs and other environmental chemicals by cytochrome P-450-mediated monooxygenase. CRC Crit. Rev. Biochem. 6: 401–437, 1979.
115. Negishi, M., and Nebert, D. W.: Structural gene products of the Ah locus. Genetic and immunochemical evidence for two forms of mouse liver cytochrome P-450 induced by 3-methylcholanthrene. J. Biol. Chem. 254: 11015–11023, 1979.
116. Nomenclature of multiple forms of enzymes. Recommendations. Biochem. J. 171: 37–39, 1978.
117. Norman, R. L., Johnson, E. F., and Muller-Eberhard, U.: 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.
118. Norman, R. L., Muller-Eberhard, U., and Johnson, E. F.: The role of cytochrome P-450 forms in 2-aminoanthracene and benzo(a)pyrene mutagenesis. Biochem. Biophys. Res. Commun. 89: 195–201, 1979.
119. Omura, T., Noshiro, M., and Haruda, N.: Distribution of electron transfer components on the surface of microsomal membrane. *In* Microsomes, Drug Oxidations and Chemical Carcinogenesis, ed. by M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. R. Gillette, and P. J. O'Brien, pp. 445–454, Academic Press, New York, 1980.
120. Omura, T., Sato, R., Cooper, D. Y., Rosenthal, O., and Estabrook, R. W.: Function of cytochrome P-450 of microsomes. Fed. Proc. 24: 1181–1189, 1965.
121. Ozawa, N., Yoshihara, S., Kawano, K., Okada, Y., and Yoshimura, H.: 3,4,5,3′,4′-Pentachlorobiphenyl as a useful inducer for purification of rat liver microsomal cytochrome P-448. Biochem. Biophys. Res. Commun. 91: 1140–1147, 1979.
122. Parke, D. V.: Induction of the drug-metabolizing enzymes. *In* Enzyme Induction, ed. by D. V. Parke, pp. 207–271, Plenum Press, London, 1975.
123. Parke, D. V.: Toxicological consequences of enzyme induction and inhibition. In Drug Toxicity, ed. by J. W. Gorrod, pp. 133–150, Taylor and Francis, London, 1979.
124. Parke, D. V.: The responsiveness of cells to various drug inducers. *In* The Induction of Drug Metabolism, ed. by R. W. Estabrook and E. Lindenlaub, pp. 101–111, F. K. Schattauer Verlag, Stuttgart, New York, 1979.
125. Parke, D. V., and Rahman, H.: The induction of hepatic microsomal enzymes by safrole. Biochem. J. 119: 53p–54p, 1970.
126. Pederson, T. C., and Aust, S. D.: Aminopyrine demethylase: Kinetic evidence for multiple microsomal activities. Biochem. Pharmacol. 19: 2221–2230, 1970.
127. Pelkonen, O.: Developmental change in the apparent kinetic properties of drug-oxidizing enzymes in the human liver. Res. Commun. Chem. Pathol. Pharmacol. 10: 293–302, 1975.
128. Philpot, R. M., and Arinc, E.: Separation and purification of two forms of hepatic cytochrome P-450 from untreated rabbits. Mol. Pharmacol. 12: 483–493, 1976.
129. Pohl, L. R., Nelson, S. D., Porter, W. R., Trager, W. F., Fasco, M. J., Baker, F. D., and Fenton, J. W.: Warfarin-stereochemical aspects of its metabolism by rat liver microsomes. Biochem. Pharmacol. 25: 2153–2162, 1976.
130. Pohl, L. R., Porter, W. R., Trager, W. F., Fasco, M. J., and Fenton, J. W.: Stereochemical biotransformation of warfarin as a probe of the homogeneity and mechanism of microsomal hydroxylases. Biochem. Pharmacol. 26: 109–114, 1977.
131. Porter, W. R., Branchflower, R. V., and Trager, W. F.: A kinetic method for the determination of the multiple forms of microsomal cytochrome P-450. Biochem. Pharmacol. 26: 549–550, 1977.
132. Powis, G., Talcott, R. E., and Schenkman, J. B.: Kinetic and spectral evidence for multiple species of cytochrome P-450 in liver microsomes. *In* Microsomes and Drug Oxidations, ed. by V. Ullrich, I. Roots, A. Hildebrandt, R. W. Estabrook, and A. H. Conney, pp. 127–135, Pergamon Press, New York, 1977.
133. Ryan, D., Lu, A. Y. H., Kawalek, J., West, S. B., and Levin, W.: Highly purified cytochrome P-448 and P-450 from rat liver microsomes. Biochem. Biophys. Res. Commun. 64: 1134–1141, 1975.
134. Ryan, D. E., Thomas, P. E., Korzeniowski, D., and Levin. W.: Separation and characterization of highly purified forms of liver microsomal cytochrome P-450 from rats treated with polychlorinated biphenyls, phenobarbital and 3-methylcholanthrene. J. Biol. Chem. 254: 1365–1374, 1979.
135. Schenkman, J. B., Remmer, H., and Estabrook, R. W.: Spectral studies of drug interaction with hepatic microsomal cytochrome. Mol. Pharmacol. 3: 113–123, 1967.
136. Serabjit-Singh, C. J., Wolf, C. R., and Philpot, R. M.: The rabbit pulmonary monooxygenase system. Immunological and biochemical characterization of enzyme components. J. Biol. Chem. 254: 9901–9907, 1979.
137. Shiverick, K. T., and Reims, A. H.: Multiplicity of testosterone hydroxylases in a reconstituted hepatic cytochrome P-450 system from uninduced male rats. Drug Metab. Dispos. 7: 290–295, 1979.
138. Sladek, N. E., and Mannering, G. J.: Evidence for a new P-450 hemoprotein in hepatic microsomes from methylcholanthrene-treated rats. Biochem. Biophys. Res. Commun. 24: 668–674, 1966.
139. Sladek, N. E., and Mannering, G. J.: Induction of drug metabolism. I. Differences in the mechanisms by which polycyclic hydrocarbons and phenobarbital produce their inductive effects on microsomal N-demethylating systems. Mol. Pharmacol. 5: 174–185, 1969.
140. Sladek, N. E., and Mannering, G. J.: Induction of drug metabolism. II. Qualitative differences in the microsomal N-demethylating systems stimulated by polycyclic hydrocarbons and by phenobarbital. Mol. Pharmacol. 5: 186–199, 1969.
141. Slaughter, S. R., Wolf, C. R., Marciniszyn, J. P., and Philpot, R. M.: 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, ed. by M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. R. Gillette, and P. J. O'Brien. pp. 171–174, Academic Press, New York, 1980.
142. Snyder, R., and Remmer, H.: Classes of hepatic microsomal mixed function oxidase inducers. Pharmacol. Ther. A 7: 203–244, 1979.
143. Strobel, H. W., Lu, A. Y. H., Heidema, J., and Coon, M. J.: Phosphatidylcholine requirement in the enzymatic reduction of hemoprotein P-450 and in fatty acid, hydrocarbon, and drug hydroxylation. J. Biol. Chem. 245: 4851–4854, 1970.
144. Sugiyama, T., Miki, N., and Yamano, T.: The obligatory requirement of cytochrome b_5 in the *p*-nitroanisole O-demethylation reaction catalyzed by cytochrome P-450 with a high affinity for cytochrome b_5. Biochem. Biophys. Res. Commun. 90: 715–720, 1979.
145. Thakker, D. R., Yagi, H., Akagi, H., Koreeda, M., Lu, A. Y. H., Levin, W., Wood, A. W., Conney, A. H., and Jerina, D. M.: Metabolism of benzo[a]pyrene. VI. Stereoselective metabolism of benzo[a]pyrene and benzo[a]pyrene 7,8-dihydrodiol to diol epoxides. Chem.-Biol. Interact. 16: 281–300, 1977.
146. Thomas, P. E., Korzeniowski, D., Ryan, D., and Levin, W.: Preparation of monospecific antibodies against two forms of rat liver cytochrome P-450 and quantitation of these antigens in microsomes. Arch. Biochem. Biophys. 192: 524–532, 1979.
147. Thomas, P. E., Lu, A. Y. H., Ryan, D., West, S. B., Kawalek, J., and Levin, W.: Immunochemical evidence for six forms of rat liver cytochrome P-450 obtained using antibodies against purified rat liver cytochromes P-450 and P-448. Mol. Pharmacol. 12: 746–758, 1976.
148. Thomas, P. E., Lu, A. Y. H., Ryan, D., West, S. B., Kawalek, J., and Levin, W.: Multiple forms of rat liver cytochrome P-450: Immunochemical evidence with antibody against cytochrome P-448. J. Biol. Chem. 251: 1385–1391, 1976.
149. Thomas, P. E., Lu, A. Y. H., West, S. B., Ryan, D., Miwa, G. T., and Levin, W.: Accessibility of cytochrome P-450 in microsomal membranes: Inhibition of metabolism by antibodies to cytochrome P-450. Mol. Pharmacol. 13: 817–831, 1977.
150. Ullrich, V., and Kremers, P.: Multiple forms of cytochrome P-450 in the microsomal monooxygenase system. Arch. Toxikol. 39: 41–50, 1977.
151. Ullrich, V., and Weber, P.: The O-dealkylation of 7-ethoxycoumarin by liver microsomes, a direct fluorometric test. Z. Physiol. Chem. 353: 1171–1177, 1972.
152. Ullrich, V., Weber, P., and Wollenberg, P.: Tetrahydrofurane—An inhibitor for ethanol-induced liver microsomal cytochrome P-450. Biochem. Biophys. Res. Commun. 64: 808–813, 1975.
153. Villeneuve, J. P. Mavier, P., and Joly, J. G.: Ethanol-induced cytochrome P-450: Catalytic activity after partial purification. Biochem. Biophys. Res. Commun. 70: 723–728, 1976.
154. Wada, F., Shimakawa, H., Takasugi, M., Kotake, T., and Sakamoto, Y.: Effects of steroid hormones on drug-metabolizing enzyme systems in liver

microsomes. J. Biochem. 64: 109–113, 1968.
155. WANG, P., MASON, P. S., AND GUENGERICH, F. P.: Purification of human liver cytochrome P-450 and comparison to the enzyme isolated from rat liver. Arch. Biochem. Biophys. 199: 206–219, 1980.
156. WARNER, M., AND NEIMS, A. H.: Multiple forms of ethoxyresorufin O-deethylase and benzphetamine N-demethylase in solubilized and partially resolved rat liver cytochromes P-450. Drug Metab. Dispos. 7: 188–193, 1979.
157. WELTON, A. F., AND AUST, S. D.: Multiplicity of cytochromes P-450 hemeproteins in rat liver microsomes. Biochem. Biophys. Res. Commun. 56: 898–906, 1974.
158. WELTON, A. F., O'NEAL, F. O., CHANEY, L. C., AND AUST, S. D.: Multiplicity of cytochrome P-450 hemoproteins in rat liver microsomes: Preparation and specificity of an antibody to the hemoprotein induced by phenobarbital. J. Biol. Chem. 250: 5631–5639, 1975.
159. WERRINGLOER, J., AND ESTABROOK, R. W.: Heterogeneity of liver microsomal cytochrome P-450: The spectral characterization of reactants with reduced cytochromes P-450. Arch. Biochem. Biophys. 167: 270–286, 1975.
160. WERRINGLOER, J., AND ESTABROOK, R. W.: The characterization of product adducts of liver microsomal cytochrome P-450 and their use as probes for the heterogeneity of cytochrome P-450 as modified by the induction of drug metabolism. *In* The Induction of Drug Metabolism, ed. by R. W. Estabrook and E. Lindenlaub, pp. 269–307, F. K. Schattauer Verlag, Stuttgart, New York, 1979.
161. WEST, S. B., HUANG, M. T., MIWA, G. T., AND LU, A. Y. H.: A simple and rapid procedure for the purification of phenobarbital-inducible cytochrome P-450 from rat liver microsomes. Arch. Biochem. Biophys. 193: 42–50, 1979.
162. WIEBEL, F. J., LEUTZ, J. C., DIAMOND, L., AND GELBOIN, H. V.: Aryl hydrocarbon (benzo[a]pyrene)hydroxylase in microsomes from rat tissues: Differential inhibition and stimulation by benzoflavones and organic solvents. Arch. Biochem. Biophys. 144: 78–86, 1971.
163. WIEBEL, F. J., SELKIRK, J. K., GELBOIN, H. V., VAN DER HOEVEN, T. A., AND COON, M. J.: Position-specific oxygenation of benzo(a)pyrene by different forms of purified cytochrome P-450 from rabbit liver. Proc. Nat. Acad. Sci. U.S.A. 72: 3917–3920, 1975.
164. WOLF, C. R., SZUTOWSKI, M. M., BALL, L. M., AND PHILPOT, R. M.: The rabbit pulmonary monooxygenase system: Characteristics and activities of two forms of pulmonary cytochrome P-450. Chem.-Biol. Interact. 21: 29–43, 1978.
165. WOOD, A. W., CHANG, R. L., LEVIN, W., YAGI, H., THAKKER, D. R., JERINA, D. M., AND CONNEY, A. H.: Differences in mutagenicity of the optical enantiomers of the diastereomeric benzo(a)pyrene 7,8-diol-9,10-epoxides. Biochem. Biophys. Res. Commun. 77: 1389–1396, 1977.