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The Relationship between the Catalytic Activities of Rabbit Pulmonary Cytochrome P-450 Isozymes and the Lung-Specific Toxicity of the Furan Derivative, 4-Ipomeanol

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SUMMARY

Metabolism of the pulmonary toxin, 4-ipomeanol, in microsomal preparations from rabbit liver and lung and in purified cytochrome P-450-dependent monooxygenase systems was investigated. The rate of formation of reactive electrophilic products from 4-ipomeanol was estimated by measuring covalent binding to protein or glutathione. Pulmonary microsomal preparations were much more active than hepatic preparations in mediating these reactions. Both of the rabbit pulmonary cytochrome P-450 isozymes, P-450$_I$ and P-450$_{II}$, were active in the metabolism of 4-ipomeanol. In the assay for covalent binding to protein, P-450$_I$ was slightly more active than P-450$_{II}$ at high substrate concentrations and significantly more active at low substrate concentrations. Incubation of either isozyme with 4-ipomeanol produced two glutathione conjugates but at quite different ratios. Rates of metabolism determined by conjugate formation in the purified systems were about 3 times the rates determined by covalent binding to protein, whereas both determinations gave the same values in the microsomal preparations. The relationship between the activities of P-450$_I$ and P-450$_{II}$ in the metabolism of 4-ipomeanol and the pulmonary toxicity of 4-ipomeanol is discussed.

INTRODUCTION

The furan derivative, 4-ipomeanol, which is produced by the fungus *Fusarium solani*, is a potent pulmonary toxin in a number of mammalian species, including rabbits (1). In these species the lethality of 4-ipomeanol is somewhat associated with pulmonary edema and hemorrhage, but is most closely correlated with necrosis of the nonciliated bronchiolar epithelial (Clara) cell of the lower airways (2). This pathology parallels the covalent binding of metabolites of 4-ipomeanol to macromolecules within the Clara cell (2).

Because the formation of reactive metabolites from 4-ipomeanol is catalyzed by the cytochrome P-450-dependent monooxygenase system (P-450 system), Boyd (2) surmised that the Clara cell contains this enzyme system and is likely a major site for cytochrome P-450-mediated metabolism in lung. Reznik-Schüller and Hague (3) have offered a similar explanation for the Clara cell-specific covalent binding of metabolites of diethylnitrosamine in lung of Syrian golden hamsters. Direct evidence for the validity of this hypothesis is the identification of the enzymes of the P-450 system in the Clara cell of rabbit lung—in both tissue sections (4, 5) and isolated cells (6)—and the detection of high levels of cytochrome P-450-dependent monooxygenase activity in isolated Clara cells (7). The results of these studies also indicate that Clara cells contain the highest concentrations of P-450 enzymes in rabbit lung, and thereby provide a plausible explanation for the cell-specific pulmonary toxicity of 4-ipomeanol.

Although the factors responsible for the localized effects of 4-ipomeanol in lung are at least partially understood, those that control the organ specificity of this toxin have not been elucidated. In most species examined, the toxicity of 4-ipomeanol is confined to lung, but in mice the kidney is also involved, as is the liver in hamsters (8). These differences in specificity correlate reasonably well with the levels of covalent binding of metabolites of 4-ipomeanol in target and nontarget tissues (8); in rabbits over 5 times more covalent binding (per milligram of tissue protein) occurs in lung as in liver (8). Among the possible factors that could contribute to

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this difference are dissimilarities between the properties of the rabbit pulmonary and hepatic P-450 systems (9).

The majority of rabbit pulmonary cytochrome P-450 consists of two isozymes, P-450$_I$ and P-450$_{II}$ (10), which are minor forms of the enzyme in liver (5). By determining the substrate specificities of these isozymes, we have been able to explain several major differences between the abilities of pulmonary and hepatic microsomal preparations to metabolize certain substrates. For example, the 20- to 30-fold higher rates (per nanomole of cytochrome P-450) of metabolism of 2-aminoanthracene and 2-aminofluorene to mutagenic products in pulmonary microsomes is a function of the high activity of P-450$_{II}$ with these substrates (11), and the higher pulmonary rates of *N*-demethylation of benzphetamine and *O*-deethylation of 7-ethoxycoumarin result from the activity of P-450$_I$ (12).

We have now investigated the metabolism of 4-ipomeanol by rabbit pulmonary and hepatic microsomal P-450 systems and by purified cytochromes P-450$_I$ and P-450$_{II}$ by measuring the covalent binding of metabolites to protein and glutathione. The results suggest that the characteristics of the rabbit pulmonary P-450 system and the substrate specificities of cytochromes P-450$_I$ and P-450$_{II}$ are major determinants of the organ specificity of 4-ipomeanol-induced toxicosis.

MATERIALS AND METHODS

Preparation of microsomal fractions and purified enzymes. Male New Zealand White rabbits (2.0–2.5 kg) were used. Pulmonary and hepatic microsomal fractions were prepared as described by Philpot *et al.* (13) and Lu *et al.* (14). Two forms of cytochrome P-450 (P-450$_I$ and P-450$_{II}$) and NADPH-cytochrome P-450 reductase (reductase) were isolated from microsomal preparations and purified by methods previously described (12, 15, 16). Reductase and P-450$_I$ were purified to apparent homogeneity (greater than 50,000 units and 17 nmoles/mg of protein, respectively) as judged by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. The P-450$_{II}$ preparation used contained 5.6 nmoles/mg of protein and did not contain any detectable reductase, P-450$_I$, or cytochrome b_5. Microsomal cytochrome P-450 concentrations were determined by the method of Omura and Sato (17), and the P-450 concentrations in purified samples were determined from the ferric cytochrome absorption peak at 418 nm, using an extinction coefficient of 106 mM^{-1} (16). Protein concentrations were estimated by the method of Lowry *et al.* (18).

Determination of the metabolism of 4-ipomeanol. Two assays were used to estimate the rate of metabolism of 4-ipomeanol to reactive product(s): (a) covalent binding to protein and (b) conjugation with glutathione. Microsomal incubations contained hepatic or pulmonary microsomal fraction (1 mg of protein) suspended in 1 ml of phosphate buffer (0.05 M, pH 7.6), [^{3}H]4-ipomeanol (18 μCi, 0.5 mM) added in 5 μl of methanol, and NADPH (1 mM). Bovine γ-globulin (3 mg) was added to incubations for covalent binding determinations, and glutathione (1 mM) was added for analysis of conjugate formation. All incubations were run in triplicate for 5 min (with the exception of time courses) at 37°.

Incubations that contained purified enzymes were mixed first by adding phospholipid (didodecyl-L-α-lecithin, 50 μg) in chloroform to tubes and evaporating to dryness. Reductase (1250 units) and cytochrome (0.1 nmole) were then added in approximately 20 μl of buffer, and the mixtures were incubated for 5 min at 37°. One milliliter of phosphate buffer (0.5 M, pH 7.6) was then added and the reactions were initiated by the addition of [^{3}H]4-ipomeanol (18 μCi, 0.5 mM) in 5 μl of methanol and NADPH (1 mM). Bovine γ-globulin (4 mg) was included for the covalent binding experiments, and glutathione (1 mM) for the conjugation experiments.

Assays for covalent binding. The assay for covalent binding to protein was similar to that used by Boyd and Burka (19). The reactions were stopped by the addition of 2 volumes of ice-cold trichloroacetic acid (10%, w/v). After centrifugation and removal of the supernatant fraction, the precipitated protein was washed with methanol (4-ml aliquots) until no radioactivity could be detected in the supernatant fraction. The washed samples were solubilized in sodium hydroxide (1 N), and aliquots were taken for scintillation counting and protein determination. Analysis of metabolite-glutathione conjugates was made by HPAEC[4] as described by Buckpitt and Boyd (20). The conjugates were isolated from the reaction mixtures after first precipitating the protein with 2 volumes of ice-cold methanol and removing it by centrifugation. The supernatant fractions were evaporated to dryness, and the residue was dissolved in 750 μl of water and extracted with water-saturated ethyl acetate until no radioactivity could be detected in the organic phase. Samples from the aqueous phase were analyzed on a Waters high-pressure liquid chromatograph (Waters Associates, Milford, Mass.) equipped with an M-6000 UA pump, U6K loop injector, and a Partisil 10 μ SAX anion exchange column (0.46 × 25 cm) (Whatman, Clifton, N. J.). The flow rate was 1 ml/min, and the fractions were collected in vials and counted after the addition of 15 ml of ACS scintillation cocktail (Amersham Searle, Des Plains, Ill.). Counting was done with a Searle Mark III liquid scintillation counter with automatic external standardization.

Chemicals. 4-Ipomeanol [1-(3-furyl)-4-hydroxypentanone] was prepared as described previously (21). [^{3}H]4-ipomeanol, specific activity 170 mCi/mmole, was synthesized using the method described by Boyd (2). The radiochemical purity of the sample used was greater than 99.5%. Other chemicals were obtained from commercial sources and were of the highest purity available.

RESULTS

As reported by Dutcher and Boyd (8), rabbit hepatic and pulmonary microsomal preparations metabolized 4-ipomeanol to products that bound covalently to protein (Fig. 1). In the absence of NADPH no binding was detected. Although the K_m for 4-ipomeanol in the pulmonary incubations was only slightly lower than that in hepatic incubations (5×10^{-5} M versus 9×10^{-5} M), the V_{max} for the pulmonary reaction was 3 times that of the

[4] The abbreviation used is: HPAEC, high-pressure anion exchange chromatography.

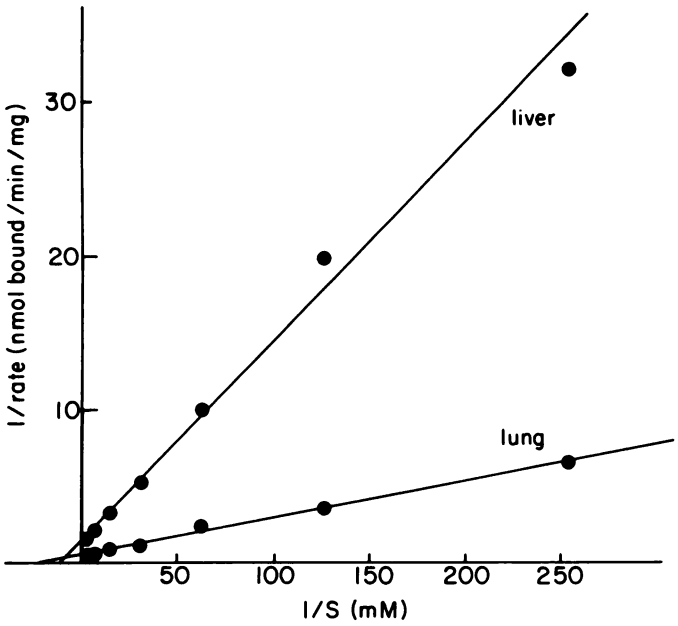

FIG. 1. *Covalent binding of metabolites of 4-ipomeanol to protein in incubations of rabbit pulmonary or hepatic microsomal preparations*

The data for covalent binding are presented as double-reciprocal plots of the rate of nanomoles of product bound per minute per milligram of microsomal protein) versus 4-ipomeanol concentration. The incubations contained microsomal protein (1 mg), bovine γ-globulin (3 mg), [^{3}H]4-ipomeanol (0.1 mM, 72,000 dpm/nmole), and NADPH (1 mM) in 1 ml of phosphate buffer (0.05 M, pH 7.6). The procedure for analysis of the covalently bound metabolites is described under Materials and Methods.

hepatic reaction per milligram of protein (2.11 versus 0.71 nmoles of product bound per minute and about 13 times faster per nanomole of cytochrome P-450 (8.44 versus 0.65 nmole of product bound per minute). The rate of metabolism determined by product conjugation with added glutathione was also faster with the pulmonary preparations (Fig. 2). Both pulmonary and hepatic incubations produced two glutathione conjugates (Conjugates 1 and 2) as has been observed by Buckpitt and Boyd (20) with hepatic preparations from rat. These conjugates, which are completely separated by high-pressure liquid chromatography, were formed in about the same ratio (1:2) by microsomal preparations from either tissue. The rates of metabolism calculated from conjugate formation (1 + 2) were 8.9 and 0.65 nmoles of product formed per minute per nanomole of P-450 for lung and liver, respectively, rates that were remarkably similar to those calculated from covalent binding to protein observed in the absence of glutathione (little or no covalent binding to protein was detected in incubations that contained 1 mM glutathione).

Both of the major isozymes of cytochrome P-450 found in rabbit lung, P-450$_I$ and P-450$_{II}$, catalyzed the metabolism of 4-ipomeanol to product(s) that bound covalently to protein in incubations that contained purified cytochrome P-450 and NADPH-cytochrome P-450 reductase, NADPH, and bovine immunoglobulin G as the acceptor protein (Fig. 3). The amount of covalent binding was proportional to the concentrations of the cytochromes from 0.02 to 0.1 nmole/ml, and the exclusion of phospholipid (500 μg/nmole of P-450) from the incubations decreased covalent binding by approximately 40% and 25% for P-450$_I$ and P-450$_{II}$, respectively (data not shown). The maximal rates (V_{max}) of covalent binding were similar for both cytochromes: 2.6 nmoles of product bound per minute per nanomole for P-450$_I$ and 2.0 for P-450$_{II}$. However, the K_m for 4-ipomeanol was nearly 5 times lower with P-450$_I$ (3.5×10^{-5} M versus 1.5×10^{-4} M for P-450$_{II}$), and the difference in reaction rate (P-450$_I$ > P-450$_{II}$) increased from approximately 1.2- to 5-fold with decreasing substrate concentration (10^{-3} M–4×10^{-5} M).

In the presence of glutathione, Conjugates 1 and 2 were formed in incubations that contained either P-450$_I$ or P-450$_{II}$. As can be seen from the HPAEC profile (Fig. 4), the ratio of 1:2 formed was different for each cytochrome. With P-450$_I$, about 5 times more Conjugate 2 than Conjugate 1 was formed, whereas nearly equal amounts of each conjugate were formed with P-450$_{II}$. These ratios of conjugate formation were the same at all concentrations of 4-ipomeanol used (10^{-5} M–10^{-3} M), although the responses of the two enzymes to changes in substrate concentration were clearly different (Fig. 5). Substrate saturation was approached in incubations that contained P-450$_{II}$, and K_m values of 8.6×10^{-5} M and 1.1×10^{-4} M were calculated for the formation of Conjugates 1 and 2, respectively—values close to that for the covalent binding to protein ($K_m = 1.5 \times 10^{-4}$ M). However, with P-450$_I$ saturation was not apparent and the estimated K_m values were greater than the highest concentration of 4-ipomeanol used (10^{-3} M) even though the K_m

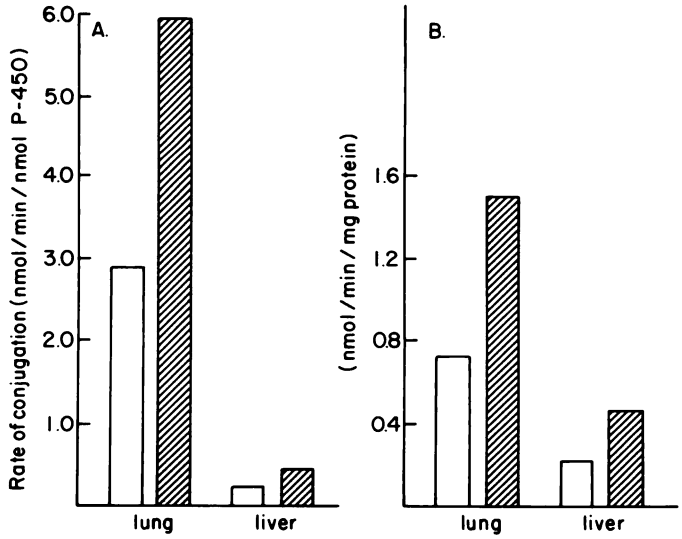

FIG. 2. *Covalent binding of metabolites of 4-ipomeanol to glutathione (conjugation) in incubations of rabbit pulmonary or hepatic preparations*

The data for the formation of Conjugate 1 (*clear bars*) and Conjugate 2 (*cross-hatched bars*) are presented as nanomoles of conjugate formed per minute per nanomole of P-450 (A) and as nanomoles of conjugate formed per minute per milligram of microsomal protein (B). The incubations were the same as those described in the legend to Fig. 1 with the exception that glutathione (1 mM) was also included. The isolation, separation, and quantitation procedures used are noted under Materials and Methods. The values reported are the means of three determinations with standard errors of less than 10% of the mean values.

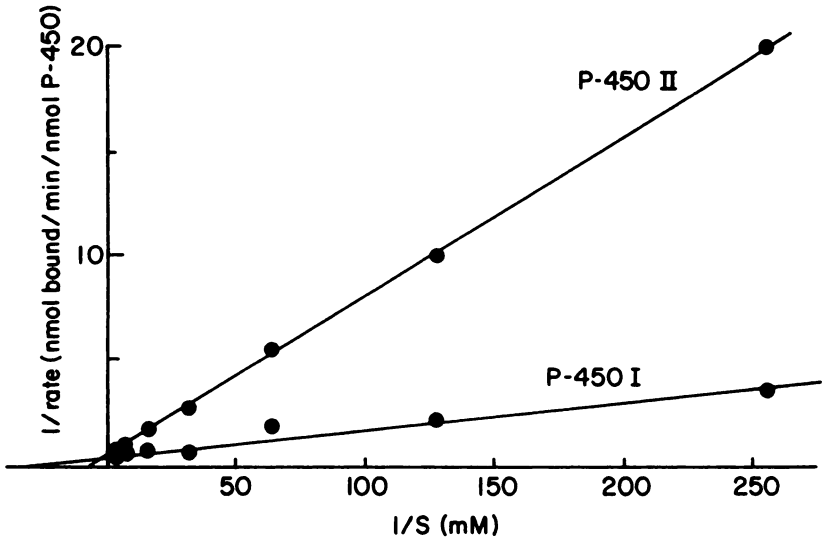

FIG. 3. *Covalent binding of metabolites of 4-ipomeanol to protein in incubations of purified cytochromes P-450$_I$ and P-450$_{II}$*

The data for covalent binding are presented as double-reciprocal plots of the rate (nanomoles of product bound per minute per nanomole of P-450$_I$ or P-450$_{II}$) versus 4-ipomeanol concentration. The incubations (1 ml) contained cytochrome P-450$_I$ or P-450$_{II}$ (0.1 nmole), NADPH cytochrome P-450 reductase (1250 units), didocecanoyl-L-α-lecithin (50 μg), bovine γ-globulin (4 mg), NADPH (1 mM), and [^{3}H]-4-ipomeanol (18 μCi, 0.5 mM) in phosphate buffer (0.05 M, pH 7.6). Analysis for covalent binding was as noted in the legend to Fig. 1.

for the covalent binding to protein with P-450$_I$ (3.5×10^{-5} M) was less than that with P-450$_{II}$.

Unlike the results obtained with microsomal preparations—approximately equal rates of metabolism determined by conjugation or covalent binding to protein—total conjugation (1 + 2) in incubations that contained either purified cytochrome was about 3 times greater than covalent binding to protein (Fig. 6; Table 1). This was not due to a loss in linearity of the covalent binding reaction, as both covalent binding and conjugation exhibited very similar time courses in the purified systems and both were linear for approximately 5 min (Fig. 6), the incubation time used for kinetic determinations. The estimated kinetic constants for the covalent binding and conjugation reactions in microsomes and purified systems are summarized in Table 1.

DISCUSSION

Rabbit pulmonary microsomal preparations metabolize 4-ipomeanol to products that bind covalently to

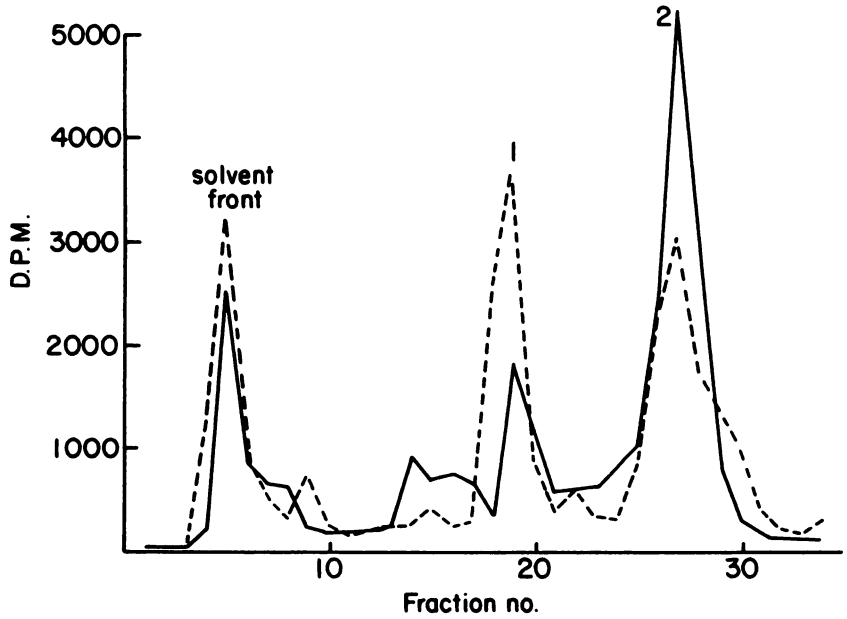

FIG. 4. *Distribution of radioactivity in fractions from HPAEC analysis of the covalent binding of metabolites of 4-ipomeanol to glutathione in incubations of purified cytochromes P-450$_I$ or P-450$_{II}$*

The profile for cytochrome P-450$_I$ is shown with the *solid line* and the profile for cytochrome P-450$_{II}$ with the *broken line*. The incubations were the same as those described in the legend to Fig. 3 with the exception that glutathione (1 mM) was used in place of the bovine γ-globulin. The results shown are from one of several experiments that gave similar results.

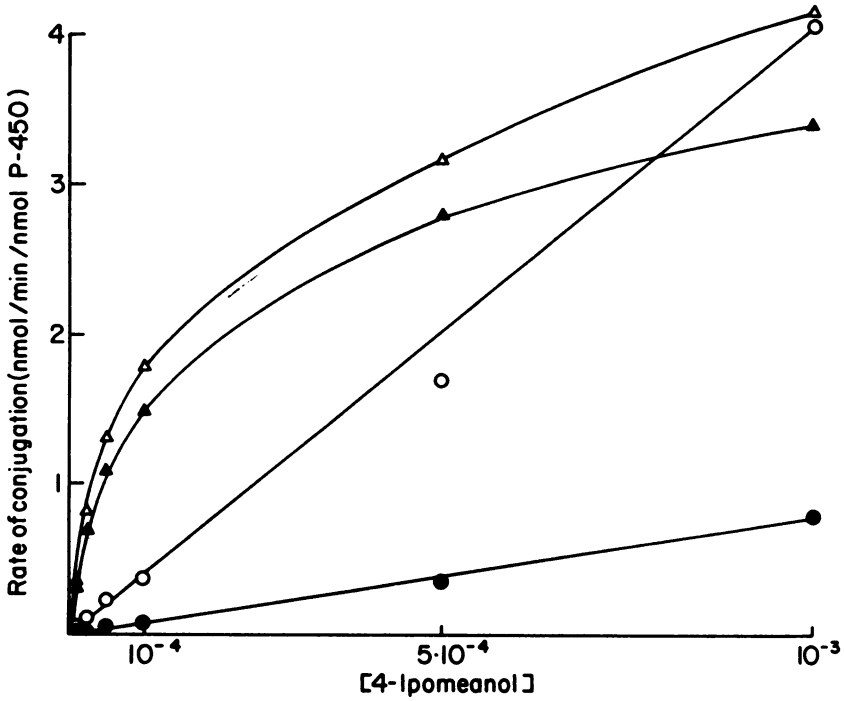

FIG. 5. *Effect of substrate concentration on the formation of glutathione Conjugates 1 and 2 mediated by cytochrome P-450$_I$ or P-450$_{II}$*

The data are presented as the rate of conjugation (nanomoles of conjugate formed per minute per nanomole of P-450$_I$ or P-450$_{II}$) versus the 4-ipomeanol concentration for the formation of Conjugate 1 by P-450$_I$ (●), Conjugate 2 by P-450$_I$ (○), Conjugate 1 by P-450$_{II}$ (▲), and Conjugate 2 by P-450$_{II}$ (△). The incubations were the same as those described in the legend to Fig. 4 except that the concentration of 4-ipomeanol was varied from 10^{-5} to 10^{-3} M.

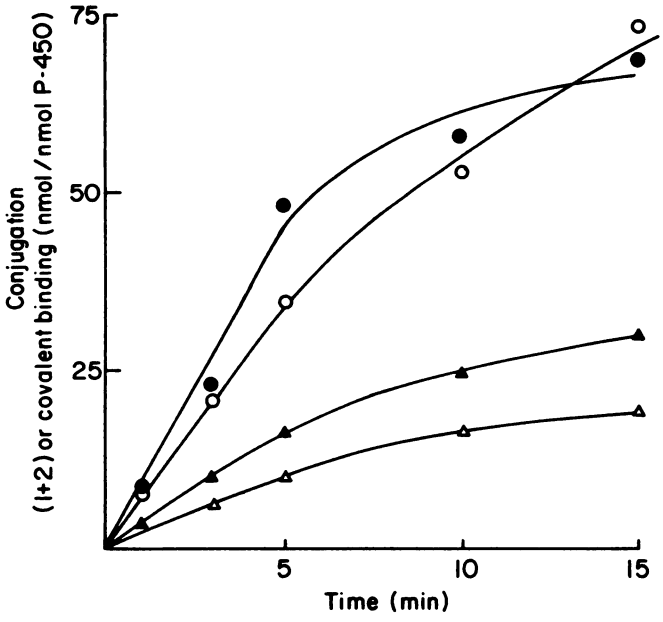

FIG. 6. *Time-dependent covalent binding of metabolites of 4-ipomeanol to protein or glutathione in incubations of purified cytochromes P-450$_I$ or P-450$_{II}$*

The data are presented as the rate of conjugation or covalent binding (nanomoles of product per minute per nanomole of P-450) versus time for the formation of Conjugates 1 and 2 by P-450$_I$ (●) or P-450$_{II}$ (○) and covalent binding to protein mediated by P-450$_I$ (▲) or P-450$_{II}$ (△). The incubations contained the same concentrations of chemicals as described in the legends to Figs. 3 and 4. The incubation volumes were 18 ml, and triplicate 1-ml samples were taken at the indicated times.

protein or glutathione at 3 times the rate mediated by hepatic microsomal preparations. This difference, which has also been reported by Dutcher and Boyd (8), is much greater than the differences (1- to 1.3-fold) between the activities of pulmonary and hepatic preparations from rats (Sprague-Dawley), mice (NIH-Swiss) or guinea pigs (Hartley), species for which 4-ipomeanol is also a lung-specific toxin (8). However, in both rats and rabbits, covalent binding of metabolites of 4-ipomeanol to protein *in vivo* is greater than 5 times more extensive in lung than in liver (8), and, in general, the difference in *in vivo* binding is a better indicator of the target organ for 4-ipomeanol toxicity than the difference *in vitro* (8). The disparity between the *in vivo* and *in vitro* results may be a function of differences in the *in vivo* and *in vitro* substrate concentrations and the relative affinities for 4-ipomeanol of the cytochrome P-450 isozymes that catalyze the activation reactions. Because the K_m for the covalent binding reaction in rat pulmonary microsomal preparations is over 10 times lower than that in rat hepatic preparations (22), binding *in vivo*, as compared with binding *in vitro* at saturating substrate concentration, should favor lung (8). However, with rabbit preparations there is less than a 2-fold difference between the K_m values in liver and lung, and this is consistent with the ratio of binding between liver and lung being similar *in vivo* and *in vitro*.

The similarity between the K_m values for 4-ipomeanol in rabbit liver and lung suggests that the activation reaction may be catalyzed primarily by the same P-450 isozymes in both tissues. The P-450 isozymes, P-450$_I$ and

TABLE 1

Kinetic constants for the covalent binding to protein and conjugation with glutathione metabolites of 4-ipomeanol in microsomal and purified systems

	Microsomes						Purified systems			
	Liver			Lung			$P\text{-}450_I$		$P\text{-}450_{II}$	
	K_m	V_{max}[a]		K_m	V_{max}[a]		K_m	V_{max}[b]	K_m	V_{max}[b]
		/mg protein	/nmole P-450		/mg protein	/nmole P-450				
	M			M			M		M	
Covalent binding	9×10^{-5}	0.71	0.65	5×10^{-5}	2.11	8.44	3.5×10^{-5}	2.56	1.5×10^{-4}	1.99
Conjugation										
Conjugate 1	—	0.27	0.25[c]	—	0.73	2.92[c]	$>10^{-3}$	~2.0[d]	8.6×10^{-5}	3.10
Conjugate 2	—	0.44	0.40	—	1.50	5.98	$>10^{-3}$	~8.0	1.1×10^{-4}	4.13

[a] The units for V_{max} are nanomoles of product bound or conjugated per minute per milligram of protein or per nanomole of cytochrome P-450.
[b] The units for V_{max} are nanomoles of product bound or conjugated per minute per nanomole of cytochrome $P\text{-}450_I$ or $P\text{-}450_{II}$.
[c] The rates for conjugation in the microsomal incubations are those observed using 5×10^{-4} M 4-ipomeanol.
[d] The kinetic constants for $P\text{-}450_I$-mediated conjugation reactions were estimated from data obtained using concentrations of 4-ipomeanol that were less than the estimated K_m values.

$P\text{-}450_{II}$, that make up the majority of the rabbit pulmonary cytochrome P-450 (10), catalyze the metabolism of 4-ipomeanol to products that bind covalently to protein at similar rates. In view of the distinctive substrate specificities of these isozymes (9), this finding was somewhat unexpected. $P\text{-}450_I$ and $P\text{-}450_{II}$ are present in liver in low concentrations (5), but antibodies to these isozymes markedly inhibit the metabolism of 4-ipomeanol in both liver and lung (23). Thus, the majority of the hepatic cytochrome P-450 appears to be relatively inactive in the metabolism of 4-ipomeanol, and the greater concentration of $P\text{-}450_I$ and $P\text{-}450_{II}$ in lung accounts for the difference between the activities of the two tissues.

Although the difference between the amounts of covalently bound metabolites of 4-ipomeanol to proteins in rabbit liver and lung is marked, both *in vivo* and *in vitro*, it is not clear that results obtained from whole-tissue studies offer any real explanation for the organ specificity of 4-ipomeanol toxicity. For example, covalent binding to lung and liver *in vivo* and *in vitro* is essentially the same in guinea pigs even though 4-ipomeanol is a lung-specific toxin in this species with an LD_{50} similar to that in rats, mice, and rabbits (8). Actually, the toxicity of 4-ipomeanol appears to be highly cell-specific (1), and results obtained from whole-lung studies are undoubtedly misleading. The covalent binding and toxicity of 4-ipomeanol are localized to nonciliated bronchiolar (Clara) cells in species for which lung is the target organ (1). Clara cells are also a major site in rabbits for cytochrome P-450 isozymes that we have now shown to be highly active in the metabolism of 4-ipomeanol to products that bind covalently to protein. The contribution of Clara cells to the activity of the pulmonary microsomal fraction is not known, but isolated Clara cells are much more active than are whole-lung digests or isolated alveolar Type II cells or macrophages in the metabolism of 4-ipomeanol (24). We believe that these results show that the highly localized covalent binding of 4-ipomeanol in lung results from the presence of relatively high concentrations of active cytochrome P-450 isozymes in Clara cells and that differences between the activities of Clara cells and hepatocytes are undoubtedly much greater than the differences that have been observed between lung and liver.

It is difficult to assess the relative contributions of $P\text{-}450_I$ and $P\text{-}450_{II}$ to the metabolism of 4-ipomeanol *in vivo*, although they do have similar V_{max} constants when examined in purified systems. At least three major questions must be answered before the activities of $P\text{-}450_I$ and $P\text{-}450_{II}$ *in vivo* can be evaluated fully. First, what concentration of 4-ipomeanol reaches the Clara cell following the administration of toxic doses? At physiological concentrations of 4-ipomeanol, the difference between the apparent K_m values of $P\text{-}450_I$ and $P\text{-}450_{II}$ may be decisive. Second, what are the relative concentrations of $P\text{-}450_I$ and $P\text{-}450_{II}$ in Clara cells? Qualitative assessments in isolated Clara cells do not indicate major differences in the concentrations of the two isozymes (6), but this question requires additional quantitative experimentation. Third, are the glutathione Conjugates 1 and 2 indicative of the formation of distinct metabolites that might have different toxic potencies? The rate of metabolism determined by conjugation in pulmonary microsomal preparations is similar to that observed in purified systems with either $P\text{-}450_I$ or $P\text{-}450_{II}$, and the ratio of formation of Conjugate 1 to Conjugate 2 (1:2) is reasonably accounted for by the combined ratios (1:4 for $P\text{-}450_I$ and 1:1.3 for $P\text{-}450_{II}$) observed with the purified enzymes. However, the validity of these data is uncertain. In the purified systems, the amount of covalent binding to protein equals only one-third the glutathione conjugate formation.

Buckpitt and Boyd (20) have reported that total conjugation is 2-fold greater than covalent binding to protein in 8-min incubations with microsomal preparations from rat liver or lung. Their results may have been due to a longer period of linearity in incubations containing glutathione because of protection of the microsomal enzymes (20). The addition of γ-globulin as an acceptor protein may have served a similar purpose in our microsomal experiments, as we observed similar amounts of covalent binding or conjugation in 5-min incubations that were linear for both determinations. However, similar

time courses for covalent binding and conjugation were also observed in the purified systems even though the rates were substantially different with either P-450$_I$ or P-450$_{II}$. It was also noted that glutathione had a marked effect on the apparent K_m values for the conjugation reactions mediated by P-450$_I$; the K_m values for conjugation were about 300 times higher than the K_m for covalent binding. With P-450$_{II}$, all of the K_m values for covalent binding and conjugation were approximately the same. It is not known whether glutathione has a similar effect on P-450$_I$ in microsomal preparations.

Although the relative activities of P-450$_I$ and P-450$_{II}$ *in vivo* are not known, both isozymes do metabolize 4-ipomeanol to products that bind covalently to protein or glutathione. The presence of these isozymes in Clara cells, the target cell for the covalent binding and toxicity of 4-ipomeanol in rabbits and other species, is clearly an important factor in the organ specificity of this toxin.

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