Regioselective and Stereoselective Hydroxylation of R and S Warfarin by Different Forms of Purified Cytochrome P-450 from Rabbit Liver

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The metabolism of the R and S enantiomers of warfarin by rabbit liver microsomes and by purified and partially purified forms of rabbit liver cytochrome P-450 (P-450\textsubscript{R}, P-450\textsubscript{S}) to yield multiple monohydroxylated products has been investigated to probe the substrate specificities and regio- and stereoselectivities of the cytochromes. The metabolism of warfarin by rabbit liver microsomes differed from that of rat microsomes with respect to the overall rates of warfarin metabolism, the relative rates of formation of the different metabolites, and the effects of inducing agents on the metabolism. These results imply that the rabbit hepatic cytochromes differ markedly from those of rat liver.

Based on their catalysis of warfarin metabolism, the purified and partially purified, reconstituted P-450\textsubscript{R} enzymes were divided into three groups; P-450\textsubscript{R1}, and P-450\textsubscript{R2} were inactive with warfarin; P-450\textsubscript{R3} and P-450\textsubscript{R4} had the same activities; and P-450\textsubscript{R5} had a markedly different activity. The regio- and stereoselectivities of P-450\textsubscript{R1} and P-450\textsubscript{R2} catalyzed metabolism of warfarin differed, and the results indicate that P-450\textsubscript{R1} is the only enzyme that approaches the enzyme catalytic site at the coumarin side of the molecule, and preferentially when warfarin is in the R configuration. P-450\textsubscript{R2} also acts on the coumarin side of warfarin, but preferentially when it is in the S configuration, and also on the phenyl ring, but preferentially when warfarin is in the R configuration. Comparison of warfarin metabolite patterns from microsomes and P-450\textsubscript{R} enzymes indicates that regio- and stereoselectivities of the P-450\textsubscript{R} enzymes were essentially maintained when the cytochromes were removed from the microsomal membrane and purified.

The broad substrate specificity of cytochrome P-450 has been partially attributed to the multiplicity of the cytochrome (e.g., Refs. 3 to 7). The concept of multiple forms of cytochrome P-450 has been supported by the results of numerous studies on the effects of inducing agents, together with immunological (6, 8, 9) and gel electrophoretic (3) evidence. However, the isolation and purification of a number of different forms of cytochrome P-450, most notably by groups in this country (10-14) and Japan (15, 16), have provided the most compelling evidence for the multiplicity of cytochrome P-450.

Highly purified and partially purified forms of cytochrome P-450 have been isolated, in one of the present laboratories, from uninduced and phenobarbital- or β-naphthoflavone-induced rabbit liver microsomes, and assigned a nomenclature based on their electrophoretic mobilities (10-12). The specificities of these purified enzymes have been investigated with a number of different substrates (11, 17), and the regiospecificities have been studied with benzo[a]pyrene and benzo[a]pyrene(-)-trans-7,8-diol as the substrates (7, 18). The substrate specificities, stereo- and regiospecificities of the purified forms of cytochrome P-450 are still not clearly understood, however, and further investigations are essential for elucidation of the multifaceted modes of action of the enzymes.

Rat P-450\textsubscript{R} catalyzes the metabolism of warfarin (Scheme IA) to yield the following as shown in the scheme: B, 6-hydroxywarfarin; C, 7-hydroxywarfarin; D, 8-hydroxywarfarin; E, 4'-hydroxywarfarin; F, benzyl hydroxywarfarin (19, 20, 22). G, dehydrowarfarin. Warfarin contains an asymmetric carbon atom (21) and the enantiomeric forms, R and S warfarin, undergo stereoselective hydroxylations catalyzed by microsomal cytochrome P-450 (22). The relative formation rates of the warfarin metabolites are markedly altered by prior induction of the microsomes (23), indicating that a number of different forms of cytochrome P-450 are involved in warfarin metabolism. We recently developed HPLC assays for the simultaneous analysis of the all the microsomal metabolites of the warfarin enantiomers (24) greatly facilitates the use of warfarin as a probe of cytochrome P-450-catalyzed metabolism.

In the present study we have investigated the effects of PB and BNF induction on the rabbit hepatic microsomal metabolism of R and S warfarin by using the HPLC assay. Regiospecificities and stereoselectivities of R and S warfarin metabolism catalyzed by purified rabbit P-450\textsubscript{R}, P-450\textsubscript{M}, P-450\textsubscript{LM}, and P-450\textsubscript{LM}, have been investigated and the results compared with those from the microsomal metabolism.

* The abbreviations used are: P-450\textsubscript{R}, liver microsomal cytochrome P-450; PB, phenobarbital; BNF, β-naphthoflavone; dilauroyl-GPC, dilauroylglyceryl-3-phosphorylcholine; HPLC, high pressure liquid chromatography.

followed by affinity chromatography on 2',5'-ADP agarose (29) as in that glycerol was not added to the buffer mixture for final suspen-
sion membrane (Millipore Corp., Bedford, Mass.) prior to use in the
MO.). All other materials were obtained as described previously (10,
NADPH was type 1 purchased from Sigma Chemical Co. (St. Louis,
Angles, Calif.), was resolved by a previously described method (21)
was loaded onto the column and the flow of the solvent mixture
maintained for 60 s to concentrate the filtrate into a narrow band on
the column. A concave gradient elution was then initiated using the
Waters Associates model 660 solvent programmer (gradient 7). The
method of analysis has been previously described (24) and subsequently modi-
fied. The solvent systems were modified as follows: Solvent I, 1.5?
acetic acid (pH 4.85 with NH,OH); Solvent II, 1.5% acetic acid (pH
4.85 with NH,OH)/acetonitrile (69/31). The column was equilibrated
for 3 min at 30°C with shaking, and the reaction was then initiated
by the addition of 1.0 μmol of NADPH. Components were
added and mixed in the manner previously described (17). Incubation
was continued for 10 to 30 min, and the reaction was terminated by
cooling in ice and filtering through a pad of filters as described above.
A series of control experiments was performed with either one com-
ponent being excluded in each case or boiled protein components
being used. The precise concentrations of the components for each
experiment are provided in the figure legends. All of the data are the
averages of duplicate experiments.

The metabolites of warfarin were analyzed by HPLC, using a
Waters Associates model 244 liquid chromatograph equipped with a
Hewlett-Packard model 3380A recording integrator. The method of
preparation of microsomes and purification of cyto-
chromes—Microsomes were prepared from New Zealand male rabbits
(2 to 2.5 kg), which were either uninduced or induced with PB or
BNF as previously described (12). Water was deionized, glass-distilled, and filtered through a 0.22-
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Hydroxylation of Warfarin by Purified Cytochrome P-450

**FIG. 1.** A, rates of formation of 4'-hydroxywarfarin (4'-OH), 6-hydroxywarfarin (6-OH), 8-hydroxywarfarin (8-OH), and 7-hydroxywarfarin (7-OH) from R or S warfarin, catalyzed by control, PB-, or BNF-induced rabbit liver microsomes. Reactions were performed using 2 mg of microsomal protein/ml at 30°C, pH 7.4. All results are the averages of two experiments. B, rates of formation of warfarin metabolites from R and S warfarin catalyzed by control, PB-, or 3-methylcholanthrene-induced rat liver microsomes. Reaction conditions were as in A. Cytochrome P-450 concentrations were 1.0, 2.2, and 1.7 nmol/mg of microsomal protein for control, PB-, or 3-methylcholanthrene-induced microsomes, respectively.

**TABLE I**

<table>
<thead>
<tr>
<th>Warfarin metabolite</th>
<th>Rate of formation</th>
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<tbody>
<tr>
<td></td>
<td>Preparation 1</td>
<td>Preparation 2</td>
</tr>
<tr>
<td>R warfarin</td>
<td>pmol product/nmol P-450Lm/min</td>
<td></td>
</tr>
<tr>
<td>4'-OH</td>
<td>50.4, 53.0</td>
<td>36.0</td>
</tr>
<tr>
<td>6-OH</td>
<td>10.3, 9.7</td>
<td>7.9</td>
</tr>
<tr>
<td>7-OH</td>
<td>3.2, 3.2</td>
<td>2.5</td>
</tr>
<tr>
<td>S warfarin</td>
<td>23.9, 25.5</td>
<td>19.3</td>
</tr>
<tr>
<td>4'-OH</td>
<td>99.5, 74.2</td>
<td>54.6</td>
</tr>
<tr>
<td>6-OH</td>
<td>2.8, 2.8</td>
<td>1.4</td>
</tr>
</tbody>
</table>

* Duplicate analyses.

The P-450Lm-catalyzed formation of all the metabolites from R warfarin was linear with time for 30 min (Fig. 3A), but with S warfarin metabolite formation was only linear for 20 min, with slight deviations being apparent after this time (Fig. 3B). In the absence of dilauroyl-GPC from the reaction mixture, rates of R warfarin metabolite formation were approximately one-fourth of the rates at the optimal dilauroyl-GPC concentration. The maximal rates of R 4', R 6-, and R 7-hydroxywarfarin formation all occurred at a ratio of approximately 30 to 45 mol of lipid/mol of P-450Lm (20 to 30 mg of lipid/nmol of P-450Lm) (Fig. 4A). When the ratio of dilauroyl-GPC to P-450Lm exceeded this ratio, the rates of all the metabolites were markedly decreased. The effects of dilauroyl-GPC on the P-450Lm-catalyzed metabolism of S warfarin were very similar (Fig. 4B) to the results with R warfarin.

In the absence of NADPH-cytochrome P-450 reductase, no metabolites were formed from R warfarin. As the reductase concentration was increased, the formation rates of all the metabolites increased until a molar ratio of reductase/P-450Lm, of approximately 1.4 was reached. At higher ratios of reductase/P-450Lm, the rates were markedly decreased (Fig. 5A). Similar results were obtained with S war-
Hydroxylation of Warfarin by Purified Cytochrome P-450

FIG. 2. Rates of formation of 4'-hydroxywarfarin (4-OH), 6-hydroxywarfarin (6-OH), 8-hydroxywarfarin (8-OH), and 7-hydroxywarfarin (7-OH) from R and S warfarin catalyzed by P-450<sub>1.9</sub> or P-450<sub>1.75</sub> in the reconstituted enzyme system. Both forms of the cytochrome and the NADPH-cytochrome P-450 reductase were isolated from microsomes from PB-treated rabbits. Reaction mixtures contained in a total of 1.0 ml: P-450<sub>1.9</sub> (1.0 nmol), reductase (1.4 nmol, 4.2 units), dilauroyl-GPC (20 µg, 30 nmol), R or S warfarin (0.9 pmol), NADPH (1.9 µmol) and Tris-HCl buffer (50 mM). Reactions were run at 30°C and pH 7.4 for 20 min. Metabolites were analyzed by HPLC. All data points are the averages of two experiments.

FIG. 3. Effect of time on the quantity of 4'-hydroxywarfarin (■), 6-hydroxywarfarin (○), and 7-hydroxywarfarin (▼) produced from (A) R warfarin and (B) S warfarin catalyzed by P-450<sub>1.9</sub> in the reconstituted enzyme system. Other conditions were as in Fig. 2.

Warfarin, but maximal rates were achieved at a somewhat higher reductase/P-450<sub>1.9</sub> ratio (Fig. 5B).

The effects of P-450<sub>1.9</sub> concentration on the rates of formation of metabolites of R and S warfarin are shown in Figs. 6 and 7. In Fig. 6, the concentrations of reductase were varied with the concentrations of P-450<sub>1.9</sub> so as to maintain the optimal ratio of reductase/P-450<sub>1.9</sub>. Rates of formation of all the metabolites from R warfarin increased linearly with increasing P-450<sub>1.9</sub> concentration until a value approaching 1 nmol of P-450<sub>1.9</sub>/ml was reached, above which the increase in rates was much less rapid (Fig. 6A). With S warfarin as substrate (Fig. 6B), however, the rates of metabolite formation deviated slightly from linearity only at low P-450<sub>1.9</sub> concentrations. When the P-450<sub>1.9</sub> concentration was varied but the reductase concentration was maintained at a constant value (which was equivalent to that producing maximal rates at a P-450<sub>1.9</sub> concentration of 1 nmol/ml), the results shown in Fig. 7 were obtained. With R warfarin (Fig. 7A) and S warfarin (Fig. 7B) rates of metabolite formation increased linearly at low P-450<sub>1.9</sub> concentrations but deviated from linearity at higher enzyme concentrations.

Warfarin concentration (0.9 mM) was saturating but not inhibiting in all of these assays as was demonstrated by the
equivalent results obtained when the warfarin concentration was altered to 0.45 mM. Addition of sodium cholate (0.2 mM) or sodium deoxycholate (0.2 mM) to the reconstituted system or the replacement of the Tris buffer by N-[tris-(hydroxymethyl)methyl]-2-aminoethanesulfonic acid buffer or phosphate buffer did not alter the rates of formation of the metabolites.

**Metabolism of Warfarin by P-450LM,-** The reconstituted system containing P-450LM,- catalyzed the formation of R warfarin to yield predominantly 6-hydroxywarfarin with lesser quantities of 8- and 7-hydroxywarfarin. S warfarin was metabolized to a much lesser extent with 6-hydroxywarfarin being the predominant product and 8- and 7-hydroxywarfarin having lower rates of formation. No 4'-hydroxywarfarin was detected from either R or S warfarin with P-450LM,-. The metabolite formation rates catalyzed by P-450LM,- from uninduced, PB-, or BNF-induced rabbits are presented in Table II. In most cases, the P-450LM,- from uninduced rabbits produced the lowest formation rates for the metabolites. The relative contributions of the formation rates of each metabolite to the total formation rates of all products from the metabolism of R or S warfarin was essentially the same with P-450LM,- from control, PB-, or BNF-treated microsomes (Table II). This suggests that the P-450LM,- from these three sources is the same enzyme. Subsequent studies were performed using the P-450LM,- from PB-induced rabbits.

The P-450LM,- catalyzed formation of all the metabolites from R warfarin deviated slightly from linearity over 20 min (Fig. 8), but the relative quantities of the metabolites were unaltered and subsequent studies were performed with reactions run for 20 min. In the absence of dilauroyl-GPC from the reaction mixture, the rate of 6-hydroxywarfarin formation was approximately three-fifths of the rate at the optimal dilauroyl-GPC concentration (Fig. 9). The maximal rate of 6-hydroxywarfarin formation occurred at the ratio of approximately 30 mol of lipid/mol of P-450LM,- and when the lipid concentration was increased, the rate of formation decreased markedly. The rates of formation of 7- and 8-hydroxywarfarin

**Table II**

<table>
<thead>
<tr>
<th>Warfarin metabolite</th>
<th>Rate of formation</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>R warfarin</td>
<td></td>
</tr>
<tr>
<td>6-OH</td>
<td>33.0, 40.9 (73)</td>
</tr>
<tr>
<td>7-OH</td>
<td>3.2, 3.9 (7)</td>
</tr>
<tr>
<td>8-OH</td>
<td>8.7, 11.4 (20)</td>
</tr>
<tr>
<td>S warfarin</td>
<td></td>
</tr>
<tr>
<td>6-OH</td>
<td>5.1, 6.1 (45)</td>
</tr>
<tr>
<td>7-OH</td>
<td>2.2, 2.9 (21)</td>
</tr>
<tr>
<td>8-OH</td>
<td>4.3, 4.3 (34)</td>
</tr>
</tbody>
</table>

* Duplicate analyses.
* Percentage of total formation rates of all products from R or S warfarin.

**Fig. 6.** Effect of P-450LM,- concentration on the rates of formation of 4'-hydroxywarfarin (■), 6-hydroxywarfarin (○), and 7-hydroxywarfarin (●) from (A) R warfarin and (B) S warfarin catalyzed by P-450LM,- in the reconstituted enzyme system. The NADPH-cytochrome P-450 reductase concentration was varied to produce a constant P-450LM,-/reductase molar ratio of 1.4. Other conditions were as in Fig. 2.

**Fig. 7.** Effect of P-450LM,- concentration on the rates of formation of 4'-hydroxywarfarin (■), 6-hydroxywarfarin (○), and 7-hydroxywarfarin (●) from (A) R warfarin and (B) S warfarin catalyzed by P-450LM,- in the reconstituted enzyme system. The NADPH-cytochrome P-450 reductase concentration was varied to produce a constant P-450LM,-/reductase molar ratio of 1.4. Other conditions were as in Fig. 2.

**Fig. 8.** Effect of time on the quantity of 6-hydroxywarfarin (○), 7-hydroxywarfarin (●), and 8-hydroxywarfarin (□) produced from R warfarin catalyzed by P-450LM,- in the reconstituted enzyme system. Other conditions were as in Fig. 2.
Hydroxylation of Warfarin by Purified Cytochrome P-450

**DISCUSSION**

The rabbit hepatic microsomal metabolism of R and S warfarin is markedly different from that of the rat with respect to the overall rates of warfarin metabolism, the relative rates of formation of the different metabolites, and the effects of inducing agents on the metabolism. Comparisons of both R and S warfarin metabolism by rat or rabbit microsomes indicate the following differences. In the rat R 7- and S 4'-hydroxywarfarin are the major uninduced microsomal metabolites from the warfarin enantiomers (22), while in the rabbit R 4'- and S 6-hydroxywarfarin are the major products. PB induction of rat microsomes results in the formation of R and S 7-hydroxywarfarin as the major metabolites (23) in contrast to rabbit microsomes where R 4'- and S 6-hydroxywarfarin predominate. BNF induction is similar to 3-methylcholanthrene or benzo[a]pyrene induction (22) in markedly enhancing the rates of formation of R 6- and R 8- and S 7-hydroxywarfarin catalyzed by rat hepatic microsomes, but in the rabbit BNF induction leads to R 6- and S 7-hydroxywarfarin as the major products. Furthermore, the metabolites dehydrowarfarin and benzylic hydroxywarfarin, which are products of the rat hepatic microsomal metabolism of the aliphatic moiety of warfarin (22), are not detectable products of rabbit
The overall rate of rat hepatic microsomal cytochrome P-450-catalyzed metabolism of R or S warfarin is approximately twice that of the rabbit with uninduced and PB-induced microsomes, and six times that of the rabbit with BNF-induced microsomes. These differences in function between rat and rabbit microsomes imply that the forms of cytochrome P-450 in the livers of the two species are different (cf Fig. 1, A and B).

The differences in the metabolic activities of the various purified and partially purified P-450\textsubscript{r,M} enzymes with warfarin, separate them into three groups: P-450\textsubscript{r,M}, P-450\textsubscript{r,LM}, and P-450\textsubscript{r,LM}, comprise a group which exhibits no activity with warfarin; P-450\textsubscript{r,M} and P-450\textsubscript{r,LM} exhibit the same activity, which is markedly different from that of P-450\textsubscript{r,LM}, which comprises the third group. From the results depicted in Fig. 2, it was determined that P-450\textsubscript{r,LM}, is stereoselective for R 4'- and S 6-hydroxywarfarin, while P-450\textsubscript{r,LM} is stereoselective for R 6- and R 8-hydroxywarfarin. When the overall metabolism was considered, the two enzymes showed opposite stereoselectivities, with P-450\textsubscript{r,LM} being stereoselective for S warfarin and P-450\textsubscript{r,LM} for R warfarin. The regioselectivities of the two enzymes are also different, with P-450\textsubscript{r,LM} being regioselective for R 4'- and S 6-hydroxywarfarin, with no detectable formation of 8-hydroxywarfarin, and P-450\textsubscript{r,LM} being regioselective for R 6- and S 6-hydroxywarfarin, with no detectable formation of 4'-hydroxywarfarin.

The regioselective and stereoselective properties of P-450\textsubscript{r,M} and P-450\textsubscript{r,M}, enzymes must arise from differing activities of the enzyme catalytic sites with differently oriented warfarin molecules. Thus, P-450\textsubscript{r,M}, which only catalyzed the formation of metabolites which have the phenyl portion of the coumarin moiety hydroxylated (6-, 7-, and 8-hydroxywarfarin), has activity with warfarin only when it is oriented to approach the enzyme at the coumarin side of the molecule. Furthermore, the activity of P-450\textsubscript{r,M} on warfarin in this orientation is greater with the R enantiomer than with the S enantiomer. In the case of P-450\textsubscript{r,LM}, there is also potential activity with warfarin oriented with the coumarin moiety toward the catalytic site (6- and 7-hydroxywarfarin formed), but the S enantiomeric configuration is preferred. In addition, P-450\textsubscript{r,LM} has the potential to interact with the phenyl substituent of warfarin, but for that interaction the R configuration of warfarin is favored.

It has been proposed that rat hepatic cytochrome P-450-catalyzed metabolism of warfarin to 8-hydroxywarfarin proceeds via formation of a 7,8-arene oxide, which preferentially opens to 8-hydroxywarfarin, and that 6-hydroxywarfarin similarly arises from a 6,7-arene oxide (22). If the same mechanism were applicable to rabbit P-450\textsubscript{r,LM}, the metabolite patterns obtained (Fig. 2) indicate that P-450\textsubscript{r,LM} only catalyzes the formation of the 6,7-arene oxide of warfarin, while P-450\textsubscript{r,LM} catalyzes the formation of both the 6,7- and 7,8-arene oxides.

Warfarin undergoes a tautomeric-driven, ring closure in solution to form a hemiketal (Scheme 1H) (32). The extent of this reaction is dependent on the polarity of the environment of the warfarin molecule. The environment of the catalytic sites of the P-450\textsubscript{r,LM} enzymes could thus influence the tautomerism to an unknown extent. The tautomeric form present could affect the metabolism of warfarin.

The fact that in the absence of added lipid, P-450\textsubscript{r,LM} catalyzed metabolism of R warfarin proceeds to a relatively much greater degree than P-450\textsubscript{r,LM} catalyzed metabolism, is consistent with results using other substrates (33). The inhibitory effect of excess lipid on the rates of P-450\textsubscript{r,LM} and P-450\textsubscript{r,LM} catalyzed R warfarin metabolism is a function of the lipid concentration, rather than the lipid/P-450\textsubscript{r,LM} ratio. This was determined from the studies of the effects of P-450\textsubscript{r,LM} concentration on the rates of formation of the warfarin metabolites. At low P-450\textsubscript{r,LM} concentrations, the ratio of lipid/P-450\textsubscript{r,LM} was greatly in excess of the optimal ratio, but the metabolite formation rates were linearly related to P-450\textsubscript{r,LM} concentrations (Figs. 6A and 11), indicating that a high ratio of lipid/P-450 was not, in itself, inhibitory. A similar argument may not be applied to the effect of lipid concentration on S warfarin metabolism by P-450\textsubscript{r,LM}, since the metabolism formation rates deviate slightly from linearity.

The variation in the effects of lipid concentration on the P-450\textsubscript{r,LM} catalyzed formation rates of the different metabolites (Fig. 9) can arise from lipid-imposed conformational restraints on the enzyme, such that its interactions with warfarin in different orientations are differentially affected. It has been previously suggested that lipids can affect microsomal cytochrome P-450 function by inducing protein conformational changes (32, 34, 35). An alternative possibility is that there is more than one form of cytochrome P-450 in the P-450\textsubscript{r,LM} preparation. Although the P-450\textsubscript{r,LM} preparations yield a single band on sodium dodecyl sulfate gel electrophoresis, and a single COOH-terminal residue (12), the NH\textsubscript{2}-terminal amino acid analysis of P-450\textsubscript{r,LM} lends some support to the possibility of more than one enzyme component (36).

The requirements for NADPH-cytochrome P-450 reductase in the P-450\textsubscript{r,LM} and P-450\textsubscript{r,LM}-reconstituted systems for the metabolism of R or S warfarin were similar (Figs. 5, A and B, and 10). In all cases, high levels of reductase apparently inhibited the reaction. We have eliminated the possibility that the NADPH levels produced with high reductase concentrations inhibited warfarin metabolism, and thus it is possible that protein-protein interactions are the source of the inhibition. Either the reductase, when in excess, binds to the P-450\textsubscript{r,LM} nonspecifically and sterically hinders the interaction of the substrate with the P-450\textsubscript{r,LM}, or the nonspecific interaction causes conformational changes in the P-450\textsubscript{r,LM}, which results in the inhibition of the reaction.

Although excess NADPH-cytochrome P-450 reductase in the reconstituted system clearly caused inhibition of warfarin metabolism at P-450\textsubscript{r,LM} concentrations of 1 mM (Figs. 5 and 10), a similar effect did not occur at low P-450\textsubscript{r,LM} concentrations. This was apparent from experiments where the reductase concentration was maintained at high levels and the P-
450,M concentrations were varied (Figs. 7 and 11B). At low P-
450,M concentrations, no inhibitory effect of the relatively high reductase concentration was observed which implies that reductase concentrations rather than reductase/P-450,M ratios govern the inhibition.

Comparisons of microsomal cytochrome P-450 and purified cytochrome P 450 catalyzed reactions must be made with caution due to the potential for different rate-controlling steps in the two systems, as a consequence of different cytochrome P-450/reductase ratios. However, the similarities between the metabolite patterns derived from the P-450,M and the PB-induced microsome catalyzed metabolism of R and S warfarin is marked. In the case of the comparison of BNF-induced microsomal metabolism with P-450,M-catalyzed metabolism, the similarity is less marked, and thus other forms of cytochrome P-450 must be making a major contribution to warfarin metabolism by BNF-induced microsomes, particularly with respect to 4'- and 7-hydroxywarfarin formation. The similarities between the purified P-450,M and microsomal results indicate that the selectivities of these two enzymes for warfarin must essentially be maintained when they are removed from the microsomal membrane on purification.

The regio- and stereoselectivities of warfarin metabolism by P-450,M enzymes have significant pharmacological implications in view of the following facts. The anticoagulation activity of S warfarin is five to eight times greater than that of R warfarin (37), and only 4'-hydroxywarfarin, of all the known metabolites, possesses significant anticoagulant activity (19). Thus, induction of P-450,M leads to diminished rates of metabolism of the more active S enantiomer, which would enhance the pharmacological potency of clinically administered racemic warfarin. In contrast, induction of P-450,M results in enhanced metabolism of S warfarin, and thus diminished pharmacological effects, but this would be offset to some extent by the formation of the active 4'-hydroxywarfarin metabolite.

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REFERENCES

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