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Accessibility of Cytochrome P450 in Microsomal Membranes: Inhibition of Metabolism by Antibodies to Cytochrome P450

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SUMMARY

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Specific antibodies against highly purified cytochrome P450 from phenobarbital (PB)-treated rats and cytochrome P448 from 3-methycholanthrene (MC)-treated rats were produced in rabbits. These antibodies are inhibitors of drug metabolism catalyzed by liver microsomes from untreated rats or from rats treated with PB, MC, or pregnenolone-16α-carbonitrile (PCN). Microsomal metabolism of five substrates was examined; the extent of antibody inhibition was dependent not only on the source of the antibody and microsomes but also on the specific substrate and the reactions catalyzed. A comparison of the antibody inhibition patterns of the various substrates examined indicates a marked difference in the proportions of the different forms of cytochrome P450 present in liver microsomes from untreated and PB-, MC-, and PCN-treated rats. Antibody inhibition of microsomal metabolism indicates that the membrane-bound terminal oxidase, cytochrome P450 or P448, is at least partially exposed to the hydrophilic environment on the exterior of microsomal membranes.

INTRODUCTION

The NADPH-mediated electron transport system in liver microsomes is capable of metabolizing a variety of compounds, such as steroids, fatty acids, drugs, and many xenobiotics (1-5). This membrane-bound enzyme system consists of two proteins (5, 6), cytochrome P450 and NADPH-cytochrome *c* reductase (also known as NADPH-cytochrome P450 reductase). Other studies have shown that phospholipid also plays an important role in microsomal electron transport and drug hydroxylation (7). Information concerning the spatial arrangement and localization of both cytochrome P450 and NADPH-cytochrome *c* reductase in the microsomal membrane is essential for an understanding of the interaction of these components and the mechanism of microsomal electron transport.

Information is available about the location of NADPH-cytochrome *c* reductase in the membrane. The hydrophilic portion of NADPH-cytochrome *c* reductase can be released from the microsomal membrane by protease treatment (8-10), and antibody against purified NADPH-cytochrome *c* reductase inhibits microsomal NADPH-mediated electron transport (11, 12). This enzyme can be classified as an amphipathic protein like cytochrome b_5 and NADH-cytochrome b_5 reductase (13-16) of the NADH-mediated electron transport system. The hydrophobic tail of these amphi-

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pathic proteins is embedded in the lipid bilayer of the microsomal membrane, whereas the bulk of the proteins, containing the active site of the enzymes, is exposed to the cytosol. On the other hand, the localization of cytochrome P450 in the microsomal membrane is still uncertain. Treatment of liver microsomes with protease does not result in the solubilization of significant amounts of either cytochrome P450 or its denatured form, cytochrome P420 (8). In addition, it has been reported that antibody produced against one of the forms of cytochrome P450 in liver microsomes from phenobarbital-treated rats inhibits the *N*-demethylation of benzphetamine in a solubilized and reconstituted hydroxylation system, but not in microsomal suspensions (17). These results suggest that, unlike other microsomal electron carriers (such as cytochrome b_5, NADH–cytochrome b_5 reductase, and NADPH–cytochrome *c* reductase), cytochrome P450 may be completely embedded in the hydrophobic environment of the microsomal membrane.

Recently we have reported on antibody to purified rat liver cytochrome P450 from PB[1]-treated rats (anti-PB-P450) (18) and antibody to purified rat liver cytochrome P448 from MC-treated rats (anti-MC-P448) (19, 20). These specific antibodies differentially inhibit the metabolism of a variety of substrates catalyzed by the purified and reconstituted system. In this report we describe the ability of these antibody preparations to inhibit metabolism of five substrates catalyzed by microsomal suspensions from rats treated with PB or MC. The inhibitory capacity of these two antibodies was examined in the absence of membrane solubilization agents in order to determine whether native microsomal cytochrome P450 is accessible to these antibodies. The effects of these antibodies on metabolism in microsomes from control (untreated) and PCN-treated rats were also examined to determine whether differences exist between the cytochrome P450 present in these microsomes and that in microsomes from PB- and MC-treated rats.

[1] The abbreviations used are: PB, phenobarbital; MC, 3-methylcholanthrene; PCN, pregnenolone-16α-carbonitrile; IgG, immunoglobulin G; PB-450, purified cytochrome P450 preparation isolated from livers of phenobarbital-treated rats, and anti-PB-P450, rabbit IgG prepared against it; MC-P448, purified cytochrome P448 preparation isolated from livers of 3-methylcholanthrene-treated rats, and anti-MC-P448, rabbit IgG prepared against it. PB microsomes refer to the microsomes from PB-treated rats; likewise for MC, PCN, and control microsomes.

MATERIALS AND METHODS

Production of antibodies. Hepatic cytochrome P450 from PB-treated rats and cytochrome P448 from MC-treated rats were solubilized with detergent and purified (21). Antibodies to each of these purified hemeproteins were produced in rabbits (18, 19). Antibody preparations were derived from pools of hyperimmune sera collected by multiple bleedings of rabbits. The IgG fractions from immune sera and control sera were isolated as previously described (19) and found to be at least 96% homogeneous by agarose gel electrophoresis at pH 8.6. Protein was estimated by the method of Lowry *et al.* (22), using bovine serum albumin as the standard.

Treatment of rats and isolation of microsomes. Immature male Long Evans rats (weighing 50–60 g) from Blue Spruce Farms, Altamont, N. Y., were divided into groups of 10 and injected intraperitoneally with PB (75 mg/kg), MC (25 mg/kg), or PCN (25 mg/kg) on each of 4 consecutive days. Twenty-four hours after the last injection the rats were decapitated and the livers were removed, pooled, and weighed. Livers were homogenized (glass-Teflon homogenizer) and microsomes prepared as previously described (23). Microsomes, containing 20 nmoles of cytochrome P450 per milliliter, were suspended in 0.25 M sucrose and stored at −70° in aliquots. Cytochrome P450 was determined in microsomes from the CO difference spectra of dithionite-reduced samples as described by Omura and Sato (24). The cytochrome P450 (P448) contents of the various microsomal preparations were 1.2, 3.1, 2.4, and 1.8 nmoles/mg of protein for microsomes obtained from control, PB-, MC-, and PCN-treated rats, respectively.

Microsomal metabolism. The appropriate antibody was first incubated with the indicated microsomes (containing 0.1–1 nmole of cytochrome P450) for 10 min at 23° in a final volume of 0.25 ml in calcium- and magnesium-free phosphate-buffered saline at pH 7.4 (25). Then the tubes were placed on ice, and substrate, together with other required components, was added to each tube. The amounts of the various components and final incubation volume used in each assay are listed in the appropriate figure legends.

The assays for the hydroxylation of benzo[*a*]pyrene (26) and testosterone (27) were performed as described. The dealkylation of 7-ethoxycoumarin was assayed by the method of Ullrich and Weber (28) as modified by Jacobson *et al*. (29). Benzphetamine *N*-demethylation and ethylmorphine *N*-demethylation were assayed by following the formation of formaldehyde (30). All values reported in this study represent the means of duplicate or triplicate determinations for each experiment. All experiments were repeated with selected concentrations of both antibodies to confirm that the observed differential inhibition patterns were reproducible.

Materials. Benzo[*a*]pyrene, NADPH, 3-methylcholanthrene, and crystalline bovine serum albumin were purchased from Sigma Chemical Company; sodium phenobarbital, from Merck and Company; 7-ethoxycoumarin and 7-hydroxycoumarin, from Aldrich Chemical Company; ethylmorphine, from Mallinckrodt Chemical Works; and [4-^{14}C]testosterone (5.75 mCi/mmole), from New England Nuclear Corporation. PCN was obtained from G. D. Searle and Company, and *d*-benzphetamine hydrochloride, from Dr. F. F. Sun of the Upjohn Company. All other chemicals were of the highest purity commercially available.

RESULTS

Figures 1–7 show the effects of anti-PB-P450 and anti-MC-P448 on metabolism by microsomes from control (untreated) rats or rats treated with PB, MC, or PCN. The amount of antibody is expressed as milligrams per nanomole of cytochrome P450 (P448), to normalize for differences in the microsomal content of cytochrome P450. Each figure is divided into two parts. The upper part (A) gives the results with anti-PB-P450, whereas the lower part (B) gives the results with anti-MC-P448. IgG from the serum of nonimmunized rabbits had no effect on the metabolism of benzo[*a*]pyrene and 7-ethoxycoumarin. However, some control IgG preparations, when tested at 20 mg of IgG per nanomole of hemeprotein, showed significant inhibition of the metabolism of ethylmorphine, benzphetamine, and testosterone. Heating these control IgG preparations at 56° for 60 min abolished all but a small percentage of this inhibition. Heating of either experimental antibody preparation did not alter its inhibitory capacity. Thus heated control IgG and heated experimental IgG were used for these studies. The turnover numbers for each substrate with each of the four microsomal preparations are given in Table 1.

Metabolism of ethoxycoumarin and benzo[a]pyrene. Anti-PB-P450 inhibited ethoxycoumarin deethylation in microsomes from PB-treated rats but not in microsomes from MC-treated rats (Fig. 1A). The patterns of inhibition of ethoxycoumarin metabolism by anti-PB-P450 were similar in microsomes from untreated rats and rats treated with PB or PCN, although microsomes from control rats could be inhibited by a maximum of only 50%. Anti-MC-P448 inhibited ethoxycoumarin deethylation most efficiently in MC microsomes (Fig. 1B). Anti-MC-P448 inhibited PCN and PB microsomal metabolism of ethoxycoumarin much less than anti-PB-P450, while metabolism in control microsomes was inhibited to a similar extent by anti-PB-P450 and anti-MC-P448. The rank order of inhibition of the microsomal preparations obtained with one antibody was the reverse of that obtained with the other antibody.

The inhibition of benzo[*a*]pyrene hydroxylation (Fig. 2) by either antibody was similar to that of ethoxycoumarin deethylation. However, there was generally less inhibition of benzo[*a*]pyrene hydroxylation with either antibody (particularly at

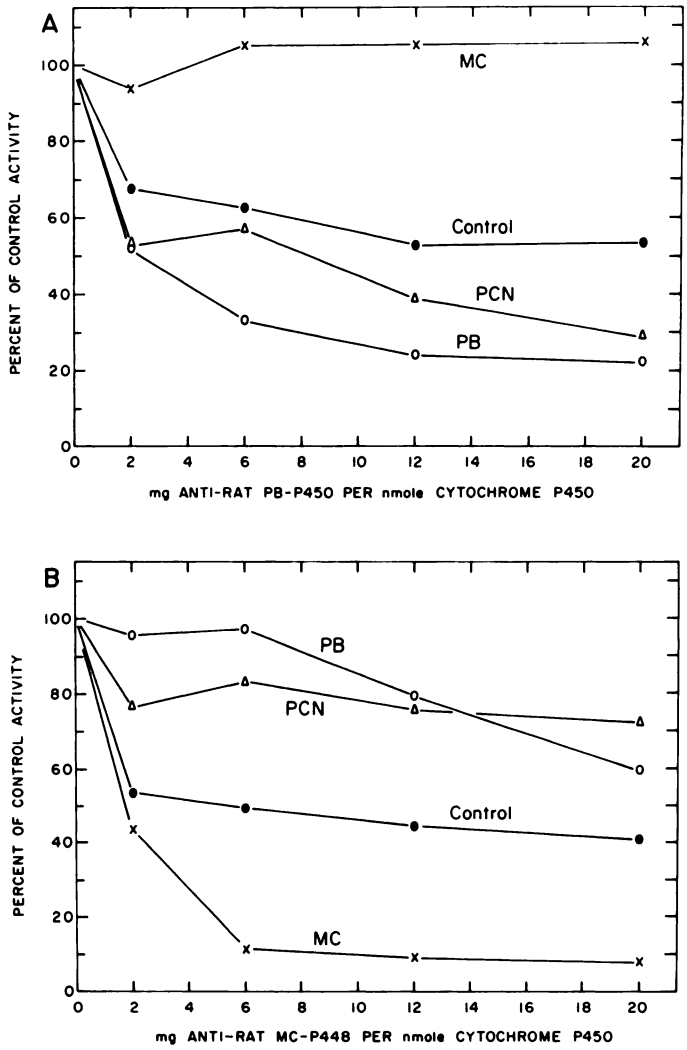

FIG. 1. *Effect of varying concentrations of anti-PB-P450 (A) and anti-MC-P448 (B) on ethoxycoumarin O-deethylation catalyzed by liver microsomes from rats treated with inducers*

The incubation mixture contained the following components in a final volume of 1 ml: the appropriate amount of microsomes containing 0.1 nmole of cytochrome P450, 0.5 mM NADPH, 0.3 mM ethoxycoumarin, 3 mM $MgCl_2$, and 50 mM potassium phosphate, pH 7.4. Tubes containing PB, PCN, or control microsomes were incubated at 37° with shaking for 10 min, while those containing MC microsomes were incubated identically, but for 5 min.

low ratios of antibody to cytochrome P450) than of ethoxycoumarin deethylation, and this difference was most evident with PCN microsomes. The inhibition of metabolism of ethoxycoumarin and benzo[*a*]pyrene indicates that membrane-bound cytochrome P450 is accessible to these antibodies. Anti-MC-P448 markedly inhibited metabolism of these substrates catalyzed by MC microsomes. The lack of inhibition of MC microsomes by anti-PB-P450 is due to the high specificity of this antibody and not to inaccessibility of cytochrome P448 in MC microsomes.

Metabolism of benzphetamine and ethylmorphine. Figure 3A shows that high levels of anti-PB-P450 (12–20 mg/nmole of cytochrome) are capable of inhibiting benzphetamine *N*-demethylation by 45–80%, depending on the microsomal prepa-

ration used to support the reaction. However, at low ratios of antibody to hemeprotein, there was more apparent antibody specificity. At 2 mg of anti-PB-P450 per nanomole of cytochrome, 55% inhibition of benzphetamine *N*-demethylation was observed with PB microsomes, compared with 30% inhibition of enzymatic activity with control and MC microsomes and no inhibition of PCN microsomes. Anti-MC-P448 was generally less inhibitory toward the metabolism of benzphetamine than was anti-PB-P450 using microsomes from control, PB- or PCN-treated animals (Fig. 3B). Microsomes from MC-treated animals were inhibited to the same extent (40–50%) with either antibody preparation.

As with the metabolism of benzphetamine, high concentrations of anti-PB-P450 markedly inhibited the *N*-demethylation

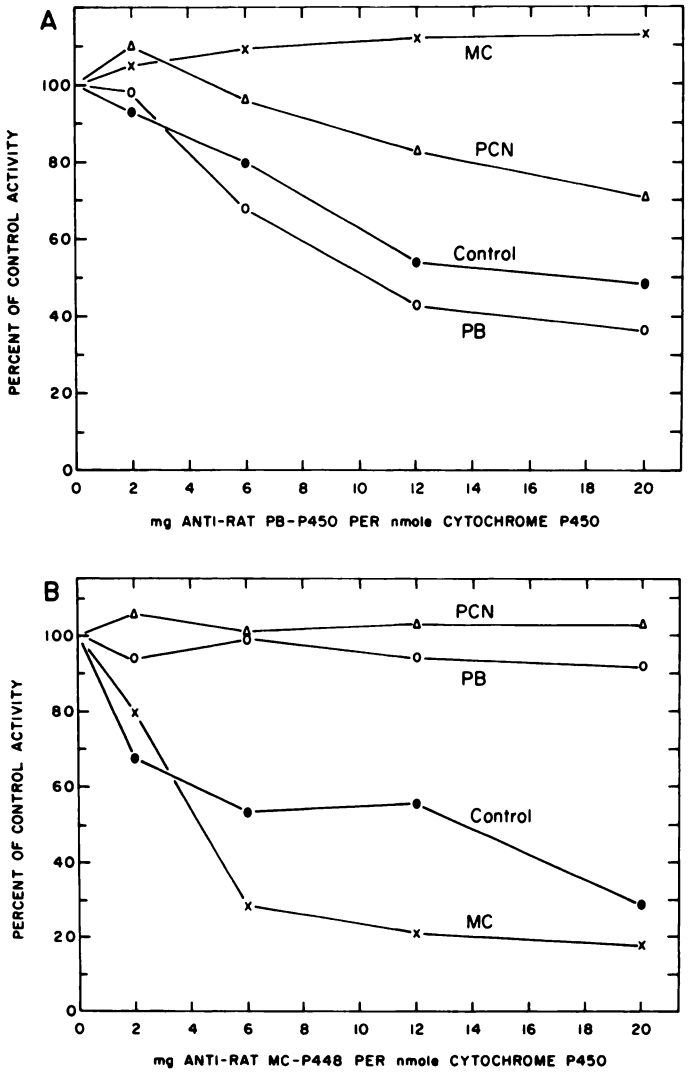

FIG. 2. *Effect of varying concentrations of anti-PB-P450 (A) and anti-MC-P448 (B) on benzol[a]pyrene hydroxylation catalyzed by liver microsomes from rats treated with inducers*

The incubation mixture contained the following components in a final volume of 1 ml: the appropriate amount of microsomes containing 0.1 nmole of cytochrome P450, 0.5 mM NADPH, 1.5 mM $MgCl_2$, 0.1 mM benzo[a]pyrene, and 100 mM potassium phosphate, pH 7.4. Assays were initiated by the addition of substrate, followed by incubation with shaking at 37° for 5 min.

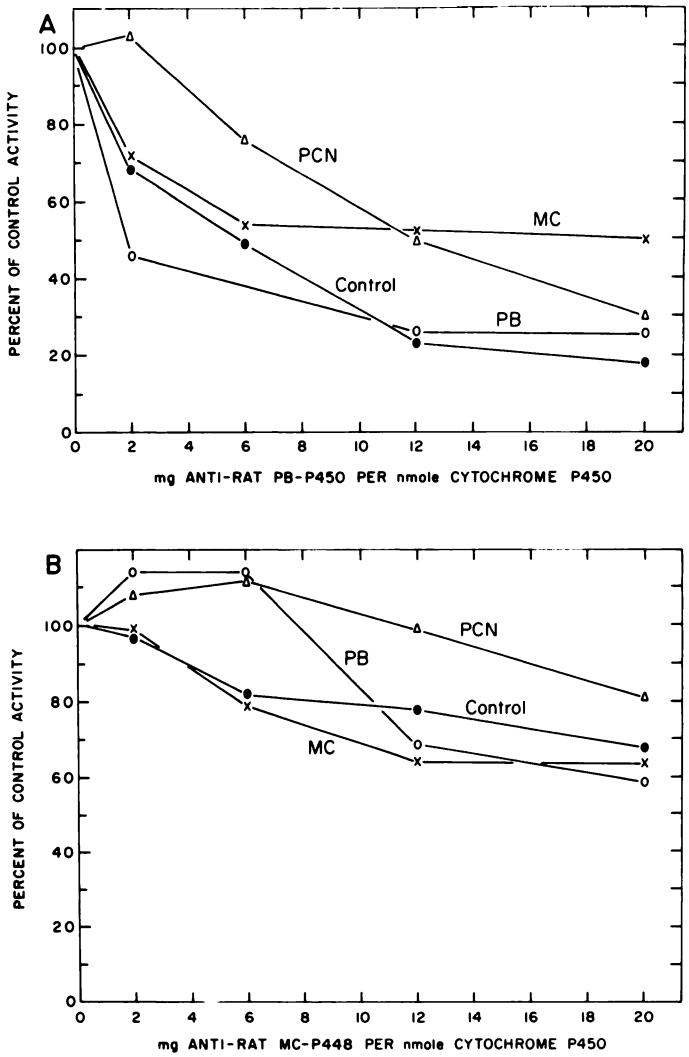

FIG. 3. *Effect of varying concentrations of anti-PB-P450 (A) and anti-MC-P448 (B) on benzphetamine N-demethylation catalyzed by liver microsomes from rats treated with inducers*

The incubation mixture contained the following components in a final volume of 1.5 ml: the appropriate amount of microsomes containing 1 nmole of cytochrome P450, 1.0 mM NADP, 5 mM glucose 6-phosphate, 0.7 unit of glucose 6-phosphate dehydrogenase, 1 mM EDTA, 5 mM semicarbazide, 1 mM benzphetamine, and 100 mM potassium phosphate, pH 7.4. Assay tubes were incubated with shaking at 37° for 15 min, except for PB microsomes, which were incubated for 10 min.

of ethylmorphine catalyzed by the four microsomal preparations (Fig. 4A). However, the effects of 2 mg of anti-PB-P450 per nanomole of hemeprotein on ethylmorphine metabolism varied considerably, depending on the source of the microsomes. Anti-PB-P450 inhibitied the *N*-demethylation of ethylmorphine supported by MC microsomes by 80%, compared with the 40% inhibition observed with PB and control microsomes. Ethylmorphine metabolism was not inhibited at this concentration of antibody when catalyzed by PCN microsomes. Figure 4B shows that anti-MC-P448 had very little effect on metabolism of ethylmorphine supported by PCN or PB microsomes but markedly inhibited the reaction supported by control or MC

microsomes. Anti-PB-P450 and anti-MC-P448 differentially affected the metabolism of ethoxycoumarin and benzo[*a*]pyrene, depending on the source of the microsomes. On the other hand, these antibodies had comparatively similar effects on the metabolism of benzphetamine when the different sources of microsomes were compared. When the two antibodies were compared for their effectiveness as inhibitors of the metabolism of both benzphetamine and ethylmorphine, anti-PB-P450 was the most effective inhibitor of both reactions. These results clearly demonstrate that the inhibition of catalytic activity is dependent not only on the antibody used and the source of the liver microsomes but also on the substrate.

Metabolism of testosterone. Figures 5, 6, and 7 show the effects of anti-PB-P450 and anti-MC-P448 on testosterone hydroxylation in the 6β-, 7α-, and 16α-positions, respectively. The 6β- and 16α-hydroxylation of testosterone supported by control,

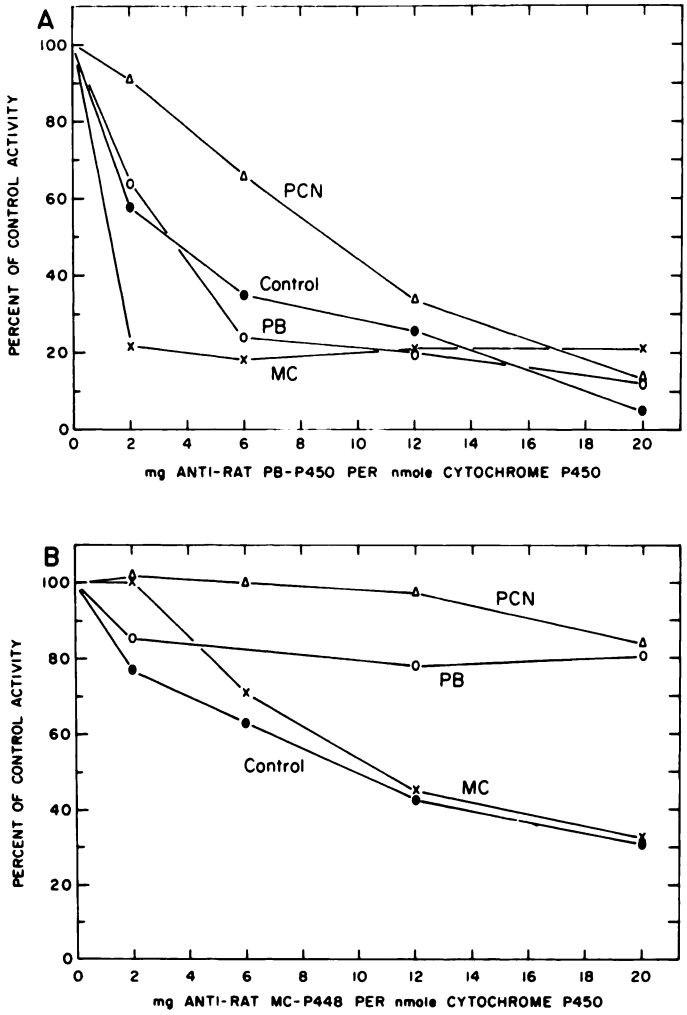

FIG. 4. *Effect of varying concentrations of anti-PB-P450 (A) and anti-MC-P448 (B) on ethylmorphine N-deethylation catalyzed by liver microsomes from rats treated with inducers*

The incubation mixture contained the following components in a final colume of 1.5 ml: the appropriate amount of microsomes containing 1 nmole of cytochrome P450, 1.0 mM NADP, 5 mM glucose 6-phosphate, 0.7 unit of glucose 6-phosphate dehydrogenase, 1 mM EDTA, 5 mM semicarbazide, 10 mM ethylmorphine, and 100 mM potassium phosphate, pH 7.4. Assay tubes were incubated with shaking at 37° for 10 min.

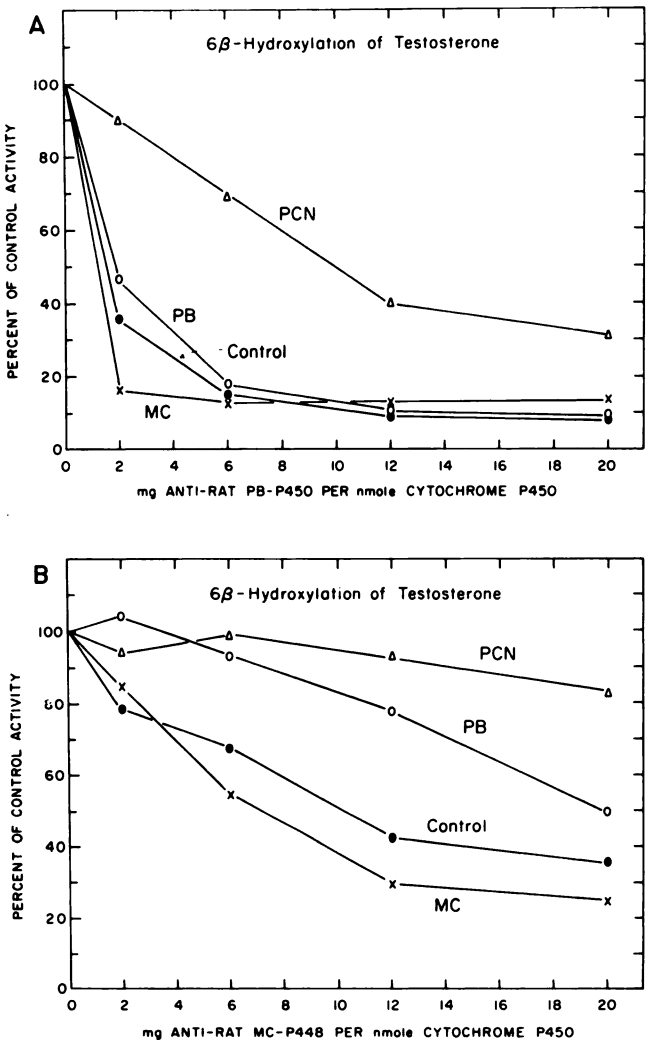

FIG. 5. *Effect of varying concentrations of anti-PB-P450 (A) and anti-MC-P448 (B) on testosterone 6β-hydroxylation catalyzed by liver microsomes from rats treated with inducers*

The incubation mixture contained the following components in a final volume of 1 ml: the appropriate amount of microsomes containing 1 nmol of cytochrome P450, 1.5 mM NADP, 3 mM $MgCl_2$, 5 mM glucose 6-phosphate, 0.7 unit of glucose 6-phosphate dehydrogenase, 400 μM [^{14}C]testosterone, and 100 mM potassium phosphate, pH 7.4. Assays were initiated by the addition of testosterone, followed by incubation with shaking at 25° for 15 min.

PB, and MC microsomes was inhibited strongly by anti-PB-P450 and more weakly by anti-MC-P448. Metabolism of testosterone by PCN microsomes was least inhibited by either antibody, compared with metabolism supported by control, PB, or MC microsomes. For example, there was no effect of either antibody on the hydroxylation of testosterone in the 16α-position catalyzed by PCN microsomes. As shown in Table 1, MC microsomes were very inefficient in catalyzing the hydroxylation of testosterone in the 16α-position. Therefore the effects of the antibodies on the hydroxylation of testosterone in this position catalyzed by MC microsomes could not be determined. It is clear from these results with antibody inhibition of testosterone metabolism that the position of metabolic attack is as important in determining sen-

sitivity to antibody inhibition as was the substrate in the preceding results.

DISCUSSION

Accessibility of microsomal cytochrome P450 to antibody. Cytochrome b_5, NADH-cytochrome b_5 reductase, and NADPH-cytochrome *c* reductase have been shown to be exposed to the cytosol, based on protease digestion and antibody inhibition (8–12, 31–33). These two approaches to locate enzymes in the transverse architecture of the microsomal membrane have been based on the inability of proteases or antibodies to penetrate the hydrophobic milieu of the membrane (10). When these two approaches were tried with liver microsomes, Ito and Sato were unable to solubilize significant quantities of cytochrome P450 or cytochrome P420 by protease treatment (8). Welton *et al.* (17), using an antibody to partially purified protease-treated cytochrome P450, were unable to inhibit the microsomal metabolism of benzphetamine. These results could be interpreted to indicate that cytochrome P450 is fully or

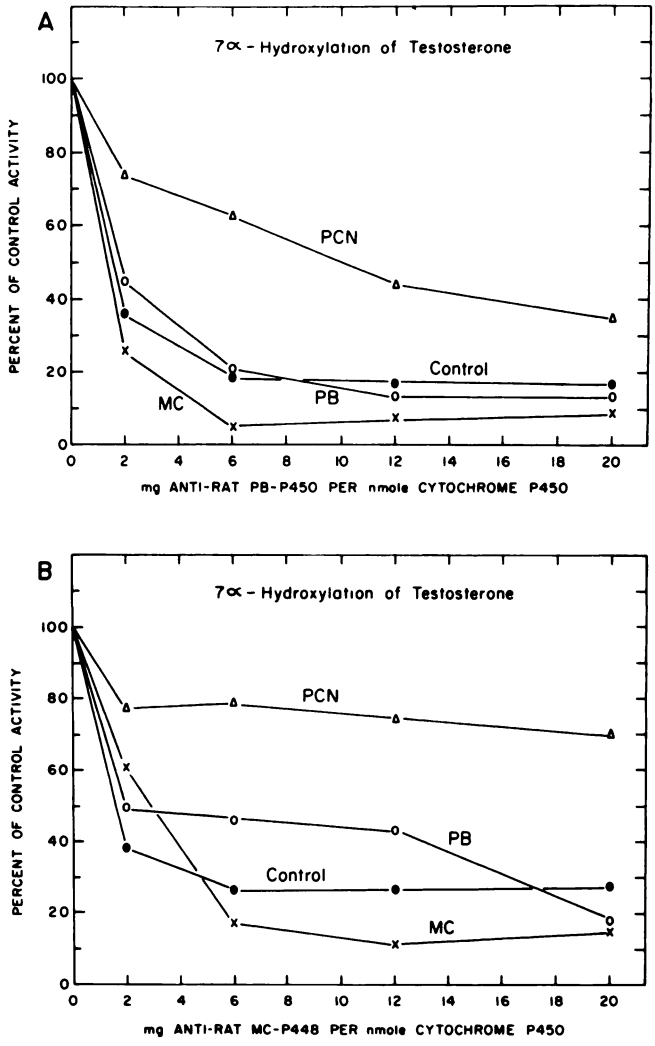

FIG. 6. *Effect of varying concentrations of anti-PB-P450 (A) and anti-MC-P448 (B) on testosterone 7α-hydroxylation catalyzed by liver microsomes from rats treated with inducers*

Metabolism to this product was determined in the same incubation mixture described in Fig. 5.

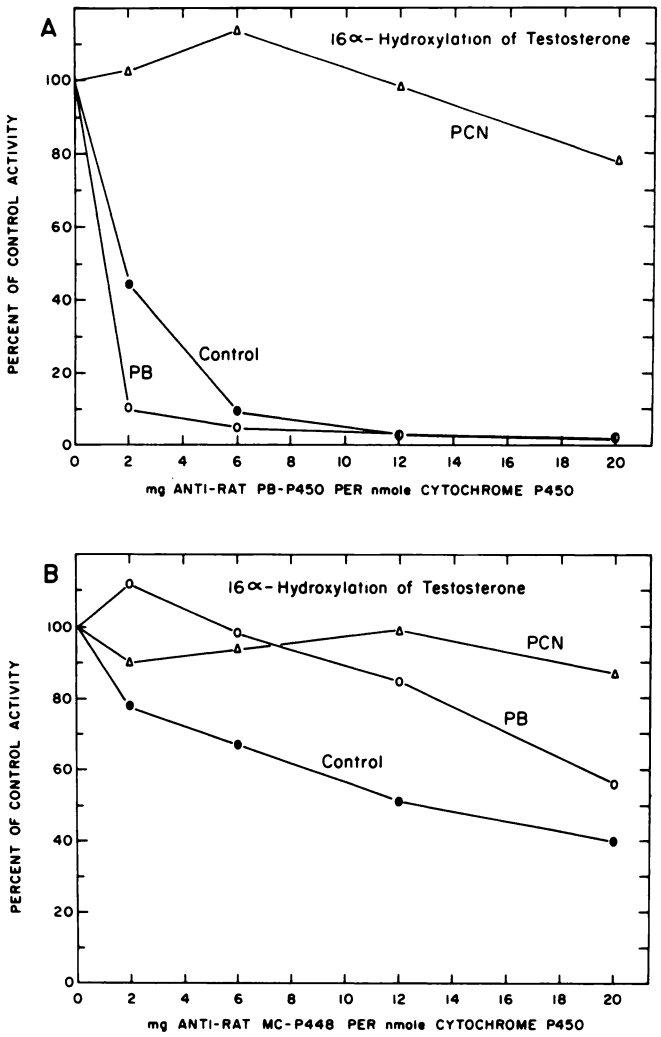

FIG. 7. *Effect of varying concentrations of anti-PB-P450 (A) and anti-MC-P448 (B) on testosterone 16α-hydroxylation catalyzed by liver microsomes from rats treated with inducers*

Metabolism to this product was determined in the same incubation mixture described in Fig. 5.

nearly fully embedded in the hydrophobic environment of the microsomal membrane. An alternative interpretation of these results is that the cytochrome P450 is exposed to the hydrophilic environment but the antibody produced against the protease-treated antigen may not recognize inhibitory antigenic sites of the native, membrane-bound cytochrome. Thus negative results do not clarify the location of cytochrome P450 in the microsomal membrane. On the other hand, positive results suggest that at least a portion of the cytochrome P450 is exposed on the cytoplasmic side of the membrane. For example, Welton and Aust (34) labeled microsomal proteins with ^{125}I, using lactoperoxidase, and showed that this label is incorporated into microsomal proteins which migrate in the 50,000 mol wt region on sodium dodecyl sulfate gels. Welton *et al.* (17) showed that antibody produced against protease-treated cytochrome P450 interacts with microsomes when assayed by a sensitive complement fixation assay, although no inhibition of catalytic activity was observed.

The inhibition of microsomal drug me-

tabolism by anti-PB-P450 and anti-MC-P448 indicates that a significant portion of the cytochrome P450 is indeed exposed to the cytosol. Based on our results, the exposed portion of cytochrome P450 must contain at least one antigenic site and, in all likelihood, more than one antigenic site. It is possible to estimate the size of an antigenic site from the three-dimensional structure at the binding region of IgG. Assuming that the antigenic site is a plane, it can be estimated that the minimum area covered by the binding region of the IgG would be a circle approximately 40 angstroms in diameter (35).

Multiplicity of microsomal cytochromes P450. Our results indicate that inhibition of microsomal metabolism by the antibodies depends on four parameters: the antibody, the source of the microsomes, the substrate, and the reaction product examined. The dependence of inhibition on the first two parameters results from the specificity of the antibody preparations and the differences in the cytochrome P450 composition of microsomes from PB- and MC-treated rats. We have eliminated the possibility that the inhibition of microsomal metabolism is due to inhibition of NADPH–cytochrome *c* reductase activity. Membrane-bound or highly purified NADPH–cytochrome *c* reductase is not inhibited by either antibody preparation, nor do these antibody preparations react with NADPH–cytochrome *c* reductase as measured by precipitin band formation on Ouchterlony plates. If microsomes from PB- or MC-treated rats contain only one form of cytochrome P450, the pattern of inhibition of metabolism by a specific antibody should not depend on the substrate. However, our results show that the degree of inhibition does depend on the substrate. The dependence of inhibition on the substrate and reaction product would be expected if multiple forms of cytochrome P450 are present in these microsomal preparations.

Recently evidence has accumulated which indicates that there are multiple forms of cytochrome P450 in rat liver microsomes (17–19, 36–38) as well as in microsomes from other laboratory animals (39–41). Our results indicate that the inhibition of the catalytic activity of control microsomes either is intermediate or parallels the inhibition observed with PB or MC microsomes. These results suggest the presence in control microsomes of forms of cytochrome P450 antigenically similar to those present in PB and MC microsomes. Metabolism in PCN microsomes is relatively resistant to antibody inhibition, suggesting that the cytochrome(s) P450 induced by PCN treatment is antigenically unlike those forms present in PB and MC microsomes. It was shown earlier by Lu *et al.* (42) that the specificity of the induction effect of PCN on the metabolism of several substrates is different from the specificity of either PB or MC. These results suggest that PCN induces a different proportion of the various forms of cytochrome P450 than PB or MC. Haugen *et al.* (40) reached a similar conclusion, based on the electrophoretic mobility of microsomal proteins. However, their conclusion was based only on protein staining and did not distinguish cytochrome P450 from other microsomal proteins.

TABLE 1

Turnover numbers for liver microsomes from rats treated with different inducers

Turnover numbers were determined at 37° except for testosterone hydroxylation, which was assayed at 25°. The specific contents of cytochrome P450 (P448) in the microsomal preparations from control, PB-, MC-, and PCN-treated rats were 1.2, 3.1, 2.4, and 1.8 nmoles/mg of protein, respectively. Values represent the means of determinations performed in triplicate.

Substrate	Control	PB	MC	PCN
	nmoles product/nmole cytochrome P450/min			
7-Ethoxycoumarin *O*-deethylation	2.8	2.5	14	2.5
Benzo[*a*]pyrene hydroxylation	0.20	0.30	1.6	1.3
Benzphetamine *N*-demethylation	2.7	10	1.6	5.4
Ethylmorphine *N*-demethylation	7.9	12	4.1	21
Testosterone				
6β-hydroxylation	0.49	0.64	0.33	1.9
7α-hydroxylation	0.20	0.18	0.24	0.32
16α-hydroxylation	0.02	0.25	0.01	0.07

Antibody specificity studies in micro-

somes compared with reconstituted system. As reported previously, anti-PB-P450 and anti-MC-P448 inhibited the metabolism of a variety of substrates catalyzed by the purified and reconstituted system (18, 19). In general, the specificity of each antibody is similar in microsomes and the reconstituted system toward the metabolism of ethoxycoumarin, benzo[*a*]pyrene, benzphetamine, and testosterone. For example, anti-MC-P448 inhibits the metabolism of ethoxycoumarin to a much greater extent when catalyzed by cytochrome P448 in the reconstituted system or MC microsomes than when catalyzed by PB-P450 in the reconstituted system or PB microsomes. However, two striking differences in antibody inhibition of metabolism by microsomes compared with the reconstituted system should be mentioned. The 6β-hydroxylation of testosterone by the cytochrome P448-containing reconstituted system was inhibited only slightly (20%) by anti-PB-P450, while more than 80% inhibition of this reaction was observed in MC microsomes with this antibody. Since most of the 6β-hydroxylase activity is lost on purification of the hemeproteins, this difference in inhibition could be due to the loss of the major form of cytochrome responsible for the 6β-hydroxylation of testosterone in MC microsomes. In the case of benzo[*a*]pyrene hydroxylation, anti-MC-P448 causes pronounced inhibition (80%) of the cytochrome P450-containing reconstituted system but does not inhibit PB microsomes. One possible explanation for this difference is that most of the benzo[*a*]pyrene hydroxylase activity in the cytochrome P450 reconstituted system is due to a quantitatively minor form of cytochrome P450 which is responsible for most of the benzo[*a*]pyrene hydroxylase activity in the reconstituted system. Alternatively, if the inhibitory antigenic site of a particular form of cytochrome P450 is not exposed to the cytosol, inhibition of catalytic activity would not be expected compared to the reconstituted system.

The extent of inhibition of microsomal metabolism was generally less than was seen in the reconstituted system. The diminished inhibition of microsomes compared with the reconstituted system was not the result of less inhibitory antibody preparations. Some of the antigenic sites of cytochrome P450 in microsomes may not be accessible to the antibodies. Any forms of cytochrome P450 present in microsomes but not present in the purified cytochrome P450 preparations would not be inhibited unless antigenic sites of those forms lost during the purification were the same as those present in the purified cytochrome preparations. At least one form of cytochrome P450 from MC- and PB-treated rats is lost during the purification procedure (36), and thus this form would not be inhibited by anti-PB-P450 or anti-MC-P448 in microsomes.

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