Inhibition of Cholesterol Side Chain Cleavage by Active Site Directed Antibody to Corpus Luteum Cytochrome P-450*

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The abbreviations used are: P-450c, cytochrome P-450c; P-450₁₆, cytochrome P-450₁₆; CSCC, cholesterol side chain cleavage; BAM, bovine adrenal mitochondria; BCLM, bovine corpus luteum mitochondria; HPM, human placental mitochondria; ISP, iron sulfur protein reductase to HPM P-450c in.

In this study, antibody to purified P-450c from bovine corpus luteum mitochondria (1) was produced in a goat and was used for immunochemical and functional comparison of the P-450c,s from mitochondria of bovine adrenal cortex, bovine corpus luteum, and human placenta. Bovine corpus luteum mitochondrion P-450c was selected because the mitochondria and microsomes of the bovine corpus luteum are thought to have only one extrahepatic catabolizing enzyme activity and partially inhibited CSCC. In this study, antibody to purified P-450c from bovine corpus luteum mitochondria (1) was produced in a goat and was used for immunochemical and functional comparison of the P-450c,s from mitochondria of bovine adrenal cortex, bovine corpus luteum, and human placenta. Bovine corpus luteum mitochondrion P-450c was selected because the mitochondria and microsomes of the bovine corpus luteum are thought to have only one steroid hydroxylating enzyme activity and avoiding contamination from other P-450s. In addition, the role of the concentration of ISP in the analysis of the antibody inhibition of the CSCC system is demonstrated. The nature of the antigenic sites is considered.

EXPERIMENTAL PROCEDURES

Materials—[26-¹⁴C]Cholesterol (54 mCi/mmole), and [¹H]H₂O (100 µCi/mmole) were purchased from New England Nuclear. Deoxycoformycin (4-pregnen-21-ol-3,20-dione), NADPH, and glucose 6-phosphate dehydrogenase were purchased from Sigma.

Preparation of Immunoglobulin G from Goat Antibody to Bovine Corpus Luteum Cytochrome P-450 Catalyzing CSCC Activity (P-450c)-Cytochrome P-450c was purified to homogeneity from bovine corpus luteum mitochondria as described previously (1). The specific content of the purified P-450 was 16.3 nmol/mg of protein.

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The purified cytochrome P-450sc (100 μg/ml in borate/saline buffer composed of 0.01 M borate and 0.154 M NaCl at pH 8), was mixed with an equal volume of Freund's complete adjuvant and was injected subcutaneously into a mature male goat. The animal was reimmunized intravenously 3 weeks later with an additional 100 μg of cytochrome P-450. Blood was collected from the jugular vein prior to immunization and 10 days after the second immunization. The serum was stored in aliquots of about 60 ml at -20°C. Globulin "G" fractions were prepared from both the preimmune and immune sera by the methods of Kekwick (6) and Levy and Sober (7). The IgG fraction was concentrated by ultrafiltration (Amicon, PM 30) and dialyzed against 0.01 M borate buffer, pH 8.0.

Preparation of Mitochondria and Cytochrome P-450 Fractions—Mitochondria were prepared from bovine corpora lutea, bovine adrenal cortices, and human placentas as described previously (8, 9). For studies with unsonicated mitochondria, the washed mitochondria were suspended in 0.25 M sucrose and 0.1 mM EDTA solution. To prepare sonicated mitochondria, the mitochondrial pellet was suspended in Buffer A containing 0.02 M potassium phosphate buffer, pH 7.4, 20% glycerol (v/v), 0.1 mM EDTA, and 0.1 mM dithioerythritol at a protein concentration of about 20 mg/ml and 56 ml aliquots were sonicated in an ice bath at 20 kC, 85 watts intermittently for a total of 5 min with a Model W 185D Sonifier Cell Disruptor (Heat Systems-Ultrasonics, Inc., Plainview, NY) (1). To prepare solubilized mitochondria, the suspension of sonicated mitochondria was adjusted to a protein concentration of about 25 mg/ml and 10% sodium cholate was added to bring the final concentration to 0.98%. For immunodiffusion tests, the cholate was removed by dialysis against Buffer A. Addition of ammonium sulfate to 35% saturation precipitated most of the P-450mc, leaving essentially P-450sc in the supernatant fraction. To prepare the "solubilized fraction," the supernatant fraction was dialyzed against Buffer A to remove the cholate and ammonium sulfate. The precipitate was dissolved in Buffer A containing 0.5% sodium cholate and was reprecipitated with ammonium sulfate at 35% saturation to prepare the P-450sc fraction. The P-450mc fraction was recovered from the supernatant fraction by precipitation with 60% saturated ammonium sulfate (P-450mc preparation).

Determination of CSCC Activity—Enzymatic activity was determined by the method of Hochberg et al. (10) as modified for whole mitochondria by Uzgiris et al. (11). Each incubation vessel contained 0.05 ml of 0.1 M potassium phosphate buffer, pH 7.4, 5 mM CaCl2, 10 mM MgCl2, 0.5 mM dithioerythritol, 1.1 mM MgCl2, 11 mM CaCl2, 1 mM NADPH in a total volume of 1.5 ml of Buffer A. Incubations were carried out at 37°C for 60 min. For studies of inhibition with IgG fractions, specified amounts of IgG fraction or which contained IgG from preimmune serum. The reaction was initiated by the addition of 0.2 ml of 2 nmol deoxycorticosterone in 0.1 M HCl. The reaction was monitored by the rate of conversion of deoxycorticosterone to corticosterone at 37°C over a period of 1 h. One nmol of P-450 in Buffer A was added to the control incubation mixture. Complete assay mixtures with added IgG fractions were preincubated for 10 min at 37°C prior to initiation of the reaction by the addition of 1 mM NADPH. The biosynthesis of [14C]Isocaproate was separated by filtration through an alumina column (G type F Merck: Woelm neutral (TCN); 2:1 (w/w)). [10]H2O was used to correct for technical error. When the radioactivity recovered as isocaproate was plotted as a function of time. CSCC activity increased linearly for 30 min (Fig. 1). Reaction rates presented in Table I were determined from the linear portion of this curve. About one-third of the cholesterol added into the flask was cleaved to isocaproate by the adrenal mitochondria within 60 min. In certain experiments, iron sulfur protein and ISP reductase isolated from bovine corpora lutea were added to the placental mitochondria. These preparations were shown to be devoid of P-450sc, by spectroscopy and devoid of enzymatic activity. Inhibition of CSCC activity by the IgG was determined by comparison with the control flask after incubation for 60 min. Inhibitory effect is expressed as per cent inhibition as described above. Unsonicated bovine adrenocortical mitochondria (△-△) were incubated under identical conditions. Preimmune IgG was added to the control incubation mixture with bovine corpus luteum mitochondria (○-○).

The [14C]Isocaproate was terminated with 0.2 ml of 1 mM HgCl2.

The P-450mc was determined by comparison with the control incubation flask after incubation for 60 min. Inhibitory effect is expressed as per cent conversion relative to that of the control which did not contain the IgG fraction or which contained IgG from preimmune serum.

Determination of 11β-Hydroxylation Activity—The 11β-hydroxylation catalyzed by bovine adrenocortical mitochondria was determined by measuring the rate of conversion of deoxycorticosterone to corticosterone at 37°C over a period of 1 h. One nmol of P-450 in Buffer A was added to the control incubation mixture. Complete assay mixtures with added IgG fractions were preincubated for 10 min at 37°C prior to initiation of the reaction by the addition of 1 mM NADPH. The biosynthesis of [14C]Isocaproate was separated by filtration through an alumina column (G type F Merck: Woelm neutral (TCN); 2:1 (w/w)). [10]H2O was used to correct for technical error. When the radioactivity recovered as isocaproate was plotted as a function of time. CSCC activity increased linearly for 30 min (Fig. 1). Reaction rates presented in Table I were determined from the linear portion of this curve. About one-third of the cholesterol added into the flask was cleaved to isocaproate by the adrenal mitochondria within 60 min. In certain experiments, iron sulfur protein and ISP reductase isolated from bovine corpora lutea were added to the placental mitochondria. These preparations were shown to be devoid of P-450sc, by spectroscopy and devoid of enzymatic activity. Inhibition of CSCC activity by the IgG was determined by comparison with the control flask after incubation for 60 min. Inhibitory effect is expressed as per cent conversion relative to that of the control which did not contain the IgG fraction or which contained IgG from preimmune serum.

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BCLM and BAM with the anti-BCL-P-450, demonstrated single clean precipitin lines without spurs. No precipitin line respective P-450m, was tested. 450, were tested against 750 pg of anti-BCLM-P-450, IgG in the

tions of BCLM P-450, BAM P-450, BAM P-450lp, and HPM P-cytochrome P-450, by spectrophotometric analysis and assay for

of the solubilized preparations of cytochrome P-450, from bovine corpus luteum mitochondria (A—A), bovine corpus luteum (●—●), and human placenta (■—■) were incubated with various amounts of anti-P-450c, as described under "Experimental Procedures." Preimmune IgG was added to the control incubation mixture with solubilized bovine corpus luteum mitochondria (×—×).

TABLE II

<table>
<thead>
<tr>
<th>IgG added</th>
<th>Corticosterone formed by sonicated bovine adrenal cortical mitochondria</th>
<th>Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg protein</td>
<td>nmol/min/nmol P-450</td>
<td>%</td>
</tr>
<tr>
<td>0</td>
<td>25.7 ± 2.2</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>27.5 ± 2.3</td>
<td>107</td>
</tr>
<tr>
<td>10</td>
<td>54.0 ± 6.2</td>
<td>210</td>
</tr>
<tr>
<td>20</td>
<td>103.5 ± 13.5</td>
<td>402</td>
</tr>
</tbody>
</table>

Double Diffusion—Ouchterlony double diffusion tests were performed in Petri dishes containing 1.0% agarose in 0.15M NaCl, 10 mm potassium phosphate, pH 7.4, and 0.02% sodium azide or on commercial Ouchterlony dishes obtained from Cappell Laboratories, Inc. Cochranville, PA. All samples were developed at 4°C. Preparations of BCLM P-450, BAM P-450, BAM P-450lp, and HPM P-450, were tested against 750 µg of anti-BCLM-P-450c, IgG in the center well. The test wells contained 200 µg of the preparations which had specific contents of 0.6, 1.8, 1.8, and 0.4 nmol/mg of protein, respectively. In other experiments, increased concentrations of the HPM P-450c, were tested as well as preparations of cholate extracts of HPM and HPM P-450c, fractionated with ammonium sulfate. Certain of the HPM fractions were augmented with BCLM-P-450c to eliminate the possibility of interference from the HPM proteins. Finally, a preparation of BCLM-P-450c prepared by heating the respective P-450c, was tested.

Preparation of ISP Reductase and ISP—Both ISP and ISP reductase were partially purified from bovine corpus luteum mitochondria as previously described (1); both preparations were free from cytochrome P-450, by spectrophotometric analysis and assay for CSCC activity. The concentrations of ISP and ISP reductase were determined spectrophotometrically (14, 15). The absorbance ratio of ISP at 414 nm and 280 nm was approximately 0.3. Units of ISP reductase activity are reported in nanomoles of 2,6-dichlorophenolin

of the content of P-450s other than P-450c, and the enzymatic activities of the three preparations reflect the different stoichiometric relationships of iron sulfur protein, ISP reductase and P-450. Nevertheless, both the sonicated and solubilized preparations of the enzyme from the BCLM and BAM were inhibited by the antibody in a concentration-dependent fashion (Figs. 2 and 3). HPM preparations were not inhibited under these conditions. On the other hand, comparable preparations were not inhibited by IgG obtained from preimmune serum. The half-maximum inhibitory concentration of the IgG (I50) was somewhat greater for the BCLM preparations than for those of adrenal origin (Table I). While the adrenal P-450c, appears to be more sensitive to the antibody at low concentrations, nevertheless, the inhibition curves are confluent at higher concentrations reaching 90 to 90% at 30 mg

RESULTS

Ouchterlony Double Diffusion—Double diffusion analysis of the solubilized preparations of cytochrome P-450c, from BCLM and BAM with the anti-BCL-P-450c, demonstrated single clean precipitin lines without spurs. No precipitin line was obtained between the anti-BCL-P-450c and the BAM-P-450lp or the HPM P-450 preparations at the available concentrations. Further concentration of the HPM P-450 was technically not possible, but it could be demonstrated that the HPM preparations did not interfere with the precipitin line obtained with BCL-P-450c, in view of the enzymatic inhibition demonstrable with the HPM P-450 preparations, it was concluded that the concentration of P-450c was too low or that there are antigenic differences in the P-450c, from the human and bovine sources. Of interest, the BCLM-P-420 did not cause a precipitin line.

Inhibition of CSCC in Intact and Sonicated Mitochondria and in Solubilized P-450, by Anti-P-450c—In the presence of 30 mg of IgG/nmol BAM-P-450, catalytic activity was inhibited about 55% in unsonicated mitochondria, 80% in sonicated mitochondria, and about 90% in a comparable solubilized P-450, preparation (Figs. 2 and 3). These data add to the evidence that P-450, is located on the inner mitochondria membrane and is, therefore, less accessible to the antibody (18, 19).

Anti-P-450c, Inhibition of P-450, from Various Organs and Species—The catalytic activities of sonicated mitochondria and solubilized P-450, from BCLM, BAM, and HPM were compared at various concentrations of antibody (Table I, Figs. 2 and 3). Preimmune serum IgG was used for control studies.

From Table I, it is evident that the native preparations from the three sources are not comparable in specific CSCC activity. The adrenal concentration of P-450 is high because of the content of P-450s other than P-450c, and the enzymatic activities of the three preparations reflect the different stoichiometric relationships of iron sulfur protein, ISP reductase and P-450. Nevertheless, both the sonicated and solubilized preparations of the enzyme from the BCLM and BAM were inhibited by the antibody in a concentration-dependent fashion (Figs. 2 and 3). HPM preparations were not inhibited under these conditions. On the other hand, comparable preparations were not inhibited by IgG obtained from preimmune serum. The half-maximum inhibitory concentration of the IgG (I50) was somewhat greater for the BCLM preparations than for those of adrenal origin (Table I). While the adrenal P-450c, appears to be more sensitive to the antibody at low concentrations, nevertheless, the inhibition curves are confluent at higher concentrations reaching 90 to 90% at 30 mg

FIG. 4. Effect of ISP on CSCC by sonicated human placental mitochondria. Each vessel contained 5 DCPIP units of ISP reductase, 0.25 nmol of human placental mitochondrial P-450, and 0, 5, 10, 15, or 30 nmol of ISP. Other incubation conditions are described under "Experimental Procedures."
(Figs. 2 and 3). Thus, the CSCC enzymes from both endocrine organs appear to be immunochemically similar.

**Effect of P-450scc on 11β-Hydroxylation**—The anti-P-450scc IgG was added in graduated amounts to a sonicated preparation of bovine adrenocortical mitochondria containing both the CSCC and 11β-hydroxylation systems (Table II). No effect on 11β-hydroxylation was obtained at IgG concentrations of 5 mg/nmol of P-450, but this amount was sufficient to inhibit over 50% of CSCC activity (Fig. 2). At higher concentrations, there was a linearly progressive stimulation of corticosterone formation until at the level of 20 mg of IgG, which inhibits 70 to 75% of CSCC, the increase in 11β-hydroxylation was 4-fold (Fig. 2, Table II). Although the rate of decrease in CSCC appears to be less than the rate of increase in 11β-hydroxylation, this difference can be explained by the requirement of 3 mol of NADPH and oxygen for CSCC compared to 1 mol required for 11β-hydroxylation. No significant changes were observed when IgG from preimmune serum was substituted for the anti-P-450scc IgG.

**Effect of ISP and ISP Reductase on CSCC in Human Placental Mitochondria**—The lack of inhibition of CSCC of HPM by anti-BCLM-P-450scc might indicate lack of common antigenic determinants because of either the difference in source or the differences in species. An alternative explanation is that the available amounts of electron carriers, ISP and ISP reductase, might be markedly less than the available cytochrome P-450scc. If this were the case, the system would not fully utilize the cytochrome P-450scc and the pool of unutilized P-450scc would bind to the antibody without apparent inhibition. In other words, to test the CSCC system properly, the limiting component should be the antigen, P-450scc. Alternatively, a lack of inhibition might result from the presence of an enzymatically inactive P-450scc fragment or other antigen, which would bind the antibody.

The effects of adding ISP reductase, ISP, and both at optimal concentrations were examined in sonicated placental mitochondria. First, ISP reductase (20 DCPIP units/nmol of P-450) was added to establish a control level of side chain cleavage which was 4 pmol of cholesterol/min/nmol of P-450 (Fig. 4). This is approximately the rate of CSCC obtained with native placental mitochondria (Table I). The addition of increasing amounts of ISP progressively increased the amount of cleavage to about 6-fold. While Fig. 4 presents data from one experiment, in replicate experiments with different placentals preparations, the maximum turnover rate ranged from 14 to 30 (22.7 ± 6.5, mean ± S.D.) pmol/min/nmol of P-450 after the addition of 10 nmol of ISP and 20

**FIG. 5. Effect of ISP reductase on CSCC by sonicated human placental mitochondria with optimal amounts of ISP.** Each vessel contained 10 nmol of ISP, 0.25 nmol of human placental mitochondrial cytochrome P-450scc, and 0, 2.5, 5, 10, 20, 30, or 40 DCPIP units of ISP reductase. Other incubation conditions are described under “Experimental Procedures.”

**FIG. 6. Effect of anti-P-450scc IgG on CSCC by human placental mitochondria in the presence of ISP reductase and ISP.** Each vessel contained 10 nmol of ISP, 20 DCPIP units of ISP reductase, 0.25 nmol of either sonicated or solubilized human placental mitochondrial P-450scc, and 0, 1, 2.5, 5, 7.5, 10, or 12.5 mg of anti-P-450scc IgG. The control vessels contained appropriate amounts of preimmune IgG (X-X-X). Other incubation conditions are described under “Experimental Procedures.”

**DISCUSSION**

The administration of homogeneously pure cytochrome P-450scc from bovine corpus luteum mitochondria into goats produced an antibody which effectively inhibited CSCC in BCLM and P-450 preparations and yielded a single, nonspurred line with BCLM-P-450scc by Ouchterlony double diffusion tests. This antibody was used to compare organ and species specificities of cytochrome P-450scc, to determine the accessibility of membrane-bound cytochrome P-450scc to the antibody, and to study the stoichiometric relationships of the electron transport proteins to the antigen-antibody reaction.
Finally, this preparation was used to demonstrate the interdependence of the various mitochondrial P-450-linked enzyme systems.

In both the corpus luteum and adrenal preparations, the antibody was more effective in inhibiting CSCC in solubilized preparations than in sonicated preparations, and it inhibited the sonicated and solubilized adrenal preparations more than unfragmented adrenal mitochondria (Figs. 2 and 3). Thus, the antigenic sites of the membrane-bound cytochrome are not as readily accessible to the antibody and the most likely reason is that some of those sites are located on the inner membrane of the mitochondrion.

The Ouchterlony test did not distinguish between P-450s from bovine adrenal and corpus luteum sources although the antibody appeared to inhibit CSCC of the BAM-sonicated and -solubilized preparations at somewhat lower concentrations than were necessary for inhibition of comparable BCLM preparations (Table I, Figs. 2 and 3). However, the CSCC activity is expressed in picomoles of isocaproate per nmol of P-450 and, for the BAM CSCC rate, the denominator includes P-450s as well as P-450s, because the two cytochromes are spectroscopically indistinguishable.

Adrenal preparations may contain somewhat less P-450s than those from the BCLM; therefore, less anti-P-450 would be required for inhibition. Alternatively, the ratios of ISP to P-450s are probably not optimal in the native preparations and the limiting component of the transport system may not be the cytochrome P-450s. In spite of these variables, the inhibition by higher concentration of anti-P-450s is sufficiently close that it is reasonable to conclude that the two bovine sources contain immunologically similar cytochrome P-450s.

The anti-P-450s did not react with the purified P-450s in the Ouchterlony double diffusion test and did not inhibit 11β-hydroxylation (Table II). Similar results were obtained by Watanuki et al. (20) and Sahara et al. (2) who used antibody raised in rabbits against BAM-P-450s and BAM-P-450s, and found no cross-reactivity with the alternative antigen. On the other hand, our data differ from that of those investigators because increasing the concentrations of IgG in adrenocortical preparations containing both P-450s and P-450s markedly increased 11β-hydroxylation (Table II). The reason for this difference is the type of system used. Those investigators used reconstituted systems containing excessive amounts of ISP and P-450s. Under optimal conditions, Sahara et al. (2) reported a turnover rate of 110 nmol of corticosterone/min/mmol of P-450. The turnover rate of our native adrenal sonicated mitochondria in the presence of 20 mg of IgG is 105. Thus, blocking P-450s made sufficient electron transport components available to the 11β-hydroxylation system to achieve maximal efficiency. This demonstrates that the two systems are intimately linked and has important implications for clinical conditions where one system or the other may appear to be either excessively productive or inhibited.

It may be that the apparent lack of stoichiometric equivalence of the components of the transport system in our native systems is artifactual. Adrenodoxin has been shown to undergo rapid autooxidation (21, 22). The ISP/P-450 ratio in separated adrenal and placental mitochondria exceeds or equals 1:1. This is less than optimal for reconstituted systems (23–25) but the ratios obtained in separated mitochondria may differ from those which occur in vivo. Nevertheless, confirmation of the interdependence of the P-450-linked systems in the regulation of adrenal steroidogenesis comes from other experiments involving specific inhibitors of the mitochondrial enzymes. Thus, Graves et al. (26) demonstrated that inhibitors of 11β-hydroxylation such as metyrapone stimulated CSCC, and conversely, Whipple et al. (27) found that inhibition of P-450s with aminoglutethimide resulted in stimulation of 11β-hydroxylation. While the precise stoichiometric conditions and topographical arrangements of the enzymes, electron transport proteins, and cofactors in vivo are not known, these data imply that there is close linkage of the mitochondrial P-450 systems and also that the terminal P-450 may not be the rate-limiting enzyme.

The data obtained with the human placental mitochondria are more complex. By Ouchterlony double diffusion, precipitation of the placental P-450s was not obtained with this antibody. Furthermore, the antibody did not inhibit placental CSCC (Figs. 2 and 3) until large amounts of ISP (of bovine origin) were added to the preparation. One explanation for the lack of enzymatic inhibition, therefore, is that the rate-limiting enzyme in the sequence was the ISP and not the P-450s. Our experience with placental microsomal and mitochondria P-450s is that these P-450s have considerably less stability than do preparations from any other tissue and that the autooxidizability of ISP clearly results in low levels of that enzyme. This, however, does not explain the negative Ouchterlony test. We attribute this either to the low specific content of our preparations or to the possibility that the placental P-450s is not immunologically identical with the bovine P-450s but does have some cross-reactivity.

These findings with placental proteins are not unique to cytochrome P-450. Barron tested the placental system with antibody to bovine adrenodoxin and found no enzymatic inhibition even though double diffusion produced clean precipitin lines (4). As pointed out by Watanuki et al. the inhibition of enzyme activity is a more sensitive indicator of antigen-antibody reaction than double diffusion (20). Perhaps the reason for Barron’s findings was the presence of relatively large amounts of inactive ferrodoxin which would react with the antibody to produce precipitin lines and would bind antibody rendering it unavailable to the active form of ISP.

It is of interest to consider the nature of the antigenic sites, especially in light of the data published by Dus et al. (5). It is clear that both his antibody preparations, and most likely ours also, consist of a mixture of antibodies directed both toward an active site of the enzyme and to other sites. Thus, antibodies which do not interfere with enzymatic function do not interfere with the active site either directly or by altering the conformation of the enzyme. Consequently, P-450s and liver P-450s might have one or more antigenic sites to bind to the anti-P-450s prepared by Dus et al. Although Dus et al. did not test for inhibition of 11β-hydroxylation activities, we found that 11β-hydroxylation in the presence of our antibody was actually increased.

One can speculate that there are two binding sites on each P-450 molecule: one being a site for ISP to reduce the P-450 and the other being a site for the substrate to be oxygenated. An antibody preparation might interfere with either site or both.

The ISP site for P-450s and P-450s are probably similar because the ISPs are interchangeable. Our antibody caused an increase in 11β-hydroxylation indicating that it was not interfering with the ISP site of P-450s and therefore probably not with the ISP site on P-450s. In fact, using the HPM P-450s as a model, the addition of ISP increased CSCC activity even in the presence of the antibody. Thus, it is unlikely that this IgG is inhibiting the system through a significant effect on either the ISP or the ISP site.

On the other hand, the anti-P-450 IgG inhibited CSCC activity by P-450s from diverse sources including the bovine adrenal, bovine corpus luteum, and human placenta. Whatever other differences these P-450s may have, it is likely that
the substrate active sites are similar. Since precipitin lines were not obtained for the human P-450, it appears that other regions of the human protein differ from those of the bovine P-450s. Similarly, P-420 derived from bovine sources did not yield a precipitin line. Thus, we conclude that at least one component of our antibody mixture is directed toward the substrate-associated binding site.

REFERENCES
Inhibition of cholesterol side chain cleavage by active site directed antibody to corpus luteum cytochrome P-450.
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