Mechanism of Inhibition by Carbonyl Cyanide m-Chlorophenylhydrazone and Sodium Deoxycholate of Cytochrome P-450-Catalysed Hepatic Microsomal Drug Metabolism

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1. Treatment of liver microsomal fraction with 0.03–0.12% sodium deoxycholate and 0.005–0.06 mM carbonyl cyanide m-chlorophenylhydrazone decreases phospholipid-dependent hydrophobicity of the microsomal membrane, assayed by the kinetics of 8-anilinonaphthalene-1-sulphonate binding and ethyl isocyanide difference spectra. 2. Sodium deoxycholate at a concentration of 0.01% lacks its detergent properties, but competitively inhibits aminopyrine binding and activates the initial rate of NADPH-cytochrome P-450 reductase. In the presence of 0.03–0.09% sodium deoxycholate the rate-limiting factor in p-hydroxylation of aniline is the content of cytochrome P-450, and that for N-demethylation of aminopyrine is the activity of NADPH-cytochrome P-450 reductase. 3. Carbonyl cyanide m-chlorophenylhydrazone has no effect on the binding and metabolism of aniline; investigation of its inhibiting effect on aminopyrine N-demethylase established that the rate-limiting reaction is the dissociation of the enzyme-substrate complex in the microsomal preparations. 4. In the mechanism of action of carbonyl cyanide m-chlorophenylhydrazone the key step may be the electrostatic interaction of its protonated form and one of the forms of activated oxygen at the catalytic centre of cytochrome P-450. 5. At least two different phospholipid-dependent hydrophobic zones are assumed to exist in the microsomal membrane, both coupled with cytochrome P-450. One of them reveals selective sensitivity to the protonation action of carbonyl cyanide m-chlorophenylhydrazone and contains the 'binding protein' for type I substrates and NADPH-cytochrome P-450 reductase; the other contains the cytochrome P-450 haem group and binding sites for type II substrates.

The problems of regulating the enzymic activity in multicomponent membrane-bound systems have currently become of interest because of the elucidation of not only the structural, but also the functional role for the lipid component of biomembranes. The essential requirement of phospholipid for microsomal cytochrome P-450 activity in the mono-oxygenase system is, in principle, characterized by three criteria, postulated by Fleischer et al. (1962), namely, decrease in the enzymic activity on removal of phospholipid (Omura & Sato, 1964; Chaplin & Mannering, 1970; Eling & DiAugustine, 1971; Leibman & Estabrook, 1971), re-activation of the enzyme on addition of phospholipid (Cater et al., 1972; Lu & Levin, 1974; Vore et al., 1974) and association of enzyme with phospholipids (Omura & Sato, 1964; Ichikawa & Yamano, 1967; Imai & Sato, 1967; Leibman & Estabrook, 1971).

Treatment leading to alterations of the phospholipid component of the microsomal membrane changes the binding and metabolism of type I and type II substrates (Schenkman et al., 1967) in various ways (Shoeman et al., 1969; Chaplin & Mannering, 1970; Leibman & Estabrook, 1971; Eling & DiAugustine, 1971; Tsyrlor & Lyakhovich, 1975), although it is known that phospholipids are essential for the hydroxylation of microsomal substrates of both types (Imai & Sato, 1960; Lu & Levin, 1974). On the basis of the data of Schenkman et al. (1967) and Remmer et al. (1968) that type I substrates bind in the lipoprotein moiety of cytochrome P-450 enzyme and that type II substrates affect the ligand of haem iron with the formation of ferrihaemochrome, it may be assumed that the sterically separated active centres of mono-oxygenase for type I and type II substrates are in association with different hydrophobic zones in the microsomal membrane.

We have attempted to determine the dependence of the various functional aspects of the microsomal mono-oxygenase system on its hydrophobic environment. This has been achieved with the aid of carbonyl cyanide m-chlorophenylhydrazone and...
sodium deoxycholate, agents different in chemical structure but both affecting hydrophobic interactions in artificial and natural membranes (Hemker, 1962; Omura & Sato, 1964; Victoria & Barber, 1969; Verma et al., 1973). We also intended to clarify the nature of the influence of these agents on the spectral and kinetic constants of the microsomal cytochrome P-450-containing mono-oxygenase.

These studies were presented in part at the 5th International Symposium on Drugs Affecting Lipid Metabolism, Milan, September 1974 (Tsyrlol & Lyakhovich, 1974) and at the 55th Meeting of the Biochemical Society, Edinburgh, September 1975 (Tsyrlol et al., 1975).

**Materials and Methods**

All procedures for the preparation of the microsomal fraction from male Wistar (150–180g) rat livers were as previously described (Tsyrlol & Lyakhovich, 1975; Tsyrlol et al., 1976).

Aminopyrine N-demethylase activity was determined essentially as described by Smuckler et al. (1967). The incubation medium contained microsomal fraction (5–6mg of protein), 0.1m-Na2HPO4/KH2PO4 buffer (pH7.5), 5mm-aminopyrine, 0.3mm-NADP+ and a NADPH-generating system consisting of 7mm-glucose 6-phosphate, 10mm-MgCl2 and 3 units of glucose 6-phosphate dehydrogenase (EC 1.1.1.49). Total volume of the incubation medium was 3ml. The amount of formaldehyde formed was measured by the procedure of Nash (1953).

Aniline p-hydroxylation activity was measured by the method of Imai et al. (1966). The incubation medium contained in final volume of 2ml the microsomal fraction (6mg of protein), 0.1m-Tris/acetic acid buffer (pH8.0), 8mm-aniline, 0.32mm-NADP+ and NADPH-generating system as described above. The amount of p-anisidinophenol formed was measured at 630nm, by using p-anisidinophenol as standard.

The amounts of cytochromes P-450 and P-420 were determined from CO-difference spectra of Na2S2O4-treated microsomal fraction as described by Omura & Sato (1964), by using ε = 91 and 111 litre·mmol⁻¹·
cm⁻¹ for cytochromes P-450 and P-420 respectively.

Ethyl isocyanide difference spectra were measured as described by Imai & Siekevitz (1971). Microsomal fraction (3.0mg of protein/ml) in 0.2m-potassium phosphate buffer at designated pH values, and 30% (v/v) glycerol were placed in both sample and reference cuvettes. Ethyl isocyanide (final concentration 0.4mm) was added to sample cuvette and minimal amounts of Na2S2O4 were added to both cuvettes. Scanning was started at 600nm 3min after Na2S2O4 addition. The magnitudes of the 455 and 430nm peaks of the ethyl isocyanide difference spectra of reduced cytochrome P-450 were expressed in terms of ΔE455(430)–500/nmol of cytochrome P-450, and the E455/E430 ratio was calculated.

Difference spectra were recorded in a Hitachi model 356 two-wavelength double-beam scanning spectrophotometer, in the split-beam mode.

The activity of NADPH-cytochrome c reductase was determined kinetically under conditions similar to those of Phillips & Langdon (1962). Results were expressed as nmol of cytochrome reduced/min per mg of protein; ε was taken as 19.1 litre·mmol⁻¹·cm⁻¹ (King, 1967).

The activity of NADPH-cytochrome P-450 reductase was measured as described by Gnosspelius et al. (1969). Only the first linear segment (rapid-reaction phase) was used to calculate the rate of reduction, conventionally assuming ε for cytochrome P-450 to be 91 litre·mmol⁻¹·cm⁻¹. The effect of the substrate on the initial reaction rate was studied in this system, and also in the system containing 3.2mm-aminopyrine from the start. The results were expressed in nmol of cytochrome reduced/min per mg of protein. Assays for the reductases were performed with a Hitachi-356 apparatus in the dual-wavelength mode.

The binding spectra of aminopyrine, a type I substrate, and aniline, a type II substrate, were determined by using microsomal fraction (5mg of protein) suspended in 0.1m-Na2HPO4/KH2PO4 buffer (pH7.5). Various increasing amounts of the substrates were added to the sample cuvette, the reference cuvette receiving equivalent volumes of solvent. The effects of carbonyl cyanide m-chloro-phenylhydrazone and sodium deoxycholate on the substrate-induced spectra were measured under the same conditions. Thereafter equal quantities of the agents were added to both cuvettes. The spectral dissociation constant (Kd) for both type I and type II substrates was determined by the method of Schenckman et al. (1967). Maximal binding of the substrate to cytochrome P-450 (constant ΔE₄₅₅) was measured as described by Kato et al. (1971).

The fluorescence of 8-anilinonaphthalene-1-sulphonate in the presence of the microsomal fraction was measured in a Spekol spectrofluorimeter. The incubation medium contained microsomal suspension at a concentration of 1.3mg of protein/ml, 0.125m-KCl, 0.02m-Tris/HCl buffer (pH7.4) and 8-anilinonaphthalene-1-sulphonate at concentrations of 1–30µm in a total volume of 2.5ml. In studies of the intensity of 8-anilinonaphthalene-1-sulphonate fluorescence in a medium containing control and experimental microsomal fractions of various protein concentrations (0.04–0.24mg of protein) the concentration of the dye was 10µm. Excitation was at 360nm and emission was at 470nm.

For protein determination the biuret method of Gornall et al. (1949) was used. Details of the experiments are in the legends to the Figures and Tables.
Results

Spectral characteristics of cytochrome P-450 and the activity of NADPH-dependent reductases

As shown in Fig. 1(a), addition of increasing concentrations of sodium deoxycholate to the sample cuvette caused a sequential, but significant (P < 0.05), decrease in the peak at 450 nm in Na₂S₂O₄-reduced CO-bubbled microsomal suspension (see also Table 2). There was no conversion of cytochrome P-450 into cytochrome P-420, an enzymically inactive form, in the presence of sodium deoxycholate until the concentration of this agent reached 0.09%, which shows the absence of a stoichiometric relationship between the disappearance of cytochrome P-450 and appearance of cytochrome P-420.

On the other hand, the presence of increasing concentrations of carbonyl cyanide m-chlorophenylhydrazone was not accompanied by any changes whatever in the cytochrome P-450 spectrum (Fig. 1b) under various incubation conditions.

Investigation of the effect of these agents on the rate of NADPH-cytochrome c reductase has shown that carbonyl cyanide m-chlorophenylhydrazone did not alter the activity of this enzyme, whereas sodium deoxycholate at concentrations of 0.03–0.09% somewhat increased the reaction velocity (Table 1).

Quite different effects were observed for NADPH-cytochrome P-450 reductase. As shown in Table 1, carbonyl cyanide m-chlorophenylhydrazone suppressed the initial phase of reductase reaction, the decrease in reductase activity being most pronounced when carbonyl cyanide m-chlorophenylhydrazone concentration reached 60 μM (39% of the control value); further increase in the amount of the agent did not change the reductase action. It is noteworthy that carbonyl cyanide m-chlorophenylhydrazone, although inhibiting NADPH-cytochrome P-450 reductase, did not remove the stimulating effect caused by the presence of type 1 substrate, such as aminopyrine.

As for sodium deoxycholate, this agent had a biphasic effect on the rate of NADPH-dependent reduction of cytochrome P-450. Thus 0.01% sodium deoxycholate stimulated reductase activity, but inhibited the same increase in the enzyme activity caused by aminopyrine (Table 1). These effects of 0.01% sodium deoxycholate could be explained by the fact that this derivative of cholesterol is a type 1 substrate for cytochrome P-450 and is characterized

![Fig. 1. Difference spectra of CO-binding haemoprotein in rat liver microsomal fraction treated with sodium deoxycholate (a) and carbonyl cyanide m-chlorophenylhydrazone (b)](image)

Incubation medium contained 0.125 M-KCl and 0.02 M-Tris/HCl buffer (pH 7.4) and microsomal fraction (1.9 mg of protein/ml). The measurements were made at 22°C. Assays were done (a) in the absence (——) and in the presence of 0.01% (---), 0.03% (-----), 0.06% (— --), 0.09% (------) and 0.12% (-----) sodium deoxycholate (b), and in the absence (——) and in the presence of 90 μM-carbonyl cyanide m-chlorophenylhydrazone (----). Cytochrome P-450 content in untreated microsomal fraction was 0.92 ± 0.01 nmol/mg of protein. The result is the mean ± S.E.M. of five to eight tests.

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by a high affinity for the haemoprotein (Greim et al., 1973). The larger concentrations of this detergent considerably decreased the rate of cytochrome P-450 reductase and completely abolished the stimulating effect of 3.2 mm-aminopyrine.

Substrate-binding capacity of cytochrome P-450

Another indication that 0.01% sodium deoxycholate serves as a type I substrate for cytochrome P-450 was its competitive inhibition of the binding of aminopyrine (Fig. 2a). A qualitatively different effect was detected when using sodium deoxycholate at 0.04% and higher concentrations: without changing the affinity of cytochrome P-450 for aminopyrine, this agent lowered the molar concentration of binding sites (constant $\Delta E_{\text{max}}$) for the substrate; that is, non-competitive inhibition of the binding of aminopyrine occurred. As for aniline, a type II substrate, sodium deoxycholate inhibited its binding, this being a so-called mixed inhibition, i.e. $K_i$ for this substrate increased and $\Delta E_{\text{max}}$ decreased simultaneously. At the same time, increasing the sodium deoxycholate concentration also enhanced its inhibiting effect (Fig. 2b).

Double-reciprocal plots illustrating the influence of carbonyl cyanide $m$-chlorophenylhydrzone on aminopyrine binding prove that this agent competitively blocked substrate binding (Fig. 3a). However, on adding carbonyl cyanide $m$-chlorophenylhydrzone to the oxidized microsomal suspension we failed to detect any spectra changes showing the agent binding to cytochrome P-450. On the other hand, Fig. 3(b) shows that carbonyl cyanide $m$-chlorophenylhydrzone did not influence the binding of aniline, since neither the affinity for cytochrome P-450 nor the molar concentration of binding sites for aniline differed from the corresponding control values.

Interaction of microsomal fraction with 8-anilinonaphthalene-1-sulphonate and ethyl isocyanide

Our tests have shown a considerable fall in the quantum yield of 8-anilinonaphthalene-1-sulphonate fluorescence in the microsomal suspension after addition of carbonyl cyanide $m$-chlorophenylhydrzone or sodium deoxycholate (Fig. 4). Maximum decreases in the intensity of this fluorescence was in the presence of 60 $\mu$m-carbonyl cyanide $m$-chlorophenylhydrzone and 0.12% sodium deoxycholate (by 60% and 87% respectively). Study of these observations by using Lineweaver–Burk plots (Fig. 5) established that sodium deoxycholate and carbonyl cyanide $m$-chlorophenylhydrzone lowered the molar concentration of the binding sites for 8-anilinonaphthalene-1-sulphonate, but did not alter the degree of affinity of this dye for microsomal phospholipids ($K_i = 5 \mu M$). Fig. 5 also gives data on the competitive inhibition of 8-anilinonaphthalene-1-sulphonate binding to microsomal fraction by lipid-soluble anion tetraphenylborate. A rise of $K_i$ for the dye in the presence of tetraphenylborate may...
apparently be explained by the displacement of 8-anilinonaphthalene-1-sulphonate by this anion from positively charged binding sites in the microsomal membrane.

Finally, comparison of ethyl isocyanide difference spectra of Na$_2$S$_2$O$_4$-reduced haemoprotein, where the ratio of relative heights of the 455 and 430 nm peaks is a sensitive indicator of the degree of hydrophobicity of the cytochrome P-450 haem-group environment (Imai & Siekevitz, 1971), did not yield any difference between the control and carbonyl cyanide m-chlorophenylhydrazone-treated microsomal fraction (results not shown).

**Kinetic constants of aminopyrine and aniline metabolism**

Table 2 shows that increasing concentrations of sodium deoxycholate gradually inhibit aminopyrine metabolism, and in the presence of 0.12% sodium deoxycholate N-demethylation of aminopyrine practically does not occur. Since we know the $V$ value and the content of the enzyme (cytochrome P-450), we determined the rate of dissociation of the enzyme-substrate complex (constant $k_{+2}$ in the control and experimental microsomal preparations. Inspection of the $k_{+2}$ values for each sodium deoxycholate concentration indicated that this reaction was not rate-limiting during metabolism of aminopyrine. This applies to an even greater extent to aniline $p$-hydroxylation, since the $k_{+2}$ values were greater than the $V$ values for each sodium deoxycholate concentration.

As shown in Table 2, carbonyl cyanide m-chlorophenylhydrazone lowered the rate of aniline $p$-hydroxylation only slightly. Moreover, this agent did not change the content of cytochrome P-450 in the microsomal suspension after incubation at 37°C for 20 min. Hence, we infer that carbonyl cyanide m-chlorophenylhydrazone does not influence the kinetics of aniline metabolism in microsomal mono-oxygenases. However, this agent considerably inhibited the rate of aminopyrine $N$-demethylation; the
Fig. 4. Intensity of 8-anilinonaphthalene-1-sulphonate fluorescence in control and experimental rat liver microsomal fractions

Incubation medium contained 0.125 M-KCl and 0.02 M-Tris/HCl buffer (pH 7.4). Microsomal protein content in control and experimental cuvettes was the same. Cuvette volume was 2.5 ml and 8-anilinonaphthalene-1-sulphonate concentration 10 μM. Excitation was at 360 nm and emission at 470 nm. Assays were done in control (○) and sample microsomal fraction treated with 10 (△) and 30 μM (△) carbonyl cyanide m-chlorophenylhydrazone, or with 0.03 (●) and 0.09% (□) sodium deoxycholate.

decrease was more than 50% of the control rate, even though the content of cytochrome P-450 was unchanged.

A study of the pH-dependence of the inhibition of aminopyrine N-demethylase by carbonyl cyanide m-chlorophenylhydrazone showed that maximum inhibition occurred at pH 5.5–6.0, with a considerable loss of inhibition when the pH of incubation medium was made more alkaline (Fig. 6).

Discussion

The present studies on the effects of sodium deoxycholate were performed because detergents are now widely used in the study of biomembranes, both to isolate components and to probe the structure of the membrane itself (Kirkpatrick et al., 1974). The conversion of cytochrome P-450 into cytochrome P-420 in the presence of sodium deoxycholate (Fig. 1a) may result from the effects of this detergent on hydrophobic bonds in the lipoprotein membrane (Victoria & Barber, 1969), with consequent unmasking of the hydrophobic phospholipid groups responsible for maintaining the spectral properties of the CO-binding haemoprotein (Omura & Sato, 1964; Imai & Sato, 1967; Ichikawa & Yamano, 1967). Sodium deoxycholate 'opens' the hydrophobic cavity, created by these phospholipid groups, making it accessible to the aqueous phase (Raykhman et al., 1972).

The inhibition of aniline binding and metabolism by sodium deoxycholate are not unexpected, since it is known that binding of this substrate takes place in the lipophilic moiety of the cytochrome P-450 molecule (Ichikawa & Yamano, 1967) and that p-hydroxylation of aniline by mono-oxygenase requires phospholipid (Imai & Sato, 1960; Lu et al., 1972).

The results shown in Table 1 and Fig. 3(a) give evidence that at concentrations exceeding 0.03%, sodium deoxycholate acts as a non-competitive inhibitor of aminopyrine binding and suppresses the activity of NADPH-dependent reduction both of cytochrome P-450 alone and of the cytochrome P-450–aminopyrine complex. It is known that the 'binding protein' for type I substrates and NADPH–cytochrome P-450 reductase is strictly dependent for activity on the state of their surrounding phospho-
Table 2. Effects of carbonyl cyanide m-chlorophenylhydrazone and sodium deoxycholate on the amount of cytochrome P-450 and kinetic constants of drug metabolism in the microsomal fraction

Experimental conditions are given in the Material and Methods section. \( V \) is expressed as nmol of formaldehyde (for aminopyrine) or \( p \)-aminophenol (for aniline) formed/min per mg of microsomal protein. \( k_+2 \) is expressed as \( V/\text{nmol of cytochrome P-450} \) for every concentration of agents used. All the values given are the means of four to six determinations, the results of which differed by less than 5%.

<table>
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<tr>
<th>Carbonyl cyanide m-chlorophenylhydrazone concn. (( \mu M ))</th>
<th>Cytochrome P-450</th>
<th>Aminopyrine</th>
<th>Aniline</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(nmol/mg of protein)</td>
<td>(% of control)</td>
<td>(% of control)</td>
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<tr>
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<td>100</td>
<td>3.35 100</td>
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<tr>
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<tr>
<td>90</td>
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<th>Sodium deoxycholate concn. (%)</th>
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<th>Aniline</th>
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Incubation media contained potassium phosphate buffers of different pH values. Carbonyl cyanide m-chlorophenylhydrazone (final concentration 90 \( \mu M \)) was used in all experiments. The insert shows dependence of aminopyrine \( N \)-demethylase activity on pH. For experimental conditions see the Material and Methods section.
phosphatidylcholine and that the 'binding protein'
of cytochrome P-450 apoenzyme is the binding site
for aminopyrine (Remmer et al., 1968), we consider
that an increase in the hydrophilicity of the phos-
pholipid component of the microsomal membrane
determines the suppression of type I-substrate bind-
ing in the presence of detergent.

Since sodium deoxycholate is an anionic detergent,
we compared its effects on binding of aniline, amino-
pyrine, and 8-anilinonaphthalene-1-sulphonate with
those of the lipid-soluble anion, tetraphenylborate,
which is also an inhibitor of microsomal mono-
oxigenase (Liberman et al., 1974). The absence of
any influence of tetraphenylborate on types I and II
binding spectra (not shown) and the competitive
inhibition of 8-anilinonaphthalene-1-sulphonate
binding (Fig. 5) apparently shows that the negatively
charged hydrophilic region of the sodium deoxy-
cholate molecule is not associated with the effects
caused by this agent.

On the strength of all the factors that determine
mono-oxygenase activity in the presence of 0.03–
0.09\% sodium deoxycholate, we conclude that in the
present case the rate-limiting factor in aniline p-
hydroxylation is the activity of cytochrome P-450.
On the other hand, the activity of NADPH-cyto-
chrome P-450 reductase can be regarded as the rate-
limiting factor in aminopyrine N-demethylation.

The ability of carbonyl cyanide m-chlorophenyl-
hydrazone to influence the mitochondrial oxidative
system and to increase the electrical conductivity of
artificial and natural membranes is because of the
presence of an ionizable proton in its structure (see
Scheme 1) (Mitchell, 1966) and its solubility in low-
polarity media (Hemker, 1962) increasing the perme-
ability of the membranes to $H^+$ ions (Liberman,
1968).

The present results show that incubation of micro-
somal fraction in the presence of increasing amounts
of carbonyl cyanide m-chlorophenylhydrazone is not
accompanied by changes in the spectrum of cyto-
chrome P-450 as well as in the binding and metabolism
of aniline. This suggests that the hydrophobic region
of the microsomal membrane, including the active
centre of mono-oxygenase for type II substrates, is
not influenced by carbonyl cyanide m-chlorophenyl-
hydrazone. Indeed, the ethyl isocyanide difference
spectra of cytochrome P-450 did not differ consider-
ably for the control and protonophore-treated
microsomal fractions, which points to the intactness
of the hydrophobic region containing the haem group
of cytochrome P-450 (Imai & Siekevitz, 1971).}

However, carbonyl cyanide m-chlorophenyl-
hydrazone inhibits 8-anilinonaphthalene-1-sulphon-
ate fluorescence, diminishing thereby the molar con-
centration of the binding sites, but without affecting
the $K_i$ for this dye (Figs. 4 and 5). Not changing the
content of type I 'binding protein' and not being a
substrate for cytochrome P-450, carbonyl cyanide
m-chlorophenylhydrazone nevertheless increases the
$K_i$ for aminopyrine. Lastly, the protonophore
suppresses the activity of such phospholipid-
dependent reactions as NADPH-cytochrome P-450
reductase, and even to a greater extent, $N$-demethyla-
tion of aminopyrine.

As shown in Table 2, the rate constant, $k_{+2}$, refers
to a rate-limiting reaction in the metabolism of amino-
pyrine in the presence of carbonyl cyanide m-chloro-
phenylhydrazone. The mechanism of cytochrome
P-450 action commonly accepted includes dissoci-
ation of 'reduced P-450-substrate-activated oxygen'
complex, subsequent incorporation of one of the
oxygen atoms into the substrate and release of the
hydroxylated product, water and oxidized cyto-
chrome P-450 (Estabrook et al., 1971).

Being a weak acid and having $pK_a5.7$, carbonyl
cyanine m-chlorophenylhydrazone is most effective
as a proton carrier in the presence of a mixture of its
anionic and undissociated forms. At pH values on
either side of its $pK_a$ (where either the agent is in
the anionic form and there are few $H^+$ ions or it is
largely undissociated) carbonyl cyanide m-chloro-
phenylhydrazone increasingly loses its ability to be a
mobile proton carrier (Skulachev, 1969). The pH
dependence of $N$-demethylation inhibition by carbonyl
cyanine m-chlorophenylhydrazone showed the maxi-
mum effect near the $pK_a$ for this agent (Fig. 6). On
the alkaline side of the $pK_a$ the degree of inhibition falls
considerably, indicating that the protonated form is
responsible for the decreased $N$-demethylation activity.

The above findings suggest that the mechanism of
carbonyl cyanide m-chlorophenylhydrazone action
consists of the electrostatic interaction of its proto-
nated form with one of the forms of activated oxygen,
i.e. it is assumed that $H^+$ ion competes with amino-
pyrine for $O_2$ or $O_2^{-}$.

Thus there may be at least two different phospho-
lipid-dependent hydrophobic zones coupled with

Scheme 1.
cytochrome $P$-450. One of them reveals selective sensitivity to the influence of carbonyl cyanide $m$-chlorophenylhydrazone and includes ‘binding protein’ for type I substrates and NADPH–cytochrome $P$-450 reductase; the other contains the cytochrome $P$-450 haem group and binding sites for type II substrates. Sodium deoxycholate is capable of influencing both zones.

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References