MOLECULAR PHARMACOLOGY, 6, 221–230

Characteristics of Guinea Pig Liver and Adrenal Monooxygenase Systems

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(Received December 22, 1969)

SUMMARY

Liver and adrenal microsomes from the guinea pig were found to catalyze the oxidative demethylation of *p*-chloro-*N*-methylaniline and aminopyrine, and the ω-oxidation of laurate. These activities were markedly higher in the liver than in the adrenals.

The level of cytochrome P-450 was higher in adrenal than in liver microsomes, whereas the rate of cytochrome P-450 reduction by NADPH was substantially lower in adrenal microsomes than in liver microsomes. This finding suggests that the reduction of cytochrome P-450 —rather than the amount of cytochrome P-450—may be the rate-determining step in the oxidative demethylation and ω-oxidation reactions.

Spectral changes induced by the addition of various compounds to liver and adrenal microsomes were examined. (a) Cortisol and cortisol 21-sodium succinate (cortisol succinate) were found to yield type I spectra with adrenal microsomes, but with liver microsomes these compounds yielded either a weak type I spectrum or no spectral changes; occasionally even a type II spectrum was evident. (b) Aminopyrine and hexobarbital gave type I spectra with liver microsomes and no spectral changes with adrenal microsomes. (c) *p*-Chloro-*N*-methylaniline gave a type II spectrum with both liver and adrenal microsomes. Despite the apparent inability of hexobarbital and aminopyrine to produce spectral changes in adrenal microsomes, these compounds diminished the magnitude of the type I spectrum produced by cortisol succinate and cortisol. It is proposed that the binding sites responsible for spectral changes and for metabolism are not necessarily identical, and that a given compound can bind at more than one site.

The effects of the compounds tested on the rates of NADPH-mediated reduction of cytochrome P-450 were different in liver and adrenal microsomes. However, the ability of a compound to enhance the rate of cytochrome P-450 reduction was found to be correlated with its capacity to induce a type I spectral change in microsomes from the respective tissues.

INTRODUCTION

The central role of cytochrome P-450 as the oxygen-activating component in a number of microsomal mixed-function oxidation (monooxygenase) reactions is now well established. More recent studies have demonstrated the direct involvement of this pigment in the binding to liver microsomes of various compounds capable of undergoing hydroxylation (1–4). Such an interaction of compounds, among them drugs, steroid hormones, and fatty acids, with a suspension of liver microsomes is characterized by two types of spectral changes, termed "type I" and "type II"; the type I spectrum has a

These studies were supported by the Swedish Medical Research Council under Project B70-13X-2471-03.

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trough at 420 nm and a peak at 385–390 nm, whereas the type II spectrum has a peak at about 430 nm and a trough at 390–400 nm (2).

In liver microsomes cytochrome P-450 is reduced by an NADPH-dependent system, which presumably involves NADPH–cytochrome *c* reductase as one of its components (5–7).[2] Furthermore, substrates causing the type I spectral change have been shown to stimulate the rate of cytochrome P-450 reduction, indicating that the cytochrome P-450–substrate complex is reduced more readily than the substrate-free cytochrome (8, 9). Subsequently it was postulated that the reduction of the cytochrome P-450–substrate complex may in fact be the rate-limiting step in liver microsomal mixed-function oxidation reactions (10).

Cytochrome P-450 is the terminal oxidase involved in the 11β-hydroxylation of corticosteroids catalyzed by adrenal cortex mitochondria (11). Important differences in the apparent composition as well as in substrate specificity of the liver microsomal and adrenal mitochondrial systems raised the question whether there exist several species of cytochrome P-450 (12). Heterogeneity within the liver microsomal pool of cytochrome P-450, based mainly on differences in the inducibility of enhanced hydroxylation activity by drugs and polycyclic hydrocarbons, has been postulated recently (9, 13–16).

In the guinea pig both adrenal and liver preparations, supplemented with NADPH, are able to hydroxylate cortisol at positions C-2 and C-6 (17–19). More recently, guinea pig liver and adrenal homogenates and their respective 9000 × *g* supernatant fractions were shown to catalyze the NADPH- and oxygen-dependent *N*-demethylation of *p*-chloro-*N*-methylaniline (20). Moreover, the *N*-demethylation of *p*-chloro-*N*-methylaniline by preparations from either tissue was inhibited by carbon monoxide. These findings suggested that the *N*-demethylating activity and possibly the cortisol-hydroxylating activity may be properties of a mixed-function oxidase system present in the adrenal and liver microsomes.

[2] T. Omura, personal communication.

The present investigation was designed to examine similarities and differences in the composition of the catalytic activity of the mixed-function oxidase system(s) of liver and adrenal microsomes from the guinea pig. Particular emphasis was placed on examination of the mode of substrate interaction with the terminal oxidase(s) of the two tissues.

MATERIALS AND METHODS

Male albino guinea pigs (local strain) weighing 450–500 g were used. On the day of experiment, guinea pigs which had been deprived of food overnight (water was given ad libitum) were killed by a blow on the head and decapitated. The livers and adrenals were immediately excised, and microsomes were isolated as described by Ernster *et al.* (21). The microsomal pellets were rinsed with ice-cold 0.25 M sucrose solution and resuspended in this medium at a protein concentration of about 10 mg/ml. To obtain "washed" microsomes, the microsomal pellet was suspended in 0.154 M aqueous KCl solution, centrifuged for 1 hr at 105,000 × *g*, and resuspended in 0.25 M sucrose as described above. Protein concentrations were determined by the method of Lowry *et al.* (22).

The rate of demethylation of aminopyrine was measured in a system containing liver or adrenal microsomes (1–2 mg of protein), 50 mM Tris-Cl buffer (pH 7.5), 5 mM $MgCl_2$, 5 mM aminopyrine, 1 mM $NADP^+$, 5 mM DL-isocitrate, 0.005 mM $MnCl_2$, and 0.4 international unit of pig heart isocitrate dehydrogenase in a final volume of 2 ml. Incubation was performed for 10 min at 37° in an atmosphere of air. Formaldehyde formed was measured by the procedure of Nash (23).

The rate of demethylation of *p*-chloro-*N*-methylaniline was determined in the incubation system described above, containing 1.5 mM *p*-chloro-*N*-methylaniline instead of aminopyrine. The formation of *p*-chloroaniline was determined as described previously (24); the adequacy of the NADPH-generating system was simultaneously assessed (25).

Sodium laurate was incubated with liver microsomes in the incubation system de-

scribed above for aminopyrine, except that aminopyrine was omitted and laurate was added in a final concentration of 0.05 mM. The rate of ω-hydroxylation of laurate was determined by separating laurate from its oxidation products by the chromatographic technique described by Kusunose *et al.* (26).

NADPH oxidation was measured in an Eppendorf fluorometer as described by Estabrook and Maitra (27). The incubation mixture contained 50 mM Tris-Cl buffer (pH 7.5), 3.3 mM $MgCl_2$, 0.05 mM NADPH, and about 1 mg of microsomal protein in a final volume of 3 ml.

NADPH–cytochrome *c* reductase activity was measured in an incubation mixture containing 0.1 mM NADPH, 0.054 mM cytochrome *c*, 0.3 mM KCN, 50 mM potassium phosphate buffer (pH 7.5), and microsomes (0.01–0.02 mg of protein) in a final volume of 1 ml.

The content of cytochrome P-450 was measured as follows. Washed microsomes (1 mg of protein) were suspended in 6 ml of 50 mM potassium phosphate buffer (pH 7.5), and a few milligrams of sodium dithionite were added. Aliquots (2.9 ml) were taken for the reference and sample cuvettes, and carbon monoxide was bubbled into the sample cuvette. The spectrum was recorded with a Phoenix dual-wavelength scanning spectrophotometer.

NADPH–cytochrome P-450 reductase activity was assayed by measuring the rate of formation of the reduced cytochrome P-450–CO complex at 450 nm minus 475 nm in a Phoenix dual-wavelength scanning spectrophotometer. The assay system consisted of liver or adrenal microsomes (0.5–2.0 mg of protein), 50 mM potassium phosphate buffer (pH 7.5), submitochondrial particles [approximately 2 mg of protein, prepared as described by Löw and Vallin (28)], 6.6 mM sodium succinate, 0.1 μM rotenone, and the appropriate concentration of the substance whose effect on the reductase was to be examined. The mixture was allowed to stand until complete anaerobiosis was achieved, as indicated by a rise in absorbance at 445 nm due to accumulation of reduced cytochrome *c* oxidase. Carbon monoxide was then bubbled through the suspension for 1 min. The rate of formation of the cytochrome P-450–CO complex was measured at 450 nm minus 475 nm after starting the reaction by the rapid addition of 5 μM NADPH. The results are given in the form of the recordings actually obtained. The validity of this method is based on the fact that the rate of cytochrome P-450 reduction is considerably slower than the rate at which CO combines with the reduced cytochrome (10).

The spectral changes caused by various substances added to a suspension of microsomes were recorded with a Phoenix dual-wavelength scanning spectrophotometer. Each cuvette contained microsomal suspension (2 mg of protein) and 50 mM Tris-Cl buffer (pH 7.5) in a final volume of 3 ml. The substance under study was added, at a specified concentration, to the sample cuvette, and an equivalent amount of the solvent was added to the reference cuvette.

All chemicals employed were standard commercial products of reagent grade quality. $NADP^+$, NADPH, cytochrome *c*, rotenone, and cortisol 21-sodium succinate were obtained from Sigma Chemical Company. Pig heart isocitrate dehydrogenase was obtained from Boehringer und Soehne, Mannheim. *p*-Chloro-*N*-methylaniline and *p*-chloroaniline were gifts from Calbiochem.

RESULTS

Guinea pig liver microsomes were found to catalyze the oxidative demethylation of aminopyrine and *p*-chloro-*N*-methylaniline at a considerably higher rate than adrenal microsomes (Table 1). The same was true for the ω-oxidation of laurate. However, the level of cytochrome P-450 in the two tissues did not parallel the rates of the oxidative metabolism of the above substrates. As shown in Fig. 1, reduction of microsomes from liver or adrenals by sodium dithionite, followed by exposure to carbon monoxide, yielded difference spectra with an absorption maximum at 450 nm, and the level of cytochrome P-450 in the adrenal microsomes was about double that in the liver microsomes (Table 1).

Whereas microsomes from both tissues catalyzed the reduction of cytochrome *c* by

TABLE 1

Oxidative demethylation of aminopyrine and p-chloro-N-methylaniline and ω-hydroxylation of laurate by guinea pig liver and adrenal microsomes

Values represent the mean and range of several experiments. Each determination was made with microsomes obtained from pooled livers and adrenals from four to eight animals.

Tissue	Oxidative demethylation activity		ω-Oxidation activity	Cytochrome P-450[a]
	Aminopyrine	*p*-Chloro-*N*-methylaniline		
	nmoles metabolized/min/mg protein		*nmoles ω-hydroxylaurate formed/min/mg protein*	$\Delta A_{450-500}$/*mg protein/ml*
Liver	14.2 (9.2–18.8)	27 (26–28)	2.18	0.095 (0.072–0.123)
Adrenals	5.6 (4.5–6.3)	14.3 (11–18)	0.16	0.191 (0.171–0.205)

[a] Washed microsomes were usually used; however, there was no difference when microsomes were not washed with 0.154 M KCl solution.

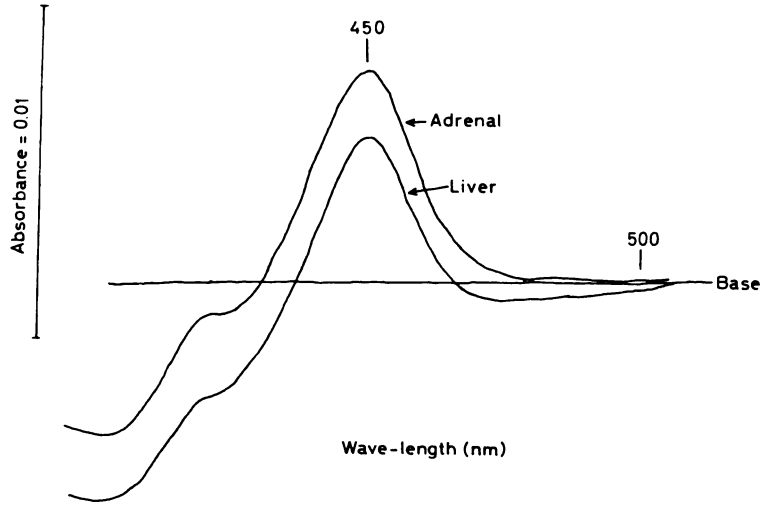


FIG. 1. *Difference spectra of dithionite-reduced, CO-exposed microsomes from livers and adrenals of male guinea pigs*

NADPH at approximately equal rates (Table 2), the distribution of NADPH oxidase activity was similar to that of the oxidative demethylation activity, being higher in liver than in adrenal microsomes (Tables 1 and 2). However, this difference was abolished in the presence of menadione, which increased the rate of NADPH oxidation many fold, resulting in roughly equal rates in microsomes from both tissues (Table 2).

The lack of correlation among oxidative demethylation activity, NADPH–cytochrome *c* reductase activity, and the concentration of cytochrome P-450 in liver and adrenal microsomes indicated that neither NADPH–cytochrome *c* reductase activity nor the amount of cytochrome P-450 present is rate-limiting in the demethylation reaction and suggested experiments to compare the rate of reduction of cytochrome P-450 in liver and adrenal microsomes.

As may be seen in Fig. 2, the initial rate of reduction of cytochrome P-450 was considerably lower in adrenal microsomes than in liver microsomes. Furthermore, whereas in liver microsomes there are two discernible phases in the rate profile of cytochrome P-

TABLE 2

Menadione-stimulated NADPH oxidation and NADPH-cytochrome c reductase activities in guinea pig liver and adrenal microsomes

Microsomes were obtained from livers and adrenals which were pooled from four to eight animals. For cytochrome *c* reduction, each determination was made with livers or adrenals pooled from two animals, and each value represents the mean of three experiments.

Tissue	NADPH oxidation	Menadione-stimulated NADPH oxidation[a]	NADPH–cytochrome *c* reductase activity
	nmoles NADPH oxidized/min/mg protein		*nmoles cytochrome c reduced/min/mg protein*
Liver	16.3 (13.4–19.1)	188	226 (191–250)
Adrenals	5.8 (5.7–5.9)	258	285 (260–307)

[a] Menadione (2-methylnaphthoquinone) was added in 10 μl of ethanol in a final concentration of 0.2 mM.

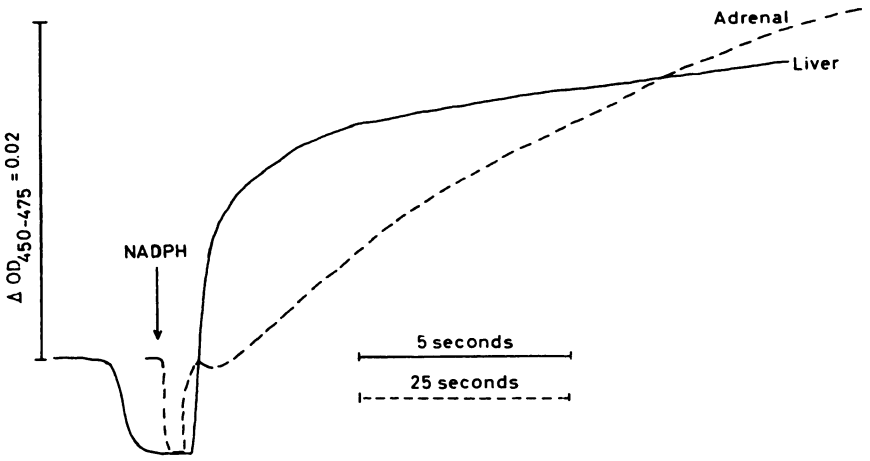


FIG. 2. *Rate of reduction of cytochrome P-450 by NADPH in adrenal and liver microsomes from male guinea pigs*

450 reduction—an initial fast phase and a second slow phase—in adrenal microsomes there seems to be, after an initial lag period, only a single phase (Figs. 2 and 4).

In agreement with previous findings in the rat (9, 10), hexobarbital enhanced the rate of reduction of cytochrome P-450 in guinea pig liver microsomes (Fig. 3). A similar, though somewhat less pronounced, enhancement of the rate of reduction of cytochrome P-450 was observed with aminopyrine (results not presented). In adrenal microsomes, however, neither of these compounds stimulated the rate of the NADPH-mediated cytochrome P-450 reduction, but actually caused inhibition when added at higher concentrations (1 mM and 5 mM, respectively). *p*-Chloro-*N*-methylaniline added at concentrations from 0.15 mM to 3.3 mM did not increase the rate of cytochrome P-450 reduction in microsomes from either tissue; in fact, at 3.0 mM this compound inhibited the rate of cytochrome P-450 reduction in adrenal microsomes. Cortisol had no measurable effect on the rate of cytochrome P-450 reduction in liver microsomes, but enhanced this activity in the adrenals (Figs. 3 and 4). Furthermore, a semilogarithmic plot of the rate of reduction of cytochrome P-450 in adrenal microsomes in the absence and presence of cortisol

reveals straight lines, suggesting first-order kinetics (Fig. 4).

These findings suggested experiments to study in some detail the interaction of the various substrates with liver and adrenal microsomes. In accordance with previous observations in rat liver (3), hexobarbital and aminopyrine yielded type I spectral changes in guinea pig liver microsomes (Table 3). Neither of these compounds, however, produced a detectable spectral change with adrenal microsomes. On the other hand, *p*-chloro-*N*-methylaniline produced type II spectra with both liver and adrenal micro-

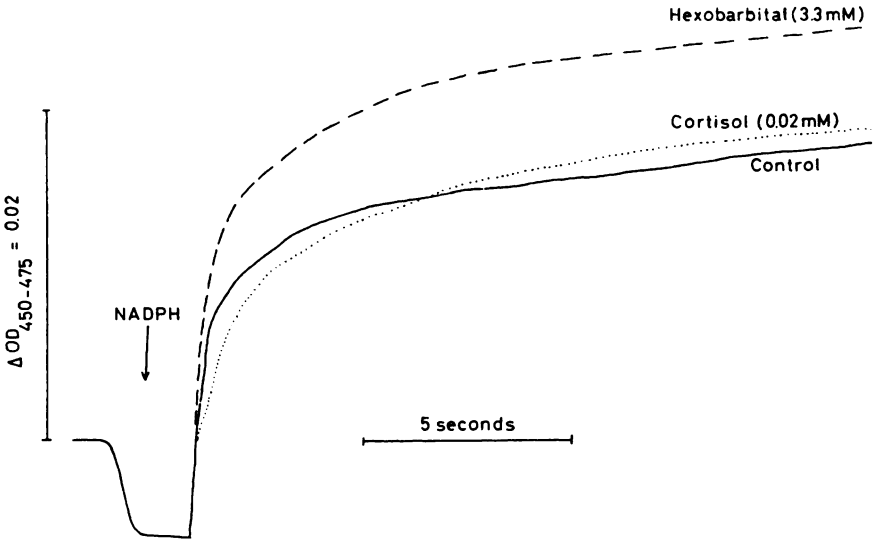


FIG. 3. *Effects of hexobarbital and cortisol on rate of reduction of cytochrome P-450 by NADPH in liver microsomes*

When dithionite is added during the progress of NADPH-cytochrome P-450 reduction, the reduction is completed almost instantaneously, yielding similar levels of absorbance in controls and in the presence of cortisol or hexobarbital.

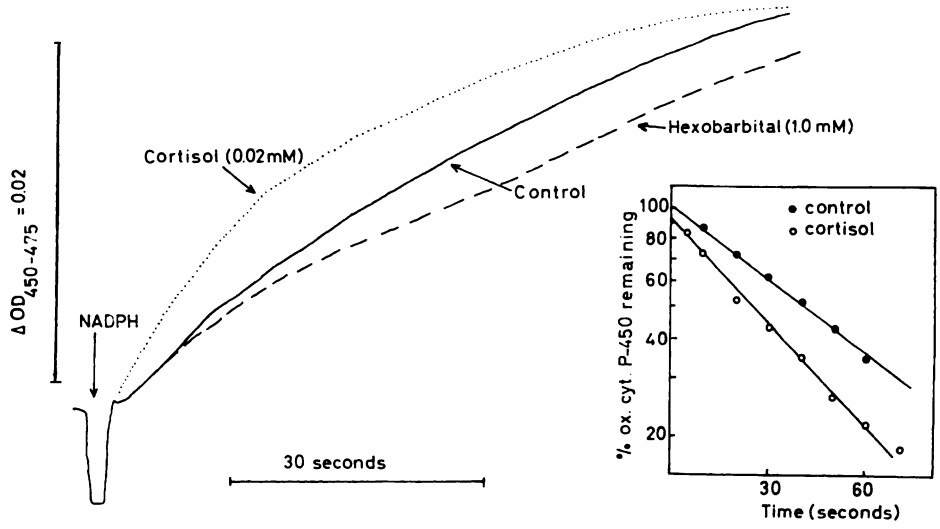


FIG. 4. *Effects of hexobarbital and cortisol on rate of reduction of cytochrome P-450 by NADPH in adrenal microsomes*

The inset shows a semilogarithmic plot of the rate of reduction of cytochrome P-450 in the presence and absence of cortisol (0.02 mM).

TABLE 3

Absorption maxima and minima of guinea pig liver and adrenal microsomes induced by various substances

All compounds, except cortisol, were added as a water solution. Cortisol was added in 10–30 μl of ethanol. The reference cuvette received the same amount of the respective solvent.

Compound	Liver			Adrenals		
	Minimum	Maximum	Type	Minimum	Maximum	Type
	nm	*nm*		*nm*	*nm*	
Hexobarbital (0.33–3.3 mM)	421	388	I	—[a]	—	
Aminopyrine (0.33–3.3 mM)	421	386–388	I	—	—	
p-Chloro-*N*-methylaniline (0.7 mM)	392[b]	421	II	393[b]	420	II
Cortisol (0.02–0.10 mM)	420	385[b]	(I)[c]	423	388	I
Cortisol 21-sodium succinate (0.2–0.8 mM)	412–416	378–383[b]	(I)[c]	421	388	I

[a] A dash denotes no visible peak.

[b] Broad plateau.

[c] Weak type I; occasionally, however, spectral changes were not observed and at times a type II spectrum was evident.

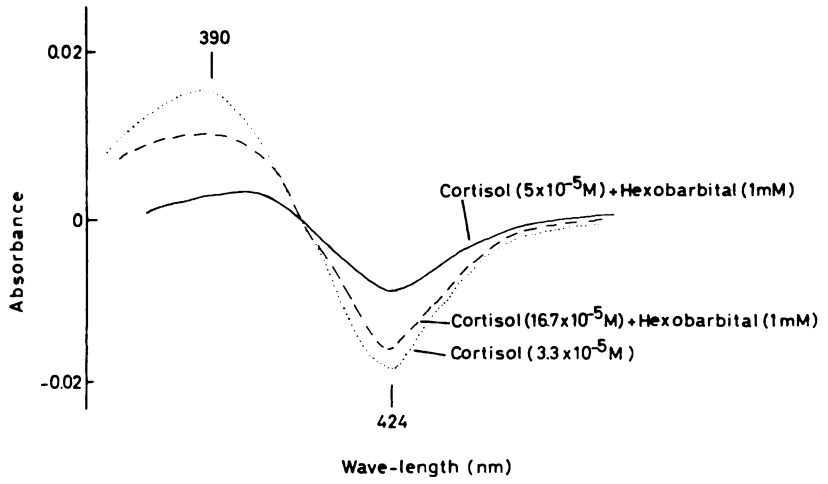


FIG. 5. *Effect of hexobarbital on magnitude of the changes in absorbance in adrenal microsomes produced by cortisol*

Each cuvette contained microsomal suspension (3.3 mg of protein) and 50 mM Tris-Cl buffer, pH 7.5, in a final volume of 3 ml. ····, cortisol (0.01 M in ethanol) was added to the sample cuvette at a final concentration of 3.3×10^{-5} M. ——, both the sample and reference cuvettes contained 1 mM hexobarbital. Cortisol was added to the sample cuvette at a final concentration of 5×10^{-5} M. – – –, both the sample and reference cuvettes contained 1 mM hexobarbital. Cortisol was added to the sample cuvette at a final concentration of 16.7×10^{-5} M.

somes. Cortisol produced a type I spectrum (minimum at 423 nm and maximum at 388 nm) with adrenal microsomes; the apparent K_s value[3] (at 424 nm) was 4.5–5.0 $\times$ 10^{-5} M.

[3] K_s is defined as the substrate concentration which yields a half-maximal absorption change at the indicated wavelength, as described previously (3).

Similarly, cortisol 21-succinate elicited a type I spectral change with adrenal microsomes, with an apparent K_s (at 421 nm) of 4.8 $\times$ 10^{-4} M. On the other hand, with liver microsomes both compounds yielded a weak type I spectrum in most experiments, although at times they elicited no spectral change or even gave a type II spectrum.

TABLE 4

Effect of hexobarbital on magnitude of cortisol-induced spectral change in adrenal microsomes

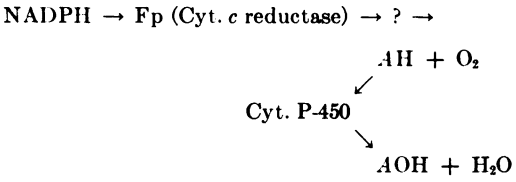
Microsomes were derived from adrenals pooled from seven male guinea pigs. Each cuvette contained 3.3 mg of microsomal protein and 50 mM Tris-Cl buffer (pH 7.5) in a final volume of 3 ml. The indicated concentrations of cortisol were obtained by the addition of varying amounts of a 0.01 M ethanolic solution of cortisol to the sample cuvette. The reference cuvette received a corresponding volume of ethanol. Where shown, 0.03 ml of a 0.1 M aqueous solution of hexobarbital was added to both cuvettes. The baseline was adjusted to equal light absorption, and cortisol was added to the sample cuvette as described above.

Cortisol	Hexobarbital (1 mM)	Absorbance	
		424 nm (trough)	388–390 nm (peak)
$\times 10^{-5}$ M			
1.67	–	0.009	0.006
3.3	–	0.013	0.010
5.0	–	0.018	0.015
7.0	–	0.019	0.017
16.7	–	0.019	0.021
3.3	+	0.0045	0.002
5.0	+	0.009	0.0025
16.7	+	0.016	0.0095
33.0	+	0.018	0.011

In another series of experiments we observed that hexobarbital, which did not produce detectable spectral changes with adrenal microsomes, nevertheless diminished the magnitude of the cortisol-induced spectral change in these microsomes (Fig. 5 and Table 4). Similarly, hexobarbital and aminopyrine interfered with the type I spectrum produced by cortisol succinate. However, the extent of hexobarbital interference diminished with increasing concentrations of cortisol (Table 4).

DISCUSSION

The available evidence suggests that cytochrome P-450 is the terminal oxidase involved in the monooxygenase reactions catalyzed by microsomes from both liver and adrenals. Thus, if it is assumed that the sequence of electron flow in guinea pig adrenal microsomes is identical with that which has been often described for the mixed-function oxidation of various lipid-soluble substrates by liver microsomes,



then the observation that the cytochrome P-450 level is higher in adrenal than in liver microsomes (see Table 1) would suggest that the total amount of this pigment is not rate-limiting in the oxidative demethylation or ω-hydroxylation reactions, since these activities are considerably higher in liver than adrenal microsomes. This is in accordance with the observation by Davies *et al.* (29) that sex and species differences in the rate of drug hydroxylation in liver microsomes are not correlated with similar differences in the distribution of cytochrome P-450. However, the possibility that species, sex, and tissue differences in the catalytic properties of cytochrome P-450, rather than its concentration, are responsible for such lack of correlation cannot be ruled out.

The present finding that, in contrast to the similar NADPH–cytochrome *c* reductase activities in liver and adrenals, the rate of reduction of cytochrome P-450 is much lower in adrenal than in liver microsomes (see Fig. 2) may be taken to indicate that cytochrome P-450 reductase and cytochrome *c* reductase are not identical entities. This in turn suggests that the rate-limiting step may involve a yet unknown factor(s) as a mediator of electron flow from the flavoprotein to cytochrome P-450. However, the possibility should also be considered that cytochrome *c* is not the optimal electron acceptor for the flavoprotein and therefore its rate of reduction cannot be used as a measure of the activity of this enzyme. Nevertheless, even with menadione as an electron acceptor, the rate of NADPH oxidation with liver microsomes was of about the same magnitude as with adrenal microsomes, providing further support for the hypothesis that the NADPH–cytochrome *c* reductase is not rate-limiting in the over-all hydroxylation reaction.

Finally, the rate of reduction of cytochrome P-450 or of the cytochrome P-450–

substrate complex may be the rate-limiting step, as indicated by the present observation of a lower rate of reduction of cytochrome P-450 in adrenal microsomes than in liver microsomes. It has been suggested by Gigon *et al.* (10) that the rate-limiting step may involve the rate of reduction of the cytochrome P-450–substrate complex and not that of the substrate-free cytochrome. Hence it is possible that the faster initial rate of cytochrome P-450 reduction in the liver as compared with the adrenals is due merely to the liver enzyme being bound to an "endogenous substrate" whereas in the adrenals it is the rate of reduction of substrate-free cytochrome P-450 that is measured. The biphasic nature of the rate of cytochrome P-450 reduction in liver microsomes and the monophasic rate in adrenal microsomes may be an indication of the presence of substrate-bound and substrate-free cytochrome P-450 in the respective microsomes. However, the absence of a biphasic rate in the adrenals after the addition of a "substrate" such as cortisol (see Fig. 4, inset) does not support the above explanation. A more likely possibility is that the cytochromes from the two organs either are structurally different or merely behave differently under the influence of their respective microenvironments.

Gigon *et al.* (10) reported that only compounds which form the type I spectra enhance the rate of cytochrome P-450 reduction in rat liver microsomes. Our observations with liver and adrenal microsomes from the guinea pig support these conclusions. Moreover, in our hands, a given compound was found to enhance the rate of cytochrome P-450 reduction in microsomes from one of the tissues while it had no effect on microsomes from the other tissue. This stimulation or lack of stimulation of the rate of cytochrome P-450 reduction could be correlated with the formation or absence of the type I spectral change in the microsomes from the respective tissue. Thus, the addition of hexobarbital or aminopyrine to liver microsomes and the addition of cortisol to adrenal microsomes produced the type I spectral change and enhanced the rate of cytochrome P-450 reduction. On the other hand, hexobarbital and aminopyrine, which did not yield spectral changes in adrenal microsomes, and cortisol and cortisol succinate, which produced only weak or no type I spectra in liver microsomes, did not augment the rate of cytochrome P-450 reduction in these microsomes. Similarly, *p*-chloro-*N*-methylaniline, which yields a type II spectrum with adrenal and liver microsomes, either had no effect or, at higher concentrations, inhibited the rate of cytochrome P-450 reduction.

It is of particular interest that a given compound, such as cortisol, can induce different spectral changes in microsomes from the same tissues in different species and in microsomes from different tissues in the same species. Whereas with guinea pig adrenal microsomes both cortisol and cortisol succinate elicit pronounced type I spectra (see Table 3), cortisol produces a weak type II spectrum in bovine adrenal cortex microsomes (30). In rat liver microsomes we were able to substantiate the findings of Schenkman *et al.* (3) that cortisol yields a "modified" type II spectrum. On the other hand, with guinea pig liver microsomes, both cortisol and cortisol succinate at times yielded type I spectra of low magnitude or elicited no spectral changes, whereas we only occasionally observed a type II spectrum.

In the guinea pig both adrenal and liver preparations are able to convert cortisol to 2α-hydroxy and 6β-hydroxy derivatives (17–19). The rate of hydroxylation of cortisol was substantially higher with adrenal preparations than with liver preparations. A possible cause of these differences may be related to the low degree of interaction of cortisol with liver microsomes compared to that with adrenal microsomes, as observed in our spectral studies.

The fact that aminopyrine is metabolized by both adrenal and liver microsomes demonstrates that spectral changes may not necessarily reflect the ability of a monooxygenase to metabolize a given compound. Like aminopyrine, hexobarbital was found to yield a type I spectrum with rat liver (3, 10) or guinea pig liver (see Table 3) but produced no spectral changes with adrenal microsomes. To our knowledge, there have been no studies which compare the rate of metabolism of hexobarbital by guinea pig liver and adrenal microsomes. However, in

preliminary experiments we observed that adrenal microsomes metabolize hexobarbital at a low rate. Furthermore, the possibility that binding of hexobarbital or of aminopyrine can occur without an apparent spectral change was investigated. The observations that in adrenal microsomes hexobarbital interferes with the induction of type I spectra by cortisol (see Fig. 5 and Table 4) and that both aminopyrine and hexobarbital interfere with the spectral changes produced by cortisol succinate[4] suggest that binding without a concomitant detectable spectral change may occur. Support for this hypothesis can also be derived from the finding that the interference with the cortisol-induced spectra in adrenal microsomes by hexobarbital can be diminished and eventually almost eliminated by increasing the concentration of cortisol. Such binding of hexobarbital or of aminopyrine may occur at the same site which normally binds cortisol, or at a different site, with a resultant alteration in the binding affinity of cortisol by adrenal microsomes. Presently we cannot distinguish between these two possibilities.

ACKNOWLEDGMENTS

The authors would like to thank Professor Lars Ernster for helpful discussions. The expert assistance of Mrs. Hjördis Thor and Miss Margareta Sparthan is gratefully acknowledged.

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[4] Unpublished observations.