MOLECULAR PHARMACOLOGY, 12, 649-657

Drug Metabolism in Liver Tumors

Resolution of Components and Reconstitution of Activity

SUSAN E. SAINE AND HENRY W. STROBEL

Department of Biochemistry and Molecular Biology, The University of Texas Medical School at Houston, Houston, Texas 77030

(Received December 10, 1975)

SUMMARY

SAINE, SUSAN E. & STROBEL, HENRY W. (1976) Drug metabolism in liver tumors. Resolution of components and reconstitution of activity. *Mol. Pharmacol.*, 12, 649-657.

The microsomal drug metabolism system has been resolved from Hepatoma 5123 t.c.(H) into its component enzymes: cytochrome P-450 and NADPH-cytochrome P-450 reductase. Reconstitution of benzphetamine hydroxylation activity with these two hepatoma proteins requires the presence of phosphatidylcholine. The hepatoma cytochrome P-450 has been purified 12-fold and can be substituted for liver cytochrome P-450 in a reconstituted system containing phosphatidylcholine and liver reductase. Hepatoma cytochrome P-450 reductase has been purified 130-fold. The purified reductase has an apparent minimum molecular weight of about 80,000 and is free of cytochromes P-450 and P-420. The purified reductase catalyzes electron transfer to the artificial electron acceptors cytochrome *c*, dichlorophenolindophenol, and ferricyanide; the K_m concentrations required by the hepatoma reductase to generate half the maximal velocity for each acceptor are similar to those required by purified liver reductase. The K_m value of the hepatoma reductase for NADPH (76 μM), however, is an order of magnitude higher than that of the purified liver reductase (7.8 μM). Hepatoma reductase can substitute for liver reductase in a reconstituted benzphetamine hydroxylation system containing liver cytochrome P-450 and phosphatidylcholine. Reconstitution of benzphetamine hydroxylation activity from hepatoma components was shown to require, like the liver system, the presence of both cytochrome P-450 and NADPH-cytochrome P-450 reductase, as well as phosphatidylcholine, to achieve the maximal hydroxylation rate. In a benzphetamine hydroxylation system containing liver cytochrome P-450, hepatoma cytochrome P-450 reductase, and phosphatidylcholine, the K_m values for benzphetamine and NADPH were shown to be 390 and 15 μM, respectively. The value for benzphetamine is similar to that obtained in a reconstituted liver system, while the value for NADPH is an order of magnitude higher than that determined for the liver system.

INTRODUCTION

The cytochrome P-450-containing, mixed-function oxidase system of liver endoplasmic reticulum has been shown to catalyze metabolism of fatty acids and steroids as well as a variety of foreign compounds, including drugs, insecticides, and carcinogens (1), in the presence of NADPH and molecular oxygen. The drug metabolism system was shown to be composed of three components: cytochrome P-450, NADPH-cytochrome P-450 reductase, and phosphatidylcholine (2-4). Furthermore, metabolism of a variety of drugs and other compounds was shown to be cata-

This research was supported by Grant DRG-1258 from the Damon Runyon Memorial Fund and by a grant from the Pat Rutherford Fund.

lyzed *in vitro* by a system reconstituted from these components (4–6). Cytochrome P-450 has been purified to homogeneity from rabbit liver (7–10) and to near homogeneity from rat liver (11). NADPH–cytochrome P-450 reductase has been prepared to about 75% purity from rat liver by Vermilion and Coon (12) and purified to homogeneity in our laboratory (13).

Cytochrome P-450-containing oxidase systems have been found in a vareity of other tissues, including yeast (14, 15), bacteria (16), kidney (17), adrenal mitochondria (18), and transplantable hepatomas (19). The demonstration of metabolism of chemotherapeutic agents by hepatoma oxidase systems suggests the importance of tumor drug metabolism systems to management of metastatic disease. Sugimura *et al.* (20) demonstrated the metabolism of aniline in Hepatomas 7794A, 7316A, 7793, and 7795. Conney and Burns (21) demonstrated induction of azo dye *N*-demethylase activity in Hepatoma 5123 by 3-methylcholanthrene, and Rogers *et al.* (22) obtained a 3-fold induction of hexobarbital metabolism in Hepatoma 7800 by phenobarbital. Watanabe *et al.* (23) studied benzpyrene hydroxylation activity and its induction under controlled feeding conditions in several hepatomas. Their studies indicated that benzpyrene hydroxylation activity was present, although at a lower level than in liver, and was inducible by 3-methylcholanthrene in Hepatomas 9618A, 7800, 7794A, and 8999. Miyake *et al.* (24) catalogued the amounts of microsomal NADH- and NADPH-dependent flavoprotein activities and the specific contents of cytochromes P-450, P-420, and b_5 in Hepatomas 5123C, 7777, and R3B. These authors, however, found no induction of the metabolism of substrates (benzphetamine, aminopyrine, or benzpyrene) in liver tumors, in contrast to other reports (21–23). This paper presents the resolution of the tumor drug metabolism system into components, the characterization of components, and the reconstitution of drug metabolism activity *in vitro*.

MATERIALS AND METHODS

Cytochrome P-450 concentration was determined by the method of Omura and Sato (25). In samples in which the presence of hemoglobin interfered with the cytochrome P-450 determination by the usual procedure, the method of Miyake *et al.* (24) was used. In this procedure, both the sample and reference cuvettes are saturated with carbon monoxide and only the sample cuvette is reduced with dithionite, thus canceling the contribution of carboxyhemoglobin to the spectrum. An extinction coefficient of 91 mM^{-1} cm^{-1} for $A_{450} - A_{490}$ was used for both procedures.

Cytochrome *c* reduction was measured at 550 nm, using an extinction coefficient of 21,000 M^{-1} cm^{-1} (26); dichlorophenolindophenol reduction, at 600 nm, using an extinction coefficient of 21,000 M^{-1} cm^{-1} (27); and ferricyanide reduction, at 420 nm, using an extinction coefficient of 1020 M^{-1} cm^{-1} (28). Epinephrine cooxidation was measured at 480 nm, using an extinction coefficient of 4020 M^{-1} cm^{-1} (29, 30). Benzphetamine hydroxylation was measured spectrophotometrically at 340 nm as benzphetamine-dependent NADPH oxidation (5), using an extinction coefficient of 6200 M^{-1} cm^{-1}. All assays were performed at 30°. One unit of reductase activity is defined as that amount of enzyme which catalyzes the reduction of 1 μmole of cytochrome *c* per minute at 30° and pH 7.7.

Cytochrome b_5 was determined from the dithionite-reduced difference spectrum, using an extinction coefficient of 185 mM^{-1} cm^{-1} for $A_{424} - A_{409}$ (31). Total heme was determined as the pyridine hemochrome (25).

Protein concentrations in microsomal preparations were determined by the method of Lowry *et al.* (32); the method of Shaffner and Weissman (33) was used to determine protein in all other preparations. Bovine serum albumin was used as a protein standard. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed by the method of Weber and Osborn (34).

Horse heart cytochrome *c*, NADPH, and alumina Cγ gel were purchased from Sigma Chemical Company. Renex 690 (polyoxyethylene alkylaryl ether) was obtained from Emulsion Engineering, Inc. Dilauroylphosphatidylcholine was purchased from Serdary Research Laborato-

ries. Benzphetamine hydrochloride was the gift of Dr. J. W. Hinman of the Upjohn Company. Phenobarbital was obtained from Merck. DEAE-Sephadex A-25 and QAE-Sephadex A-25 were purchased from Pharmacia; hydroxylapatite (Bio-Gel HTP) and Agarose (Bio-Gel A-0.5m) were obtained from Bio-Rad Laboratories.

Hepatoma 5123 t.c.(H) was obtained from Dr. Robert A. Hickie of The University of Saskatchewan and grown as subcutaneous implants in the inguinal and axillary regions of 100-g Buffalo rats. Tumor-bearing rats were treated with phenobarbital (75 mg/kg of body weight) by a single injection daily for 3 days, followed by two injections per day for 2 days immediately prior to death. The animals were killed by decapitation, tumors were removed and freed of connective and necrotic tissue, and large clumps were dispersed by passage through a tissue press. Cells were ruptured in phosphate-buffered NaCl by passage through a French pressure cell. Microsomes were prepared by differential centrifugation, suspended in 0.25 M sucrose, and used immediately for preparation of enzymes.

The drug metabolism system of hepatoma 5123 t.c.(H) was solubilized with 1% Renex 690, and the protein components were resolved by DEAE-Sephadex A-25 chromatography. Hepatoma cytochrome P-450 reductase was prepared as previously described (13), with the inclusion of 0.1 mM phenylmethyl sulfonyl fluoride in all buffers. For preparation of hepatoma cytochrome P-450, the flow-through and wash from the DEAE-Sephadex A-25 column were loaded on a 20-ml hydroxylapatite column equilibrated with 0.01 M potassium phosphate buffer, pH 7.7, containing 20% glycerol, 0.1 mM dithiothreitol, 1.0 mM EDTA, 0.1 mM PMSF,[1] and 0.1% Renex 690. All buffers used throughout the procedure contained 20% glycerol, 0.1 mM dithiothreitol, 1.0 mM EDTA, and 0.1 mM PMSF. The hydroxylapatite column was washed with equilibration buffer, and the P-450 was eluted with 0.3 M potassium phosphate buffer, pH 7.7, containing 0.1% Renex 690. The P-450-containing fractions were pooled, diluted with 5 volumes of 20% glycerol, 0.1 mM dithiothreitol, 1.0 mM EDTA, and 0.1 mM PMSF, and loaded on a 5-ml hydroxylapatite column which was equilibrated with 0.01 M potassium phosphate buffer, pH 7.7. The flow-through and wash contained about 50% of the cytochrome P-450 and were concentrated by adsorption on alumina Cγ gel and elution with 0.3 M potassium phosphate buffer, pH 7.7, containing 0.2% sodium cholate. The P-450 adsorbed to the hydroxylapatite column was eluted with 0.3 M potassium phosphate buffer, pH 7.7, containing 0.1% sodium cholate.

Liver cytochrome P-450 was prepared by the method of Lu *et al.* (35), and liver cytochrome P-450 reductase was prepared as described previously (13).

[1] The abbreviation used is: PMSF, phenylmethyl sulfonyl fluoride.

RESULTS

Resolution of tumor drug metabolism system. The phenobarbital-induced hepatoma microsomes used in these studies showed a 2–4-fold induction of the components of the drug metabolism system, NADPH–cytochrome P-450 reductase and cytochrome P-450. Cytochrome P-450 reductase activity, measured as the rate of reduction of cytochrome *c*, was induced from 18 to 76 nmoles of cytochrome *c* reduced per minute per milligram, and cytochrome P-450 content, from 0.2 to 0.9 nmole/mg. The hepatoma microsomes were solubilized, treated with protamine sulfate, and resolved by DEAE-Sephadex A-25 column chromatography into cytochrome P-450 and NADPH–cytochrome P-450 reductase fractions as shown in Fig. 1 and described under MATERIALS AND METHODS. The scheme in Fig. 1 outlines the further purification steps for the reductase and cytochrome P-450 fractions. The resolution of cytochrome P-450 and cytochrome P-450 reductase is based on the binding of the reductase but not of the cytochrome P-450 to the DEAE-Sephadex column under the conditions employed.

Hepatoma cytochrome P-450. The fraction with the highest specific content of cytochrome P-450 (1.0 nmole/mg) was eluted from the second hydroxylapatite column with 0.3 M potassium phosphate

buffer. The absorption maximum of the reduced carbon monoxide difference spectrum of this fraction (Fig. 2) occurs at 451 nm. This fraction was purified 12-fold over the starting microsomes (0.08 nmole/mg). The cytochrome P-450 preparation had a heme content of 1.74 nmoles/mg of protein, indicating the presence of some additional heme protein, and also contained low but detectable amounts of NADPH- and NADH-dependent cytochrome *c* reductase activities. A fraction with a lower specific content of cytochrome P-450 was obtained by alumina Cγ gel concentration of the cytochrome P-450 fraction washed through the second hydroxylapatite column. This fraction had a specific content of 0.73 nmole/mg. The total yield of cytochrome P-450 was about 6%.

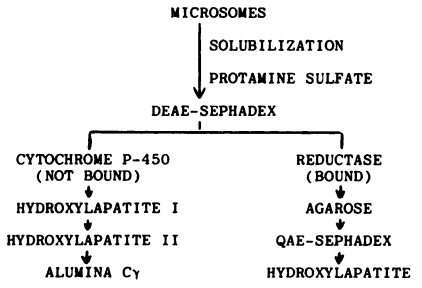

FIG. 1. *Scheme for resolution and purification of hepatoma drug metabolism system*

Hepatoma cytochrome P-450 reductase. The purification procedure for hepatoma NADPH-cytochrome P-450 reductase is summarized in Table 1. Solubilization with Renex 690 and protamine sulfate treatment of hepatoma reductase were ac-

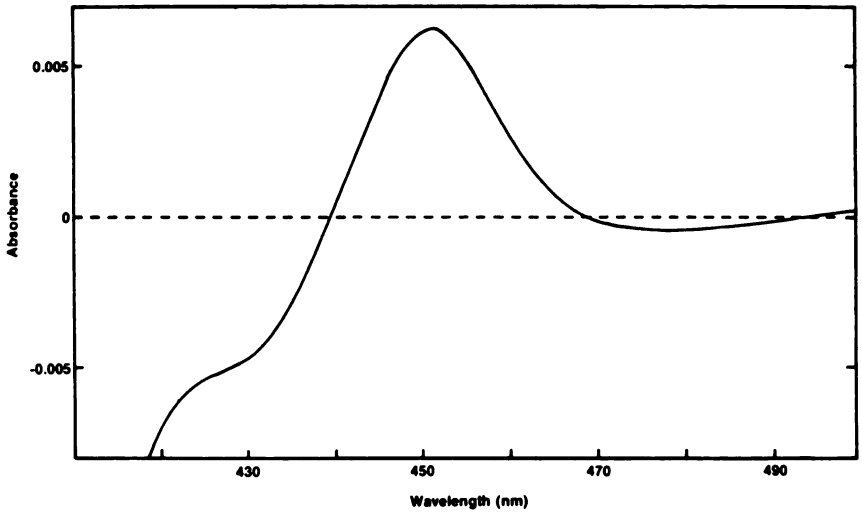

FIG. 2. *Carbon monoxide difference spectrum of Hepatoma 5123 t.c.(H) cytochrome P-450 (0.0703 nmole, 0.0677 mg of protein)*

TABLE 1

Purification of NADPH-cytochrome P-450 reductase from Hepatoma 5123 t.c.(H) microsomes

Preparation	Protein	Total activity	Specific activity	Yield (%)
	mg	*μmoles cytochrome c reduced/min*	*μmoles cytochrome c reduced/min/mg protein*	
Microsomes	1070	72.9	0.068	100
Solubilized microsomes	726	72.9	0.1004	100
DEAE-Sephadex A-25 eluate	36.8	31.9	0.868	44
Agarose A-0.5m	3.47	23.9	6.9	33
QAE-Sephadex A-25; hydroxylapatite eluate	0.792	6.86	8.66	9.6

complished without loss of activity. The hepatoma reductase was purified 130-fold, with a 10% yield, by this procedure. In experiments not shown here, sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the purified hepatoma reductase showed a single major band, accounting for more than 65% of the protein, along with some minor bands. Electrophoresis on gels calibrated with standards of known molecular weight indicated that the major polypeptide has an apparent minimum molecular weight of about 80,000. The properties of the hepatoma reductase are shown in Table 2. NADH-cytochrome *c* reductase activity is absent from this preparation. Neither cytochrome P-450 nor cytochrome P-420 could be detected, although some cytochrome b_5 was identifiable as the heme contaminant in this preparation. The ability of the purified reductase to transfer electrons to various acceptors is shown in Table 3. The K_m values of the hepatoma reductase for electron transfer to the artificial acceptors cytochrome *c*, dichlorophenolindophenol, and potassium ferricyanide and the cooxidant epinephrine are almost identical with the values for purified liver reductase, although the calculated V_{max} values of the hepatoma reductase are lower than those for liver reductase. The hepatoma reductase, however, has a K_m for the electron donor, NADPH, which is an order of magnitude higher than that of the liver reductase (7.75 μM) when cytochrome *c* is used as the electron acceptor.

Reconstitution of drug metabolism activity. The reconstitution of drug metabolism activity from the resolved hepatoma reductase and cytochrome P-450 fractions, as judged by benzphetamine-dependent NADPH oxidation, is shown in Table 4. Full benzphetamine hydroxylation activity requires the presence of both protein components and phosphatidylcholine. When one of the components is omitted, the resultant activity is markedly reduced or eliminated. The low levels of activity observed in the absence of reductase or lipid are consistent with the presence of small amounts of reductase or lipid in the cytochrome P-450 fraction. It is clear, however, that the reconstituted hepatoma system, like the reconstituted drug metabolism system from liver, does require the presence of the lipid factor phosphatidylcholine (5, 6).

The partially purified hepatoma cytochrome P-450 and cytochrome P-450 reductase are interchangeable with liver cytochrome P-450 and reductase (Table 5). Hepatoma cytochrome P-450 can catalyze

TABLE 2

Properties of partially purified Hepatoma 5123 t.c.(H) cytochrome P-450 reductase

Component	Concentration or activity
	(mg protein)$^{-1}$
NADPH-cytochrome *c* reductase	8.66 μmoles/min
NADH-cytochrome *c* reductase	ND[a]
Cytochrome P-450	ND
Cytochrome P-420	ND
Cytochrome b_5	1.91 nmoles
Total heme	2.0 nmoles

[a] Not detectable.

TABLE 3

Kinetic properties of Hepatoma 5123 t.c.(H) NADPH-cytochrome P-450 reductase in artificial systems

Reaction mixtures contained 300 μmoles of potassium phosphate buffer (pH 7.7), reductase as indicated, and the acceptor in a final volume of 1.0 ml. The reaction was started by the addition of NADPH, and the rate was measured spectrophotometrically at 30°. The specific activity of the reductase was 3.4 μmoles of cytochrome *c* reduced per minute per milligram of protein.

Substrate	K_m	V_{max}
	M	*μmoles/min/ mg protein*
Cytochrome *c*[a]	1.19×10^{-5}	4.63
Dichlorophenol-indophenol[a,b]	1.81×10^{-5}	4.28
Potassium ferricyanide[b,c]	2.53×10^{-5}	17.7
Epinephrine[d]	3.33×10^{-4}	0.642
NADPH[e]	7.57×10^{-5}	4.31

[a] 0.00475 unit, 1.58 μg of protein.

[b] The reaction mixtures contained 0.1 mg of bovine serum albumin.

[c] 0.0178 unit, 5.92 μg of protein.

[d] 0.119 unit, 39.5 μg of protein.

[e] 0.00592 unit, 1.97 μg of protein. Cytochrome *c* (40 μM) was used as the electron acceptor.

TABLE 4

Benzphetamine hydroxylation by reconstituted enzyme system resolved from Hepatoma 5123 t.c.(H) microsomes

Reaction mixtures contained 100 μmoles of potassium phosphate buffer (pH 7.7), cytochrome P-450 (0.137 nmole, 0.187 mg of protein), reductase (0.086 unit, 0.010 mg of protein), dilauroylphosphatidylcholine (30 μg), 1.0 μmole of benzphetamine, and 0.15 μmole of NADPH in a final volume of 1.0 ml. NADPH oxidation was measured spectrophotometrically at 30°. Activities are corrected for the rate of NADPH oxidation in the absence of benzphetamine.

System	Activity	Relative activity
	nmoles NADPH oxidized/min	%
Complete	0.644	100
No P-450	0	0
No reductase	0.161	25
No lipid	0.241	37
No benzphetamine	0	0

TABLE 5

Interchangeability of cytochrome P-450 and cytochrome P-450 reductase components partially purified from liver and Hepatoma 5123 t.c.(H) microsomes in reconstituted benzphetamine hydroxylation system

Reaction mixtures contained 100 μmoles of potassium phosphate buffer (pH 7.7), 30 μg of dilauroylphosphatidylcholine, reductase and cytochrome P-450 as indicated, 1.0 μmole of benzphetamine, and 0.15 μmole of NADPH in a final volume of 1.0 ml. NADPH oxidation was measured spectrophotometrically at 30°. Activities are corrected for the rate of NADPH oxidation in the absence of benzphetamine.

Hepatoma 5123 t.c.(H)		Liver		Activity
Reductase[a]	P-450	Reductase[b]	P-450[c]	
				nmoles NADPH oxidized/min
+	+[d]	−	−	0.644
+	−	−	+	2.74
−	+[e]	+	−	1.13
−	−	+	+	7.54

[a] 0.086 unit, 0.010 mg of protein.
[b] 0.28 unit, 0.0068 mg of protein.
[c] 0.20 nmole, 0.0475 mg of protein.
[d] 0.137 nmole, 0.187 mg of protein.
[e] 0.121 nmole, 0.212 mg of protein.

drug metabolism when reconstituted *in vitro* with liver cytochrome P-450 reductase and lipid. Liver cytochrome P-450 can catalyze benzphetamine hydroxylation when electrons are transferred to cytochrome P-450 by liver or hepatoma reductase in a system reconstituted in the presence of phosphatidylcholine. The data in Fig. 3 show that the rate of NADPH oxidation by a reconstituted system containing liver cytochrome P-450 and phosphatidylcholine in excess is proportional to the amount of hepatoma cytochrome P-450 reductase present. The addition of reductase in concentrations higher than those shown in Fig. 3 caused an inhibition of the rate of hydroxylation.

The kinetic properties of the reconstituted benzphetamine hydroxylation system containing hepatoma cytochrome P-450 reductase and liver cytochrome P-450 are shown in Table 6. The K_m value for

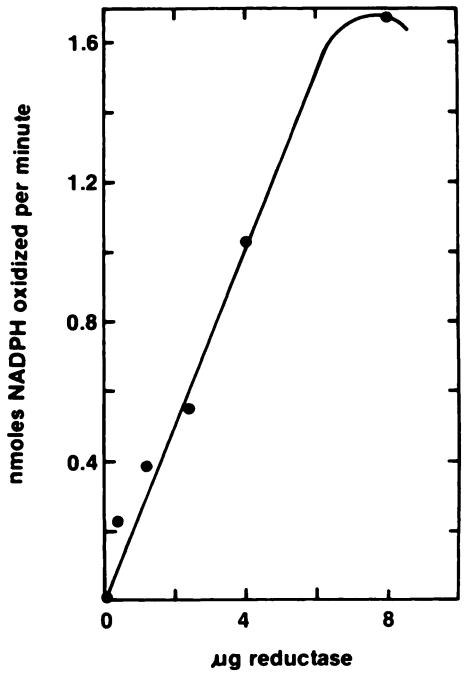

FIG. 3. *Benzphetamine hydroxylation as a function of Hepatoma 5123 t.c.(H) reductase*

Reaction mixtures contained 30 μg of dilauroylphosphatidylcholine and 0.02 nmole of liver cytochrome P-450 (47.5 μg of protein) while the reductase was varied. Activites are corrected for the rate of NADPH oxidation in the absence of reductase.

TABLE 6

Kinetic properties of benzphetamine hydroxylation system reconstituted from liver cytochrome P-450 and hepatoma reductase

Reaction mixture contained 100 μmoles of potassium phosphate buffer (pH 7.7), 30 μg of dilauroylphosphatidylcholine, and rat liver cytochrome P-450, hepatoma reductase, NADPH, and benzphetamine as indicated in a final volume of 1.0 ml. NADPH oxidation was measured spectrophotometrically at 30°.

Component	K_m
	M
Cytochrome P-450[a]	2.7×10^{-7}
NADPH[b]	1.54×10^{-5}
Benzphetamine[c]	3.94×10^{-4}

[a] 11.85 μg of reductase, 1.0 μmole of benzphetamine, and 0.15 μmole of NADPH.

[b] 7.9 μg of reductase, 47.5 μg of P-450, and 1.0 μmole of benzphetamine.

[c] 19.75 μg of reductase, 47.5 μg of P-450, and 0.15 μmole of NADPH.

NADPH in this system is an order of magnitude higher than the value for a system reconstituted from liver cytochrome P-450 and liver reductase (1.25 μM) confirming the elevated reductase K_m for NADPH observed with artificial electron acceptors. On the other hand, the K_m value for benzphetamine shown in Table 6 is in good agreement with previously published values for a benzphetamine hydroxylation system reconstituted from liver cytochrome P-450 and cytochrome P-450 reductase (6).

DISCUSSION

Not only has the presence of drug and polycyclic hydrocarbon metabolism been observed in a variety of liver tumors by several laboratories (19, 23), but the induction of these activities by phenobarbital and 3-methylcholanthrene has also been demonstrated (22, 23). Induction of drug or polycyclic hydrocarbon metabolism in liver microsomes by phenobarbital or 3-methylcholanthrene is accompanied by a several-fold increase in cytochrome P-450 or cytochrome P-448, respectively (36, 37), and in cytochrome P-450 reductase activity (5). Our results indicate that phenobarbital induction causes a 3–4-fold increase in Hepatoma 5123 t.c.(H) cytochrome P-450, measured by carbon monoxide difference spectroscopy, and in hepatoma cytochrome P-450 reductase, measured by cytochrome *c* reduction. This induction of reductase activity and cytochrome P-450 content in hepatoma microsomes constitutes a considerable increase over the uninduced levels of these activities. However, both cytochrome P-450 content and cytochrome P-450 reductase in the induced and uninduced hepatoma are lower than in the induced and uninduced liver.

Although induction of hepatoma mixed-function oxidase activity has been observed by other workers, to our knowledge this paper constitutes the first report of resolution and reconstitution *in vitro* of hepatoma drug metabolism, as judged by benzphetamine hydroxylation. After solubilization with Renex 690, the two enzyme components were resolved by their differing affinities for DEAE-Sephadex A-25 and identified as cytochrome P-450 and cytochrome P-450 reductase by their spectral properties and enzymatic activities. The response of the hepatoma system to Renex 690 solubilization and DEAE-Sephadex chromatography is highly similar to that of the microsomal system of liver (13). Further similarity between the liver and hepatoma systems is apparent in the requirement of phosphatidylcholine to reconstitute benzphetamine hydroxylation activity in a system *in vitro* containing hepatoma cytochrome P-450 and hepatoma cytochrome P-450 reductase.

The highly purified hepatoma cytochrome P-450 reductase has an apparent minimum molecular weight (80,000) identical with that determined for homogeneous liver reductase (13). Furthermore, the hepatoma reductase can catalyze the reduction of many artificial electron acceptors, requiring about the same concentrations of acceptors to achieve half-maximal rates as the liver enzyme. The maximal rates of reduction catalyzed by the hepatoma reductase are lower than those catalyzed by the liver reductase. The significance of lower V_{max} values must, however, await further purification of the hepatoma reductase.

The hepatoma reductase requires an order of magnitude more NADPH to support reduction than does liver reductase. That this altered K_m for NADPH is indeed a property of the reductase is shown by the lack of dependence of this requirement on the acceptor utilized. When liver cytochrome P-450 and hepatoma cytochrome P-450 reductase are reconstituted with phosphatidylcholine in a benzphetamine hydroxylation system *in vitro*, the K_m of NADPH (Table 6) is also an order of magnitude higher than for a system containing liver cytochrome P-450 and liver reductase, while the K_m for benzphetamine (Table 6) is identical with that of the all-liver system (6). These data suggest that although the hepatoma reductase is similar to the liver reductase in many ways, its binding site for NADPH may be altered in such a way that the hepatoma reductase has a reduced affinity for the electron donor. The reduced affinity of the reductase for NADPH could be a significant cause of the lowered level of activity of the tumor drug metabolism system.

The data presented in this paper demonstrate the presence of drug metabolism activity in hepatoma 5123 t.c.(H) by the resolution and partial purification of the component enzymes, cytochrome P-450 and cytochrome P-450 reductase, and the reconstitution of benzphetamine hydroxylation *in vitro*, using the resolved purified components and phosphatidylcholine. These data are in agreement with the reports of others who also concluded that various hepatomas have competent drug and carcinogen metabolism systems (19–23), and are at variance with the suggestion that the drug metabolism system is not present or not functional in hepatomas (24, 38–40).

ACKNOWLEDGMENTS

We wish to acknowledge the skillul technical assistance of Mr. Tracy Lanagan.

REFERENCES

1. Gillette, J. R., Davis, D. C. & Sasame, H. A. (1972) *Annu. Rev. Pharmacol.*, 12, 57–84.
2. Lu, A. Y. H. & Coon, M. J. (1968) *J. Biol. Chem.* 243, 1331–1332.
3. Lu, A. Y. H., Junk, K. W. & Coon, M. J. (1969) *J. Biol. Chem.* 244, 3714–3721.
4. Strobel, H. W., Lu, A. Y. H., Heidema, J. & Coon, M. J. (1970) *J. Biol. Chem.*, 245, 4851–4854.
5. Lu, A. Y. H., Strobel, H. W. & Coon, M. J. (1969) *Biochem. Biophys. Res. Commun.*, 36, 545–551.
6. Lu, A. Y. H., Strobel, H. W. & Coon, M. J. (1970) *Mol. Pharmacol.*, 6, 213–220.
7. van der Hoeven, T. A. & Coon, M. J. (1974) *J. Biol. Chem.*, 249, 6302–6310.
8. van de Hoeven, T. A., Haugen, D. A. & Coon, M. J. (1974) *Biochem. Biophys. Res. Commun.*, 60, 569–575.
9. Haugen, D. A., van der Hoeven, T. A. & Coon, M. J. (1975) *J. Biol. Chem.*, 250, 3567–3570.
10. Imai, Y. & Sato, R. (1974) *Biochem. Biophys. Res. Commun.*, 60, 8–14.
11. Ryan, D., Lu, A. Y. H., Kawalek, J., West, S. B. & Levin, W. (1975) *Biochem. Biophys. Res. Commun.*, 64, 1134–1141.
12. Vermilion, J. L. & Coon, M. J. (1974) *Biochem. Biophys. Res. Commun.*, 60, 1315–1322.
13. Dignam, J. D. & Strobel, H. W. (1975) *Biochem. Biophys. Res. Commun.*, 63, 845–852.
14. Lebeault, J. M., Lode, E. T. & Coon, M. J. (1971) *Biochem. Biophys. Res. Commun.*, 42, 413–419.
15. Duppel, W., Lebeault, J. M. & Coon, M. J. (1973) *Eur. J. Biochem.*, 36, 583–592.
16. Tyson, C. A., Lipscomb, J. D. & Gunsalus, I. C. (1972) *J. Biol. Chem.*, 247, 5777–5784.
17. Ellin, A., Jakobsson, S. B., Schenkman, J. B. & Orrenius, S. (1972) *Arch. Biochem. Biophys.*, 150, 64–71.
18. Omura, T., Sato, R., Cooper, D. Y., Rosenthal, O. & Estabrook, R. W. (1965) *Fed. Proc.*, 24, 1181–1189.
19. Morris, H. P. (1965) *Adv. Cancer Res.*, 9, 227–302.
20. Sugimura, T., Ikeda, K., Horota, K., Hozumi, M. & Morris, H. P. (1966) *Cancer Res.*, 26, 1711–1716.
21. Conney, A. H. & Burns, J. J. (1963) *Adv. Enzyme Regul.*, 1, 189–214.
22. Rogers, L. A., Morris, H. P. & Fouts, J. R. (1967) *J. Pharmacol. Exp. Ther.*, 157, 227–244.
23. Watanabe, M., Potter, V. R. & Morris, H. P. (1970) *Cancer Res.*, 30, 263–273.
24. Miyake, Y., Gaylor, J. L. & Morris, H. P. (1974) *J. Biol. Chem.*, 249, 1980–1987.
25. Omura, T. & Sato, R. (1964) *J. Biol. Chem.*, 239, 2379–2385.
26. Williams, C. H. & Kamin, H. (1962) *J. Biol. Chem.*, 237, 587–595.
27. Steyn-Parve, E. P. & Beinert, H. (1958) *J. Biol. Chem.*, 233, 843–852.
28. Schellenberg, K. A. & Hellerman, L. (1958) *J.*

Biol. Chem., **231**, 547–556.
29. McCord, J. M. & Fridovitch, I. (1969) *J. Biol. Chem.*, **244**, 6049–6055.
30. Green, S., Mazur, A. & Shorr, E. (1956) *J. Biol. Chem.*, **220**, 237–255.
31. Omura, T. & Sato, R. (1964) *J. Biol. Chem.*, **239**, 2370–2378.
32. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265–275.
33. Shaffner, W. & Weissman, C. (1973) *Anal. Biochem.*, **56**, 502–514.
34. Weber, K. & Osborn, M. (1969) *J. Biol. Chem.*, **244**, 4406–4412.
35. Lu, A. Y. H., Kuntzmann, R., West, S. & Conney, A. H. (1971) *Biochem. Biophys. Res. Commun.*, **42**, 1200–1206.
36. Sladek, N. E. & Mannering, G. J. (1969) *Mol. Pharmacol.*, **5**, 174–185.
37. Sladek, N. E. & Mannering, G. J. (1969) *Mol. Pharmacol.*, **5**, 186–199.
38. Adamson, R. H. & Fouts, J. R. (1961) *Cancer Res.*, **21**, 667–672.
39. Bowers, J. A. J. & Emmelot, P. (1960) *Exp. Cell Res.*, **19**, 467–474.
40. Neubert, D. & Hoffmeister, I. (1960) *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.*, **239**, 234–244.