Biochemical Pharmacology, Vol. 24, pp. 227–233. Pergamon Press, 1975. Printed in Great Britain.

COMPARATIVE STUDIES ON THE OXIDATION AND REDUCTION OF DRUG SUBSTRATES IN HUMAN PLACENTAL VERSUS RAT HEPATIC MICROSOMES*

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(*Received* 22 *March* 1974; *accepted* 14 *June* 1974)

Abstract—Human placental microsomes prepared by conventional methods were compared with analogous preparations from adult, male rat livers with respect to biochemical components and systems which could affect rates of mixed-function oxidation and reduction of drugs and steroids *in vitro*. Each of the electron transport components required for the mixed-function oxidation of drugs in hepatic systems was present in lower concentrations in placental than hepatic microsomes. In contrast to hepatic microsomes, placental microsomes which exhibited unusually high aryl hydrocarbon hydroxylase activities did not contain increased concentrations of the electron transport components. Evidence was provided to indicate that rapid degradation of initial electron donors (reduced pyridine nucleotides) was not responsible for the observance of low or negligible drug metabolic activities observed in incubations with placental microsomes. Cytochromes P-450 and b_5 and their corresponding reductases were shown to be present in human placental preparations. NADPH and NADH-dependent cytochrome c reductase activities in placental microsomes were somewhat lower but comparable to those determined in hepatic preparations. However, cytochromes b_5 and P-450 and contaminating hemoglobin and methemoglobin accounted for less than 56 per cent of the total heme present in placental microsomes. Rapid degradation of placental cytochrome P-450 was observed at 37° in the presence of sodium hydrosulfite, but conversion to cytochrome P-420 was minimal after incubation for 1 hr in the presence of NADPH at the same temperature. It was considered probable that the low rates of drug biotransformation observed would be explicable in terms of high substrate specificities of the placental enzymic components.

Considerable confusion appears to exist concerning the capacity of the human placenta to catalyze mixed-function oxidation or reduction reactions with various typical foreign compounds as substrates because of seemingly conflicting reports in the literature. For an extensive discussion of this topic, the reader is referred to a recent review [1]. In view of the uncertainty in this area of research, surprisingly few studies have appeared in the literature in which attempts were made to evaluate possible reasons for failures to detect significant drug metabolic activity in cases in which negative results were obtained. A number of possible explanations for observed low or negligible activities have been alluded to but not investigated thoroughly. These include the reported inability to detect cytochrome P-450 in microsomal fractions of placental homogenates at term [2–5] and during early gestation [6]. Others have reported, however, that this important cytochrome is present in human placental microsomal subfractions [7–10] and that its presence in these preparations does not result from cross-contamination of the microsomal fraction with mitochondrial cytochrome P-450 [11].

Inability to detect significant drug oxidation or reduction in placental homogenates or subfractions might also be explained on several other bases including: rapid degradation of reduced pyridine nucleotides in incubation vessels; rapid degradation of cytochrome P-450 during incubation; lack or instability of specific flavoproteins involved in requisite electron transport; absence of cytochrome b_5 from placental microsomes; high specificity of placental enzyme systems for endogenous substrates; and improper membrane-phospholipid environment for adequate binding of drug substrates to enzyme sites. The purpose of the present study was to investigate drug oxidation and reduction reactions in human placental microsomes with a consideration of several of the above-mentioned factors. The parameters investigated in the placental microsomes were quantitated in comparison with the same parameters measured in the extensively investigated microsomes of male rat livers. In addition, attempts were made to determine whether positive or negative

* This investigation was supported by Research Grants HD-04839, PHS, NIH; CRBS-250 National Foundation (March of Dimes); and Training Grant GM-01160, NIH.

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correlations could be discerned between placental aryl hydrocarbon hydroxylase activity and any of the other measured placental parameters.

MATERIALS AND METHODS

Tissues. Human placentas were obtained at term from the delivery room of the University Hospital, Seattle, Wash., after routine vaginal deliveries or cesarean sections. Homogenization and differential centrifugation procedures were carried out immediately according to schedule 3 described by Juchau and Smuckler [12]. Hepatic microsomes were prepared from adult male Sprague–Dawley rat livers according to the method described by Mazel [13].

Chemicals. All chemicals and solvents utilized were reagent grade and were of the highest purity commercially available. $NADP^+$, NADPH, NADH, FMN, FAD, cytochrome c, glucose 6-phosphate, methemoglobin, hemin and glucose 6-phosphate dehydrogenase were obtained from Sigma Chemical Co., St. Louis, Mo. Benzo(α)pyrene, *p*-nitrobenzoic acid (PNBA), *p*-aminobenzoic acid (PABA), *p*-aminophenol hydrochloride, aniline hydrochloride, coumarin, 7-hydroxycoumarin, aminopyrine, 4-aminoantipyrine and sulfanilamide were obtained from Eastman Organic Chemical Co., Rochester, N.Y. Neoprontosil was obtained as a gift from the Sterling–Winthrop Research Institute, Rensselaer, N.Y. Carbon monoxide (CO) was obtained from Matheson Chemical Co., Los Angeles, Calif., and *p*-(methylamino)-benzoic acid and *p*-(dimethylamino)-benzoic acid were purchased from Aldrich Chemical Co., Milwaukee, Wis.

Assay procedures. Analyses of the total flavin, FAD and FMN content of tissue microsomal fractions were performed spectrofluorometrically according to the procedures of Bessey *et al.* [14]. Concentrations of total microsomal heme were assayed according to methods described by Gilbert [15] except that the absorbance differences between the spectral maxima near 556 nm and minima near 540 nm were utilized in the calculations. The concentration of ferrous hemoglobin in microsomes was determined by dividing fraction samples into two stoppered cuvettes, bubbling CO through the sample cuvette for 60 sec, recording the difference spectrum in the Soret region and determining the absorbance difference between 419 and 433 nm. A molar extinction coefficient of $570{\cdot}8\ mM^{-1}\ cm^{-1}$ was utilized to calculate the concentration of carbon monoxyhemoglobin [16]. In order to determine the concentration of methemoglobin in microsomes, the isotonic KCl solution used to wash the same fraction (in the second wash) was first analyzed for ferrous hemoglobin content by the procedure described above. The percentage of methemoglobin in the wash was then determined by adding a few mg dithionite to the sample cuvette and calculating the difference between concentrations of carbon monoxyhemoglobin before and after the addition of dithionite. This percentage was extrapolated to the microsomes.

NADPH and NADH-dependent cytochrome c reductase activities were determined according to the procedures cited in *Methods in Enzymology* [17]. NADPH oxidase activity was measured spectrophotometrically according to the methods described by Gillette *et al.* [18].

Determinations of microsomal hemoproteins were performed by analyses of difference spectra with a model DW-2 recording spectrophotometer (American Instrument Co.). Each spectrum was calibrated with a holmium oxide filter. The methods described by Greim [19] (dithionite–CO minus CO) and by Omura and Sato [20] (dithionite–CO minus dithionite) were utilized for the analysis of cytochrome P-450. Cytochrome b_5 concentrations were determined by measuring reduced (NADH) minus oxidized absorbance differences between 427 and 409 nm [20].

Typical reaction mixtures for determinations of rates of reduction of PNBA to PABA consisted of 0·4 ml of a suspension of placental microsomes (25–30 mg protein/ml), 1·2 ml of 0·1 M potassium phosphate buffer (pH 7·35), $2{\cdot}3 \times 10^{-3}$ M PNBA and 10^{-3} M NADPH (final concentrations) in a total volume of 2 ml. For measurements of rates of reduction of neoprontosil to sulfanilamide, the reaction flasks contained the same components except that $1{\cdot}7 \times 10^{-3}$ M neoprontosil was utilized as the substrate. In addition, 5×10^{-6} M FMN was present in some of the reaction vessels. Both reactions were allowed to proceed for 30 min at 37·5° under an atmosphere of 100% nitrogen. The reactions were stopped by additions of 5 ml ice-cold 6·67% trichloroacetic acid. Amounts of PABA or sulfanilamide formed were assayed colorimetrically as previously described [21].

Quantities of PABA formed from the oxidative *N*-demethylations of *p*-(methylamino)benzoic acid and *p*-(dimethylamino)-benzoic acid were also determined by the method cited above [21]. A typical incubation mixture consisted of 0·8 ml placental microsomes (25–30 mg protein/ml, resuspended in potassium phosphate buffer), 0·2 ml of 0·1 M potassium phosphate buffer at pH 7·35, $3{\cdot}3 \times 10^{-3}$ M substrate, 92 μmoles glucose 6-phosphate (G-6-P), 40 μmoles nicotinamide, 20 μmoles magnesium chloride, 2 units G-6-P dehydrogenase and $2{\cdot}5 \times 10^{-3}$ M NADPH in a final volume of 2·0 ml. Several flasks also were incubated in the absence of added nicotinamide. Incubations continued for 2 hr at 37·5° under an atmosphere of 100% oxygen in a Dubnoff metabolic shaker (40–50 rev/min). The reaction was stopped by the addition of 5 ml ice-cold trichloroacetic acid.

Quantitative estimations of the amounts of *p*-aminophenol formed from aniline hydrochloride were assayed by methods described by Schenkman *et al.* [22]. Reaction flasks were incubated for 2 hr and contained 1·0 ml of a suspension of placental microsomes (20–25 mg protein/ml), 1·6 ml of 0·1 M potassium phosphate buffer (pH 7·35), $1{\cdot}4 \times 10^{-2}$ M aniline hydrochloride and $1{\cdot}2 \times 10^{-3}$ M NADPH in a total

volume of 3·3 ml. Several incubation flasks also contained 10^{-5} M FMN. Determinations of rates of hydroxylation of benzo(α)pyrene were performed according to previously described methods [23].

Rates of 7-hydroxylation of coumarin and of the *O*-dealkylation of 7-ethoxycoumarin or 7-methoxycoumarin were determined by spectrophotofluorometric assay of concentrations of 7-hydroxycoumarin (umbelliferone) formed in reaction vessels according to methods described by Creaven *et al.* [24] and by Ullrich and Weber [25]. Typical reaction vessels contained 0·6 ml of a suspension of placental microsomes (20–25 mg protein/ml), 1·0 ml of 0·1 M Tris–HCl buffer at pH 7·35, 9 μmoles G-6-P, 1 unit G-6-P dehydrogenase, 10^{-3} M NADPH and $1{\cdot}3 \times 10^{-4}$ M, 1×10^{-4} M and 5×10^{-4} M ethoxycoumarin, methoxycoumarin and coumarin respectively. These mixtures were incubated in air at 37·5° for 20–60 min, and the formation of the fluorescent 7-hydroxycoumarin (umbelliferone) was determined at 5-min intervals in an Aminco–Bowman spectrophotofluorometer.

Rates of aminopyrine *N*-demethylation and ω-oxidation of laurate were determined according to methods described by Juchau and Pedersen [26] and Lu *et al.* [27], respectively, except that higher concentrations of NADPH ($1–2 \times 10^{-3}$ M final concentrations) were present in the reaction vessels, and prepared microsomal fractions were employed in each case. For comparison and standardization purposes, determinations of rates of drug biotransformation in rat hepatic microsomes were performed under analogous reaction conditions and with the same substrate and cofactor concentrations as utilized for corresponding analyses of placental microsomes, except that a standard NADPH-regenerating system consisting of $2{\cdot}2 \times 10^{-2}$ M G-6-P, 10^{-2} M nicotinamide, $5{\cdot}3 \times 10^{-2}$ M magnesium sulfate (final concentrations) and 2 units G-6-P dehydrogenase was added to each reaction vessel. Reaction vessels contained 2–3 mg hepatic microsomal protein. Incubations were carried for 20 min in each case. The rates of all measurable reactions were linear with time and with protein concentrations.

$NADP^+$ pyrophosphatase activities were determined according to the method of Kornberg [28]. A typical reaction mixture contained microsomes (1 mg protein), 2×10^{-3} M NADPH and 0·1 M potassium phosphate buffer (pH 7·8) in a total volume of 1·0 ml. Sample flasks were incubated in air at 37° for 20 min. The reaction was stopped by placing the flasks in ice water. A 0·1-ml aliquot of the incubation mixture was added to a cuvette containing 2·8 ml of $2{\cdot}3 \times 10^{-2}$ M G-6-P. Absorbance readings at 340 nm were determined. Four units G-6-P dehydrogenase was added, and a second reading was taken after 5 min. Control flasks were treated in a similar fashion except that they were incubated at 0–4° rather than at 37°. Specific acitivites were determined by computing absorbance differences between sample and control flasks.

$NADP^+$ nucleosidase activities were determined by the method described by Kaplan [29]. Incubation flasks contained 0·4 mg microsomal protein, $8{\cdot}5 \times 10^{-4}$ M $NADP^+$ and 0·1 M potassium phosphate buffer (pH 7·35) in a total volume of 0·6 ml. The samples were incubated in air at 37° for 8 min. The samples were then transferred to a cuvette containing 2·0 ml of 1 M KCN and the absorbance at 325 nm was compared against a distilled water blank. Control samples were added directly to the solution of KCN.

Lipid peroxidase activities were determined according to the method of Ernster and Nordenbrand [30]. Reaction vessels contained 19 mg microsomal protein, 5×10^{-5} M NADPH and 0·05 M potassium phosphate buffer (pH 7·4) in a total volume of 4·0 ml and were incubated in air at 37° for 30 min. In addition, various flasks were incubated which contained both 1×10^{-4} M ADP and $1{\cdot}8 \times 10^{-5}$ M $FeCl_3$ (final concentrations). Reactions were stopped by the addition of 1 ml of 25% trichloroacetic acid. A 2·5-ml aliquot of the incubation mixture was then added to a flask containing 0·5 ml of 0·6 N HCl and 2·0 ml thiobarbituric acid reagent. This mixture was heated at 100° for 10 min and the absorbance of the sample was determined at 535 nm using distilled water as the blank. Control flasks were incubated concomitantly and contained heat-inactivated (100°, 5 min) microsomes.

All enzyme activities and concentrations of tissue components were expressed in terms of microsomal protein concentrations. Protein analyses were performed according to the method of Lowry *et al.* [31].

RESULTS

The results of assays of flavins and flavoproteins in human placental and rat hepatic microsomes are presented in Table 1. The content of FAD in placental microsomes was approximately 25 per cent of that observed in hepatic microsomes on the basis of protein concentrations. The relative concentration of placental microsomal FMN, however, was approximately 36 per cent of that present in rat hepatic microsomes. Placental preparations also exhibited relatively high cytochrome c reductase activities with both NADH and

Table 1. Analysis of flavins and flavoprotein activities in microsomes*

	Human placenta	Rat liver
Total flavin†	120 ± 12	383 ± 52
FAD†	79 ± 7	322 ± 61
FMN†	39 ± 5	109 ± 12
NADPH cytochrome *c* reductase‡	71 ± 13	117 ± 18
NADH cytochrome *c* reductase‡	200 ± 11	1016 ± 32

* Numbers in the table represent means (± S. E.) of eight separate analyses.

† Concentrations in n-moles/g of microsomal protein.

‡ Specific activities expressed as μmoles/g of microsomal protein/min.

Table 2. Analysis of rates of drug oxidation and reduction in microsomes*

Substrates tested	Product measured	Specific activities (n-moles product formed/g protein/hr) Human placenta Without added FMN	With added FMN	Rat liver
Neoprontosil	Sulfanilamide	ND†	ND	36,800 ± 7900
p-Nitrobenzoic acid	*p*-Aminobenzoic acid	290 ± 90	780 ± 20	91,800 ± 7200
p-(Methylamino)-benzoic acid	*p*-Aminobenzoic acid	54 ± 30	‡	3550 ± 268
p-(Dimethylamino)-benzoic acid	*p*-Aminobenzoic acid	ND	‡	2120 ± 187
Ethoxycoumarin	7-Hydroxycoumarin	ND	‡	316,500 ± 25,700§
Methoxycoumarin	7-Hydroxycoumarin	ND	‡	131,700 ± 19,500§
Coumarin	7-Hydroxycoumarin	ND	‡	45,220 ± 6980§
Aniline hydrochloride	*p*-Aminophenol	ND	55 ± 10	1400 ± 160
Aminopyrine	4-Aminoantipyrine	ND	‡	860 ± 190
3,4-Benzo(α)pyrene	3-Hydroxybenzpyrene	5479 ± 1950	‡	86,500 ± 11,900

* Numbers in the table represent means (± S. E.) of four separate analyses.
† ND indicates that activities could not be detected.
‡ Determinations were not made.
§ Determined on preparations of rabbit liver microsomes.

NADPH as electron donors. The measured specific activities were 20 and 61 per cent, respectively, of those detected in rat hepatic microsomes.

In spite of the use of high concentrations of NADPH and/or placental microsomal protein in reaction flasks, assays for mixed-function oxidation of xenobiotic substrates yielded negative results in most cases (Table 2). Exceptions to this general rule were provided by aryl hydrocarbon hydroxylase and methylaminobenzoic acid *N*-demethylase activities. *N*-demethylating activity was very low (although easily measurable) and did not appear to correlate with aryl hydrocarbon hydroxylase activities. Significant rates of aniline *p*-hydroxylation was observed only if flavin compounds were added to reaction vessels. Rates were extremely low and comparable to those observed in tissue-free model systems with comparable concentrations of hemoproteins and flavins [32]. Placental microsomes exhibiting unusually high aryl hydrocarbon hydroxylase activities also were inactive with respect to aniline *p*-hydroxylation unless supplemented with flavins. Likewise, the $\omega(\omega\text{-}1)$ oxidation of laurate could not be detected in these studies.

In view of the relatively high cytochrome c reductase activities observed in placental microsomes, it was somewhat surprising that significant enzymic catalysis of the reduction of the azo linkage of neoprontosil could not be detected in incubation flasks containing these same microsomal preparations. A rapid, nonenzymic reduction of neoprontosil by NADPH [33, 34] could be observed in the presence of placental microsomes, indicating that rapid degradation of NADPH was not a significant factor in the observance of negative results with respect to placental microsomal drug metabolic activities. A comparison of rates of neoprontosil reduction by NADPH in the presence of human placental vs rat hepatic preparations from which NADPH-regenerating systems were omitted from both preparations revealed that the rate of reduction was considerably more rapid in preparations containing placental microsomes than in those containing hepatic microsomes. Additions of FMN or FAD to the incubation flasks containing placental microsomes also did not result in the detection of observable enzymic reduction. For these experiments, it should be emphasized that incubation mixtures containing placental microsomes exhibited no capacity to reduce NADP.

Significant rates of reduction of PNBA to PABA were observed in incubation flasks containing human placental microsomes. Since heat inactivation (100°, 5 min) decreased the rate of the reaction by 20–30 per cent, it was assumed that enzymic activity was responsible for that percentage of the reaction rate. It should be noted, however, that rates of reduction of PNBA to PABA in flasks containing placental microsomes were 2–3 orders of magnitude less than rates observed in rat hepatic microsomes.

Additional experiments designed to determine whether high rates of catabolism (oxidation or hydrolysis) of NADPH in incubation flasks containing placental microsomes could account for low or undetectable drug metabolic activities (Table 3) indicated that this was probably not a significant factor. NADPH oxidase activity was not detectable in any of the placental microsomal preparations unless androstenedione or testosterone was added to the cuvettes. Additions of these steroids then resulted in only very slow rates of NADPH oxidation as determined by absorbance decreases at 340 nm. (Details of these studies will

Table 3. Analysis of human placental and rat liver microsomal enzymes involved in the degradation of pyridine nucleotides*

Enzyme	Specific activities Human placenta	Rat liver
Nucleosidase†	ND‡	0·9 ± 0·3
Pyrophosphatase†	3·5 ± 1·4	4·0 ± 0·7
Lipid peroxidase§	ND	2·8 ± 0·2
NADPH oxidase‖	ND	3·1 ± 0·5

* Means of a minimum of four determinations with standard errors.

† Specific activities expressed as nmoles $NADP^+$ hydrolyzed/mg of microsomal protein/min.

‡ ND indicates that detectable activities were observed.

§ Specific activities expressed as nmoles malonaldehyde formed/mg of microsomal protein/min. Specific activities of the hepatic system were increased 2 to 3-fold by additions of ADP and $FeCl_3$ (see Methods), but no activity could be detected in placental microsomes.

‖ Expressed as nmoles NADPH oxidized/mg of microsomal protein/min.

be published elsewhere.) Additions of a number of drugs, i.e. hexobarbital, pentobarbital, chlorpromazine, aminopyrine and desmethylimipramine, did not result in measurable rates of NADPH oxidation. In agreement with the above observations on placental NADPH oxidase activity was the lack of measurable lipid peroxidase activity in the placental microsomes under each of the reaction conditions utilized. Significant nucleosidase activity likewise could not be detected. On the other hand, placental microsomes exhibited pyrophosphatase activities which were very similar to those observed in rat hepatic microsomes. Since the pyrophosphatase assay system measures the total disappearance of the nucleotide from the reaction mixture, hydrolytic catabolism catalyzed by non-specific enzymes (i.e. alkaline phosphatase) also would be accounted for in the reactions.

Measurements of heme and hemoproteins (Table 4) appeared to indicate that hemoglobin was a significant contaminant of placental microsomes prepared in this fashion. Analyses of the microsomal washes also provided evidence that the hemoglobin bound to placental microsomes could be converted quite readily to methemoglobin in air. The percentage of methemoglobin varied between 12 and 55 per cent in the isotonic KCl solutions utilized in the washing of the microsomes. This seemed to depend on the duration and temperature of storage of the microsomes.

Table 4. Analysis of total heme and hemoproteins in microsomes*

	Concentrations (n-moles/g microsomal protein) Human placenta	Rat liver
Total heme	337 ± 66	2033 ± 90
Hemoglobin	79 ± 10	8 ± 5
Cytochrome b_5	60 ± 5	827 ± 65
Cytochrome P-450	51 ± 7	1120 ± 92

* Numbers represent means of a minimum of five determinations with standard errors.

The presence of cytochrome b_5 in placental microsomes was evident in difference spectra determined after the additions of NADH to sample cuvettes (Fig. 1). Additions of 5×10^{-4} M NADH (final concentrations) to microsomes resuspended in potassium phosphate buffer appeared to completely reduce placental cytochrome b_5, since additions of higher concentrations did not result in an increased absorbance difference between 427 and 409 nm. Introduction of a few grains of sodium dithionite into the sample cuvette produced an increase in the absorbance difference between 427 and 409 nm. This was probably accounted for by a superimposed deoxyhemoglobin minus oxyhemoglobin/methemoglobin difference spectrum, as well as reduced minus oxidized difference spectra of contaminating mitochondrial cytochromes. Addition of excess sodium dithionite to the sample cuvette followed by the bubbling of both the sample and reference cuvettes with CO resulted in a marked increase in absorbance near 427 nm. Superimposition of a carbon monoxyhemoglobin minus methemoglobin difference spectrum plus a CO–cytochrome P-420 minus ferric cytochrome P-420 spectrum and perhaps a CO–cytochrome oxidase minus ferric cytochrome oxidase spectrum probably accounted for most of the changes in the observed absorption spectrum in the Soret region. NADH appeared to partially reduce cytochrome P-450 (Fig. 1); however, NADPH reduced this

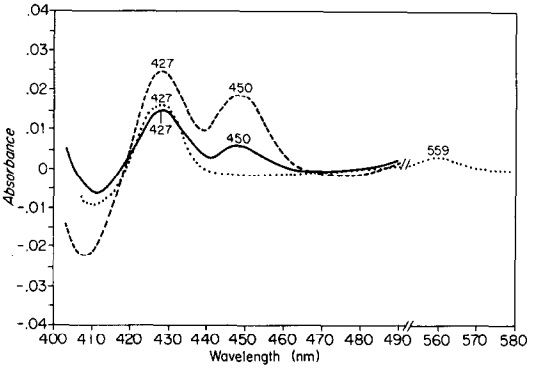

Fig. 1. Optical absorption spectra of human placental microsomes. Key: ·····, 5×10^{-4} M NADH (final concentration) added to the sample cuvette; ——, the sample cuvette contained 5×10^{-4} M NADH plus CO and the reference cuvette contained CO; – – –, the sample cuvette contained 5×10^{-4} M NADH, sodium hydrosulfite and CO and the reference cuvette contained CO. Protein concentrations in the cuvettes were 3 mg/ml.

cytochrome to a greater extent than NADH when microsomes were resuspended in phosphate buffer. When resuspended in 20 per cent glycerol solutions, NADH was a more effective reducing agent than NADPH [11].

Hemoglobin, methemoglobin, cytochrome b_5 and cytochrome P-450 did not account for all of the heme pigment present in the placental microsomes, but did account for essentially all of the heme content of rat hepatic microsomes (Table 4). Cytochrome P-420 may account for at least a portion of the remainder, since some cytochrome P-450 appears to be converted to cytochrome P-420 during preparation of placental microsomes [11]. (However, measurements of cytochrome P-450 before and after incubation for 1 hr at 37° in the presence of NADPH, 10^{-3} M, final concentration, indicated that less than 8 per cent of the cytochrome P-450 was converted to cytochrome P-420 during the course of the incubation.) Contaminating mitochondrial cytochromes also would be expected to account for a small percentage of the remainder.

Additions of 0·2% bovine serum albumin to the washing solutions of isotonic KCl appeared not to remove significant additional quantities of hemoglobin from the microsomal fractions. The perfusion of placentas was variably effective, but comparable quantities of hemoglobin still remained bound to the placental microsomes due to the inability to completely remove blood from placental tissues by perfusion techniques.

All of the placental microsomal fractions investigated also were assayed for aryl hydrocarbon hydroxylase activities in order to determine whether any correlations could be drawn between hydroxylase activities and any of the other measured placental parameters. No such correlations could be detected. A negative correlation between aryl hydrocarbon hydroxylase activity and microsomal cytochrome P-450 content appeared to exist, but it was not statistically significant at the 0·05 level of probability.

DISCUSSION

From the results presented, it would appear that the lack of measurable drug metabolic activity in human placental microsomes would not be due to the following factors: (1) rapid oxidative or hydrolytic degradation of NADPH or conversion of NADPH to NADH during incubations; (2) lack of NADPH-cytochrome *c* reductase activity or of the capacity of NADPH to readily reduce placental cytochrome P-450; (3) lack of cytochrome b_5 reductase; or (4) lack of cytochrome P-450 (since concentrations of this pigment in incubation vessels can be increased markedly by the simple expedient of reconstituting a highly concentrated microsomal fraction). In addition, experiments performed during the present investigations indicate that conversion of cytochrome P-450 to cytochrome P-420 during the course of typical incubations was minimal. It should be pointed out, however, that calculations of concentrations of the hemoproteins in placental microsomes were performed on the basis of extinction coefficients determined in liver microsomes. Preliminary results in our laboratory tend to indicate that human placental microsomal cytochrome P-450 has a much higher extinction coefficient. If confirmed, this would provide for an even lower concentration of cytochrome P-450 in placental microsomes than the figures in Table 4 indicate. Using the same extinction coefficient, Bergheim *et al.* [9] calculated very similar values for cytochrome P-450 in human placental microsomes in a very recent study; however, in view of some unusual characteristics of the placental cytochrome which have been reported [9–11], it may be more reasonable to expect that low or negligible rates of mixed-function oxidation of most of the conventional "type I" drug substrates in placental homogenates or subfractions could be explained in terms of substrate specificities. This will require additional study for confirmation.

Previous studies of the kinetics of the human placental aryl hydrocarbon hydroxylase system [22] indicated that relatively high concentrations of NADPH were required to obtain maximal specific activities. The apparent K_m (NADPH) in human placental microsomes was $1{\cdot}1 \times 10^{-4}$ M as compared with $3{\cdot}6 \times 10^{-5}$ M in rat hepatic microsomes. It was speculated that the affinity of the placental hydroxylase system for NADPH might be lower than that of the rat hepatic system. The studies presented here tend to support this speculation inasmuch as increased degradation of the reduced pyridine nucleotide in placental microsomes (as compared to rat liver microsomes) seems to be ruled out as a possible factor.

A statistically significant correlation between concentrations of placental microsomal cytochrome P-450 and specific acitivity of placental aryl hydrocarbon hydroxylase could not be observed in studies with ten placentas. This observation is in agreement with previously reported preliminary results [11]. A trend toward a negative correlation again was observed but was not statistically significant. In addition, it was observed that while the hydroxylase system is comparatively stable after repeated freezing and thawing, placental microsomal cytochrome P-450 is less stable. Comparatively high rates of hydroxylation could be observed after cytochrome P-450 had been converted almost entirely to cytochrome P-420. These observations tend to question the involvement of spectrally observable placental cytochrome P-450 in the aryl hydrocarbon hydroxylase system present in this organ. We are currently studying this aspect.

Acknowledgements—The authors gratefully acknowledge the technical assistance of Paula Hopkins and Mark Pedersen. Also, we thank the Obstetrics Ward of the University Hospital, University of Washington, for their cooperation in the procurement of placental tissues.

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