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COMPONENTS OF THE HEME BIOSYNTHETIC PATHWAY AND MIXED FUNCTION OXIDASE ACTIVITY IN HUMAN FETAL TISSUES

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Abstract—Components of the enzyme systems involved in heme biosynthesis and mixed function oxidase activity were examined in tissues from human fetuses between the ages of 10·8 and 17·5 weeks, aborted by hysterotomy. The activity of δ-aminolevulinic acid synthetase (ALAS) was highest in liver, and successively lower in adrenal, placenta, kidney and lung. The mean level of ALAS activity in fetal liver [61·9 ± 13·4 (S.E.) nmoles ALA/g/hr] was three times higher than the level reported in human adult liver. ALAS activity was directly correlated with the concentrations of porphyrins in liver, lung and placenta ($r = 0{\cdot}96$). Protoporphyrin predominated in liver, while coproporphyrin predominated in lung and kidney. Ferrochelatase measured in livers from two fetuses (47·5 and 60·2 nmoles heme/g/hr) was sufficient to account for complete conversion to heme of the ALA produced by ALAS. As with other species, ALAS and ferrochelatase were found mainly in the mitochondrial subfraction of cells. Aryl hydrocarbon hydroxylation (AHH) could be measured in liver, adrenal, lung, kidney, intestine and placenta, but aminopyrine demethylation could not. In the adrenal, the concentration of cytochrome P-450 (1·92 nmoles/mg of microsomal protein) and mean AHH activity [258·2 ± 36·6 (S.E.) pmoles 3-OH benzo(a)pyrene/mg of protein/hr] were both four times higher than in the liver. In the fetal liver, the mean concentration of P-450 was within the range reported for human adult liver, but the activity of AHH [55·1 ± 17·3 (S.E.) pmoles/mg of protein/hr] was only 2 per cent of the activity reported for the adult. Cytochrome P-450 could not be detected in kidney or lung, though low levels of AHH were found (7·1 and 7·2 pmoles/mg of protein/hr). The presence of higher levels of ALAS and lower levels of cytochrome P-450 and AHH in the fetal liver than in the adrenal indicates that the activity of the heme biosynthetic pathway is not the main determinant of hemoprotein concentration and the extent of mixed function oxidase activity in human fetal tissues. The data also suggest that limitations in heme and hemoprotein synthesis may contribute to the very low mixed function oxidase activity in human fetal lung and kidney, but that other factors must account for the disparity in hepatic mixed function oxidase activity between the human fetus and adult.

Yaffe *et al.* [1] first showed that human fetal liver, in contrast to fetal liver of other mammalian species, contained substantial amounts of cytochrome P-450 and NADPH cytochrome c reductase activity and could hydroxylate lauric acid and testosterone, endogenously produced substrates of the mixed function oxidase enzyme system. Subsequently, human fetal liver was also shown to be capable of metabolizing foreign compounds. Hydroxylation of benzo(a)pyrene [2] and aniline [3], demethylation of ethylmorphine [3], *N*-methylaniline [2] and diazepam [4], sulfoxidation of chlorpromazine [5], and reduction of *p*-nitrobenzoic acid [6] have been demonstrated to occur in human fetal liver homogenates. Some capacity for drug metabolism has been detected in fetuses as young as 6 weeks. Except for the hydroxylation of aniline, for which higher activities were found in human fetal than in human adult liver [3], other hepatic drug-metabolizing reactions have been reported to proceed at a slower rate in the human fetus than in the human adult.

The adrenal gland may also serve as an important site for drug metabolism in the human fetus. Juchau and Pedersen [7] recently reported that the concentration of cytochrome P-450 was higher, and that several drug biotransformations proceeded more rapidly in the human fetal adrenal than in the fetal liver.

The microsomal cytochromes P-450 and b_5 consist of heme prosthetic groups attached to apoproteins. It is possible that the capacity of fetal tissues to synthesize heme will affect the concentration of microsomal hemoproteins which will, in turn, affect fetal drug-metabolizing capacity. There is evidence that certain chemicals which stimulate δ-aminolevulinic acid synthetase (ALAS) activity also increase microsomal hemoprotein content and mixed function oxidase activity [8–10], while chemicals which inhibit heme synthesis prevent the induction of microsomal hemoproteins and stimulation of mixed function oxidase activity by such agents as phenobarbital [8, 11]. It is not clear, however, whether the extent of a cell's capacity for hemoprotein biosynthesis is commensurate with its capacity for carrying out mixed function oxidase reactions, or whether in those cells which have the capacity for mixed function oxidation, the intracellular regulation of heme biosynthesis, in the absence of foreign chemicals, affects the rate of mixed function oxidase activity. There is some evidence that the concentration of microsomal hemoproteins is not rate limiting in mixed function oxidase reactions [12, 13], suggesting that the biosynthesis of hemoproteins, though contributory, may not be the most important factor determining the extent of mixed function oxidase activity.

We have examined the relationship between steps in heme and hemoprotein biosynthesis and mixed func-

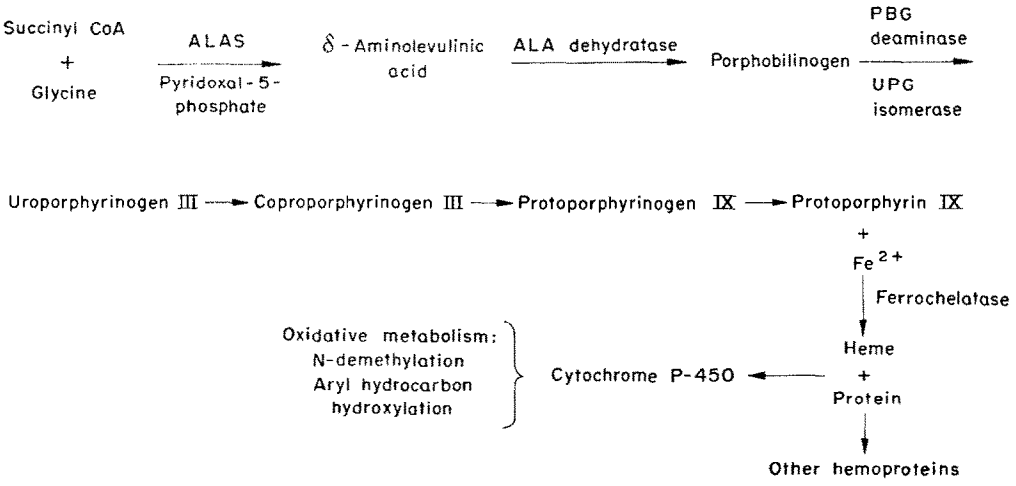

Fig. 1. Biosynthetic and functional relationship between heme synthesis and mixed function oxidase activity.

tion oxidase activity in human fetal tissues. Here we report on measurements of the activity of ALAS, the rate-limiting enzyme in heme synthesis, ferrochelatase (EC 4.99.1.1), which catalyses the insertion of ferrous iron into protoporphyrin, and is the final enzyme in heme biosynthesis, the concentration and type of porphyrins formed and the relationship between those factors, cytochrome P-450 concentration and microsomal mixed function oxidase activities in tissues from human fetuses aborted by hysterotomy. The biosynthetic and functional relationship of the parameters examined is shown in Fig. 1.

METHODS

Tissue samples. Tissues were obtained from eleven fetuses aborted by hysterotomy. Tissues were excised immediately after hysterotomy, placed in cold 0·1 M phosphate buffer at pH 7·4 and brought directly to the laboratory where they were promptly weighed and processed. The time between hysterotomy and beginning of enzyme assays was between 0·5 and 2 hr. All determinations, except for measurements of porphyrin content of the tissues, were performed on the day of the hysterotomy.

Tissues were homogenized in 0·1 M phosphate buffer at pH 7·4 to a concentration of 250 mg/ml, except for adrenal glands, which were made 50 mg/ml.

To prepare subfractions of tissues, homogenates were first spun at 100 *g* for 5 min to remove nuclei, red cells and cell debris. The supernatants were then spun at 9000 *g* for 15 min in a Sorvall RC2-B refrigerated centrifuge. The 9000 *g* pellets and supernatants were separated. For studies in which separation of activities between the 9000 *g* pellet and supernatant was of interest, the pellets were washed with 0·1 M phosphate buffer, pH 7·4, and spun again at 9000 *g* for 15 min. Otherwise, the pellets were used without further washing. Microsomes were prepared from the 9000 *g* supernatants by spinning at 105,000 *g* for 60 min in a Beckman model L ultracentrifuge. The microsomal pellets were suspended in 30% glycerol in 0·1 M phosphate buffer at pH 7·4, since glycerol has been shown to increase the stability of cytochrome P-450 [14]. Protein was measured by the method of Lowry *et al.* [15].

Delta-aminolevulinic acid synthetase (ALAS). ALAS was measured in homogenates by a modification of the method of Marver *et al.* [16], and in the mitochondrial subfractions, using the method of Sassa and Granick, as shown in Poland and Glover [17]. The procedure of Marver *et al.* [16] calls for larger amounts of tissue than are readily available in the fetus, so the assay was scaled down to one-tenth the original volume to require less tissue. By this modification, it was possible to measure ALAS in homogenate from as little as 5 mg tissue. Reaction mixtures included 0·1 ml containing 20 μmoles glycine, 2·0 μmoles EDTA, 15 μmoles Tris-HCl and 0·04 μmole pyridoxal phosphate, and 0·1 ml homogenate equivalent to 5–25 mg tissue. Incubations were carried out in 75 × 12 mm test tubes. Five replicate tubes were prepared for each tissue specimen. Reactions were stopped with 0·2 ml of 3·3% (w/v) trichloroacetic acid (TCA) in two tubes at zero time and in three tubes after incubation at 37° in a shaking water bath in air. The tubes were centrifuged and the δ-aminolevulinic acid (ALA) formed was measured in the supernatants. Then 0·1 ml of 1·0 M sodium acetate and 0·01 ml acetyl acetone were added to 0·3 ml of each of the supernatants in 75 × 10 mm test tubes. The tubes were then placed in a boiling water bath for 10 min. Aminoacetone was extracted with 1 ml dichloromethane. (Dichloromethane removes 92 per cent of aminoacetone pyrrole and less than 9 per cent of ALA pyrrole [17].) The upper aqueous phase (0·3 ml) was removed and added to 0·3 ml modified Ehrlich's reagent [18]. The absorbance at 552 nm (ALA pyrrole formed) was measured after 15 min, and the mean value for the zero time blanks was subtracted from the mean value for the incubated samples. The concentration of ALA/g of tissue was calculated using the extinction coefficient for ALA pyrrole at 552 nm: 58 cm^{-1}, mM^{-1} [19]. Using chick embryo liver, measurements made by the micro and standard macro assays gave equivalent results.

Porphyrins. Porphyrins were measured by the spectrofluorometric method of Granick [19] as modified by Sassa [20]. Tissue homogenate (0·5 ml, equivalent to 125 mg) was extracted in a total volume of 5 ml, 1 M perchloric acid–methanol (1:1, v/v). After 30 min, the extracts were filtered through Whatman

paper No. 42. The procedures were carried out in the dark. Porphyrins were measured in the filtrates using a Hitachi MPF 3 spectrophotofluorimeter (The Perkin Elmer Corp.) equipped with an R-446 photomultiplier tube to enhance red sensitivity. Emission spectra were obtained between 560 and 700 nm, at an excitation wavelength of 402 nm. A coproporphyrin III reference standard was a gift of Dr. S. Sassa. Protoporphyrin IX was prepared from protoporphyrin IX dimethyl ester (Sigma) by the method of Porra and Jones [21]. The concentration of protoporphyrin was determined spectrophotometrically in acid solution [22]. A standard solution for spectrophotofluorimetry was prepared by diluting the protoporphyrin to 10^{-8} M in 1 M perchloric acid–methanol (1:1, v/v).

Ferrochelatase. Ferrochelatase was measured by subtracting preformed tissue heme from heme formed anaerobically in the presence of protoporphyrin and iron, in the 9000 *g* pellet (mitochondrial fraction), after first removing nuclei, red cells and cell debris by centrifuging the homogenate at 100 *g* for 10 min. An assay based on the method of Porra *et al.* [23] was used. The reaction mixtures included 30 nmoles protoporphyrin IX in 0·1 M Tris buffer, pH 8·2 (prepared as above), 100 nmoles ferrous sulfate, 10 μmoles glutathione (Nutritional Biochemicals), 0·1 ml of 1% (v/v) Tween 80 and 0·1 ml ethanol, brought to a total volume of 2·1 ml with 0·1 M Tris buffer, pH 8·2. Tubes were capped with anaerobic test tube stoppers (Fisher Scientific Co.). The tubes were evacuated for 5 min using a 12 arm glass manifold attached at one end to a vacuum pump and to the test tubes at the other, by means of needles inserted into the stoppers. Nitrogen (Matheson Gas Co.) was bubbled into the tubes, and a second needle was inserted into each cap to allow gas exchange. Incubations were started by adding tissue equivalent to 100 mg wet weight (usually mitochondrial fraction) in 0·4 ml of 0·1 M Tris buffer, pH 8·2, through the second needle to bring the total volume to 2·5 ml. The second needle was then removed. Nitrogen was bubbled into the tubes for a final min, and then the valves were closed. Tubes were incubated in a water bath at 38° for periods up to 120 min. Duplicate tubes were incubated with and without protoporphyrin and iron. Reactions were stopped by adding 0·5 ml of 0·4 M iodoacetamide to the tubes and placing them in an ice bath. Heme was measured by the pyridine hemochromogen method, using the millimolar extinction coefficient of 20·7 [22] for the difference in the absorbance between the peak at 557 nm and the trough between the α and β peaks. The amount of heme present in mitochondria was subtracted from the amount of heme formed in the presence of protoporphyrin and iron to calculate ferrochelatase activity.

Cytochrome P-450. Cytochrome P-450 could not be measured by means of the carbon monoxide (CO) reduced vs reduced difference spectra when there were large amounts of interfering hemoglobin. Use of the CO reduced vs CO difference spectra according to Estabrook *et al.* [24] enabled measurement of cytochrome P-450 in liver microsomes and in homogenates and microsomes of other tissues. In adrenal microsomes, distinct peaks in the 450 nm region were obtained with both CO reduced vs reduced and CO reduced vs CO difference spectra. The concentrations of P-450, calculated using millimolar extinction coefficients of 91 for the former [25], and 100 for the latter [24], were identical. In some experiments, to eliminate the possible interference of mitochondrial cytochromes, succinate (5 mM) was first added to suspensions of microsomes to reduce mitochondrial cytochrome oxidase, before obtaining the CO reduced vs CO difference spectra [26].

N-demethylation. *N*-demethylation was studied by measuring the formaldehyde produced [27] after the demethylation of aminopyrine by tissue homogenates. A reaction mixture which we found to maximize human fetal liver production of formaldehyde from aminopyrine was used. The reaction mixture, a modification of that used by Kato and Onoda [28], contained 10 mM nicotinamide, 5 mM $MgCl_2$, 10 mM semicarbazide, 2 mM NADP, 5 mM glucose 6-phosphate, 1 U glucose 6-phosphate dehydrogenase (Sigma), 4 mM aminopyrine, and tissue equivalent to 25–50 mg wet weight, in a total volume of 1 ml. Incubations were carried out in 100 × 13 mm test tubes in a shaking water bath in air at 37° for 30 min. Five tubes were prepared for each determination, including a zero time control, two tissue blank controls in which water was substituted for aminopyrine and two experimental tubes. Reactions were stopped with 0·5 ml of 15% TCA (w/v). Using chick embryo liver homogenates, activity was linear to 30 min, in reaction mixtures containing tissue equivalent to between 10 and 50 mg wet weight.

Aryl hydrocarbon hydroxylase. The conversion of benzo(a)pyrene to 3-OH benzo(a)pyrene by tissue homogenates was used as a measure of aryl hydrocarbon hydroxylase activity, according to the method of Nebert and Bausserman [29]. For maximum sensitivity, 3 ml of the organic phase were extracted in a final volume of 0·5 ml sodium hydroxide. Extraction of 3-OH benzo(a)pyrene added to the organic phase was complete in 0·5 ml sodium hydroxide. Reactions were carried out in a shaking water bath in air at 37°, usually for 10 min. 3-OH benzo(a)pyrene was first standardized against quinine sulfate, and a quinine sulfate standard was included in each experiment to permit quantification of the hydroxylated benzo(a)pyrene. 3-OH benzo(a)pyrene was a gift of Dr. H. Gelboin, National Cancer Institute, National Institutes of Health, Bethesda, Md.

Statistics. Means, standard errors, correlation coefficients (r) and P values to evaluate whether r differed significantly from zero were calculated according to established methods.

RESULTS

Clinical data. Abortions were performed by hysterotomy because the patients desired tubal ligations. Each of the women had had three to seven previous pregnancies. Fetal age was estimated by the crown rump length [30] and the patient's history (last menstrual period). Age and sex of the fetuses, maternal age and maternal cigarette-smoking history are shown in Table 1.

No drugs other than vitamins had been prescribed for any of the pregnant women during early gestation. All of the women were given secobarbital or pentobarbital the night before hysterotomy, according to the nursing notes. Meperidine and atropine were administered 2 hr prior to hysterotomy. At hysterotomy,

Table 1. Clinical data

Fetus	Gestational age (weeks)	Sex	Maternal age	Maternal cigarette smoking history
1	10·8	M	23	No smoking
2	11·0	M	26	7–10 Cigarettes/day
3	11·8	F	31	3–5 Cigarettes/week
4	12·5	F	35	*
5	14·5	M	31	10 Cigarettes/day
6	15·1	M	28	
7	15·8	F	25	20 Cigarettes/day
8	16·0	F	37	
9	16·5	F	34	10 Cigarettes/day
10	17·5	F	25	No smoking
11	18·0	M	45	

* Blanks, in this and in all subsequent tables, indicate that information was not obtained.

sodium pentothal, curare, succinyl choline and, in one instance (fetus 2), fentanyl were injected.

ALAS. ALAS activity could be detected in five of six livers, four of four adrenals, two of five lungs, three of three kidneys and five of six placentas (Table 2). The tissue concentrations and incubation times used were in the linear range of activity for liver, adrenal and placenta. In lung and kidney, linearity of activity with time and tissue concentration could not be established because of low activity or limited tissue available.

Levels of ALAS were highest in the liver and adrenal, lowest in the lung and kidney and intermediate in the placenta. Hepatic levels of ALAS from different fetuses fell within a narrow range, except for the one fetus in which the enzyme was not detected. The mean hepatic level of ALAS (±S.E.) was 61·9 ± 13·4 nmoles ALA/g of liver/hr. Adrenal ALAS activity was more variable and in three of four fetuses was lower than hepatic activity; the mean activity of adrenal ALAS (±S.E.) was 58·3 ± 30·6 nmoles ALA/g of adrenal/hr. Although mean placental levels of ALAS were lower than mean hepatic or adrenal levels, in one fetus (fetus 7), ALAS was higher in the placenta than in any other tissue (123 nmoles ALA/g of placenta/hr).

Extra hepatic tissue levels of ALAS (adrenal, lung and placenta) in fetus 7 were higher than in the other fetuses, although hepatic ALAS in fetus 7 was within the range of the hepatic levels in the other fetuses.

No developmental changes in ALAS were observed during the time period examined, and no correlations were observed between the extent of ALAS activity in the liver and other tissues.

The distribution of ALAS between the 9000 *g* pellet and supernatant was examined in liver and adrenal from two fetuses. Ninety per cent of ALAS activity was found in the 9000 *g* pellet in both liver and adrenal, suggesting that ALAS is localized in the mitochondrial fraction of cells of the human fetus, as in other species [19].

Porphyrins. With coproporphyrin and protoporphyrin in 0·1 M perchloric acid–methanol (1:1, v/v), at an excitation wavelength of 402 nm, emission peaks for coproporphyrin III were at 598 and 650 nm, and for protoporphyrin IX, at 606 and 660 nm. Certain qualitative information can, therefore, be derived from the porphyrin fluorescence emission spectra.

Emission spectra characteristic of porphyrins were observed in all the tissue samples examined (liver, lung and placenta) (Table 3). Except for one placenta (from fetus 7), in which the porphyrin concentration exceeded that in the liver, the highest levels of porphyrins were found in the liver.

The emission spectra of porphyrins from liver, lung and placenta differed from each other, suggesting that the predominant type of porphyrins in the tissues differed. In the liver, the peaks were at 606 and 656 or 659 nm, indicating a preponderance of protoporphyrin. In two of three fetuses, lung appeared to contain mainly coproporphyrin. In placenta, the porphyrin emission spectra were not strictly characteristic of either coproporphyrin or protoporphyrin; the location of the spectral peaks suggested that placenta contained a mixture of porphyrins with coproporphyrin predominating. In the lung and placenta of fetus 7 (in both of which ALAS was higher than in the other fetuses), there was a shift in the fluorescent peaks of the porphyrin formed to higher wavelengths, indicating that a greater proportion of protoporphyrin was formed.

Table 2. δ-Aminolevulinic acid synthetase in human fetal tissue*

Fetus No.	Fetal age (weeks)	Liver	Adrenal	Lung	Kidney	Placenta
1	10·8	57·8	18·4	10·0	11·5	3·7
2	11·0	0		0		15·7
3	11·8	90·1				1·3
5	14·5	87·4	65·3	0	6·5	5·9
7	15·8	63·0	142·0	25·7		123·0
10	17·5	73·3	7·7	0	7·8	0
Mean ± S.E.		61·9 ± 13·4	58·3 ± 30·6	7·1 ± 5·0	8·6 ± 1·5	24·9 ± 19·7

* Expressed as nmoles ALA/g of tissue/hr.

Table 3. Porphyrin content of human fetal tissues

Fetus	Age (weeks)	Liver			Lung			Placenta		
		Emission peaks (nm)*	Porphyrin type	(pmoles/ tube)	Emission peaks (nm)	Porphyrin type	(pmoles/ tube)	Emission peaks (nm)	Porphyrin type	(pmoles/ tube)
1	10·8							603, 654	mixture†	20·0
5	14·5	606, 656	proto	185	598, 650	copro	22·5	603, 654	mixture	16·6
7	15·8	606, 659	proto	74	604, 657	proto	52·9	606, 659	proto	245
10	17·5	606, 656	proto	110	598, 650	copro	17·2	603, 654	mixture	15·0

* Excitation wavelength was 400 nm. Each tube contained tissue equivalent to 125 mg wet weight tissue.
† The porphyrin contents of the type designated "mixture" were calculated as if they contained mainly coproporphyrin.

For those tissues in which both ALAS and porphyrins were measured, porphyrin content of tissues was positively correlated with ALAS activity ($r = 0{\cdot}96$, $P < 0{\cdot}001$).

Ferrochelatase. Ferrochelatase activity (Table 4) was measured in mitochondria from the livers of two fetuses and from the placenta of one of those fetuses. In liver of both fetuses, activity was linear for 90 min of incubation. Mean hepatic activity was 53·9 nmoles heme formed/g of liver/hr. Activity, at a lower level, was also detected in mitochondria from a placenta. No activity was found in the 9000 *g* supernatant (postmitochondrial fraction) from liver tissue. After aerobic incubation, activity was depressed by about 50 per cent. Thus, ferrochelatase in human fetal liver is similar to the enzyme in other mammalian species both in intracellular localization and anaerobic enhancement [31].

Cytochrome P-450. Cytochrome P-450 (Table 5) was detected in homogenates and microsomes of all liver, adrenal and placental tissues examined, though not in lung or kidney. The mean hepatic level of cytochrome P-450 in liver microsomes from three fetuses, derived from CO reduced vs CO difference spectra, was $0{\cdot}28 \pm 0{\cdot}05$ nmole/mg of microsomal protein ± S.E. In the CO reduced vs CO difference spectrum, the peak was at 448 nm. We have, however, observed a shift from a peak of 450 nm to 448–449 nm when comparing CO reduced vs reduced and CO reduced vs CO difference spectra in microsomes from chick embryo liver, so the occurrence of a peak at 448 nm in the CO reduced vs CO difference spectrum of human fetal liver microsomes cannot be taken as evidence that the spectral form of human fetal hepatic cytochrome P-450 differs from that commonly found in liver of other species.

The concentration of cytochrome P-450 in the adrenal glands was higher than in the liver. In one fetus, in which cytochrome P-450 was measured in microsomal fractions from both liver and adrenal, the concentration of the cytochrome in the adrenal was eight times higher than in the liver. Because interference of hemoglobin was no problem, cytochrome P-450 could be easily measured in adrenal homogenates as well as in microsomes; in three homogenates, the

Table 4. Ferrochelatase in human fetal tissues

Fetus	Age (weeks)	Tissue	Fraction	Incubation	Incubation time (min)	Heme (nmoles formed/*g* tissue)
5	14·5	Liver	9000 *g* pellet	Anaerobic	60	47·5
		Liver	9000 *g* pellet	Anaerobic	90	64·9
		Placenta	9000 *g* pellet	Anaerobic	120	16·2
7	15·8	Liver	9000 *g* pellet	Anaerobic	60	60·2
		Liver	9000 *g* pellet	Anaerobic	90	79·7
		Liver	9000 *g* pellet	Aerobic	90	38·7
		Liver	9000 *g* supernatant	Anaerobic	90	0

Table 5. Cytochrome P-450 in human fetal tissues*

Fetus	Age (weeks)	Tissue fraction	Liver	Adrenal	Lung	Kidney	Placenta
4	12·5	homogenate		0·56	0†	0	
5	14·5	homogenate		0·59	0		
		105,000 *g* pellet	0·21				0·18
7	15·8	homogenate		0·49			
		105,000 *g* pellet	0·24	1·92	0		0·09
8	16·0	homogenate				0	
10	17·5	105,000 *g* pellet	0·38		0	0	0·27
11	18·0	105,000 *g* pellet			0	0	

* Expressed as nmoles/mg of homogenate protein for homogenates and nmoles/mg of microsomal protein for 105,000 *g* pellets.
† Zero indicates that no absorbance in the 450 nm region was detected in the presence of a reducing agent (sodium dithionite) and carbon monoxide.

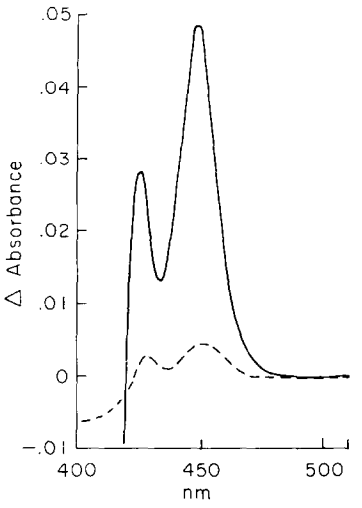

Fig. 2. CO reduced vs CO difference spectrum of human fetal liver microsomes. One ml of a microsomal suspension was added to each of two cuvettes. CO was bubbled into both the sample and reference cuvettes for 1 min. A baseline, reading zero at all points from 510 to 400 nm, was then recorded. A few grains of sodium dithionite were added to the sample cuvette and the difference spectrum was recorded after 15 min. The spectrum (——) was recorded using the 0·1 sensitivity slide wire, while the spectrum (– – – –) represents the full scale reading. The concentration of protein in the microsomal suspension was 1·29 mg/ml.

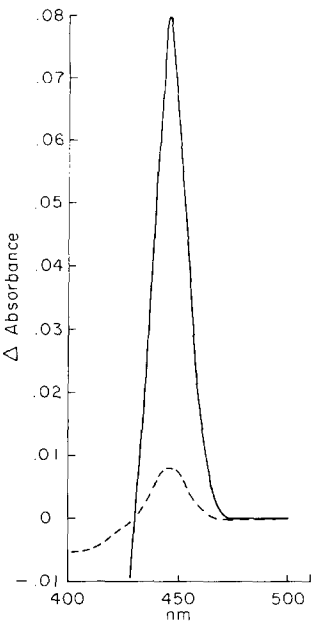

Fig. 3. CO reduced vs CO difference spectrum of human fetal adrenal microsomes. The procedure was the same as described in the legend to Fig. 2. The concentration of protein in the microsomal suspension was 0·77 mg/ml.

mean concentration of cytochrome P-450 was 0·54 nmole/mg of protein. In the standard CO reduced vs reduced difference spectrum, the spectral peak for the CO binding hemoprotein in the three adrenals studied was located at 447–448 nm. In the CO reduced vs CO spectrum, the peak was at 447 nm. Typical CO reduced vs CO difference spectra obtained with liver and adrenal microsomes are shown in Figs. 2 and 3.

In placenta microsomes, there was always a small, but distinct, shoulder or peak in the 450 nm region, using the CO reduced vs CO difference spectrum. The exact location of the peak could not be determined. The calculation of the content of cytochrome P-450 in placenta microsomes (Table 5) should, therefore, be regarded as only approximate.

No evidence of a substance absorbing in the 450 nm region was ever observed with lung or kidney tissues, though attempts were made to measure the cytochrome in lung tissues from five fetuses and in kidney tissues from four fetuses. The precaution of adding succinate to microsomes prior to carbon monoxide did not result in the appearance of a peak in the region of 450 nm in fetal lung or kidney microsomes.

N-*demethylation*. In homogenates of liver, adrenal, lung, kidney and placenta from eight fetuses studied, the amount of formaldehyde formed in the presence of the substrate, aminopyrine, exceeded the amount of formaldehyde formed in the tissue blanks for any given incubation period. In the absence of NADP, glucose 6-phosphate or nicotinamide, formaldehyde production was reduced by 75 per cent. Moreover, no formaldehyde production occurred with boiled tissue, suggesting that the process was enzymatic. However, linearity of formaldehyde production with time or tissue concentration could not be demonstrated for any tissue. Thus, although there was some evidence for *N*-demethylase activity, the amount of activity could not be satisfactorily measured.

Aryl hydrocarbon hydroxylation (*AHH*). Aryl hydrocarbon hydroxylase activity, using benzo(a)pyrene as a model substrate, could be measured in all tissues studied. Tissue concentrations and incubation times used were in the linear range of enzyme activity for each tissue. The mean activities in pmoles 3-OH benzo(a)pyr-

Table 6. Aryl hydrocarbon hydroxylase in human fetal tissues*

Fetus	Age (weeks)	Liver	Adrenal	Lung	Kidney	Intestine	Placenta
1	10·8	85·9	169·2	3·8	2·2	15·7	2·6
2	11·0	24·1	211·7	21·2	20·2		41·7
3	11·8	51·1	347·9	2·6	6·0		12·2
5	14·5	17·8	259·9	8·9	0·1		9·3
7	15·8	124·9	382·9	3·7	7·9	16·6	35·9
10	17·5	26·5	177·8	3·0	6·2		3·5
Mean ± S.E.		55·1 ± 17·3	258·2 ± 36·6	7·2 ± 3·0	7·1 ± 2·9	16·2	17·5 ± 6·9

* Expressed as pmoles 3-OH benzo(a)pyrene/mg of protein/hr.

ene/mg of protein/hr ± S.E. (Table 6) in descending order were: adrenal, 258·2 ± 36·6; liver, 55·1 ± 17·3; placenta, 17·5 ± 6·9; intestine, 16·2; lung, 7·2 ± 3·0; kidney, 7·1 ± 2·9. The mean activities in nmoles/g of wet weight tissue/hr ± S.E. were: adrenal, 19·4 ± 1·3; liver, 5·7 ± 1·3; intestine, 1·3; placenta, 1·0 ± 0·5; kidney, 0·4 ± 0·1; and lung, 0·2 ± 0·1.

In all fetuses, benzo(a)pyrene hydroxylase activity was higher in the adrenal than in any other tissue, ranging between two and fourteen times the hepatic levels per unit weight. However, the total capacity for aryl hydrocarbon hydroxylation was always greater in the liver than in the adrenal because the larger size of the liver more than compensated for its lower activity per unit weight.

No developmental changes in aryl hydrocarbon hydroxylation were noted for any tissue between 10·8 and 18 weeks gestation. The adrenal was most active with respect to benzo(a)pyrene hydroxylase activity, while the liver was most active with respect to ALAS. However, for both benzo(a)pyrene hydroxylase and ALAS, lowest activities were found in the lung and kidney. No correlation was observed between hepatic ALAS and benzo(a)pyrene hydroxylase, but in the extra hepatic tissues a significant correlation was observed ($r = 0{\cdot}69$, $P < 0{\cdot}005$). There was also a significant correlation between porphyrin content and benzo(a)pyrene hydroxylase in extra hepatic tissue ($r = 0{\cdot}86$, $P < 0{\cdot}01$).

DISCUSSION

We have shown that human fetal tissues can carry out several of the reactions involved in heme biosynthesis and mixed function oxidation during the first half of pregnancy. In tissues in which there is active heme synthesis (liver and adrenal), there is greater mixed function oxidase activity than in tissues in which heme synthesis is relatively inactive (lung and kidney). However, cytochrome P-450 concentration and AHH activity were higher in the adrenal than in the liver, despite the fact that ALAS activity was usually greater in the liver than the adrenal, indicating that the activity of the heme biosynthetic pathway cannot be precisely correlated with hemoprotein concentration or mixed function oxidase activity.

ALAS, as the rate-limiting enzyme, is ordinarily the most important factor in determining the rate of heme biosynthesis [19]. The mean hepatic activity of ALAS observed in human fetal liver was three times higher than that reported in human adult liver [32, 33] and three times higher than the levels we have observed in 11-day chick embryo livers [10]. In the rat fetus, comparable [34] and even higher [35] levels of ALAS than those we observed in the human have been reported near the end of gestation, but there are no figures available for ALAS during the first half of gestation.

The fact that ALAS in human fetal liver is considerably higher than in the human adult liver suggests that in humans the early stages in hepatic heme biosynthesis proceed more actively in the fetus than in the adult. High ALAS in fetal liver may, however, be a consequence of the hematopoietic function and high erythroid cell content of human fetal liver, or alternatively, of drug induction of enzyme activity. The latter is possible because the women whose fetuses were studied had been offered barbiturates the night before hysterotomy and received anesthetic premedication before the procedure.

The activity of ALAS appears to lead to synthesis of porphyrins in fetal tissues, as indicated by the high correlation between ALAS activity and tissue porphyrin concentration. In addition to the amount of porphyrin, the type of porphyrin formed was also related to ALAS activity. The liver and those specimens of lung and placenta with the highest ALAS contained mostly protoporphyrin; those tissues with lower ALAS contained more coproporphyrin.

Heme synthesis could be restricted even in the presence of high ALAS activity and high porphyrin levels if ferrochelatase, the final enzyme in the heme biosynthetic pathway, became rate limiting. We found that ferrochelatase activity in the fetal liver was comparable to that reported in adult human liver [33] and was higher than the mean activity that has been reported in the liver of the adult rat [36]. The ferrochelatase activity in human fetal liver is more than sufficient to account for complete conversion to heme of the ALA produced by hepatic ALAS, suggesting that it is unlikely to be rate limiting for heme synthesis in human fetal liver.

The concentrations of cytochrome P-450/mg of microsomal protein in human fetal liver were within the range reported in adult human liver [12, 37, 38], and agree closely with the few other values for the human fetus that have been recently reported [39]. They are slightly higher than the values originally reported by Yaffe *et al.* [1], but individual variation and elimination of the distortion of the spectral peak by hemoglobin could account for any differences.

The fetal adrenal gland had higher levels of both cytochrome P-450 and aryl hydrocarbon hydroxylase activity than the liver, and the spectral peak of the CO-binding hemoprotein in the adrenal was at 447–448 nm, in agreement with others [7, 39]. The cytochrome in the adrenal, therefore, has different spectral properties from the CO-binding hemoprotein commonly present in the liver of most species studied so far (peak at 450 nm), and resembles the spectral form of the cytochrome induced in liver of other species after exposure to polycyclic hydrocarbons [40] and polychlorinated biphenyls [41]. A report of cytochrome P-450 concentration in microsomes from an adult human adrenal gland [42] also indicated that the CO-binding spectral peak was not at 450 but at 447 nm; however, the concentration of the cytochrome was about one-eighth of the level we observed.

Like Jakobsson and Cinti [26], no CO-binding hemoprotein could be detected in human fetal kidney, nor was it found in human fetal lung. Nevertheless, low levels of aryl hydrocarbon hydroxylase activity could be measured in both human fetal lung and kidney.

Taken together, the high levels of ALAS, porphyrin formation, ferrochelatase and cytochrome P-450 in the liver, and of ALAS and P-450 in the adrenal, suggest that there are no restrictions to the synthesis of the hemoprotein cytochrome P-450 in the human fetal liver or adrenal. In the lung and kidney, however, ALAS is much less active, and low levels of heme and hemoprotein could limit mixed function oxidase activity.

Despite the apparent high activity of the heme biosynthetic pathway in the fetal liver, and the relatively high levels of cytochrome P-450, mixed function oxidase activity is much lower than in the liver of the

human adult. Aminopyrine demethylase, for example, which can be easily measured in chick embryo and in adult rat and human liver by assays like the one used here, was too low to quantitate in the human fetal liver. Other authors using similar assays have also been unable to measure aminopyrine demethylation in human fetal tissues [1, 7]. Benzo(a)pyrene hydroxylase was about 2 per cent of the levels reported in the human adult [43], and most other drug-metabolizing reactions described in human fetal liver have also been reported to be less active than in the liver of the human adult [2, 4, 5].

Among the possible explanations to account for the low fetal liver mixed function oxidase activity in the presence of levels of cytochrome P-450 that approximate those in the adult liver are: (1) cytochrome P-450 may not be rate limiting for mixed function oxidase activity in the human fetal liver, as has been suggested for livers of the human adult and other species [12, 13]; (2) fetal cytochrome P-450 may be relatively ineffective catalytically and could differ from the cytochrome P-450 of the human adult and other species in structure, binding properties or turnover rate; (3) as has been suggested with respect to other mammalian species, mixed function oxidase activity may be competitively inhibited by endogenous substrates such as steroids [44]; and (4) the high concentrations of hemoglobin in human fetal liver may be inhibitory to mixed function oxidase reactions.

The existence in the fetus of a capacity to carry out mixed function oxidase reactions, though less than in the adult, means that the fetus can, to a limited extent, detoxify certain chemicals, but also that it can generate compounds that may be harmful to itself. Epoxide intermediates, formed during aryl hydrocarbon hydroxylation [45], have been implicated in causing tissue injury and in acting as mediators in chemical carcinogenesis [46]. The factors regulating heme synthesis and mixed function oxidase reactions in fetal tissues *in vivo*, the extent to which microsomal mixed function oxidase activity is beneficial or harmful to the fetus, and the factors that protect the fetus or make it particularly susceptible to injury from chemical exposure have still to be studied.

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