Biochemical Pharmacology, Vol. 22, pp. 2357–2364. Pergamon Press, 1973. Printed in Great Britain.

# DRUG METABOLISM AND DRUG-INDUCED SPECTRAL INTERACTIONS IN HUMAN FETAL LIVER MICROSOMES

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(*Received* 3 *January* 1973; *accepted* 13 *March* 1973)

**Abstract**—Properties of a mono-oxygenase system in human fetal liver microsomes were studied. The levels of cytochrome P-450 and NADPH-cytochrome *c* reductase were 20 and 30 per cent, respectively of rat liver microsomal levels. Corresponding percentages for homogenates were 13 and 18 per cent, respectively. Hepatic 12,000 *g* supernatants from human fetuses were found to catalyze the hydroxylation of 3,4-benzpyrene and aniline and the *N*-demethylation of aminopyrine. These activities were 2, 12 and 11 per cent of those in adult rat liver. The cytochrome P-450- and NADPH-cytochrome *c* reductase-related turn-over-numbers for aminopyrine and aniline were of the same order of magnitude in human fetal and adult livers and in rat liver. The fetal turn-over-number for 3,4-benzpyrene was small compared with adult and rat values. Spectral changes induced by the addition of various compounds to liver microsomes were studied. Aminopyrine and hexobarbital were found to yield type I spectral changes with rat or adult human microsomes, but with fetal microsomes these compounds yielded a type II spectral change except in some cases when hexobarbital in low concentration yielded a type I change. Aniline and *n*-octylamine induced type II spectral changes with both adult and fetal microsomes. The relative magnitudes of spectral changes differed greatly between fetal and adult microsomes.

RECENTLY, evidence has been presented that the human fetal liver is able to metabolize a number of drugs and other foreign compounds.[1–12] The presence of cytochrome P-450 and other components of a microsomal mono-oxygenase system has also been demonstrated in human fetal liver microsomes.[3,6,9]

The addition to liver microsomes of various substances, such as hexobarbital or aniline, causes two types of spectral change.[13–14] With hexobarbital, there is a decrease in optical density around 420 nm and an increase around 390 nm (a type I spectral change), with aniline there is an increase around 430 nm and decrease around 390 nm (a type II spectral change). The induction of a difference spectrum by the addition of substances is considered to be indicative of the binding of the substance to the terminal oxidase, cytochrome P-450, of the microsomal electron transport chain, which has been shown to participate in the oxidative metabolism of numerous exogenous and endogenous compounds.[15,16]

Studying human fetal liver microsomes, Yaffe *et al.*[6] found the type I spectral change with testosterone and laurate and the type II spectral change with aminopyrine. Rane and Ackermann[10] reported that ethylmorphine induced a "reversed type I spectral change" with human fetal liver microsomes.

In this paper we report studies on the components of a monooxygenase system in human fetal liver microsomes, on the metabolism of 3,4-benzpyrene, aniline and aminopyrine and on the spectral changes associated with the binding of aniline,

aminopyrine, hexobarbital and *n*-octylamine to cytochrome P-450. Some comparison with adult human and rat liver parameters are made.

## MATERIALS AND METHODS

*Biologic material.* Livers were obtained from 16 fetuses, removed in hysterotomy during the interruption of pregnancy for social-medical reasons. The weight of fetuses varied from 68 to 325 g and the age from 12 to 22 weeks as estimated from the last menstruation of the mother. The preparation of the microsomes was started within 30 min of abortion. Livers were homogenized in 10 vol. of 0·1 M potassium phosphate buffer, pH 7·4, and centrifuged at 900, 12,000 and 100,000 *g* for 10, 20 and 60 min, respectively. The last centrifugation was repeated and the final pellet was suspended in phosphate buffer at a protein concentration of about 10 mg/ml. Based on the determinations of NADPH-cytochrome *c* reductase and cytochrome P-450 in both homogenates and microsomes, a 40–60 per cent recovery of microsomes was attained.

Biopsies of adult human livers were taken in connection with surgical operations (Department of Surgery, University of Oulu) from patients with uncomplicated cholelithiasis and samples were transferred to the Department of Pharmacology. Adult male rats of Sprague–Dawley strain weighing 250–300 g were used to obtain control material. Both adult human liver samples and rat livers were processed in exactly the same way as fetal livers.

*Enzyme assays.* One ml of cofactor mixture consisted of KCl 200 μmoles, $MgCl_2$ 10 μmoles, glucose-6-phosphate 6 μmoles, NADP 0·25 μmole, and glucose-6-phosphate dehydrogenase (Fluka) 40 units. For determination of aniline hydroxylation and aminopyrine demethylation, 0·25 ml of cofactor mixture, substrate (aniline 1·25 μmole, aminopyrine 2·5 μmole), enzyme preparation (the 12,000 *g* supernatant fraction from 50 mg of liver wet wt) and 0·1 M potassium phosphate buffer were added so that the final volume was 1 ml. Incubation was started with enzyme preparation after a 5-min preincubation period and took place in a metabolic shaker under atmospheric air at 37°. Incubation lasted 20 min and the linearity of reactions was maintained for this time. Determinations were made in duplicate whenever possible. The formation of formaldehyde from aminopyrine was measured by the method of Nash.[17] The *p*-hydroxylation of aniline was measured according to Kato and Gillette.[18] 3,4-Benzpyrene hydroxylase activity was measured according to the method of Kuntzman *et al.*[19] as described earlier by our group.[4]

Cytochrome P-450 was determined according to Omura and Sato[20] when using microsomes and according the Greim *et al.*[21] and Schoene *et al.*[22] when using homogenates or other fractions.

NADPH-cytochrome *c* reductase activity was measured by the slightly modified method of Masters *et al.*[23]

Protein was measured by the Biuret reaction.

*Ligand interactions.* For determination of ligand interactions, the microsomal suspension was diluted with phosphate buffer to yield a protein concentration of about 2 mg/ml. All spectra were measured at room temperature in 1-cm cells in a Shimazu model MPS-50L recording spectrophotometer. Baselines were traced. Difference spectra were obtained by addition of microliter quantities of a solution of the ligand to one of the cuvettes, while an equal volume of buffer solvent was added to the

reference cuvette. The final concentrations of aniline (12 mM), aminopyrine (10 mM), hexobarbital (6·7 mM), *n*-octylamine (1·5 mM) and ethyl isocyanide (3·6 mM) gave maximal absorbance changes. The extent of the type I and II spectral changes was measured by the difference in the absorption between the wavelengths of minimum and maximum absorption. Spectra were traced within a few minutes.

## RESULTS

Drug metabolizing enzyme activities in 16 human fetal and 4 adult livers and comparison with rat liver values are presented in Table 1. The mean concentration of cytochrome P-450 in human fetal liver was 0·15 nmole/mg of microsomal protein

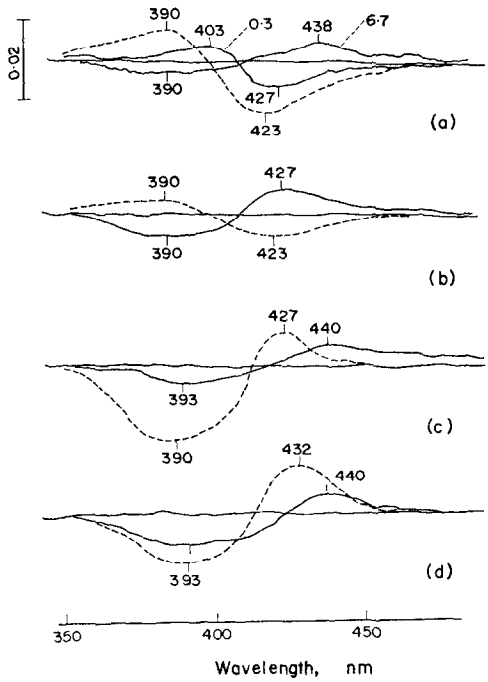


FIG. 1. Spectral changes induced by hexobarbital (a), aminopyrine (b), aniline (c), and *n*-octylamine (d) in human fetal and adult liver microsomes. (Fetus—solid lines, adult—dotted lines). Liver microsomes were prepared from one fetus, and two adult patients. Concentrations of ligands were as follows: hexobarbital 0·3 and 6·7 mM, aminopyrine 10 mM, aniline 12 mM, and *n*-octylamine 1·5 mM. Protein concentration was 3·2 mg/ml except with *n*-octylamine where it was 1·5 mg/ml.

and 3·92 nmoles/g of liver wet wt. A four-fold variation was observed in cytochrome P-450 concentration. The activity of NADPH-cytochrome *c* reductase was 37 nmoles cytochrome *c* reduced/mg of microsomal protein/min and 910 nmoles/g of liver wet wt/min. Aminopyrine demethylase and aniline hydroxylase activities correlated well with NADPH-cytochrome *c* reductase activities ($r = 0{\cdot}72$, $P < 0{\cdot}005$ and $r = 0{\cdot}68$, $P < 0{\cdot}001$), but no correlation with cytochrome P-450 concentrations was found ($r = 0{\cdot}30$ and $0{\cdot}31$, $P > 0{\cdot}05$). 3,4-Benzpyrene hydroxylase activities did not correlate with NADPH-cytochrome *c* reductase activities ($r = 0{\cdot}27$, $P > 0{\cdot}05$), but with cytochrome P-450 concentrations an almost significant correlation was found ($r = 0{\cdot}53$,

TABLE 1. COMPARISON OF DRUG METABOLISM IN LIVERS FROM HUMAN FETUS, HUMAN ADULT AND RAT

| | Cytochrome P-450 (nmole/mg) | NADPH-cyt. *c* reductase (nmole/mg/min) | Benzpyrene hydroxylase (pmole/g/min) | Aniline hydroxylase (nmole/g/min) | Aminopyrine demethylase (nmole/g/min) |
|---|---|---|---|---|---|
| Fetus ($n = 16$) | 0·15 ± 0·06 (16)* (0·06–0·29) | 37 ± 7 (16) (26–90) | 3·4 ± 1·2 (16) (1·1–5·7) | 2·8 ± 1·4 (16) (1·1–5·7) | 12·7 ± 5·8 (16) (6·1–24·3) |
| Adult ($n = 4$)† | 0·51 (4) (0·14–1·16) | 106 (4) (67–159) | 123 (4) (40–236) | 18 4 (3) (7·0–34·2) | 68 (4) (32–162) |
| Rat ($n = 8$) | 0·67 ± 0·06 (8) (0·42–0·91) | 125 ± 15 (8) (100–177) | 150 ± 42 (8) (98–229) | 23·3 ± 4·7 (8) (18·3–30·5) | 124 ± 22 (8) (101–191) |
| Fetus/rat (%) | 20 | 30 | 2·2 | 12 | 11 |

* Mean ± standard deviation (number of livers studied); range in parentheses.
† Standard deviations were not calculated because of a small number of samples studied.

TABLE 2. CYTOCHROME P-450- AND NADPH-CYTOCHROME *c* REDUCTASE-RELATED TURNOVER NUMBERS IN HUMAN FETAL, HUMAN ADULT AND RAT LIVERS

| | Fetal liver | Adult liver | Rat liver |
|---|---|---|---|
| Cytochrome P-450 -related turnover numbers | | | |
| Cytochrome P-450 (nmole/g liver) | 3·92 (8)* | 16·8 (4) | 30·5 (8) |
| Benzpyrene hydroxylase | 0·86 | 7·30 | 5·01 |
| Aniline hydroxylase | 0·73 | 1·10 | 0·75 |
| Aminopyrine demethylase | 3·26 | 4·06 | 3·67 |
| NADPH-cytochrome *c* reductase -related turnover numbers | | | |
| NADPH-cytochrome *c* reductase (nmole cyt. *c* reduced/g liver/min) | 910 (11) | 3548 (4) | 3730 (8) |
| Benzpyrene hydroxylase ($\times 10^{-3}$) | 3·7 | 35·0 | 40·2 |
| Aniline hydroxylase ($\times 10^{-3}$) | 3·1 | 5·2 | 6·2 |
| Aminopyrine demethylase ($\times 10^{-3}$) | 14·0 | 19·4 | 33·2 |

Turnover numbers are expressed as nanomoles (picomoles of benzpyrene) metabolite formed per nanomole of cytochrome P-450 or per nanomole of cytochrome *c* reduced per minute.
* Number of livers studied for cytochrome P-450 or NADPH-cytochrome *c* reductase in the homogenate.

TABLE 3. COMPARISON OF DRUG-INDUCED SPECTRAL INTERACTIONS IN LIVERS FROM HUMAN FETUS, HUMAN ADULT AND RAT

| | Spectral changes with | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Aniline | | Aminopyrine | | Hexobarbital | | *n*-Octylamine | | Ethyl isocyanide peak ratio 455/430 |
| | Type | OD/P-450* | Type | OD/P-450 | Type | OD/P-450 | Type | OD/P-450 | |
| Fetus | II | 18 ± 5 (9)† (11–25) | II | 25 ± 6 (12) (18–32) | II (I)‡ | 9 (2) (8–10) | II | 54 ± 16 (13) (37–79) | 0·28 ± 0·06 (13) (0·22–0·40) |
| Adult | II | 39 (4) (32–59) | I | 6 (4) (6–7) | I | 22 (4) (19–26) | II | 107 (4) (78–171) | 0·43 (4) (0·38–0·45) |
| Rat | II | 43 ± 5 (8) (36–48) | I | 5 ± 0·5 (8) (4–7) | I | 22 ± 2 (8) (18–25) | II | 69 ± 5 (8) (60–81) | 0·62 ± 0·12 (8) (0·41–1·07) |

* Difference in the absorption (OD × $10^3$) between the wavelengths of minimum and maximum absorption per nanomole of cytochrome P-450.

† Mean ± standard deviation (number of livers studied); range in parentheses.

‡ Hexobarbital-induced spectral change was determined in four fetal livers. In three livers hexobarbital in a concentration of 0·3 mM yielded a type I spectral change. The magnitude of the spectral change (type II) was calculated in two cases.

$P < 0{\cdot}05$). NADPH-cytochrome *c* reductase activities and cytochrome P-450 concentrations did not correlate with each other ($r = 0{\cdot}17$, $P > 0{\cdot}05$).

Turnover numbers for different substrates used relating to NADPH-cytochrome *c* reductase and cytochrome P-450 are presented in Table 2. The turnover numbers for aminopyrine and aniline were of the same order of magnitude in all livers allowing for the small number of human livers. The notable exception was the turnover number for benzpyrene, which was much smaller for human fetal liver enzyme than for adult human or rat liver enzyme.

Characteristic example of ligand interactions in human fetal and adult liver microsomes are presented in Fig. 1. In fetal liver microsomes, a "modified" type II spectral change was induced by both type I (aminopyrine and hexobarbital) and type II (aniline and *n*-octylamine) compounds. Type I spectral changes were observed in three fetal livers induced by a low concentration of hexobarbital (0·3 mM), but not by aminopyrine. The hexobarbital-induced type I spectral change faded away, when larger amounts of hexobarbital was added and a type II spectral change appeared with concentrations above 1 mM. All substrate-induced spectral changes per unit concentration of cytochrome P-450, except the type II change induced by aminopyrine, were smaller than in human adult or rat liver microsomes (Table 3). The pH intercept in the ethyl isocyanide-induced spectrum (maxima at 427–432 and 455 nm) in fetal liver microsomes was at about 7·8–7·9 and the ratio of peak heights at pH 7·4 was from 0·22 to 0·40. In adult human liver microsomes, the pH intercept was at 7·6–7·8 and peak ratio about 0·45. *n*-Octylamine gave a spectrum with a peak at 435–445 nm and a trough at 393 nm, which resembles a spectrum produced in microsomes from control or phenobarbital-treated rats. In three fetal livers aniline and *n*-octylamine caused a time-depended change from the characteristic difference spectrum to a new spectrum characterized by a deep trough at 418–420 nm and a broad peak at 360–380 nm resembling a type I spectral change.

## DISCUSSION

The results presented in this study show that a monooxygenase system in human fetal liver microsomes is capable of interacting with different ligands, both type I and type II compounds, resulting in type II spectral changes first described by Imai and Sato[13] and Schenkman *et al.*[14] Only in a few cases hexobarbital in low concentration induced a spectral change resembling a type I spectral change which perhaps indicates the presence of type I binding sites in human fetal liver microsomes.[15] Besides the shape, substrate-induced interactions in fetal microsomes were anomalous also with respect to their relative magnitudes. The magnitude of hexobarbital-induced spectral change in rat and adult human liver microsomes was three to four times larger than that of aminopyrine-induced spectral change and both interactions were of the type I variety. In fetal liver microsomes, aminopyrine induced a larger spectral change than hexobarbital and both interactions were mostly of the type II variety. Aniline, a strong inducer of a type II spectral change in rat and adult human liver microsomes, resulted in a smaller type II spectral change than aminopyrine in fetal liver microsomes. Leibman *et al.*[24] reported that the magnitudes of substrate-induced difference spectra produced by type I substrates were reduced when a modifier, either type I or II was added to microsomes. The type II response was also reduced by type II modifiers, but it was enhanced by type I modifiers. The results obtained with human fetal liver

microsomes could be explained by assuming that there is an "endogenous" type II ligand present in human fetal liver microsomes. Another explanation could be that there is a qualitative difference between cytochrome P-450 in fetal liver on the one hand and that in rat and adult human liver on the other. The presence of an "endogenous" ligand has also been suggested by Rane *et al.*[8]

Despite differing spectral interactions between fetal and adult livers, the turnover numbers for aminopyrine and aniline were similar in respect to both cytochrome P-450 and NADPH-cytochrome *c* reductase. This fact indicates that the capacity of the substrate to induce spectral change and the type and magnitude of the spectral change does not necessarily reflect the capacity of the microsomes to metabolize the substrate. In the case of 3,4-benzpyrene, the fetal turnover number was very small compared with those of rat and adult human livers. There are reports,[25,26] that the partially purified cytochrome P-450 fraction from phenobarbital induced rat liver actively supports benzphetamine *N*-demethylation but not 3,4-benzpyrene hydroxylation, whereas the reverse was true with cytochrome P-448 prepared from microsomes of rats treated with 3-methylcholanthrene. The low metabolism of 3,4-benzpyrene by human fetal liver and the characteristics of ethyl isocyanide and *n*-octylamine-induced interactions may be taken to indicate that the cytochrome P-450 in human fetal liver microsomes resembles the hemoprotein from untreated or phenobarbital-treated rat liver microsomes.

*Acknowledgements*—The indispensable help of Dr. Pentti Jouppila (Department of Obstetrics and Gynecology), Dr. Eero Kaltiala and Prof. Teuvo Larmi (Department of Surgery, University of Oulu) is gratefully acknowledged. This study was supported in part by the grant from the Yrjö Jahnsson Foundation, Finland.

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