## Notes

[Chem. Pharm. Bull. 21(10)2329—2331(1973)]

UDC 577.15.02

# Difference in the Substrate Specificities of Drug Metabolizing Enzymes in Human Term Placenta and Fetal Liver

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(Received February 12, 1973)

There are so many reports on the drug metabolizing enzymes in experimental animals, but only a few in human. It has been proved that drug metabolizing enzymes in human liver had similar function to those in livers of experimental animals.[2–6] The presence of cytochrome P-450 in human liver microsomes was first reported by Alvares, *et al.*[7] followed by Darby, *et al.*[5] and the authors.[8,9] However, the authors found rather abnormality in substrate P-450 binding spectra and in activities of drug metabolizing enzymes with human liver microsomes, that is, hexobarbital, aminopyrine and SKF 525-A, which induced type I spectra in liver microsomes from experimental animals, induced type II spectra in microsomes from post-mortem human livers, whereas aniline induced type II spectrum being similar to that of experimental animals. Moreover, in human liver, aminopyrine N-demethylase activity and hexobarbital hydroxylase activity were lower than that of rat liver, whereas aniline hydroxylase activity was similar to that of rat liver. Therefore, it was concluded that there may be smaller amount of type I binding site in human liver microsomes than those in experimental animals. Regarding to the drug metabolizing enzymes in liver microsomes of fetuses or of newborns, it is well known that the activities of drug metabolizing enzymes are low or absent in experimental animals during fetal and newborn life. Yaffe, *et al.*[10] demonstrated the presence of cytochrome P-450 and drug metabolizing activities to oxidize laurate and testosterone, but not to oxidize 3,4-benzpyrene and aminopyrine in microsomes from human fetal livers. Furthermore, they found that laurate and testosterone induced similar P-450 binding spectra to those in liver microsomes of experimental animals, whereas aminopyrine induced type II spectrum which was different from that in experimental animals. On the other hand, Juchau[11] and Juchau, *et al.*[12,13] measured considerable benzpyrene hydroxylase activity in human placenta and human fetal tissue homogenates. Studies on drug metabolizing enzymes in human placenta and human fetal liver are very important problem

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10) S.J. Yaffe, A. Rane, F. Sjöquist, L.O. Boréus, and S. Orrenius, *Life Sciences*, **9**, 1189 (1970).
11) M.R. Juchau, *Toxicol. Applied Pharmacol.*, **18**, 665 (1971).
12) M.R. Juchau, M.G. Pedersen, and K.G. Symms, *Biochem. Pharmacol.*, **21**, 2269 (1972).
13) M.R. Juchau and K.G Symms, *Biochem. Pharmacol.*, **21**, 2053 (1972).

to protect fetuses. The present study was initiated in order to clarify whether the ability of human fetal liver to metabolize exogenous compounds is present or not.

## Experimental

**Preparation of Enzyme Source**——We got liver samples from three fetuses, which were obtained by the interuption of pregnancy for medical reasons. All of their mother had taken no drugs during pregnancy. Livers and term placentas were used for experiments within two hours after handling over to us. They were homogenized with ice-cold 1.15% KCl solution in a Teflon-glass homogenizer. The homogenate of placenta was centrifuged at $9000 \times g$ for 20 min. The homogenates of both fetal liver and placenta, and $9000 \times g$ supernatant fraction of placenta were used as enzyme source.

**Enzyme Assay**——Incubation mixture consisted of 1 ml homogenate or $9000 \times g$ supernatant fraction, 0.4 μmole of NADP, 20 μmoles of glucose-6-phosphate, 18.75 μmoles of $MgCl_2$, 2.5 μmoles of substrates (except dimethylaniline, 8 μmoles) and 1 ml of 0.2M phosphate buffer pH 7.4 in a final volume of 2.5 ml. Activity of aniline hydroxylase was estimated by determining *p*-aminophenol according to the method of Brodie and Axelrod[14] as modified by Kato and Gillette.[15] Oxidative demethylation activity was assayed with aminopyrine, morphine, dimethylaniline, ethylmorphine and imipramine as substrates and the amount of formaldehyde formed was measured by the Nash reaction.[16]

## Result and Discussion

Table I shows gestational age, grown-rump length, liver weight of fetal subjects and the activities of aniline hydroxylase by their livers. In all livers, we were able to find considerable activity of aniline hydroxylase, which were about a half of the activity of that in adult rat liver (approximately 500 mμmole/g/30 min). In addition, liver of No.2 was used for measuring the activities of N-demethylases of aminopyrine, morphine, dimethylaniline, ethylmorphine and imipramine, but there were no activities of N-demethylases.

Table I. Description of Human Fetal Subjects, and Aniline Hydroxylating Activity of Human Fetal Liver

| Sample No. | Gestational age (week) | Grown-rump length (cm) | Liver weight (g) | Aniline hydroxylation (mμmole/g/30 min) |
|---|---|---|---|---|
| 1 | 12 | 3.5 | 0.185 | 235.1 |
| 2 | 16 | 9.5 | 2.200 | 242.0 |
| 3 | 28 | — | 25.000 | 183.2 |

The concentration of aniline was 1.0 mM.

The finding that there were no activities of N-demethylases in human fetal liver, was in accordance with those of Yaffe, *et al.*[9] The presence of only aniline hydroxylase activity in fetal liver was presumed to suggest that drugs might be selectively metabolized at the site of placenta, for instance, aniline does not undergo hydroxylation but do other drugs by placenta. To test this hypothesis the authors measured the activities of drug metabolizing enzymes in human term placenta. As expected, the results presented in Table II showed lower activity of aniline hydroxylase than those of N-demethylases of aminopyrine and dimethylaniline.

The fact that type I compound is more preferentially metabolized than type II compound in human term placenta, led us to suppose that most of aniline cross through placental membrane without undergoing hydroxylation, to the fetal liver, where they undergo oxidative

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Table II. Activities of Dimethylaniline (DMA) N-Demethylation, Aminopyrine (AM) N-Demethylation and Aniline (AN) Hydroxylation in Human Term Placenta

| Measurement | Enzyme source | | |
|---|---|---|---|
| | Whole homogenate | | 9000×*g* sup. |
| | (I)[a] | (II)[a] | (II)[a] |
| DMA N-demethylation (mμmole/g/30 min) | 11.1 | 41.0 | 61.1 |
| AM N-demethylation (mμmole/g/30 min) | 33.5 | 51.3 | 53.9 |
| AN hydroxylation (mμmole/g/30 min) | 4.5 | 4.7 | 2.7 |

*a*) Numbers in parentheses represent sample No. employed.

reaction, whereas other drugs may undergo demethylation reaction at the placental membrane. Another explanation to account for the fact that significant aniline hydroxylase activity is detected in the fetal liver, is that aniline hydroxylase is inducible in the fetal livers and the other enzymes are not. But the authors could not examine this speculation that aniline hydroxylase is inducible in the fetal liver since there have been no practical methods to support this speculation. Moreover, it is well established that hydroxylation of 3,4-benzpyrene, which exhibits a type II difference spectrum, is measurable in human placenta, particularly if the pregnant female has been exposed to inducing agents such as tabacco.[11,13] Meigs, *et al.*[17] have reported the existence of cytochrome P-450 in human placenta, however, the authors could not find any cytochrome P-450 peak probably because the authors could not remove blood completely from the placental tissues. Further investigations are needed to resolve such questions described above.

**Acknowledgement** We wish to express our appreciation to Dr. Koshiro Itahashi, Kawasaki Seietsu Hospital, Chiba for kind offer of human term placentas and fetal livers.

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[Chem. Pharm. Bull. 21(10)2331—2335(1973)]

*UDC 547.594.4'532.057 : 547.333.04.09*

# Stereochemical Studies. XXVIII.[1] Some Aspects of the Asymmetric Synthesis of (*R*)-(+)-4-Methyl-4-phenyl-2-cyclohexenone *via* an Enamine

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(Received March 10, 1973)

Previously, the authors reported the new asymmetric synthesis of (*R*)-(+)-4-methyl-4-phenyl-2-cyclohexenone ((*R*)-(+)-I) which featured the alkylation of the enamine (II) prepared from racemic 2-phenylpropanal (III) and L-proline derivative (IV) with methyl vinyl ketone, followed by hydrolysis and ring closure.[3]

1) Part XXVII: G. Otani and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **21**, 2130 (1973).
2) Location: *Hongo, Bunkyo-ku, Tokyo.*
3) *a*) S. Yamada and G. Otani, *Tetrahedron Letters*, **1969**, 4237; *b*) G. Otani and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **21**, 2112, 2119 (1973).