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Foetomaternal relationships of serum bile acid pattern estimated by high-pressure liquid chromatography

Susumu ITOH,* Shoju ONISHI,*† Kenichi ISOBE,‡ Masahiro MANABE‡ and Kazuhisa INUKAI‡
**Department of Pediatrics, Kagawa Medical School, Mikicho Kitagun, Kagawa 760-07, Japan, and*
‡Department of Pediatrics, Nagoya City University, Medical School, Kawasumi Mizuhoku, Nagoya 467, Japan

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The bile acid patterns in the maternal and umbilical vein and artery serum samples were analysed by a two-step chromatographic method involving group separation by piperidinohydroxypropyl-Sephadex LH-20 and high-pressure liquid chromatography using immobilized 3α-hydroxy steroid dehydrogenase. Glycochenodeoxycholate predominates in the maternal blood and taurochenodeoxycholate in the umbilical blood. In cases where a free bile acid was detected in the maternal blood, the same bile acid was also demonstrated in the corresponding cord blood. The concentrations of taurocholate and taurochenodeoxycholate were found to be significantly higher in the umbilical artery than in the corresponding umbilical vein. Our data suggest that there is a bidirectional placental transfer of free bile acids and that there is a transfer of taurine-conjugated primary bile acids from the foetus to the mother.

In recent years, bile acid metabolism in the perinatal period has been investigated in a number of species. It has been demonstrated that in the rhesus monkey, the foetal hepatic excretory mechanism of bile acids is maturing near term and that there is a bile acid exchange across the placenta (Little *et al.*, 1975). In view of the marked species specificity previously shown for other foetal hepatic excretory mechanisms, it is essential to study foetal bile acid metabolism also in man. Simultaneous analysis of bile acid patterns in maternal and umbilical-cord blood samples has not been performed in detail (Laatikainen, 1977; Barbara *et al.*, 1980). In the present work an accurate and sensitive h.p.l.c. method was used to establish the relationship between maternal and foetal bile acid metabolism and to elucidate the placental transfer of free and conjugated bile acids.

Materials and method

Patients

Eight maternal peripheral and umbilical vein and artery blood samples were simultaneously collected immediately after the delivery of the baby. All the pregnancies but one toxaemia of pregnancy were uncomplicated, terminating in labour at 37–41 weeks of gestation. All the four male (mean birth weight 3150g) and four female (mean birth weight 3050g) babies were in good condition. Blood samples were centrifuged and the serum so obtained was deep-frozen at −20°C until analysis. Informed consent was obtained from the participating families. In no case was there gastrointestinal or hepatic disease.

H.p.l.c.

Apparatus and principle, chromatographic operation and sample preparation for h.p.l.c. These were described in detail in the preceding paper (Onishi *et al.*, 1982). The amount of serum samples used was 2.5 ml. Therefore the detection limit was of the order of 10ng, equivalent to 0.01 μM.

Results and discussion

Free and conjugated bile acid concentrations in the maternal and umbilical vein and artery serum samples were estimated by the h.p.l.c. method. Although maternally derived secondary bile acids have been demonstrated in the human foetal bile, the vast majority of the secondary bile acids isolated were identified as sulphated derivatives at the 3α-hydroxy position. Therefore, these bile acids cannot be detected by the present method. The chromatograms are shown in Fig. 1. The mean

Abbreviations used: CA, cholate; CDCA, chenodeoxycholate; DCA, deoxycholate; LCA, lithocholate; a G or T prefix before the aforementioned abbreviations indicates the glycine or taurine conjugate of the respective bile acid; PHP, piperidinohydroxypropyl; h.p.l.c., high-pressure liquid chromatography.

† To whom reprint requests should be sent.

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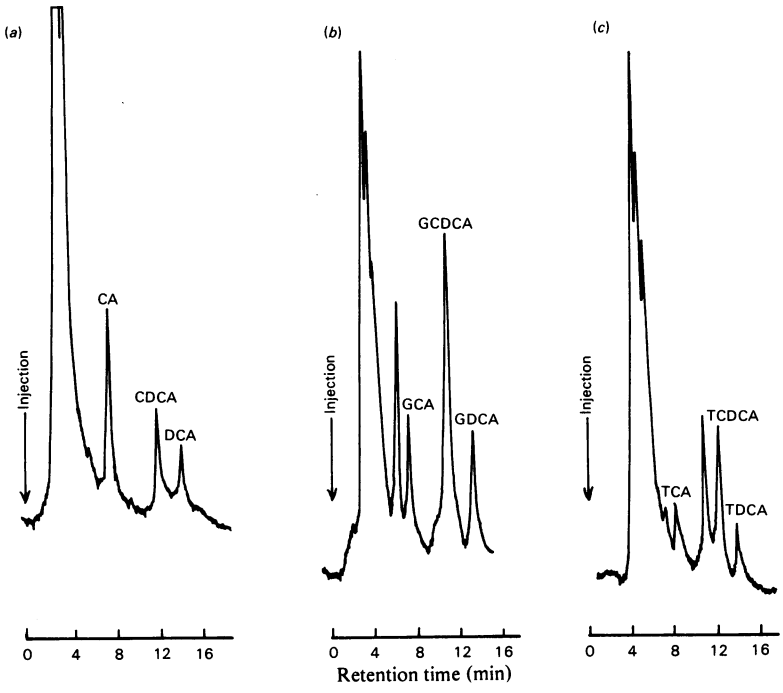

Fig. 1. *H.p.l.c. of each group of free (a) and glycine- (b) and taurine-conjugated (c) bile acids in maternal vein sample*

concentrations and the range are given in Tables 1 and 2. The relative concentration ratios of the primary bile acids CA and CDCA showed a tendency for predominance of CDCA, especially in the umbilical blood. The result was consistent with the previous report that human foetal bile contains more CDCA than CA (Laatikainen, 1977; Barbara *et al.*, 1980), suggesting a deficiency of 12α-hydroxylase in human foetal liver (Sharp & Mirkin, 1972). Conjugated DCA was detected in the maternal serum, but not in the cord serum samples. Therefore, it is likely that there is no detectable transfer of conjugated DCA from mother to foetus. Comparisons of the foetal and maternal concentrations of the serum free CA, CDCA and DCA are shown in Fig. 2. The mean concentrations in the umbilical blood were lower than those in the maternal blood. In cases where a free bile acid was detected in the maternal blood, the same bile acid was also demonstrated in the corresponding cord blood, suggesting a maternal-to-foetal transfer of the free bile acids. It has been demonstrated in dog, sheep and rhesus monkey that there is no foetal synthesis of DCA from cholesterol or CA (Lester *et al.*, 1972; Smallwood *et al.*, 1973; Little *et al.*, 1975). Presumably, the mother is the source of the free secondary bile acid, DCA, in humans. Transplacental transfer of the free primary bile acids CA and CDCA also appears probable. It would be clinically important if excessive transplacental transfer of bile acids from the mother may endanger the foetus when cholestasis of pregnancy or maternal liver disease is present.

Comparisons of the foetal and maternal concentrations of the serum taurine conjugates of CA, CDCA and DCA are shown in Fig. 3. Those of glycine conjugates are also shown in Fig. 4. The concentrations of TCA and TCDCA in the umbilical artery were found to be significantly higher than in the umbilical vein. The mean concentrations of taurine conjugates of the two primary bile acids CA and CDCA in the umbilical blood were higher than those in the mother. The mean concentrations of the glycine conjugates were similar in the umbilical and maternal blood. Taurine conjugates predominated in the foetal samples over glycine conjugates. This finding was in agreement with the higher taurine content of the foetal liver compared with the adult liver in man (Sturman & Gaull, 1975), because bile acid conjugation can be altered by manipulating taurine supply and that bile acids are preferentially conjugated with taurine (Hardison, 1978).

The permeability of the placenta to conjugated bile acids in human subjects has not yet been described (Smallwood *et al.*, 1972). Recently, it was

Table 1. *Comparison of the mean concentrations and the ranges of free and glycine- and taurine-conjugated bile acids in the maternal and umbilical vein and artery serum samples*
Results are means ($n = 8$) with ranges shown in parentheses.

		Maternal vein serum (μM)	Umbilical vein serum (μM)	Umbilical artery serum (μM)
Free	CA	0.056 (0–0.283)	0.018 (0–0.062)	0.017 (0–0.074)
	CDCA	0.041 (0–0.174)	0.021 (0–0.090)	0.013 (0–0.069)
	DCA	0.049 (0–0.144)	0.009 (0–0.028)	0.006 (0–0.028)
Total		0.146 (14.5%)	0.048 (5.4%)	0.036 (3.1%)
Glycoconjugate	CA	0.130 (0.041–0.360)	0.082 (0.062–0.312)	0.089 (0.062–0.242)
	CDCA	0.364 (0.087–0.919)	0.188 (0.038–0.384)	0.238 (0.045–0.684)
	DCA	0.135 (0.029–0.277)	0	0
Total		0.629 (62.6%)	0.270 (30.4%)	0.327 (28.4%)
Tauroconjugate	CA	0.059 (0–0.136)	0.160 (0.085–0.233)	0.213 (0.103–0.459)
	CDCA	0.128 (0.038–0.223)	0.411 (0.149–0.787)	0.577 (0.172–1.559)
	DCA	0.043 (0.005–0.098)	0	0
Total		0.230 (22.9%)	0.571 (64.2%)	0.790 (68.5%)

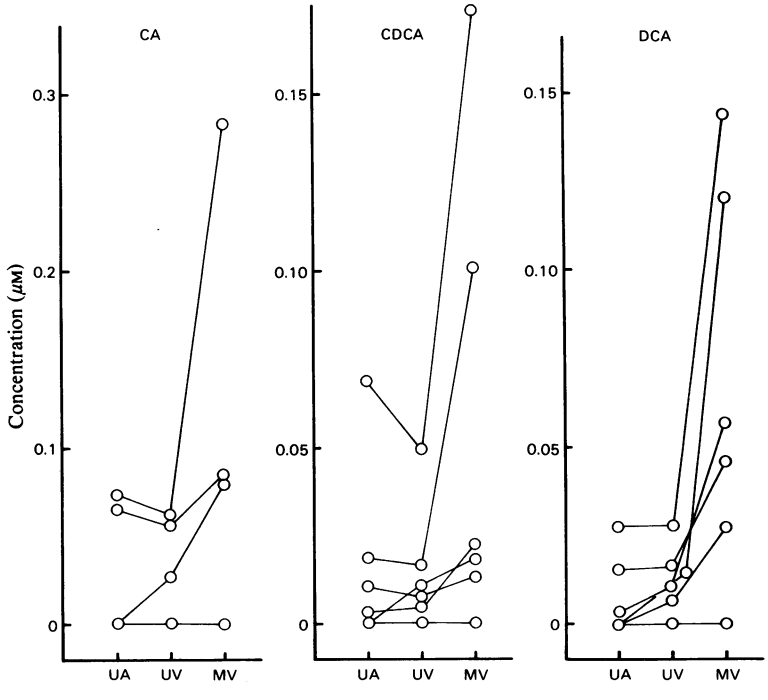

Fig. 2. *Comparison of the concentrations of free CA, CDCA and DCA in maternal and umbilical vein and artery serum samples*

Abbreviations used: UA, umbilical artery; UV, umbilical vein; MV, maternal vein.

demonstrated that in sheep the transfer from the mother, at term, contributes little if any TCA to the foetal pool, but the placenta may be an important excretory organ for foetal bile acids (Sewell *et al.*, 1980). Our data suggest a foetal-to-maternal transfer of taurine-conjugated primary bile acids.

Table 2. *Comparison of the mean concentrations of CA, CDCA and DCA in the maternal and umbilical vein and artery serum samples*

Results are means ($n = 8$).

		Maternal vein serum (μM)	Umbilical vein serum (μM)	Umbilical artery serum (μM)
Cholate	CA	0.056	0.018	0.017
	GCA	0.130	0.082	0.089
	TCA	0.059	0.160	0.213
Total		0.245 (24.4%)	0.260 (29.3%)	0.319 (29.7%)
Chenodeoxycholate	CDCA	0.041	0.021	0.013
	GCDCA	0.364	0.188	0.238
	TCDCA	0.128	0.411	0.577
Total		0.533 (53.0%)	0.620 (69.7%)	0.828 (71.8%)
Deoxycholate	DCA	0.049	0.009	0.006
	GDCA	0.135	0	0
	TDCA	0.043	0	0
Total		0.227 (22.6%)	0.009 (1.0%)	0.006 (0.5%)

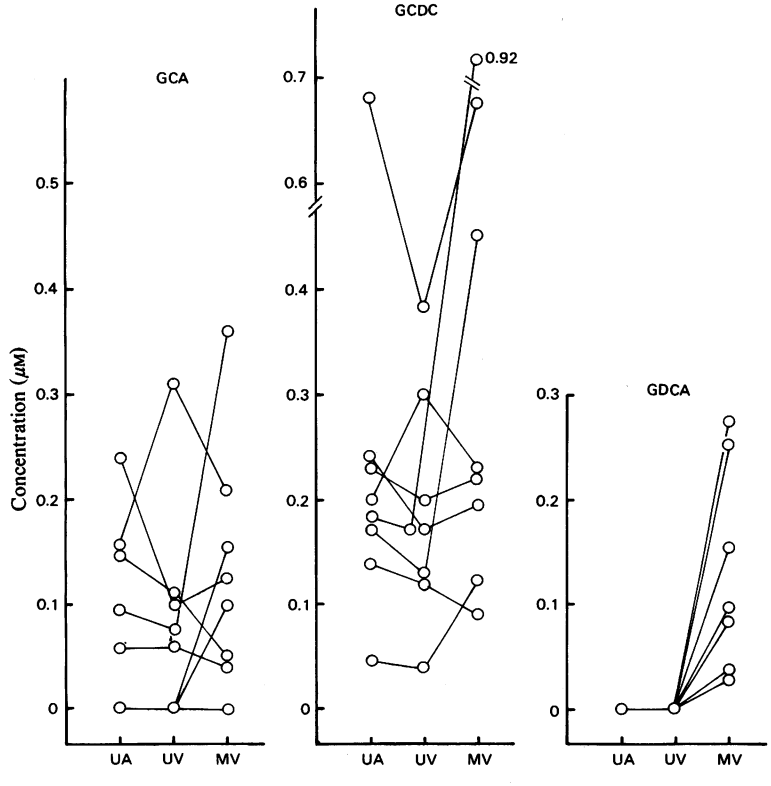

Fig. 3. *Comparison of the concentrations of glycine-conjugated CA, CDCA and DCA in maternal and umbilical vein and artery serum samples*

For definition of abbreviations used, see the legend to Fig. 2.

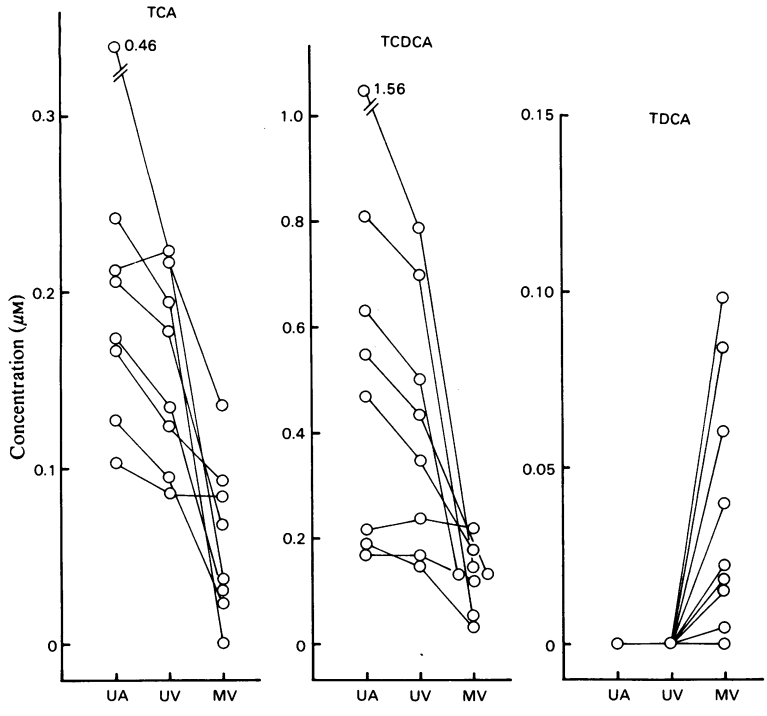

Fig. 4. *Comparison of the concentrations of taurine-conjugated CA, CDCA and DCA in maternal and umbilical vein and artery serum samples*

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