The Amino-acid Pattern in Human Foetal and Maternal Plasma at Delivery

By H. R. CRUMPLER, C. E. DENT AND O. LINDAN
Medical Unit, University College Hospital, London, W.C. 1

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Christensen & Streicher (1948) have now confirmed beyond reasonable doubt the earlier view that differences occurred between the total free α -amino nitrogen concentrations of foetal and maternal plasmas, the foetal being always higher than the maternal. In guinea pigs, for example, the ratio between the two was about 5, in the rabbit 1.5–2.0 and in the human 1.7.* The conclusion reached was that the placenta acted as a pump, withdrawing amino-acids from the mother and then transferring them at a higher concentration into the foetal plasma. Christensen & Streicher (1948) thought that this should facilitate rapid protein synthesis in the foetus on a simple mass action basis, since they were able to show in animal experiments that the raised foetal plasma concentration was associated with a raised concentration of amino-acids in the foetal tissues. Their findings also explained the low plasma α -amino nitrogen (average 3.2 mg./100 ml.) found in pregnant women by Bonsnes (1947), and its return to a normal level (average 4.3 mg./100 ml.) soon after delivery.

In the present investigation we have determined the total α -amino nitrogen of human foetal and maternal plasma and have analysed the differences in amino nitrogen in terms of individual amino-acid concentrations of the foetal and maternal plasmas. In this way the validity of Christensen's hypothesis could be checked, for it is obvious that protein synthesis in the foetus could be facilitated, in the way suggested, only if all the requisite amino-acids were found to be concentrated. If the rise in total α -amino nitrogen was caused entirely by a few amino-acids then the suggestion would have to be made that these few were involved in some process unconnected with the provision of protein-building blocks.

A preliminary report of this work has already appeared (Crumpler, Dent & Lindan, 1950).

METHODS

Cases studied. Some details of the cases chosen are shown in Table 1. All were taken from the labour ward of the Obstetric Hospital, University College Hospital, London.

Collection of blood samples. As soon as the child was born and the umbilical vein clamped and cut, blood was collected

from the placental end of the cord directly into a tube containing heparin. Most of this blood drained from the engorged veins of the cord, and was therefore mainly blood that had just traversed the placenta. This is called 'foetal blood'. The placenta was always *in utero* when the blood was collected, but the placenta may, of course, have become partly detached.

Blood was drawn from the antecubital vein of the mother immediately after the foetal sample was obtained, heparin again being used as anticoagulant.

Chromatographic analysis of plasma. The plasma, separated by centrifugation, was deproteinized by ultrafiltration through a collodion bag as described by Dent & Schilling (1949). The ultrafiltrates were desalted in a micromodification of the electrolytic apparatus described by Consden, Gordon & Martin (1947). They were then ready for paper chromatographic analysis (Consden, Gordon & Martin, 1944), two-way chromatograms on Whatman no. 4 filter paper with phenol- NH_3 and collidine-lutidine as solvents being exclusively used (Dent, 1948). To detect methionine and cystine further chromatograms were carried out on specimens previously treated with H_2O_2 and ammonium molybdate (Dent, 1948). Altogether some sixty chromatograms were prepared. The strength of the ninhydrin colour given by each amino-acid spot was expressed in terms of an arbitrary scale (Dent, 1947). Alanine and glutamine sometimes overlapped and had to be taken together. Leucine and isoleucine, which always overlap with the solvents used here, were taken together and are alluded to as 'leucines'. It must be emphasized that the numerical values assigned to colour strengths of individual amino-acid spots are not directly proportional to the amounts present on the chromatogram; nevertheless, with any one amino-acid, equal colours mean approximately equal amounts.

In five cases, chromatograms on the foetal and maternal plasmas were run using constant volumes (125 $\mu\text{l.}$) so as to compare the total amino-acid concentrations, and to confirm the trends shown by gasometric determinations. In all cases, pairs of plasmas were also compared, when the volume taken was adjusted to contain a constant total of α -amino-N (13 or 26 $\mu\text{g.}$) as obtained from the result of the gasometric analysis (see p. 224). In this way relative concentrations of amino-acids could be compared. The corresponding pairs of foetal and maternal plasmas were always run together in the same cabinet with all procedures being identical. The Cu method of Crumpler & Dent (1949) was used to confirm the identity of the γ -aminobutyric acid.

Analysis for total free α -amino nitrogen. The ninhydrin- CO_2 method of Hamilton & Van Slyke (1943) was used, either on the plasma or on the plasma ultrafiltrate. The ultrafiltrates were treated with picric acid in the same way as the plasmas, before addition of ninhydrin. With every specimen the

* See footnote to Table 4.

analysis was carried out at least in duplicate and an average of closely agreeing values is given. The results are uncorrected for urea.

RESULTS

Eight pairs of foetal and maternal blood were successfully collected and subjected to chromatographic and gasometric analysis. One further pair of specimens (patient M. M.) was analysed only by the gasometric method and another pair (patient S.) only chromatographically. The results are shown in Tables 2-4. Five further unpaired samples of foetal or maternal blood were obtained and analysed. The results fell within the range of the other figures and are not given here. The strengths of the spots for cysteic acid and for methionine sulphone are taken from the additional chromatogram run on the specimen previously oxidized with hydrogen peroxide.

As the original chromatograms are much easier to interpret than the tables, some are reproduced in Figs. 1 and 2, where are shown analyses from patient

M. L. which had the highest (3.0) foetal/maternal α -amino nitrogen ratio. The increased foetal concentration of amino-acids is readily seen in Fig. 1, where a constant volume of plasma ultrafiltrate has been taken. The similarity in the patterns when a constant amount of α -amino nitrogen has been taken can be seen from Fig. 2.

DISCUSSION

It can be seen from Tables 2 and 3 and Fig. 1 that in nearly every case the foetal plasma had a higher total concentration of amino-acids than the maternal, whether the determination was by the roughly quantitative chromatographic or by the gasometric method. This therefore confirms the findings of Christensen & Streicher (1948). The only exception was in the case of J. W. which showed a foetal/maternal α -amino nitrogen ratio of only 1.03 by the gasometric method, while the chromatographic comparisons indicated that amino-acids had been concentrated in the foetal plasma. We cannot under-

Table 1. *Details of patients*

(Patients arranged in order of decreasing foetal/maternal α -amino nitrogen ratio ($\text{NH}_2\text{-N}$ ratio). B.P. = blood pressure; Hb = haemoglobin.)

Patient	Age (years)	Parity	Pre-partum complications	Post-partum complications	Foetus		Foetal- maternal $\text{NH}_2\text{-N}$ ratio
					Wt. (kg.)	Sex	
M. L.	22	0	? Infective arthritis	? Rheumatic carditis	3.32	F	3.0
J. R.	33	2	Thrombophlebitis	Severe varicose veins	3.60	M	1.6
J. B.	20	0	None	None	3.40	M	1.6
I. F.	21	1	None	Anaemia (64% Hb)	3.47	M	1.6
D. H.	25	1	Mild hypertension	Haemorrhage	3.70	M	1.6
I. L.	36	0	Toxaemia (B.P. 158/90)	None	3.15	F	1.4
M. M.	20	0	Pre-eclamptic toxaemia	None	3.40	M	1.3
G. A.	32	1	Pre-eclamptic toxaemia	Anaemia (65% Hb)	3.37	M	1.2
J. W.	25	0	Mild toxaemia and pyelitis	None	3.05	F	1.0

Table 2. *Total α -amino nitrogen ($\text{NH}_2\text{-N}$) of human maternal and foetal plasma*

Patient	Foetal $\text{NH}_2\text{-N}$ (mg./100 ml.)	Maternal $\text{NH}_2\text{-N}$ (mg./100 ml.)	Ratio: foetal $\text{NH}_2\text{-N}$ maternal $\text{NH}_2\text{-N}$
M. L.	6.70*	2.23*	3.00
J. R.	5.19†	3.16†	1.64
J. B.	3.94*	2.40*	1.63
I. F.	5.03†	3.10†	1.62
D. H.	4.32*	2.78*	1.55
I. L.	4.69*	3.37*	1.39
M. M.	4.30†	3.31†	1.30
G. A.	4.42*	3.70*	1.23
	4.62†		
J. W.	2.94*	2.85*	1.03
Range	2.94-6.70	2.23-3.70	1.03-3.00

Values given by other workers

Christensen & Streicher (1948)†	2.91-4.93	2.70-2.98	1.44
Bonsnes (1947)	—	2.4-3.7	—

* Determined on the ultrafiltrate of the plasma.

† Determined directly on the plasma.

‡ In the original paper by Christensen & Streicher (1948) the figures were not for total $\alpha\text{-NH}_2\text{-N}$ but for 'non-glycine non-glutamine $\alpha\text{-NH}_2\text{-N}$ '. These figures gave a foetal/maternal ratio of 1.66. The figures above were kindly provided by Dr H. N. Christensen and are from four of the cases previously reported. The cases were not classified clinically.

stand this discrepancy, the gasometric analysis agreed well (foetal, 2.84, 3.01, 2.96 and maternal, 2.89, 2.81 mg./100 ml.) and so also did duplicate chromatograms.

With regard to the patterns of amino-acid distribution in the pairs of plasmas (Table 4) it seems clear that all the amino-acids present in maternal plasma are present in higher concentrations in the foetal plasma. The same amounts of total α -amino nitrogen were taken for these chromatograms, except in the case of J. W. where the samples were unfortunately too small. Three of the eight pairs were practically

the foetal plasmas do not necessarily prove concentration by the placenta. These compounds are probably synthesized by the foetus from other amino-acids.

With regard to the possible clinical significance of these results there does not appear to be any clear connexion between the value of the foetal/maternal α -amino nitrogen ratio or the foetal α -amino nitrogen concentration, and the size of the foetus (Table 1).

Fig. 1. Diagrams of chromatograms from foetal (a) and maternal (b) plasma ultrafiltrate (patient M. L.). Each sample has been placed at the bottom right-hand corner, phenol- NH_3 has been run from right to left as first solvent, followed by collidine-lutidine in an upward direction. 125 μl . of ultrafiltrate have been taken in each case corresponding to 8.4 μg . of $\alpha\text{-NH}_2\text{-N}$ in the foetal and 2.8 μg . in the maternal plasma. The numbers represent the strengths of the spots on the arbitrary scale used.

identical, while one differed in showing a slightly higher relative concentration of taurine in the foetus. One showed an unusually high concentration of γ -aminobutyric acid. These compounds have not been found in any protein and are presumably not required as protein-building blocks. The remaining three pairs showed minor but real differences which do not appear to show a consistent trend. It seems fair to say, therefore, that the placenta concentrates to fairly equal extents all the amino-acids found in maternal plasma. This supports the theory that the placental 'pump' facilitates protein synthesis in the foetus. The increased concentrations of taurine and of α - and γ -aminobutyric acids found in some of

Fig. 2. Diagrams of chromatograms prepared as described under Fig. 1 from foetal (a) and maternal (b) plasma ultrafiltrate (patient M. L.). 26 μg . of $\alpha\text{-NH}_2\text{-N}$ have been taken in each case corresponding to 390 and 1120 μl . of foetal and maternal plasma ultrafiltrate respectively. The cysteic acid spot in (a) was taken from another chromatogram in which the ultrafiltrate had been previously oxidized with H_2O_2 and ammonium molybdate.

It is interesting that the four definitely toxæmic patients were the only ones with a ratio of 1.4 or less. This is reminiscent of the theories on the connexion between toxæmia and infarcts of the placenta (Young, 1914). It may be that the small ratio indicates a defective placental function, but the number of cases here is too small for it to be more than suggestive.

Table 3. *Paper chromatographic comparison of concentrations of individual amino-acids in foetal and maternal plasma*

(The figures refer to colour strengths of the ninhydrin reaction on an arbitrary scale of 10 units, proline strengths are indicated by plus signs; a dash (-) indicates that the amino-acid was not found in the amount of fluid used.)

Patient	...	...	M. L.*		J. B.		D. H.		J. W.		S.	
Vol. of ultrafiltrate (μl.)			125		125		125		125		125	
Foetal (F) or maternal (M)			F	M	F	M	F	M	F	M	F	M
Cysteic acid (from cystine)	-	-	-	-	-	-	-	-	-	-	-	-
Aspartic acid	6	1	-	-	-	-	-	-	-	-	-	<1
Glutamic acid	7	2	2	1	<1	<1	<1	<1	1	<1	2	2
Serine	4	1	<1	2	<1	2	<1	-	<1	-	2	<1
Taurine	2	1	1	-	<1	-	<1	-	<1	-	-	-
Glycine	4	2	4	2	2	2	1	3	2	4	<1	-
Threonine	-	-	-	-	-	-	-	-	-	-	-	-
Alanine	.	.	6	3	7	7	7	5	6	3	-	-
Alanine + glutamine	7	5	.	.	.	.	.	.	.	.	.	.
Glutamine	.	.	5	<1	5	4	5	4	5	-	-	-
Tyrosine	<1	-	-	-	-	-	-	-	-	-	-	-
α-Amino-n-butyric acid	-	-	-	-	<1	-	-	-	-	-	<1	-
Methionine sulphone (from methionine)	-	-	-	-	-	-	-	-	-	-	-	-
Valine	7	3	3	2	6	4	6	3	5	1	-	-
γ-Aminobutyric acid	5	-	-	-	-	-	-	-	-	-	-	-
Lysine	2	-	2	<1	3	<1	2	<1	1	-	-	-
Phenylalanine	-	-	-	-	-	-	-	-	-	-	-	-
Leucines	4	2	3	1	3	2	5	2	3	<1	-	-
Proline	-	-	-	-	<+	<+	-	-	-	-	-	-
Arginine	-	-	-	-	-	-	-	-	-	-	-	-

* Diagrams of these chromatograms are shown in Fig. 1.

Table 4. *Paper chromatographic comparison of amino-acid patterns in foetal and maternal plasma*

(The figures refer to colour strengths of the ninhydrin reaction on an arbitrary scale of 10 units, proline strengths are indicated by plus signs; a dash (-) indicates that the amino-acid was not found in the amount of fluid used.)

Patient	...	...	M. L.*		J. R.		J. B.		I. F.		D. H.		I. L.		G. A.		J. W.	
Amount of amino-nitrogen taken† (μg.)			13		26		26		26		13		26		26		25	
Foetal (F) or maternal (M)			F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M
Cysteic acid (from cystine)	-	-	1	-	-	-	1	-	-	-	-	2	3	-	-	-	-	-
Aspartic acid	1	1	5	<1	1	<1	-	3	1	1	-	-	-	1	1	1	<1	<1
Glutamic acid	2	2	9	5	3	2	8	4	5	4	4	5	3	4	3	4	4	6
Serine	1	3	7	7	2	2	7	4	2	2	3	3	7	4	2	3	4	8
Taurine	2	2	4	3	2	1	6	-	2	1	5	2	6	2	3	1	2	1
Glycine	2	2	7	6	4	4	7	4	6	5	2	1	8	4	6	4	4	5
Threonine	-	2	4	4	1	<1	-	-	-	-	2	1	7	-	-	1	1	5
Alanine	.	5	.	10	6	6	.	.	10	.	.	.	.	.	.	4	6	8
Alanine + glutamine	5	.	9	.	.	.	10	?	10	.	9	10	10	10	10	.	.	.
Glutamine	.	5	.	9	5	5	.	.	.	9	.	.	.	.	.	6	5	7
Tyrosine	<1	1	1	1	1	1	2	-	1	1	<1	<1	2	2	1	1	1	3
α-Amino-n-butyric acid	-	<1	-	<1	1	1	-	-	-	-	1	<1	<1	-	-	1	<1	4
Methionine sulphone (from methionine)	-	-	-	-	-	-	-	-	-	-	-	<1	-	-	-	-	-	-
Valine	5	8	8	10	5	4	9	8	8	8	7	7	10	8	8	5	5	9
γ-Aminobutyric acid	3	<1	5	2	-	-	-	-	-	-	-	-	-	-	-	<1	<1	-
Lysine	<1	<1	4	3	3	2	7	3	8	7	4	3	7	1	7	3	3	7
Phenylalanine	-	-	1	2	1	1	3	3	1	1	-	-	2	3	2	1	1	4
Leucines	3	5	7	9	4	4	9	9	8	8	5	5	10	10	8	4	5	8
Proline	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arginine	-	-	-	-	-	-	<1	-	-	-	-	<1	-	<1	<1	<1	<1	3

* Diagrams of these chromatograms (26 μg. strength) are shown in Fig. 2.

† As determined by the gasometric method.

Comments: M. L., unusually high concentration of γ-aminobutyric acid especially in foetus. Considerable concentration of aspartic and glutamic acids in foetus; J. R., I. F. and G. A., foetal and maternal patterns almost identical; J. B., more tyrosine and taurine in foetus; D. H., more taurine, lysine and threonine in foetus; I. L., almost identical foetal and maternal patterns except for more taurine in foetus; J. W., several amino-acids stronger in foetus, probably more than accountable by the greater NH₂-N taken for analysis.

SUMMARY

1. Samples of foetal and maternal blood collected at the moment of birth have been analysed by chemical means for total α -amino nitrogen and by paper chromatography for their content of individual amino-acids.

2. In nearly every case the foetal plasma had a higher concentration of α -amino nitrogen than the maternal. The increase involved all the amino-acids commonly found in these fluids.

3. These results are taken to support the view that the placenta acts as an amino-acid pump assisting the foetus to synthesize proteins.

4. A low foetal/maternal ratio for the plasma α -amino nitrogen occurred only in four out of nine cases. In these four cases the mother had definite toxæmia of pregnancy. It is suggested tentatively that this may indicate that some impairment of placental function occurs in toxæmia.

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REFERENCES

- Bonsnes, R. W. (1947). *J. biol. Chem.* **168**, 345.
 Christensen, H. N. & Streicher, J. A. (1948). *J. biol. Chem.* **175**, 95.
 Consden, R., Gordon, A. H. & Martin, A. J. P. (1944). *Biochem. J.* **38**, 224.
 Consden, R., Gordon, A. H. & Martin, A. J. P. (1947). *Biochem. J.* **41**, 580.
 Crumpler, H. R. & Dent, C. E. (1949). *Nature, Lond.*, **164**, 441.
 Crumpler, H. R., Dent, C. E. & Lindan, O. (1950). *Biochem. J.* **46**, xx.
 Dent, C. E. (1947). *Biochem. J.* **41**, 240.
 Dent, C. E. (1948). *Biochem. J.* **43**, 169.
 Dent, C. E. & Schilling, J. A. (1949). *Biochem. J.* **44**, 318.
 Hamilton, P. B. & Van Slyke, D. D. (1943). *J. biol. Chem.* **150**, 231.
 Young, J. (1914). *Proc. R. Soc. Med.* **7**, 307.

Reactivation and Protection of Cytochrome Oxidase Preparations

By H. BOREI*

Molteno Institute, University of Cambridge

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Working with isolated cytochrome systems Keilin & Hartree (1947*a, b*, 1949) showed that the loss of activity caused by treatments which affect the colloidal structure of the enzyme material could be counteracted and the activity restored by addition of proteins, or by otherwise causing formation of flocculent precipitates in the medium. They suggested that the loss of activity is due to reduced mutual accessibility between the components of the system. The first part of the present paper deals with that gradual drop in activity of the cytochrome oxidase system, which occurs when the oxidase preparation is stored, and it will be shown that this activity can also be restored by the addition of protein.

It was observed by Keilin & Hartree (1938, 1949) that the ability of cytochrome oxidase preparations to oxidize aromatic amines or succinate is rapidly destroyed by drying. The second part of the paper shows that the addition of protein before desiccation will greatly, and in some cases completely, protect the preparations against this inactivation.

* Now at Wenner-Grens Institut, University of Stockholm, Sweden.

The activity of the oxidase system in tissue preparations is measured by the rate of oxidation of *p*-phenylenediamine in presence of added cytochrome *c*.

MATERIAL AND METHODS

Units. All Q_{O_2} values are in μ l. O_2 /mg. dry wt./hr.

Test system. The oxidation of *p*-phenylenediamine was followed at 39° in differential manometers; shaking frequency 120 times/min.; total fluid volume 3 ml. The substrate (final concn. 0.03M) was tipped at the beginning of the measurements from a dangling cup into the main compartment. Proteins and other substances were brought in contact with the oxidase (with or without cytochrome *c*) in the main compartment at least 30 min. before the addition of the substrate. The initial velocity was obtained from the difference between the readings at 3 and 13 min. Phosphate buffer of pH 7.2 and of final concentration 0.08M was used. In the left-hand flask a similar sample was set up, the only difference being that the oxidase preparation was boiled. By this means the autoxidation of the substrate under the conditions of the experiment was largely compensated for.

Cytochrome oxidase preparations. Two different oxidase preparations from horse-heart muscle were used: (1) the high-activity preparation (HA) according to Keilin &