Plasma Amino Acid Concentrations in Pregnant Rats and in 21-Day Foetuses

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Plasma amino acid concentrations were determined in virgin female rats, in pregnant rats (12 and 21 days after impregnation) and in 21-day foetuses. The total amino acid concentration in plasma decreases significantly with pregnancy, being lower at 12 than at 21 days. Alanine, glutamine+glutamate and other 'gluconeogenic' amino acids decrease dramatically by mid-term, but regain their original concentrations at the end of the pregnancy. With most other amino acids, mainly the essential ones, the trend is towards lower concentrations which are maintained throughout pregnancy. These data agree with known nitrogen-conservation schemes in pregnancy and with the important demands on amino acids provoked by foetal growth. In the 21-day foetuses, concentrations of individual amino acids are considerably higher than in their mothers, with high plasma foetal/maternal concentration ratios, especially for lysine, phenylalanine and hydroxyproline, suggesting active protein biosynthesis and turnover. All other amino acids also have high concentration ratios, presumably owing to their requirement by the foetuses for growth. Alanine, glutamine+glutamate, asparagine+aspartate, glycine, serine and threonine form a lower proportion of the total amino acids in foetuses than in the virgin controls or pregnant rats, probably owing to their role primarily in energy metabolism in the adults. The results indicate that at this phase of foetal growth, the placental amino acid uptake is considerable and seems to be higher than immediately before birth.

There has been considerable work on the effect of pregnancy and foetal development with regard to carbohydrate and lipid metabolism as well as their hormonal regulation (Scow *et al.*, 1964; Herrera *et al.*, 1969; Knopp *et al.*, 1970; Metzger *et al.*, 1970, 1971), but little has been done on the general aspects of protein metabolism in this physiological situation.

Amino acid metabolism is of crucial importance in the close relationship that exists between mother and foetus during pregnancy. The mother must respond to the ever-increasing demands of foetal growth, supplying all the materials needed for synthesis and to meet energy requirements through the placenta. Amino acids cross the placenta and are concentrated on the foetal side, giving rise to high foetal/maternal amino acid concentration ratios (Glendening *et al.*, 1961; Butterfield *et al.*, 1962; Ackermann & Kheim, 1964; Ghadimi & Pecora, 1964). These amino acids are likely to be used mainly for foetal growth. Foetal requirements increase exponentially, and the mother must cope with this situation by increasing the availability of amino acids for foetal growth with a consequent decrease in her own concentrations of plasma amino acids (Beaton *et al.*, 1954; Felig *et al.*, 1972).

Most available data are from humans (Crumpler *et*

al., 1950; Glendening *et al.*, 1961; Ghadimi & Pecora, 1964; Felig *et al.*, 1972), for the pregnant female and for the infant after birth or in different pathological states (Berry *et al.*, 1975); obtained to assist early detection of inborn errors of amino acid metabolism.

Accurate micromethods for the determination of amino acids and protein in small tissue samples are required if investigations in this area are to be pursued in the common laboratory animals under controlled conditions. The adaptation of simple available methodology (Arola *et al.*, 1976, 1977) has enabled us to investigate this problem.

The objective of the present work has been to examine the changes in amino acid concentrations during pregnancy and to measure the amino acid concentrations in foetal plasma before birth, at a stage preceding the well-described dramatic decrease in the plasma amino acid concentrations that occurs with and after birth (Girard *et al.*, 1973).

Materials and Methods

Albino Wistar female rats were used. The day of gestation was determined by means of vaginal smears and the detection of spermatozoa. Pregnant females were kept in individual cages with food and water *ad libitum* in a controlled environment (constant

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temperature 23°C, and a light cycle of 12 h on, 12 h off). Adult rats were killed by decapitation and samples of blood were immediately obtained from the neck into heparinized glass capillary tubes. Foetuses were rapidly dissected and freed from the foetal envelopes. They also were killed by decapitation and up to 0.3 ml of blood was obtained with capillary tubes from the neck.

Tubes filled with blood were plugged with Cryto-Seal (Fisher Scientific Co., Pittsburgh, PA, U.S.A.) and centrifuged for 20 min at 2000g; the haematocrit values were determined in this way for each individual sample. The plasma was separated for total plasma protein determination (Lowry *et al.*, 1951; Wang & Smith, 1975) with de-fatted bovine serum albumin as the standard, and for the determination of individual amino acids by a highly sensitive radiochemical assay based on the t.l.c. of dansyl*-amino acids, labelled in their dansyl moiety with ¹⁴C

* Abbreviation: dansyl, 5-dimethylaminonaphthalene-1-sulphonyl.

(Arola *et al.*, 1976) after deproteinization with acetone (Arola *et al.*, 1977). With this system, most amino acids were individually quantified, except for methionine+ornithine and leucine+isoleucine pairs, which were not properly distinguished. Glutamine and asparagine were partially deamidated during storage of samples, giving higher glutamate and aspartate concentrations (Arola *et al.*, 1976a). For this reason they are given as composite values. Cysteine, cystine and cysteic acid were properly separated in the chromatographic system, but in view of the possibility of interconversion during manipulation it was considered advisable to present a composite value in terms of 'cysteine'.

To distinguish better the effects of pregnancy on the different amino acids, they have been allocated to six physiological and structural groups: aromatic amino acids (tyrosine, phenylalanine and tryptophan); sulphur amino acids [taurine, 'cysteine' (containing also cysteate and twice the cystine value) and methionine+ornithine (regardless of the latter

Table 1. Amino acid concentrations ($\mu\text{mol/litre}$) in the plasma of 12- and 21-days-pregnant rats, virgin female controls and 21-day foetuses

Each value is the mean $\pm$ S.E.M. for seven to nine different individuals.

Amino acids	Control	Pregnant 12 days	Pregnant 21 days	Foetuses 21 days
'Gluconeogenic' total	2733 $\pm$ 90	2284 $\pm$ 45	2890 $\pm$ 145	4389 $\pm$ 314
Ala	778 $\pm$ 69	501 $\pm$ 27	892 $\pm$ 64	1508 $\pm$ 153
Glu+Gln	977 $\pm$ 50	838 $\pm$ 17	945 $\pm$ 56	1507 $\pm$ 99
Asp+Asn	187 $\pm$ 13	156 $\pm$ 10	220 $\pm$ 9	225 $\pm$ 25
Gly	272 $\pm$ 25	230 $\pm$ 16	221 $\pm$ 22	423 $\pm$ 43
Ser	387 $\pm$ 25	358 $\pm$ 14	392 $\pm$ 26	528 $\pm$ 38
Thr	230 $\pm$ 12	200 $\pm$ 17	220 $\pm$ 18	182 $\pm$ 13
Imino acids total	415 $\pm$ 28	244 $\pm$ 19	246 $\pm$ 16	732 $\pm$ 41
Pro	362 $\pm$ 30	212 $\pm$ 17	203 $\pm$ 13	577 $\pm$ 34
Hyp	54 $\pm$ 4	32 $\pm$ 2	43 $\pm$ 4	156 $\pm$ 14
Basic total	934 $\pm$ 31	843 $\pm$ 30	673 $\pm$ 65	2101 $\pm$ 109
Lys	515 $\pm$ 48	467 $\pm$ 17	349 $\pm$ 47	1407 $\pm$ 93
Arg	221 $\pm$ 36	179 $\pm$ 21	162 $\pm$ 20	409 $\pm$ 37
His	113 $\pm$ 14	82 $\pm$ 8	85 $\pm$ 5	224 $\pm$ 25
Cit	85 $\pm$ 7	96 $\pm$ 10	81 $\pm$ 8	79 $\pm$ 9
Branched-chain total	514 $\pm$ 24	546 $\pm$ 14	537 $\pm$ 19	982 $\pm$ 77
Val	202 $\pm$ 14	187 $\pm$ 15	151 $\pm$ 14	229 $\pm$ 15
Leu+Ile	312 $\pm$ 18	359 $\pm$ 14	386 $\pm$ 19	753 $\pm$ 69
Sulphur total	714 $\pm$ 41	643 $\pm$ 60	498 $\pm$ 37	1106 $\pm$ 68
'Cysteine'	107 $\pm$ 6	90 $\pm$ 4	85 $\pm$ 7	119 $\pm$ 9
Met+Orn	127 $\pm$ 11	165 $\pm$ 18	141 $\pm$ 10	321 $\pm$ 23
Taurine	479 $\pm$ 55	389 $\pm$ 44	271 $\pm$ 29	643 $\pm$ 52
Aromatic total	464 $\pm$ 18	415 $\pm$ 10	318 $\pm$ 26	899 $\pm$ 66
Tyr	146 $\pm$ 16	151 $\pm$ 8	130 $\pm$ 14	268 $\pm$ 39
Trp	256 $\pm$ 18	210 $\pm$ 9	137 $\pm$ 13	262 $\pm$ 24
Phe	62 $\pm$ 4	54 $\pm$ 2	45 $\pm$ 4	191 $\pm$ 10
Combined total	5814 $\pm$ 125	5009 $\pm$ 149	5278 $\pm$ 210	10288 $\pm$ 472

belonging to the basic amino acid group)]; branched-chain amino acids (valine and leucine+isoleucine); basic amino acids (lysine, arginine, histidine and citrulline); imino acids (proline and hydroxyproline); 'gluconeogenic' amino acids (alanine, glutamate+glutamine, aspartate+asparagine, serine, threonine and glycine). The last set is derived from the consideration that these amino acids constitute the bulk of gluconeogenic amino acid substrates (Felig, 1973; Aikawa *et al.*, 1973) and it has been decided to group them under the name 'gluconeogenic' amino acids regardless of other amino acids being possible substrates for gluconeogenesis (e.g. proline, valine, phenylalanine).

Tests for significant differences between means were done by using Student's *t* test. All calculations were done on a Compucorp 445 Statistician desk computer.

Results

Tables 1 and 2 summarize the concentrations observed for individual amino acids in plasma of 12- and 21-days-pregnant rats, virgin female controls and 21-day foetuses. The foetuses have significantly higher concentrations both of total plasma amino acids and of most individual amino acids. Pregnant rats, in general, have lower concentrations of amino acids than their controls. The differences depend on the time of gestation; 12-days-pregnant rats have a significantly lower total amino acid concentration than virgin controls and than 21-days-pregnant rats. The latter also have lower values than controls.

Foetuses have total amino acid concentrations significantly higher than the controls and than the 21-days-pregnant rats.

Considering the amino acids by groups of physio-

Table 2. Statistical evaluation of the mean differences in amino acid concentrations shown in Table 1
↑ indicates significant increase versus the group compared; ↓ indicates significant decrease versus the group compared; — indicates no significant changes (*P*>0.05); ↑ or ↓ = *P*<0.05; ↑↑ or ↓↓ = *P*<0.01; ↑↑↑ or ↓↓↓ = *P*<0.001.

Amino acids	Pregnant 21 days			Foetuses 21 days		
	Pregnant versus controls	versus controls	versus pregnant 12 days	versus controls	versus pregnant 21 days	
'Gluconeogenic' total	↓↓↓	—	↑↑	↑↑↑	↑↑	
Ala	↓	—	↑↑↑	↑↑↑	↑↑↑	↑↑↑
Glu+Gln	↓	—	↑↑↑	↑↑↑	↑↑↑	↑↑↑
Asp+Asn	—	—	↑↑↑	—	—	—
Gly	—	—	—	↑	↑	↑↑
Ser	—	—	—	↑	↑	↑
Thr	—	—	—	↓	—	—
Imino acids total	↓↓↓	↓↓↓	—	↑↑↑	↑↑↑	
Pro	↓↓↓	↓↓↓	↓	↑↑	↑↑↑	↑↑↑
Hyp	↓↓↓	—	—	↑↑↑	↑↑↑	↑↑↑
Basic total	—	↓↓	↓	↑↑↑	↑↑↑	
Lys	—	↓	—	↑↑↑	↑↑↑	↑↑↑
Arg	—	—	—	↑↑↑	↑↑↑	↑↑↑
His	—	—	—	↑↑↑	↑↑↑	↑↑↑
Cit	—	—	—	—	—	—
Branched-chain total	—	↓↓	↓	↑↑↑	↑↑↑	
Val	—	↓	—	—	—	↑↑
Leu+Ile	—	↑	—	↑↑↑	↑↑↑	↑↑↑
Sulphur total	—	↓↓	—	↑↑↑	↑↑↑	
'Cysteine'	↓	↓	—	—	—	↑
Met+Orn	—	—	—	↑↑↑	↑↑↑	↑↑↑
Taurine	—	↓↓	—	↑	↑↑↑	↑↑↑
Aromatic total	↓	↓↓↓	↓	↑↑↑	↑↑↑	
Tyr	—	—	—	↑	—	↑↑
Trp	—	↓↓↓	↓↓↓	—	—	↑↑↑
Phe	—	↓	—	↑↑↑	↑↑↑	↑↑↑
Combined total	↓↓	↓	—	↑↑↑	↑↑↑	

logical affinity shows that the concentrations of the 'gluconeogenic' amino acids tend, in general, to be lower in the 12-days-pregnant rats than in the virgin controls. At 21 days of pregnancy the differences from the controls are no longer significant. The most important amino acids in this group, as indicated by their concentrations, are glutamine+glutamate and alanine; alanine appears to have an even slightly higher concentration in 21-days-pregnant rats than in controls, but this difference is not significant. In the foetuses, the concentrations of alanine and glutamate+glutamine are very close and considerably higher than in controls and 12- and 21-days-pregnant rats. All other amino acids included in this group show changes similar to that of alanine. The foetal/maternal and foetal/control ratios of amino acid concentrations are given in Table 3. The value of the foetal/maternal ratio for combined 'gluconeogenic' amino acids is 1.52. With threonine, however, the ratio is considerably lower, reflecting a lower threonine value in foetuses than in their mothers.

Table 3. *Foetal/maternal and foetal/control ratios for plasma amino acid concentrations*

Values are calculated from the data in Table 1.

Amino acid	Foetal/ maternal ratio	Foetal/ control ratio
'Gluconeogenic' total	1.52	1.61
Ala	1.69	1.94
Glu+Gln	1.59	1.54
Asp+Asn	1.02	1.21
Gly	1.91	1.55
Ser	1.33	1.37
Thr	0.83	0.79
Imino acids total	2.98	1.76
Pro	2.84	1.59
Hyp	3.65	2.90
Basic total	3.12	2.25
Lys	4.03	2.73
Arg	2.52	1.85
His	2.76	1.98
Cit	0.97	0.92
Branched-chain total	1.83	1.91
Val	1.51	1.13
Leu+Ile	1.95	2.41
Sulphur total	2.22	1.55
Taurine	2.37	1.34
'Cysteine'	1.39	1.11
Met+Orn	2.28	2.52
Aromatic total	2.83	1.94
Tyr	2.06	1.83
Trp	1.92	1.02
Phe	4.21	3.08
Combined total	1.95	1.77

Aspartate+asparagine also have a relatively low (1.02) foetal/maternal ratio and glycine a higher value (1.91). The foetal/control concentration ratios for the 'gluconeogenic' amino acids are rather similar to the foetal/maternal values, but higher than the latter for total 'gluconeogenic' amino acids, alanine and aspartate+asparagine, and lower for glycine and threonine.

The foetal-maternal difference for the imino acids is more pronounced. The concentration decrease for the combined imino acids is abnormally large with pregnancy and is sustained to 21 days, and there is also a sharp increase in the foetal concentration. As a consequence, the foetal/maternal ratios of concentrations for these imino acids are abnormally high (for combined imino acids, 2.98). Pregnancy-induced changes in the hydroxyproline concentration resemble those observed for alanine and glutamate+glutamine, but in the foetuses hydroxyproline increases extraordinarily in comparison with both controls and 21-days-pregnant rats. Proline continues to decrease during gestation and also shows a sharp rise in the foetuses. The foetal/maternal control ratios are considerably lower than foetal/maternal ratios for both proline and hydroxyproline.

The combined concentration of basic amino acids is lower in the 21-days-pregnant rat than in the virgin controls or in the 12-days-pregnant animals. The values for foetuses are extremely high (about 20% of the total). The foetal/maternal ratio is the highest (3.12), denoting a very active concentration process. The major share in this group belongs to lysine, with a foetal/maternal ratio of 4.03 and about 14% of the total foetal amino acids (versus about 9% in the controls). Arginine and histidine show the same pattern of change, but citrulline was in very low concentrations throughout gestation and in the foetuses. The foetal/control ratios are lower than the foetal/maternal, except with citrulline, for which very similar ratios are observed.

Branched-chain amino acids show a steady concentration throughout pregnancy owing to the complementary behaviour of valine and leucine+isoleucine. Valine shows a continuing decrease, significant at 21 days (versus the controls), whereas leucine+isoleucine shows a slight increase, also significant versus virgins at 21 days. Foetal/maternal concentration ratios of 1.83 are observed for the combined branched-chain amino acids. The foetal/control and foetal/maternal ratios are very similar for the total branched-chain amino acids, owing to the compensatory changes of higher leucine+isoleucine and lower valine concentration ratios.

Sulphur amino acids show as a whole a decrease with pregnancy, and the combined value became significantly lower than the controls at 21 days. This behaviour is observed both in 'cysteine' and methionine (+ornithine) and taurine, the latter show-

Table 4. *Plasma total proteins and haematocrit values of virgin females, pregnant rats and their foetuses*

Results are mean $\pm$ S.E.M. of six to eight adult rats or of 22 foetuses. Significance versus controls: * $P < 0.05$; ** $P < 0.001$. Significance of foetuses versus their mothers: † $P < 0.001$. Significance of 12-days pregnant versus 21-days pregnant: ‡ $P < 0.001$.

	Control	Pregnant 12 days	Pregnant 21 days	Foetuses 21 day
Total plasma proteins (g/100ml)	5.89+0.45	6.08+0.45	5.96+0.21	2.24+0.08**†
Haematocrit value (%)	48.86+1.16	43.65+0.83*	36.14+0.67**‡	36.78+0.78**

ing the highest concentration. In the foetuses all values are higher than in controls or pregnant animals. The foetal/maternal concentration ratio is 2.22 for the combined sulphur amino acids; 'cysteine' shows a lower value (1.39). The foetal/control concentration ratios for sulphur amino acids are considerably lower than the foetal/maternal concentration ratios, except for methionine+ornithine which is higher.

Considered as a group, the aromatic amino acids show changes resembling those for the sulphur amino acids; their combined concentration is considerably and significantly lower in the 12- and 21-days-pregnant rats than in the controls. Tryptophan and phenylalanine follow this same pattern, but tyrosine concentration does not decrease with pregnancy. In the foetuses there is a considerably higher concentration of these amino acids, with a foetal/maternal ratio for the combined amino acids of 2.83, and of 4.21 for phenylalanine. The foetal/control ratios for aromatic amino acids are considerably lower than the foetal/maternal ratios.

The total plasma protein concentration (Table 4) is rather constant during the pregnancy, and apparently not different from the control value, but foetuses show a very much lower concentration, the value being significantly lower than that of the virgin controls and of the pregnant rats. Haematocrit values show a constant and significant decline with pregnancy. Foetuses also show a significantly lower value than controls, practically equal to that found in their mothers.

Discussion

The amino acid concentrations found in this work for virgin female rats are in general agreement with other published plasma amino acid concentrations (Scharff & Wool, 1966; Minemura *et al.*, 1970; Christophe *et al.*, 1971; Ferris & Clark, 1972; Arola *et al.*, 1976). The concentrations of 'gluconeogenic' amino acids in the controls are close to those reported by Minemura *et al.* (1970), Christophe *et al.* (1971) and Itoh *et al.* (1974) and those of branched-chain amino acids agree with values of Scharff & Wool (1966) and Bloxam & Fisher (1975). Proline values are similar to those found by Ferris & Clark (1972),

but considerably higher (Bloxam & Fisher, 1975) or lower (Itoh *et al.*, 1974) than other reported data. For the sulphur amino acids, there is general agreement with Scharff & Wool (1966), but our concentrations are higher than other published values. Tryptophan values are considerably higher than the sparse data found in the literature (Tagliamonte *et al.*, 1974), probably owing to a better recovery technique (Arola *et al.*, 1976). The other amino acids are found in concentrations similar to those already described in the literature, except for lysine. Our lysine values agree with those of Christophe *et al.* (1971), Ferris & Clark (1972) and Bloxam & Fisher (1975), but are higher (Scharff & Wool, 1966; Minemura *et al.*, 1970) or lower (Itoh *et al.*, 1974) than others.

For pregnancy the data are more sparse, and mostly limited to values found in humans, typically at term. We found that the concentrations of most plasma amino acids decrease with pregnancy, in accordance with published observations (Beaton *et al.*, 1954), and this trend is more pronounced at 12 than at 21 days of gestation. Alanine, possibly the main gluconeogenic substrate, shows a considerable decrease with pregnancy at 12 days, increasing later to a value slightly higher than in the controls. Glutamate+glutamine, serine and threonine, as well as the 'gluconeogenic' amino acids as a whole, behave in a similar way, suggesting a change in the utilization of these amino acids by the mother (the size of the combined foetuses is comparatively small). In the initial phase of pregnancy there may well be an increase in demand for glucose and other materials to meet the needs of foetal growth and metabolism, which imposes a considerable strain on the metabolic resources of the mother. These increased nutrient requirements are not fulfilled by an increased food consumption, as there is no increase in food ingestion until after about day 12 of pregnancy (Knopp *et al.*, 1975). At this stage of pregnancy it is postulated that gluconeogenesis must be enhanced, partly by the use of gluconeogenic amino acids, which may account for the sharp decrease in their plasma concentrations. The last phase of pregnancy brings with it the need to increase nitrogen conservation (Freinkel *et al.*, 1972), to avoid the excessive utilization of amino acids for gluconeogenesis; and by that time gluconeogenesis has become normal

(compared with virgin control rats) in the fed state (Herrera *et al.*, 1969). This finding is supported by the return to normal concentrations of these amino acids, with significant increases in alanine, aspartate+asparagine and in the combined 'gluconeogenic' amino acids at 12 compared with 21 days of pregnancy.

Most other amino acids studied show a tendency towards lower concentrations with pregnancy. This is shown significantly by proline, lysine, valine, 'cysteine', taurine, tryptophan and phenylalanine, as well as by the composite values for imino acids and for the basic, sulphur and aromatic amino acid groups. Branched-chain amino acids do not show this trend, as the significant decrease in valine is compensated by a significant rise in leucine+isoleucine. There is a general trend towards a decrease in the concentrations of essential amino acids, probably due to their continuous accumulation in the foetuses.

In agreement with other authors (Beaton *et al.*, 1954; Morgan, 1973) we have observed a decrease in haematocrit value with pregnancy. It appears, however, that haemodilution is accompanied by a compensating synthesis of plasma proteins since no significant change in total plasma protein concentration was found, regardless of the described variations in individual concentrations of certain plasma proteins (Morgan, 1973) during pregnancy.

In the foetuses, the blood is considerably diluted in comparison with the controls, as indicated by their low plasma protein concentration. The haematocrit value is very similar to that of their mothers.

The concentrations of amino acids in the plasma of the rat foetuses are considerably higher than in their mothers or controls. This is in general agreement with published data (Crumpler *et al.*, 1950; Glendening *et al.*, 1961; Ghadimi & Pecora, 1964; Berry *et al.*, 1975), indicating an active process of concentration of amino acids by the placenta (Crumpler *et al.*, 1950). Foetal values are close to those of the controls in the relative amounts of the individual amino acids, as shown by the relatively greater uniformity of the foetal/control concentration ratios than of the foetal/maternal ones. The percentage distribution of amino acids in the foetuses is closer to that of the controls than to that of their mothers, probably because of differences in efficiency of the placenta in the process of concentration of the individual amino acids from the maternal plasma, as suggested by the different foetal/maternal ratios of concentrations for the different amino acids. Our ratios are in general accordance with previously reported values (Glendening *et al.*, 1961; Butterfield *et al.*, 1962; Ghadimi & Pecora, 1964; Berry *et al.*, 1975), but most of these data refer to humans at term and thus are not fully comparable with ours. Increases in concentrations for the individual amino acids with respect to controls are significant for all amino acids except for

aspartate+asparagine, citrulline, valine, 'cysteine' and tryptophan, and foetal concentration is lower only for threonine. In comparison with their mothers, the situation is similar, but all amino acids except aspartate+asparagine, citrulline and threonine are at significantly higher concentrations in the foetuses.

In the pregnant rat there is a marked tendency towards diminished amino acid catabolism, which serves to prevent nitrogen losses during the last part of pregnancy (Freinkel *et al.*, 1972). The foetus also shows evidence of nitrogen conservation in its low gluconeogenic ability (Girard *et al.*, 1975). As there is a considerable degree of amino acid concentration across the placenta, it can be suggested that most of these amino acids serve the purpose of protein biosynthesis in the foetus.

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References

- Ackerman, P. G. & Kheim, T. (1964) *Clin. Chem.* **10**, 32-40
- Aikawa, T., Matsutaka, H., Takezawa, K. & Ishikawa, E. (1973) *J. Biochem. (Tokyo)* **74**, 1003-1017
- Arola, L., Palou, A., Herrera, E. & Alemany, M. (1976) *Biochimie* **58**, 1221-1226
- Arola, L., Herrera, E. & Alemany, M. (1977) *Anal. Biochem.* in the press
- Beaton, G. H., Beare, J., Ryu, M. H. & McHenry, E. W. (1954) *J. Nutr.* **54**, 291-304
- Berry, H. K., Poncet, I. B., Sutherland, B. S. & Burkett, R. (1975) *Biol. Neonate* **26**, 102-108
- Bloxam, D. L. & Fisher, M. M. (1975) *Lab. Invest.* **31**, 705-711
- Butterfield, L. J., Walker, D. M. & O'Brien, D. (1962) *Ann. Meet. Atlantic City*, p. 134
- Christophe, P., Winand, J., Kutzner, R. & Hebbelinck, M. (1971) *Am. J. Physiol.* **221**, 453-457
- Crumpler, H. R., Dent, C. E. & Lindan, O. (1950) *Biochem. J.* **47**, 223-227
- Felig, P. (1973) *Metabolism* **21**, 179-207
- Felig, P., Kim, Y. J., Lynch, V. & Hendler, R. (1972) *J. Clin. Invest.* **51**, 1195-1202
- Ferris, G. M. & Clark, J. B. (1972) *Biochim. Biophys. Acta* **273**, 73-79
- Freinkel, N., Metzger, B. E., Nitzan, M., Hare, J. W., Shambaugh, G. E., Marshall, R. T., Surmaczynska, B. Z. & Nagel, T. G. (1972) *Isr. J. Med. Sci.* **8**, 426-439
- Ghadimi, H. & Pecora, P. (1964) *Pediatrics* **33**, 500-506
- Girard, J. R., Cuendet, G. S., Marliss, E. B., Kervran, A., Rieutort, M. & Assan, R. (1973) *J. Clin. Invest.* **52**, 319-322
- Girard, J. R., Guillet, I., Marty, J. & Marliss, E. B. (1975) *Am. J. Physiol.* **229**, 466-473
- Glendening, M. B., Margolis, A. J. & Page, E. W. (1961) *J. Obst. Gynecol.* **8**, 591-593
- Herrera, E., Knopp, R. H. & Freinkel, N. (1969) *J. Clin. Invest.* **48**, 2260-2272

- Itoh, H., Kishi, T. & Chibata, I. (1974) *J. Nutr.* **104**, 386-394
- Knopp, R., Herrera, E. & Freinkel, N. (1970) *J. Clin. Invest.* **49**, 1438-1446
- Knopp, R., Boroush, M. A. & O'Sullivan, J. B. (1975) *Metabolism* **24**, 481-493
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Metzger, B. E., Agnoli, F. S. & Freinkel, N. (1970) *Horm. Metab. Res.* **2**, 367-368
- Metzger, B. E., Hare, J. W. & Freinkel, N. (1971) *J. Clin. Endocrinol.* **33**, 869-872
- Minemura, T., Lacy, W. W. & Crofford, O. B. (1970) *J. Biol. Chem.* **245**, 3872-3881
- Morgan, E. H. (1973) *Aust. J. Exp. Biol. Med. Sci.* **51**, 667-678
- Scharff, R. & Wool, I. G. (1966) *Biochem. J.* **99**, 173-178
- Scow, R. O., Chernick, S. S. & Brinley, M. S. (1964) *Am. J. Physiol.* **206**, 796-804
- Tagliamonte, A., Gessa, G., Biggid, G., Vargiu, L. & Gessa, G. L. (1974) *Life Sci.* **14**, 349-354
- Wang, C. S. & Smith, L. R. (1975) *Anal. Biochem.* **63**, 414-417