Foetal amino acids

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Maternal factors

Maternal dietary protein is the major source of foetal amino acids when maternal nutritional intake and absorption are adequate. In states of maternal protein undernutrition or malnutrition foetal amino acid supplies are maintained at the expense of maternal tissue breakdown. Rates of placental amino acid transfer are not readily measurable in man, but measurements of foetal to maternal ratios of individual amino acids have been used to give an indication of the efficiency of placental transport systems for amino acids (Kerr & Waisman, 1967). Maternal malnutrition is associated with increased plasma glycine/valine quotients in mother and foetus (Lindblad, Rahimtoola, Said, Haque & Khan, 1969). In pre-eclampsia with retarded foetal growth there is a generalized increase in maternal plasma amino acid concentrations towards non-pregnant values and the foetal to maternal plasma amino acid ratios, particularly of the branched-chain amino acids, are lower than normal (Cockburn, Blagden, Michie & Forfar, 1971). Higher maternal plasma branched-chain amino acid concentrations may reflect a release of these amino acids from maternal muscle protein.

The normal fall found in maternal plasma amino acid concentrations during pregnancy may be mediated through changes in circulating hormones. Oestrogen, progesterone, cortisol and insulin influence plasma amino acid concentrations (Zinneman, Seal & Doe, 1967; Dancis, Money, Springer & Levitz, 1968) and the fall in maternal plasma concentrations may, in part, be due to an increased urinary excretion (Zinneman, Johnson & Seal, 1963) or to an increased rate of transfer of amino acid into cells of uterus and into placenta and foetus (Bjorenesjo, 1968).

Placental factors

Oxender & Christensen (1959) postulated that amino acids are transported across cells against concentration gradients from an active uptake site on one side of the membrane to produce intracellular accumulation, while on the other side of the membrane simple diffusion down a concentration gradient was thought to occur. Such a system may function in the placental syncytiotrophoblast since...
Pearse & Sornson (1969) have described high concentrations of amino acid in the human placental parenchyma. Hill & Young (1973) demonstrated that in the guinea pig transfer from placental parenchyma to foetal plasma was blocked when foetal plasma acid concentrations exceeded the free amino acid concentrations of the placental parenchyma.

There is controversy about the role of insulin in the regulation of placental amino acid transfer (Villee, 1953; Battaglia, Meschia, Blechner & Barron, 1961; Freinkel, 1965; Grimaldi, Jung & Mahler, 1966; Szabo & Grimaldi, 1970; Demers, Gabbe, Villee & Greep, 1972). The demonstration of insulin receptors in human and animal placentas, with characteristics similar to those in established insulin target tissues such as fat cells and liver, supports the argument that insulin may play a role in the regulation of placental function (Posner, 1974).

Changes in placental blood flow on maternal and foetal sides will influence transport rates. Gross variations in human maternal plasma concentrations of individual amino acids can disturb foetal amino acid metabolism (Cockburn, Farquhar, Forfar & Robins, 1972), but lesser degrees of imbalance in the ewe do not seem to influence unduly foetal lamb values, and there appear to be group transport systems in the trophoblast similar to those of small intestine and renal tubule (Young & McFadyen, 1973).

**Foetal factors**

The degree to which the foetus can control his own supply of nutrient material is unknown. In a recent Symposium (Elliott & Knight, 1974) on foetal size the maternal and foetal factors controlling foetal growth were reviewed. From the increased rates of growth of infants of diabetic mothers (Persson, 1974) and severe growth retardation in infants lacking pancreatic islets (Liggins, 1974) it appeared that foetal insulin might be a major foetal growth promoting factor. There is evidence in preterm human infants that infusions of insulin can increase the rates of entry of glucose, potassium and amino acids into tissues (Cockburn, 1976). Conversely in rhesus monkey foetuses starved by interfering with placental blood flow, foetal muscle and other tissue protein breakdown occurs and this catabolic state may be associated with catecholamine, glucagon and cortisol release (Hill, 1974).

A small quantity of amino acids may reach the foetus from swallowed amniotic fluid. At term the human infant swallows approximately 450 ml of amniotic fluid per day (Plentl, 1966). The amino acid composition of amniotic fluid changes throughout pregnancy (Cockburn, Robins & Forfar, 1970; Cockburn, Giles, Robins & Forfar, 1973) and this is only in part related to dilution with increasing volumes of urine. Table 1 shows the fall in total mean amino acid concentrations of maternal (MV) and foetal (UA) plasma water, foetal urine (FU) and amniotic fluid (AF) from mid to late pregnancy. In mid pregnancy FU and AF individual free amino acid concentrations correlate but by term these correlations are lost and the fluids are dissimilar (Table 2).
Table 1. Total mean amino acid concentrations (μmol/l) in fluids from eight normal human pregnancies between 15 and 20 weeks gestation compared with values from eight normal human pregnancies terminated electively by Caesarean section between 39 and 40 weeks gestation.

(Values for maternal vein plasma water (MV), umbilical arterial plasma water (UA), foetal urine (FU) and amniotic fluid (AF) free amino acid concentrations were obtained by summation of individual free amino acid concentrations measured by column chromatography.)

<table>
<thead>
<tr>
<th></th>
<th>15-20 weeks</th>
<th>Ratio/AF</th>
<th>39-40 weeks</th>
<th>Ratio/AF</th>
<th>% Change in mean amino acid concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV</td>
<td>2127</td>
<td>0.89</td>
<td>1807</td>
<td>1.19</td>
<td>15</td>
</tr>
<tr>
<td>UA</td>
<td>4509</td>
<td>1.88</td>
<td>3190</td>
<td>2.10</td>
<td>29</td>
</tr>
<tr>
<td>FU</td>
<td>2056</td>
<td>0.86</td>
<td>1459</td>
<td>0.96</td>
<td>29</td>
</tr>
<tr>
<td>AF</td>
<td>2393</td>
<td>1</td>
<td>1517</td>
<td>1</td>
<td>37</td>
</tr>
</tbody>
</table>

Table 2. Correlations of 24 to 29 individual free amino acid concentrations in maternal vein plasma (MV), umbilical artery plasma (UA), amniotic fluid (AF) and foetal urine (FU) obtained from eight normal human pregnancies between 15 and 22 weeks gestation and eight normal human pregnancies terminated electively by Caesarean section between 39 and 40 weeks' gestation.

<table>
<thead>
<tr>
<th></th>
<th>MV:AF (15-20 weeks)</th>
<th>UA:AF (15-20 weeks)</th>
<th>UA:AF (39-40 weeks)</th>
<th>FU:AF (15-20 weeks)</th>
<th>FU:AF (39-40 weeks)</th>
</tr>
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<tbody>
<tr>
<td>TRY</td>
<td>26</td>
<td>27</td>
<td>25</td>
<td>29</td>
<td>28</td>
</tr>
<tr>
<td>HIS</td>
<td>THR</td>
<td>SER (neg)</td>
<td>—</td>
<td>TAU</td>
<td>VAL</td>
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<tr>
<td></td>
<td>CYS</td>
<td></td>
<td></td>
<td>VAL</td>
<td>LYS</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>MET</td>
<td>1-MeHIS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CYTH</td>
<td>ILEU</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>LEU</td>
<td>TYR</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>PHE</td>
<td>HIS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3-MeHIS</td>
<td></td>
</tr>
</tbody>
</table>

Ratios of amino acid concentrations between foetal UA plasma and urine indicate that the foetal kidney at 15–20 weeks gestation can effectively conserve amino acids, possibly reaching an adult level of competence in this respect (Cockburn et al. 1970).

Perfusion of a gravid human uterus at 18 weeks gestation through the uterine arteries for 30 min with blood containing L-[3H]phenylalanine confirmed that after very avid uptake of amino acid in syncytiotrophoblast high foetal plasma concentrations were quickly achieved but very small quantities reached foetal urine. Autoradiographs demonstrated radioactivity in the proximal tubules but this was absent from the distal and collecting tubules (Robins, Baird, Cockburn, Livingston & Smith, 1971).
Placental transfer and foetal uptake of amino acids have been examined in the catheterised foetal lamb after infusions of unlabelled amino acids (Young & McFadyen, 1973; Young, Soltesz, Noakes, Joyce, McFadyen & Lewis, 1975). Clearance rates for different amino acid groups were calculated and the postoperative changes in amino acid metabolism determined. In virtually all studies of amino acid transfer in man, monkey, sheep and guinea pig, concentrations of individual free amino acids measured in plasma water of mother and foetus have been compared. The same is true of experiments employing labelled amino acids where activity is measured in the supernatant plasma water and the precipitated plasma proteins are discarded. However, when labelled amino acid is infused into animals a greater portion of radioactivity is found to be associated with plasma protein than in plasma water. Fig. 1 shows the proportions

![Graph showing relative concentrations of radioactivity in maternal plasma (○), maternal plasma water (●), foetal plasma (△) and foetal plasma water (▲) during and after the infusion of L-[U-14C]phenylalanine into a jugular vein of a 126 d pregnant sheep whose single foetus had foetal UV and UA catheters inserted at 106 d gestation. 200 µCi of uniformly L-[U-14C]phenylalanine was infused in 20 ml saline (9 g NaCl/l) at a constant rate of 50 µCi/h for 4 h after a priming injection of 50 µCi in 5 ml saline.](https://www.cambridge.org/core/coreimage.png)
of radioactivity found in 1 ml of maternal plasma compared with that found in plasma water from 1 ml of deproteinized plasma (plasma–0.6 m-sulphosalicylic acid, 1:1 v/v). Samples were obtained during and after the infusion of L-[U-\textsuperscript{\textbf{14}}C]phenylalanine into the jugular vein of a 126 d pregnant sheep whose single foetus had foetal UV and UA catheters inserted under general anaesthesia at 106 d gestation (see Mellor & Matheson, 1975 for the techniques of catheterisation and care). 200 \( \mu \)Ci of [U-\textsuperscript{\textbf{14}}C]phenylalanine was infused in 20 ml saline (9 g NaCl/l) at a constant rate of 50 \( \mu \)Ci/h for 4 h after a priming injection of 50 \( \mu \)Ci in 5 ml saline. Maternal blood samples were obtained from the other jugular vein and foetal samples from the UA catheter. It is evident that plasma water activity is at a much lower level than that of whole plasma and that whereas plasma water values reach low concentrations after 12 h, high activity is retained in association with protein during the 9 d of study.

Foetal plasma water activity is higher than maternal and this is in accord with the high foetal to maternal ratios found in other studies; and foetal plasma protein activity is high in comparison with foetal plasma water, though it does not reach the values of maternal plasma protein. Maternal and foetal plasma protein activities are converging by the 9th day after infusion. When specific activities are considered for phenylalanine in maternal plasma water there is a fall from 79\( \times \)10\(^3\) dpm/\( \mu \)mole at 2 h to 1\( \times \)10\(^3\) dpm/\( \mu \)mole at 22 h. Values remained at about this level during the next 7 days. In foetal plasma water phenylalanine specific activity was 6\( \times \)10\(^3\) dpm/\( \mu \)mole at 5 h, 5\( \times \)10\(^3\) dpm/\( \mu \)mole at 11 h and 0.1\( \times \)10\(^3\) dpm/\( \mu \)mole at 3 d. Maternal plasma water ratios of tyrosine specific activity to phenylalanine specific activity increased fairly rapidly from 0.008 at 30 min to 0.7 by 22 h.

Acid hydrolysis of the plasma protein sulphosalicylic acid precipitates confirms that 70–110% maternal plasma protein-associated activity in the samples so far analysed is accounted for by tyrosine and phenylalanine.

From the end of the infusion until the end of the experiment phenylalanine activity averaged 222 dpm/mg plasma protein in maternal plasma and 174 dpm/mg foetal plasma protein. Mean maternal plasma protein concentration was 47 g/l whereas in the foetus this value was only 25.3 g/l.

Identical patterns of dissociation between plasma water and plasma protein activities have been found during [\textsuperscript{\textbf{14}}C]leucine infusions into the ewe.

Infusions of [\textsuperscript{\textbf{14}}C]phenylalanine into the foetus demonstrate (a) the very rapid association of radioactivity with foetal plasma protein, (b) that foetal plasma water concentrations exceed maternal and (c) that maternal protein-associated activity exceeds maternal plasma water activity (Fig. 2).

When [\textsuperscript{\textbf{14}}C]phenylalanine or [\textsuperscript{\textbf{14}}C]leucine is added to blood in a test tube or in a tonometer maintaining the blood at a ‘physiological’ temperature, pH, \( P_{\text{O}_2} \) and \( P_{\text{CO}_2} \), the activity remains in the plasma water fraction. Within 10 min in vivo a large proportion of radioactive amino acid is protein-associated. Phenylalanine and leucine are firmly attached to or are incorporated into protein or peptides and the nature of this association is currently under investigation.
Fig. 2. Relative concentrations of radioactivity in foetal plasma (△), foetal plasma water (●), maternal plasma (○) and maternal plasma water (□) during and after the infusion of L-[U-14C]phenylalanine into an umbilical vein of a 130 d single foetus which had UV and UA catheters inserted at 110 d gestation. 200 μCi of uniformly labelled L-phenylalanine was infused in 20 ml saline at a constant rate of 50 μCi/h for 4 h after a priming injection of 50 μCi in 5 ml saline.

The role of insulin in placental amino acid transfer

After a meal containing protein or amino acid, maternal plasma amino acid concentrations rise and normally increased insulin secretion will result. This rise in maternal insulin will increase insulin attachment at receptor sites on the maternal aspect of syncytiotrophoblast and encourage the transfer of amino acids into placental parenchyma. From placental parenchyma amino acids could diffuse down a concentration gradient to foetal plasma water and in turn be removed from foetal plasma water to the intracellular compartments of the foetus at a rate mediated by foetal insulin secretion.

Information gathered about foetal amino acid metabolism in different animal species is beginning to shed light on factors which control foetal amino acid
distribution, protein synthesis and growth. More information is necessary before attempts can be made to improve the well being of a foetus suffering from intrauterine malnutrition.

REFERENCES


