Models for nutritional research on the fetus: problems and pitfalls

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The concept of providing nutritional models of fetal development appears superficially to be inappropriate as the nutritional environment of prenatal life is thought to be highly controlled (Battaglia & Meschia, 1978; Bassett & Fletcher, 1982; Meschia, 1982). Nevertheless, the fetus responds to changes in maternal diet, to starvation and to reductions in maternal placental blood flow (Girard *et al.* 1977; Battaglia & Meschia, 1978; Phillips *et al.* 1978; Lafeber *et al.* 1979; Jones, 1982; Rolph & Jones, 1982; Chalk *et al.* 1983). The responses are usually adaptive (and sometimes precocious) changes in enzyme development and hence in metabolic substrates used by the fetus (Girard *et al.* 1977; Jones, 1982).

These observations are therefore indicative of the fact that the fetus is able to influence its nutritional environment and modify its metabolic capacity accordingly. Such an interpretation is exemplified by the fact that the secretion of fetal hormones in the long-term and short-term is influenced by fetal nutrition (Bassett & Madill, 1974; Girard *et al.* 1977; Lafeber *et al.* 1979; Robinson *et al.* 1980) and by the fact that acute changes in the fetal plasma concentration of hormones such as insulin have marked effects on fetal substrate utilization.

Thus in many respects the fetus behaves as the adult, but with placental rather than alimentary supply of substrate and with a substantially different metabolic capacity. For instance, the fetal tissues have limited or no capacity for glucose synthesis or for fatty acid oxidation and, in some species, for amino acid oxidation (Battaglia & Meschia, 1978; Jones, 1982; Snell, 1982) but have high rates of substrate turnover characteristic of growing cells (Scornik, 1982).

Investigation of the nutritional requirements and adaptive responses of prenatal life therefore requires an assessment of the pathways associated with the metabolism of a particular substrate, of the responses of such pathways to changes in nutritional state, of the control of hormone secretion in the fetus and of the inter-relationship between nutrition and fetal growth.

Biochemical pathways in the fetus

Apart from the relatively poorly developed state of some of the biochemical pathways in the fetal tissues as indicated previously (Girard *et al.* 1977; Battaglia & Meschia, 1978; Jones, 1982; Snell, 1982), it is becoming increasingly apparent that the pathways in fetal tissues may not be organized in ways characteristic of the adult. As indicated later (p. 181) for glycogen metabolism, a sound knowledge of the fetal functions of a pathway becomes particularly important when building models for nutritional research as erroneous conclusions may arise in the absence

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Table 1. *Glyoxylate cycle enzyme activities (units/g) in fetal liver*

(Mean values and standard deviations for four determinations. Assays performed at 25°)

	Fetal age (d)	Malate synthetase (*EC* 4.1.3.2)		Isocitratase (*EC* 4.1.3.1)	
		Mean	SD	Mean	SD
Guinea-pig	35–40	0·19	0·06	0·26	0·14
	45–50	1·17	0·63	2·62	1·28
	55–60	3·05	0·99	4·16	1·38
Sheep	60–80	<0·1		<0·2	
	80–110	0·51	0·30	0·38	0·19
	120–140	0·92	0·42	1·10	0·46

of detailed information. There are two major ways in which this problem is apparent. The fetus may possess pathways which are not found in adult tissues, or the absense of or presence of low enzyme activities at a key-point in a pathway may change the function of that pathway or those associated with it. A good example of the former (Jones, 1980*a*) is the presence of the enzymes of the glyoxylate cycle in the fetal liver (Table 1), a pathway thought not to occur in adult mammalian tissues. The function of this pathway, which disappears just after birth, is not entirely clear in the fetus. However, the susceptibility of the fetus to hypoglycaemia, the demands that the fetus makes on the pregnant mother to supply glucose and the ready availability of fatty acids in many species (Bassett & Madill, 1974; Jones, 1976*a*; Girard *et al.* 1977; Battaglia & Meschia, 1978; Phillips *et al.* 1978; Lafeber *et al.* 1979; Meschia, 1982) suggests that the glyoxylate cycle could be used in the fetal liver to convert fatty acids into hexose. In the fetal guinea-pig liver, small rates of incorporation of [^{14}C]palmitate into glycogen have been detected (Table 2). Although these are low, they are likely to be significant as the endogenous pool of fatty acids is high (Jones, 1976*a*). Moreover, inhibition of fatty acid entry into the mitochondrion causes a marked fall in hepatic intermediates of the Krebs cycle and glycolysis and of flux into the hexose monophosphate pool (Jones, 1980*a*). Thus, in the fetal liver, fatty acids could represent an important anabolic fuel (Jones, 1981).

An example of the way in which altered enzyme activities change the essential function of a pathway and the control thereof is provided by an analysis of the pathways of gluconeogenesis and glycogen synthesis in the fetal liver. Although there have been suggestions for a role of the gluconeogenic pathway in fetal glucose production, none have been convincing (Prior & Christenson, 1977; Warnes *et al.* 1977; Battaglia & Meschia, 1978; Hodgson *et al.* 1980; Jones, 1982). Moreover, the low activity of the glucose-6-phosphatase (*EC* 3.1.3.9) system (Jones & Rolph, 1981) would make such a role a quantitatively minor one. However, quite modest activities of most of the gluconeogenic enzymes have been detected in the fetal liver of a variety of species (Girard & Ferre, 1982; Jones, 1982). The function of

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Table 2. *Conversion of palmitate and glucose into glycogen by the perfused liver of the 50 d fetal guinea-pig*

(Livers were perfused through the umbilical vein for 40 min in a non-recirculating mode with Krebs' bicarbonate buffer at 37°. Results are mean values and standard deviations; no. of determinations in parentheses)

	Incorporation into glycogen (pmol/min per g)		
	Mean	SD	
0·5 mM-[U-^{14}C]palmitate	4·3	0·4	(6)
0·2 mM-[U-^{14}C]glucose	1965	473	(5)
0·2 mM-[2-^{3}H]glucose	246	74	(6)

this is indicated by a variety of different types of experiments. (1) Glycogen synthesis is inhibited by inhibition of gluconeogenic flux (Jones, 1981). (2) High rates of production of hexose monophosphates from endogenous precursors occur (Band & Jones, 1980; Jones, 1981). (3) Agents such as glucagon, that normally enhance gluconeogenic flux and glycogenolysis in the fetal liver, divert substrates to glycogen and hence cause a net increase in both synthesis and breakdown of glycogen (Tables 3 and 4).

This latter fact illustrates one very important point about the organization and responses of pathways in the fetus: the interpretation of the metabolic response to a change in hormonal or nutritional state derived simply from 'conventional' wisdom based on adult studies can be erroneous. For example, it has been considered in several species that the late fetal liver is relatively refractory to the action of glycogenolytic agents (Snell & Walker, 1973; De Vos & Hers, 1974; Kawai & Arinze, 1981; Jones, 1982). The basis for this interpretation can be understood by studying the responses of the perfused fetal rabbit liver to a physiological glucagon concentration (Table 3). However, constancy of glycogen pool size after perfusion with glucagon coincides with an increase in glucose incorporation into glycogen and a much increased glucose output (Table 3). The increased glucose output is much greater than the capacity of the fetal liver to synthesize glucose *de novo* (Jones *et al.* 1980*b*). Thus the absence of a change in liver glycogen concentration occurs at a time when there can be simultaneously high rates of glycogen synthesis and breakdown (Tables 3 and 4). This is no longer true after birth, when a very large increase in glucose-6-phosphatase activity Ballard & Oliver, 1965; Dawkins, 1966; Jones & Ashton, 1976) allows the gluconeogenic flux to be siphoned-off to extracellular glucose (Jones *et al.* 1980*b*).

Table 4 illustrates one of the further caveats in drawing simple conclusions about developing systems. It shows that in a hormone-activated system there may be a poor relationship between receptor number and physiological response, and signal transduction system, in this case adenylate cyclase (*EC* 4.6.1.1) and cAMP.

Thus model systems for nutrition research on the fetus must not only be selected carefully but investigated extensively before reliable conclusions can be drawn.

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Table 3. *Effects of glucagon on glycogen synthesis in perfused 29 d fetal rabbit liver*

(Livers were perfused through the umbilical vein for 40 min in a non-recirculating mode with Krebs' bicarbonate buffer at 37°. Results are mean values and standard deviations; no. of determinations in parentheses)

	Control		Glucagon (1 ng/ml)	
	Mean	SD	Mean	SD
Glycogen (nmol/g)	5813	2769 (10)	6855	2810 (10)
[U-^{14}C]glucose incorporation into glycogen (nmol/min per g)	21·6	8·7 (5)	35·2	10·1 (5)
Glucose output (nmol/min per g)	337	241 (12)	796	368 (12)

Biochemical responses of the fetus to altered nutrition

There are several different approaches to changing prenatal nutrition. The simplest is to starve a pregnant animal or to feed a diet in which the balance of the constituents are altered (Lee & Chow, 1965; Bassett & Madill, 1974; Miguel & Abraham, 1976; Girard *et al.* 1977; Shambaugh *et al.* 1977; Schreiner *et al.* 1980; Jones, 1982; Chalk *et al.* 1983). More profound changes in nutritional state can be achieved by restricting blood flow to the placenta (Wigglesworth, 1964; Roux *et al.* 1970; Nitzan & Groffman, 1971; Jones & Robinson, 1979; Lafeber *et al.* 1979) reducing placental size (Alexander, 1964; Robinson *et al.* 1979, 1980) or reducing the size of the litter (Van Marthens *et al.* 1972; Fletcher *et al.* 1982).

Table 4. *Glycogenolytic effects of glucagon in the developing rabbit liver*

(Livers were perfused through the umbilical vein for 40 min in a non-recirculating mode with Krebs' bicarbonate buffer at 37°. Results are mean values and standard deviations; no. of determinations in parentheses)

	Control		Glucagon (1 ng/ml)	
	Mean	SD	Mean	SD
		29 d fetal liver		
Total cAMP production (pmol/min per g)	20	7 (8)	514	195 (8)
Perfusate cAMP output (pmol/min per g)	19	6 (8)	65	26 (8)
Adenylate cyclase (*EC* 4.6.1.1) activity (pmol cAMP/min per mg protein)	2·9	1.6 (4)	4.1	2·9 (4)
Glucagon receptors (binding; pmol/mg protein)	79	47 (5)		
Glycogenolysis (nmol/min per g)	331	127 (4)	896	294 (4)
		6 d newborn liver		
Total cAMP production (pmol/min per g)	30	13 (8)	391	127 (8)
Perfusate cAMP output (pmol/min per g)	25	10 (8)	251	139 (8)
Adenylate cyclase activity (pmol cAMP/min per mg protein)	10·3	8·7 (4)	43·7	12·8 (4)
Glucagon receptors (binding; pmol/mg protein)	287	73 (5)		
Glycogenolysis (nmol/min per g)	146	51 (5)	1046	503 (5)

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Fetuses are thought to depend largely on glucose and possibly lactate and, to a lesser extent, amino acids and fatty acids as metabolic fuels (Battaglia & Meschia, 1978; Meschia, 1982). Moreover, the pathways associated with catabolic states are often relatively inactive (Battaglia & Meschia, 1978; Girard & Ferre, 1982; Jones, 1982; Snell, 1982). Thus it is not surprising that maternal starvation is associated not just with a reduced supply to the fetus of glucose and increased supply of fatty acids and ketone bodies (Simmons *et al.* 1974; Girard *et al.* 1977; Shambaugh *et al.* 1977; Elphick *et al.* 1978; Schreiner *et al.* 1980); there is also a precocious induction of enzymes (Table 5) associated with fatty acid and ketone body metabolism, of amino acid metabolism and of gluconeogenesis and a reduction of those of lipogenesis (Miguel & Abraham, 1976; Girard *et al.* 1977; Shambaugh *et al.* 1977; Girard & Ferre, 1982; Jones, 1982; Chalk *et al.* 1983). In addition the associated increase in mobilization of fatty acids from maternal adipose tissue leads to an elevated deposition in fetal tissues, particularly the liver (Elphick *et al.* 1978).

These responses are often not different from those of the adult, except in that induction of absent or very-low activity pathways is involved. The responses to a high-fat or high-carbohydrate diet, for instance, are essentially similar (Miguel & Abraham, 1976; Elphick *et al.* 1978; Susa *et al.* 1979; Jones, 1980*a*; Chalk *et al.* 1983). The important exception is that anabolic pathways are not necessarily suppressed. Thus a high-fat diet does not necessarily suppress lipogenesis in the fetal liver (Miguel & Abraham, 1976; Jones, 1982; Chalk *et al.* 1983) and a high-carbohydrate diet is unable to induce fetal hepatic glucokinase (*EC* 2.7.1.2) (Faulkner & Jones, 1976). However, just as in the adult, high-carbohydrate regimens for the fetus are associated with enhanced deposition of fat and induction of the enzymes of fatty acid synthesis (Susa *et al.* 1979; Jones *et al.* 1980*a*).

The responses of the fetus nutritionally deprived because of reduced uterine blood flow are strikingly different from those outlined previously although, as in

Table 5. *Fetal liver enzyme activity (units/g) in response to maternal starvation or glucose loading*

(Results are mean values and standard deviations; no. of determinations in parentheses. Assays were carried out at 30°. Maternal glucose load was given as an intravenous injection of 4 ml glucose (500 g/l) every 2–4 h)

	Pyruvate carboxylase (*EC* 6.4.1.1)		Phosphoenolpyruvate carboxylase (cytosolic) (*EC* 4.1.1.49)	
	Mean	SD	Mean	SD
Fetal guinea-pig (55–60 d)				
Control	0.51	0.24 (5)	0.23	0.11 (5)
48 h maternal starvation	1.20	0.43 (5)	0.92	0.34 (5)
48 h maternal glucose loading	0.20	0.04 (6)	0.07	0.02 (6)
Fetal rabbit (29–30 d)				
Control	0.12	0.05 (5)	0.19	0.07 (5)
48 h maternal starvation	0.53	0.26 (5)	0.74	0.23 (5)
48 h maternal glucose loading	<0.05		<0.05	

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starvation, fetal weight is reduced and the fetuses are hypoglycaemic (Wigglesworth, 1964; Roux *et al.* 1970; Nitzan & Groffman, 1971; Jones & Robinson, 1979; Lafeber *et al.* 1979; Robinson *et al.* 1979, 1980; Rolph & Jones, 1982). Their supply of lipid is now limited but marked elevation of plasma amino acid concentrations occur (Jones & Robinson, 1979; Lafeber *et al.* 1979; Robinson *et al.* 1979). Instead of precocious induction of enzyme activities, the opposite occurs (Table 5) and in many instances enzyme development is delayed substantially (Lafeber *et al.* 1979; Rolph & Jones, 1982). Some responses, however, remain well developed and surprisingly, in contrast to the effects of starvation, hepatic glycogen deposition can be maintained or enhanced (Lafeber *et al.* 1979).

Such undernourished fetuses have a substantially-reduced capacity for anabolic pathways such as fatty acid synthesis, whilst in maternal starvation these pathways may be well sustained (Lafeber *et al.* 1979). Also some of the catabolic pathways such as that for amino acid oxidation are much more poorly developed than normal. Although the endocrine responses observed in these two models are in someways different they do not appear sufficiently diverse, except for the plasma thyroxine (T_4) and prolactin changes, to account easily for the major dissimilarities in biochemical responses.

The results of surgical reduction in litter size are interesting as increased fetal growth rate and presumably consumption of substrates, particularly glucose, occur (Van Marthens *et al.* 1972; Fletcher *et al.* 1982). Unfortunately little detailed biochemical information is yet available on the responses to this condition but hyperinsulinaemia may be a major stimulus to growth.

In the fetus, therefore, the biochemical responses to altered nutrition appear to be dominated by two considerations: the preprogrammed sequence of enzyme induction (Jones, 1982) and the requirement of the fetus for high rates of growth. Another important aspect of the fetal responses to nutritional changes is the interaction between the fetus and placenta. A detailed analysis is beyond the scope of this article but a number of important aspects of this interaction should be mentioned. The placenta, through its own metabolism, actively produces substrates such as lactate and amino acids for the fetus (Battaglia & Meschia, 1978; Meschia, 1982). Also, when substrate supply to the feto–placental unit is compromized, consumption by the placenta of glucose, for instance, appears to be maintained at the expense of the fetus (C. T. Jones, unpublished work).

Fetal nutrition and endocrine state

The fetus is characterized by high plasma growth hormone concentrations and low levels of T_4 and particularly 3,5,3′-triiodothyronine (Bassett & Fletcher, 1982). The concentration of most of the remainder of the 'conventional' plasma hormones are not substantially different in adult life. There is considerable fetal autonomy of hormone secretion; thus fetal hypoglycaemia reduces insulin secretion and stimulates adrenaline, glucagon, ACTH and cortisol production (Bassett & Madill, 1974; Jones, 1976*b*; Girard *et al.* 1977; Mellor *et al.* 1977; Phillips *et al.* 1978;

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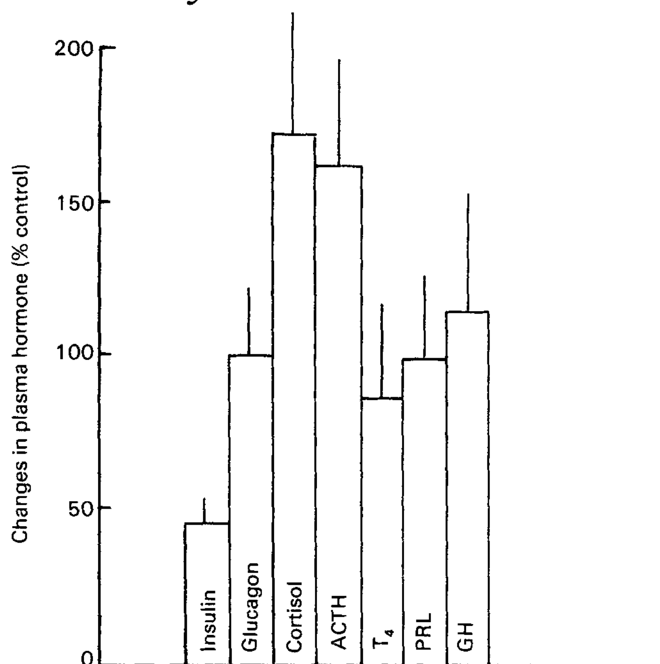

Fig. 1. Relative changes in plasma hormone concentrations (% control) in 125–135 d fetal sheep in response to 36–48 h of maternal starvation. Values are means and standard deviations for four to eight observations. T_4, thyroxine; PRL, prolactin; GH, growth hormone.

Schreiner *et al.* 1980; Jones, 1980*b*). Moreover, the infusion of insulin or elevations of plasma insulin concentration are associated with increased fetal glucose consumption and fetal growth (Picon, 1967; Van Mathens *et al.* 1972; Susa *et al.* 1979; Fletcher *et al.* 1982), whilst infusion of adrenaline depresses peripheral glucose consumption (Jones *et al.* 1980*b*). Thus, superficially, the endocrine control of substrate utilization in the fetus has many of the characteristics of that of the adult. However, the relationship for the fetuses is not as clear-cut as supposed since in the fetal sheep, under some circumstances, hypoglycaemia and hypoinsulinaemia are associated with enhanced rather than reduced glucose consumption (Harding *et al.* 1984). Destruction of the fetal pancreas is probably associated with a reduction in fetal growth and hence peripheral glucose consumption (Felix & Jaquot, 1976; Hill, 1976), whilst prolonged insulin infusion has the converse effect (Picon, 1967; Susa *et al.* 1979).

Maternal starvation leads to more or less the expected changes (Bassett & Madill, 1974; Jones, 1976*b*; Girard *et al.* 1977; Mellor *et al.* 1977; Phillips *et al.* 1978; Schreiner *et al.* 1980; Jones, 1980*b*) in fetal plasma hormone concentrations with the major effects being a fall in plasma insulin and rise in plasma glucagon, adrenaline, ACTH and cortisol (Fig. 1), although the change in plasma T_4 was small. By contrast, nutritional deprivation of the fetus by uterine artery ligation causes a somewhat different collection of hormone responses (Jones & Robinson, 1979; Lafeber *et al.* 1979; Robinson *et al.* 1980; Jones *et al.* 1980*a*). There is a sharp fall in insulin, cortisol, T_4 and prolactin and a very large increase in

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Table 6. *The effect of serum from undernourished fetal guinea-pigs on the growth in culture of hepatocytes from 50 d fetal guinea-pigs*

(Serum was diluted 1:10 in the final incubation medium. Results are mean values and standard deviations; no. of determinations in parentheses. Cells were incubated for 6 h in medium containing insulin, cortisol, thyroxine, glucagon and epidermal growth factor)

	[^{3}H]thymidine incorporation (disintegrations/min per 10^5 cells)			
	Control		+Serum	
	Mean	SD	Mean	SD
Normal fetal serum	37000	12500 (5)	98600	34100 (6)
Fetal serum after 48 h starvation	41300	16300 (5)	67340	25200 (5)
Fetal serum after uterine artery ligation	35900	10800 (5)	16300	4800 (5)

glucagon. Whether these differences in endocrine response produce the variety of biochemical responses outlined previously remains to be established. However, the accurate assessment of the regulation of metabolism in the fetus in response to nutritional changes requires the identification of as yet uncharacterized factors. For instance, whilst fetal plasma normally contains factors that promote cell growth and proliferation (Brinsmead & Liggons, 1979), this does not appear to be so for plasma from nutritionally-deprived fetuses (Table 6). Moreover, in such conditions it appears that as yet uncharacterized inhibitory agents become evident. This is comparable to the effects of nutritional deprivation in the postnatal rat.

Conclusions

The protected state of the fetus and the relatively meagre understanding of the regulation of its nutrient supply at present have limited the development of the detailed knowledge of the nutrition of the fetus. Umbilical balance studies have been useful but have not extended our understanding of mechanisms or of quantitative metabolism (Battaglia & Meschia, 1978; Meschia, 1982).

Thus most studies have been largely indirect and concentrated on simple model systems such as enzyme or pathway development or on the action of specific hormones. Such studies require the accurate assessment of the functions in the fetus of the components of the model system, which may be different from those in the adult. Moreover, it is probable that many humoral factors influencing such model systems are yet to be identified.

The physiological models that have been the most useful developed so far, that cause the greatest changes in biochemical development and are additional to the conventional methods of altering dietary intake, are those involving long-term disturbances of utero-placental or umbilical circulations.

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