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Plenary Lecture

Protein nutrition of the neonate

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The period of growth and development between birth and weaning is crucial for the long-term well-being of the organism. Protein deposition is very rapid, is achieved with a high nutritional efficiency, and is accompanied by marked differences in the growth rates of individual tissues and a series of maturational processes. These important aspects of development occur while the neonate is consuming a single and highly-specific food source, milk. Surprisingly, although there is a clear relationship between the nutrient density of milk and the growth rate of its recipient, this relationship does not apply to the overall amino acid composition of mixed milk proteins. Some amino acids, notably glycine and arginine, are supplied in milk in quantities that are much less than the needs of the neonate. The milk-fed neonate is therefore capable of carrying out a tightly-regulated transfer of N from amino acids in excess to those that are deficient. The rapid growth of the neonate is supported by a high rate of tissue protein synthesis. This process appears to be activated by the consumption of the first meals of colostrum. Recent research has identified that skeletal muscle and the brain are specifically responsive to an unidentified factor in colostrum. Following the initial anabolic response the rate of protein synthesis in some tissues, notably muscle, falls from birth to weaning. This decrease reflects a progressively smaller anabolic response to nutrient intake, which not only involves an overall fall in the capacity for protein synthesis, but also in responses to insulin and amino acids. The study of growth and protein metabolism, and their regulation in the neonate is not only important for pediatrics, but may provide important pointers to more general aspects of regulation that could be applied to the nutrition of the mature animal.

Neonate: Milk composition: Amino acid: Protein synthesis: Insulin

In some senses, the sucking period can be regarded as a transition from the protected existence of the fetus to the vulnerable life of the free-living organism. At birth the route of nutrition shifts from parenteral to enteral, necessitating among other things a rapid rate of postnatal intestinal development, and the diet that the newborn receives is of relatively (although not completely) constant composition. At weaning the nature of the diet changes to one that is variable in physical form, nutrient composition and amount. The combination of these factors necessitates the development of functions that allow the organism to adapt and accommodate to the uncontrolled changes in diet availability and composition that inevitably follow weaning. Indeed, it could be argued that much of the functional maturation that occurs over the sucking period is a preparation for the independent life of the weaned individual. In the present paper we will consider three main aspects of the protein nutrition of the neonate. First, we will discuss amino acid requirements from a factorial and compositional perspective, and examine the degree to which predicted requirements are satisfied by milk. Second, we will consider some of the intermediary metabolic implications of the differences between the composition of mixed body and milk proteins. Finally, we will consider more dynamic aspects of protein metabolism and discuss recent work on nutritional and endocrine factors that

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regulate protein turnover, factors that potentially influence the efficiency with which the neonate utilizes its dietary protein.

Protein deposition, milk composition and amino acid needs

Irrespective of the stage of development, the quantification of the dietary protein and amino acid requirements of any organism demands three items of information. First, quantitative data on the rates of the pathways that consume individual amino acids, which defines the basal biological need. Second, information on the bioavailability of the amino acids from a given protein source, which defines the efficiency with which a given diet satisfies the biological needs. Third, estimates of the inter-individual variability in, and environmental influences on, amino acid needs. These factors influence the efficiency with which a given individual is able to utilize available amino acids and, we would argue, are critical pieces of information if requirements, as defined by the first two factors, are to be converted into practical dietary recommendations.

The processes that utilize amino acids can be loosely placed into two categories, protein deposition and the so-called maintenance pathways of amino acid utilization. Largely because of limitations of space, we will not consider in detail the biological basis of maintenance amino acid needs. Nonetheless, it should be emphasized that this aspect of amino acid nutrition is of specific quantitative importance to the human subject. Furthermore, a strong case can be made in favour of the idea that pathways of amino acid utilization, associated with tissue function and immune protection rather than with protein turnover, are critical to this aspect of amino acid nutrition. Discussions of this subject can be found in Dewey *et al*. (1996) and Reeds & Beckett (1996).

In principle it is possible to quantify the amino acid needs for protein deposition simply from the product of its rate and amino acid composition. For older animals the estimate of amino acid requirement calculated in this way is very similar to that defined from N balance trials (Fuller *et al*. 1989). However, one important characteristic of growth in the early postpartum period is the markedly different rates of growth among the tissues, with intestinal growth being particularly rapid (Fig. 1). Since the proteins deposited at each site differ in amino acid composition (Table 1), it is possible that the relative need for each amino acid will vary over the sucking period. In rats (Davis *et al*. 1993) and pigs (Mahan & Shields, 1998) there are some differences in the amino acid composition of protein gain from birth to weaning, which seem to be largely a function of the varying contribution of collagen to whole-body protein. However, these differences are quite small, an observation that probably reflects the dominating influence of skeletal muscle protein deposition on whole-body amino acid accretion.

From a strictly dietetic perspective the hallmark of the sucking period is the consumption of a highly-specific diet (milk) of relatively, although not absolutely, constant composition. As one might hypothesize that milk and its recipients have co-evolved, it might be predicted that the composition of a given species' milk would reflect the peculiar nutritional and developmental needs of the neonate of that species. In a qualitative sense, the idea of co-evolution is supported by observations that milks of

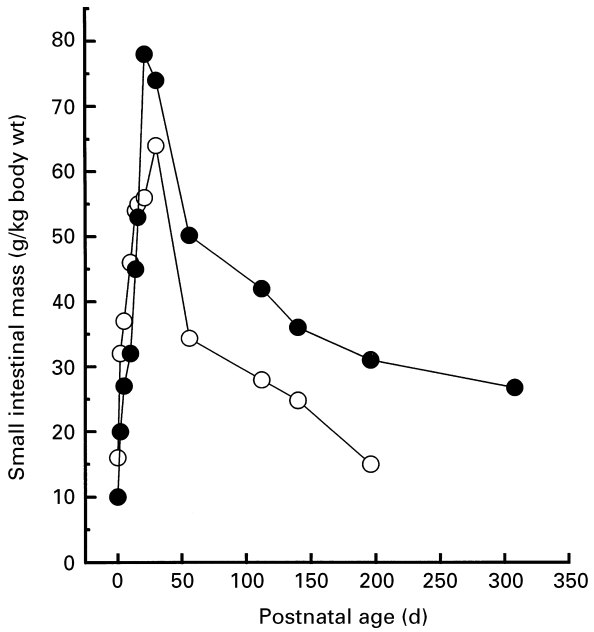

Fig. 1. Relationship between postnatal age and small intestinal mass in rats and pigs. (●), Rat data taken from Goldspink *et al.* (1984) and Burrin *et al.* (1991); (○), pig data from McMeekan (1940) and DG Burrin, R Jiang and B Stoll, unpublished results.

Table 1. Amino acid composition (mg amino acid/g protein) of the mixed proteins of different porcine tissues

	Tissue*			
Amino acid	Skeletal muscle	Liver	Intestinal mucosa	Skin
Glycine	48	76	94	195
Arginine	63	58	57	79
Alanine	59	71	78	95
Threonine	46	46	54	30
Aspartate†	94	87	92	73
Valine	55	63	58	40
Phenylalanine	41	45	42	32
Lysine	92	74	70	45
Histidine	41	23	16	12
Tryptophan	12	14	ND	2
Leucine	82	79	94	54
Methionine	27	25	17	8
Cysteine	13	19	ND	10
Isoleucine	48	49	45	23
Serine	42	55	58	42
Proline	41	55	54	119
Tyrosine	35	29	36	20
Glutamate†	159	122	120	124

*Data for skeletal muscle and skin were calculated from US Department of Agriculture nutrient composition data accessed via www.nal.usda.gov/fnic/foodcomp

Values for liver and intestinal mucosa are from B Stoll, DG Burrin and PJ Reeds, unpublished results.

†The sum of asparagine and aspartates, and glutamine and glutamate.

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different species contain different panels of growth factors and hormones (Klagsbrun, 1978; Francis *et al*. 1988; Odle *et al*. 1996; Burrin *et al*. 1997*b*) and that milk contains quite high concentrations of endproducts of other metabolic pathways. These endproducts include complex oligosaccharides (Newburg, 1997), nucleotides (Uauy *et al*. 1994) and other endproducts of amino acid metabolism (Table 2). Moreover, there is a linear relationship between the nutrient density (exemplified by the protein density in Fig. 2) of a given species' milk and the growth rate of the suckling. The protein concentration of milk and the weight-specific rate of protein deposition also tend to parallel one another as the period of lactation progresses (for example, see Fiorottto *et al*. 1991; Dewey *et al*. 1996; Dourmad *et al*. 1998).

Since different species exhibit markedly different weight-specific postnatal growth rates (Fig. 2), and because maintenance amino acid requirements are a function of body weight, there are quite marked differences in the relative contributions of protein deposition and N maintenance to total amino acid requirements among different species. For example, even though the body protein mass of the sucking rat increases by an order of magnitude over the first 3 weeks of life, during this period the maintenance protein requirement is less than 5 % of the total. In contrast, even during the first month of postnatal growth 35 % of the protein requirement of the human infant is associated with the maintenance of body N equilibrium. In growing animals, the relative amino acid requirements for growth and maintenance of N equilibrium appear to be markedly different (Fuller *et al*. 1989). It might be expected, therefore, that there would be parallels between the amino acid composition of a given milk and rate of protein deposition of the neonate. However, while an extensive survey of the amino acid composition of different mammalian milks (Davis *et al*. 1993, 1994, 1995) revealed some interesting interspecific differences in the concentrations of individual amino acids (e.g. the high tryptophan content of human milk, the high arginine content of felid milk and the high cysteine and serine content of rat milk) there appears to be no systematic relationship between the rate of growth of the neonate of a given species and the amino acid composition of the mixed milk proteins that it receives.

On the other hand, when data for milk amino acid composition are compared with the amino acid composition of mixed body protein (Fig. 3), observations of potential biological importance emerge. First, across species, threonine appears to be the first limiting essential amino acid for the protein accretion of the sucking neonate. Other essential amino acids are provided in milk at rates that either closely match (valine, phenylalanine, lysine and histidine) or exceed their utilization for protein deposition (e.g. leucine, methionine and isoleucine). In this sense milk is a relatively efficient vehicle for the delivery of essential amino acids for protein deposition. However, in other respects the amino acid mixture of milk is imbalanced. Irrespective of the species glutamate + glutamine contribute 20 % of total milk amino acids, and 40 % of the total amino

Table 2. Non-protein nitrogenous components of human milk

Component	Concentration (μmol/l) Mean	SD	Reference
Glutamine	580	46	Rassin *et al.* (1978)
Taurine	410	70	Rassin *et al.* (1978)
Creatine	140	17	Macy (1949)
Polyamines	8	2	Romain *et al.* (1992)
Nucleotides	69	12	Sugawara *et al.* (1995)
GSH	300	56	J Henry and PJ Reeds (unpublished results)

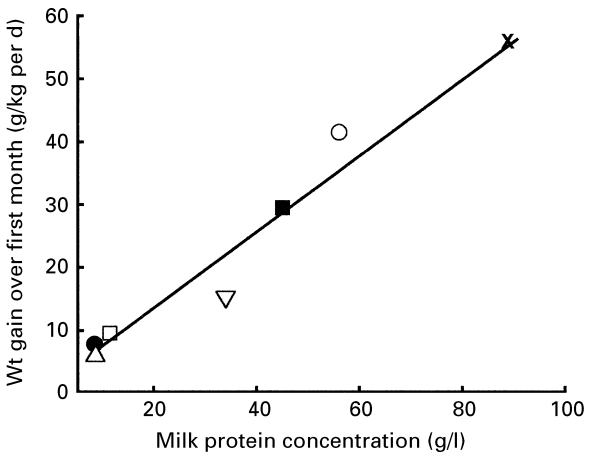

Fig. 2. Milk protein and postnatal weight gain in different coronal species. Milk protein concentration taken from Davis *et al.* (1994). Animal growth data: (●), man (Dewey *et al.* 1996); (△), (□), chimpanzee and gorilla respectively (Smith & Leigh, 1998; S Leigh, personal communication); (▽), cattle (Anderson *et al.* 1999); (■), sheep (Greenwood *et al.* 1998); (X) rat (Fiorotto *et al.* 1991); (○), pig.

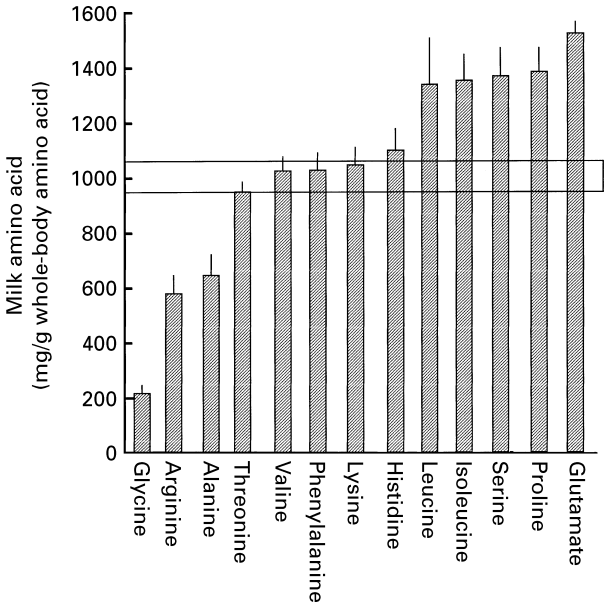

Fig. 3. Relationship between the amino acid composition of the milk and mixed body protein of six mammalian species. Data for the composition of human, gorilla, cattle, sheep, pig and rat milk were obtained from Davis *et al.* (1993, 1994). Values are means and 1 SD represented by vertical bars.

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acid intake of the milk-fed neonate is accounted for by glutamate+glutamine, proline and leucine. On the other hand, arginine, alanine and particularly glycine are remarkably deficient in milk.

Amino acid metabolism in the neonate

The presence of substantial excesses (e.g. glutamate, glutamine, leucine and isoleucine) and deficiencies (arginine, alanine and glycine) of specific amino acids in mixed milk proteins has some interesting implications. First, in order to deposit protein at a high rate, the milk-fed neonate has to carry out the regulated transfer of N from amino acids that are in substantial excess to those that are deficient. Thus, while the efficiency of utilization of individual amino acids for protein deposition varies in proportion to the degree to which their intake satisfies the need for protein deposition, the naturally-fed sucking mammal exhibits a high efficiency of utilization of milk protein-N for protein deposition (Table 3). Interestingly, when an estimate of maintenance N needs (approximately 100 mg N/kg body weight; see Dewey *et al*. 1996) is included in the calculation, then the incremental efficiency with which milk amino acids are deposited in body protein is not only very high, but also similar across the species considered in Table 3.

Table 3. The efficiency of utilization of milk total nitrogen during the first month of postnatal life*

	N for whole period (g)			Efficiency of utilization (% intake)	
Species	Total intake	Total deposited	Maintenance	Gross†	Net‡
Rat	1·25	0·98	0·06	78	82
Man	27·4	15·5	9·7	56	87
Pig	185	136	18	74	81
Sheep	290	197	28	68	78

*Data are normalized to a litter of ten for rats and pigs, and ovine and human data are for single offspring. Milk output is from Davis *et al.* (1993) for rats, Dourmad *et al.* (1998) for pigs, AW Bell (personal communication) for sheep and Dewey *et al.* (1996) for human subjects. Body protein gain (normalized to 30d) is from Fiorotto *et al.* (1991) and Davis *et al.* (1994) for rats, Mahan & Shields (1998) and Wu *et al.* (1999) for pigs, Greenwood *et al.* (1998) for sheep and Dewey *et al.* (1996) for human infants. It was assumed that maintenance N intake was 100 mg N/kg body weight per d.
†Percentage of total protein intake deposited.
‡Percentage of protein intake above maintenance that was deposited.

A direct illustration of the ability of the neonate to 'balance' the N among amino acids is provided by the experiments of TA Davis (unpublished results). In these studies (Table 4) 7-d-old piglets were infused with insulin at a rate that achieved an insulin concentration similar to that found in the fed state, glucose and essential amino acid concentrations were maintained at their fasting values, and leucine turnover and oxidation were measured with continuous intravenous infusions of [1-^{13}C]leucine. In separate experiments the amino acid mixture used to maintain amino acid concentrations was either a conventional paediatric parenteral nutrition solution, which contains relatively low amounts of non-essential amino acids, or a solution that had been supplemented with glutamine, glutamate, glycine and alanine. During the infusion of the imbalanced amino acid mixture plasma urea concentrations did not change, so that all the infused amino acid-N was utilized for anabolism. Nevertheless, leucine-C catabolism was increased 2·4-fold by insulin infusion, critically under conditions in which the plasma leucine concentration had not changed. In other words, leucine-N must have been transferred to those non-essential amino acids that were infused in low amounts. However, during the infusion of insulin together with the supplemented amino acid mixture leucine catabolism increased by only 1·5-fold, less leucine had to be infused to maintain leucine concentrations at their fasting value and a higher proportion of the additional leucine was stored. These results suggest that the neonate possesses mechanisms that allow it to sense an imbalance in the amino acid supply, and not only allow it to mount acute changes in the metabolism of specific amino acids, but to do so via a mechanism that is independent of changes in the circulating concentration of the amino acid in question. The nature of the mechanism remains unknown. It clearly allows the neonate to utilize its total amino acid intake with a high degree of efficiency. These observations have broader implications for the regulation of amino acid metabolism at other stages of development and merit closer attention.

The experiment described earlier was carried out under artificial circumstances, in as much as the amino acids were administered intravenously. Of course, under natural conditions the interconversion of amino acids must be achieved from the enteral amino acid input. In fact, we would go further, and argue that metabolism in the small intestine is critically important to this N-balancing

Table 4. Leucine utilization in 7-d-old piglets receiving intravenous infusions of insulin and glucose, with the circulating concentrations of essential amino acids (EAA) being maintained at fasting values with the infusion of isonitrogenous mixtures of amino acids containing different proportions of non-essential amino acids (NEAA) and EAA. Data from TA Davis, PR Beckett, ML Fiorotto, H Nguyen and DG Burrin (unpublished results)*

	Leucine kinetics (μmol/kg per h)			
Condition	Infused	Oxidized	Balance	Storage of infused leucine (% infused)
Fasting	0	196	–196	NA
Low NEAA : EAA	366	488	–122	20
Balanced NEAA : EAA	241	309	–69	53
Pooled SD	66	87	65	8
Effect of amino acid mixture	P<0·01	P<0·01	NS	P<0·01

*It is critical to note that the circulating leucine concentration was the same during all three infusions.

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phenomenon. In the context of the milk-fed neonate it is critical to note that glutamine (Windmueller & Spaeth, 1980) and glutamate (Stoll *et al*. 1999; Table 5) appear to be crucial energy substrates for the intestinal mucosa. In view of this factor, and the demonstrated ability of the intestine of piglets and infants to use considerable quantities of dietary glutamate and glutamine (Reeds *et al*. 1996; Darmaun *et al*. 1997), it seems reasonable to come to the teleological conclusion that the high concentration of glutamate (+glutamine) in milk may have evolved specifically to support the metabolism of the mucosa. Furthermore, the high rate of utilization of dietary glutamate by the intestinal tissues implies that virtually all the systemic glutamate (and probably glutamine) derives from endogenous synthesis. This factor, as we have shown, has consequences for the utilization of leucine (see Table 4) and isoleucine, two amino acids that are not only important N donors for glutamate and glutamine synthesis but are in excess in milk.

The high concentrations of glutamate and glutamine in milk also have a bearing on the arginine and alanine status of the milk-fed neonate. It has been known for many years that the intestinal mucosa is capable of synthesizing alanine from dietary glutamate (Neame & Wiseman, 1957; Windmueller & Spaeth, 1980), and we have confirmed the presence of this process in the piglet (Stoll *et al*. 1999). Furthermore, the intestine is such a critical site for citrulline synthesis (Windmueller & Spaeth, 1980), that arginine, which is synthesized from this citrulline, becomes an essential amino acid following massive gut resection (Wakabayashi *et al*. 1995), as well as in parenterally-nourished individuals (Motil *et al*. 1980). Recent work suggests, moreover, that the intestinal mucosa of the newborn is capable of the complete synthesis of arginine (Blachier *et al*. 1993; Wu & Knabe, 1995; Stoll *et al*. 1999), and that inhibition of ornithine transaminase, a key enzyme in mucosal arginine synthesis, substantially compromises circulating arginine concentrations in the neonatal piglet (Wu *et al*. 1997). Thus, the high concentrations of milk glutamate and glutamine not only support the metabolic needs of a crucially important tissue, but glutamate metabolism in the gut makes the dominant contribution to the synthesis of two of the amino acids that are in short supply in milk.

In many respects the most remarkable feature of the amino acid composition of milk is the substantial glycine deficit. Indeed, both on the basis of simple mass balance calculations and on the basis of the results of studies of glycine-N metabolism in infants (Jackson *et al*. 1981), it can be concluded that glycine is a specific limitation to the growth of the normal neonate. The dietary glycine deficit is even more notable because glycine is a precursor for a number of nutritionally-significant metabolic pathways that are functionally, but not directly, related to protein deposition. Thus, glycine is an obligatory precursor for purine base synthesis. This factor is important because recent data have suggested that mammalian nucleic acid synthesis relies on intracellular purine synthesis *de novo* (Berthold *et al*. 1995; Boza *et al*. 1996; Perez & Reeds, 1998). Glycine is also a precursor for the synthesis of creatine, a factor that is crucial for the maintenance of energy flow both in skeletal muscle and the central nervous system. Finally, glycine is also a component amino acid of GSH, a tripeptide that is a critical factor in the maintenance of peroxidative defences and cellular integrity, and is also the N source of haem. Thus, the true requirement for glycine is in excess of that consumed in protein deposition, and the synthesis of glycine in the milk-fed neonate, irrespective of species, must be very high.

Surprisingly, despite the critical importance of glycine synthesis to a number of aspects of the function of the neonate, and even though we know on the basis of measurements of the glycine flux that glycine must be synthesized, the pathway that is utilized remains obscure. On the basis of the literature, two pathways might be utilized.

The first is the synthesis of glycine from serine. However, it is important to note that the quantities of serine in milk are insufficient to support even the minimum estimate of glycine synthesis necessary to explain glycine deposition during the period. Thus, serine synthesis, presumably from pyruvate, could be of high nutritional importance to the neonate (Miller *et al*. 1996). The other pathway of glycine synthesis is via the transamination of glyoxylate with alanine. The necessary peroxisomal enzyme has been identified in the liver of newborn rats (Snell & Walker, 1974), but the precursor for glyoxylate synthesis is unknown. Hopefully, given the renewed interest in single C metabolism associated with the revitalization of folate research, more information on pathways of glycine synthesis will emerge.

Table 5. The contribution of dietary and systemic substrates to energy generation in the portal-drained viscera of 4-week-old milk-fed piglets (Data from Stoll *et al.* 1999)
(Values are means and 1 SD)

	Percentage of uptake oxidized		Contribution to visceral CO_2 production (%)	
Substrate	Mean	1 SD	Mean	1 SD
Dietary glucose	2	1	6	2
Dietary glutamate	52	3	36	3
Arterial glucose	27	9	29	3
Arterial glutamine	70	8	15	2

Feeding, protein turnover and growth

The high rate of protein deposition of the neonate is, as one might expect, associated with a high rate of whole-body protein synthesis (Table 6), and this rate falls logarithmically from birth to the attainment of sexual maturity. However, the changes in whole-body protein synthesis that occur over the sucking period obscure a number of critical observations with regard to tissue protein synthesis and growth. First, in rats, pigs and sheep (and presumably in other species) there is a rapid activation of tissue protein synthesis during the first day of postnatal life (Fig. 4; see also Patureau-Mirand *et al*. 1990). At this time the natural food of the neonate is colostrum, a mammary secretion that has a higher nutrient density than mature milk. Thus, it is reasonable to ascribe a significant proportion of the increased protein synthesis to a response to nutrient intake itself. However, colostrum also contains particularly high

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Table 6. The relationship between age and whole-body protein synthesis (PS; g/kg per d) in the fed state in various mammalian species (Values are means with 1SE)

Rat*			Pig†			Sheep‡			Man§		
	PS			PS			PS			PS	
Stage	Mean	1 SE	Stage	Mean	1 SE	Stage	Mean	1 SE	Stage	Mean	1 SE
20 d fetus	45	4	2·5kg	31	1	4·5kg	32	1	Preterm	11	2
21 d old	34	1	7·5kg	24	7	25kg	8	1	Term	8	1
56 d old	20	1	30kg	18	4	45kg	5	1	18 months old ‖	6	1
Adult	13	1	90kg	7	2	60kg	4	1	Adult ‖	3·5	0·4

*Goldspink & Kelly (1984).
† Reeds *et al.* (1980); TA Davis, ML Fiorotto and DG Burrin (unpublished results); JB van Goudoever and B Stoll (unpublished results).
‡Pell *et al.* (1986); MacRae *et al.* (1988); Attaix *et al.* (1988); Harris *et al.* (1992).
§ de Benoist *et al.* (1984); Denne *et al.* (1991, 1992, 1994, 1995); Kandil *et al.* (1991); Beaufrere *et al.* (1992); van Goudoever *et al.* (1995).
‖ The 18-month-old children had recovered from malnutriton and were studied with [^{15}N]glycine. Adult data are a summation of the literature on leucine flux measurements.

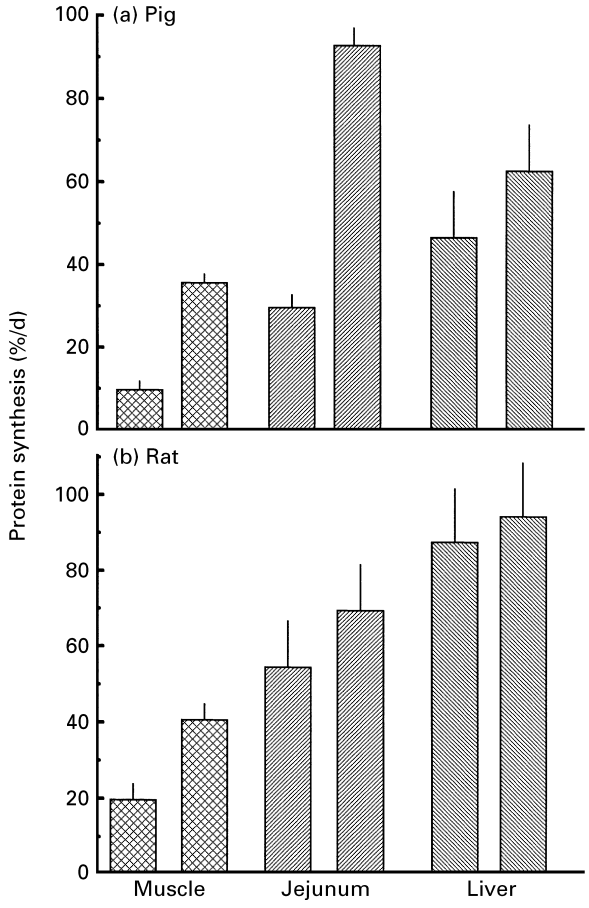

Fig. 4. Changes in tissue protein synthesis of (a) pigs and (b) rats over the first day of postnatal life. Pig data from Burrin *et al.* (1995); rat data from Goldspink *et al.* (1984), Lewis *et al.* (1984), Davis *et al.* (1991) and Burrin *et al.* (1992). Values are means with SE represented by vertical bars.

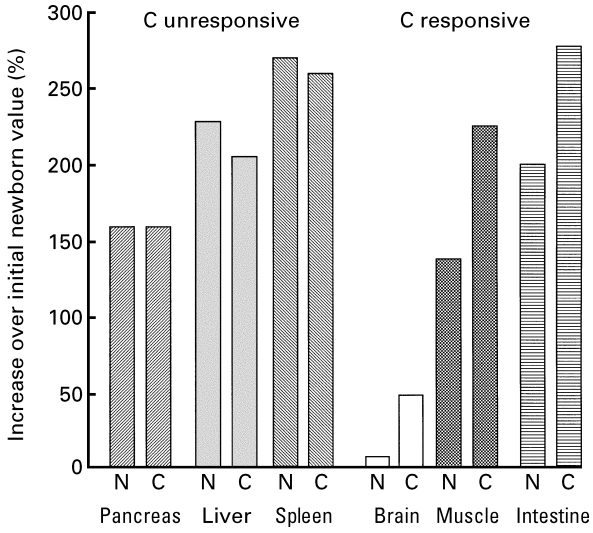

Fig. 5. Nutrient (N)- and colostrum(C)-stimulated increases in tissue protein synthesis in newborn pigs. Values are means and 1 SD represented by vertical bars. Data taken from Burrin *et al.* (1995, 1997*a*).

concentrations of a wide variety of potential growth-regulatory molecules, and studies in piglets (Burrin *et al.* 1995, 1997*a*; Fig. 5) have shown that some, but not all, tissues increase their rate of protein synthesis to a greater extent when the animal receives colostrum than when it receives an equal amount of nutrients provided in a formula. In some respects the stimulatory effect of colostrum on small intestinal protein synthesis is to be expected, because this tissue will be exposed directly to the hormones and peptide growth factors in colostrum. However, these data also show that peripheral tissues, notably the skeletal musculature (Burrin *et al.* 1995) and the brain (Burrin *et al.* 1997*a*), are specifically responsive to colostrum. Moreover, in skeletal muscle the response to colostrum appears to be confined to the synthesis of myofibrillar proteins (Fiorotto *et al.* 1995). Although such experiments have not been performed in other species, it is noteworthy that in the newborn rat the synthesis of myofibrillar protein is higher than that of the sarcoplasmic proteins. Unfortunately, at this stage the mechanism that mediates these apparently specific effects of colostrum is not known, although it appears that neither insulin nor insulin-like growth factor 1 are involved directly. Indeed, given the response in the central nervous system, it is tempting to speculate that the colostrum stimulation of protein synthesis is not a response to a soluble growth factor or hormone absorbed by the neonate, but that

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the ingestion of colostrum generates regulatory (perhaps neural) signals from the small intestine.

The general fall in whole-body protein synthesis that occurs over the sucking period also conceals quite marked differences in individual tissues. Thus, as we show in Fig. 6, in the rat during the first 3 weeks of life the fractional rate of protein synthesis in the small intestine rises, the rate of protein synthesis in the liver remains essentially constant, and it is only in skeletal muscle and the skin (data not shown), which have particularly high rates of protein synthesis at 1 d after birth, that the fractional rate of protein synthesis falls between birth and weaning. In this regard, it is interesting to note that, although these changes appear to be largely a function of a reduction in the concentration of ribosomes, there are indications, in the rat at least, that as the animal approaches weaning the translational activity of the ribosomes increases. Essentially identical data have been found in the pig (Davis *et al*. 1996), and it seems likely to us that similar changes occur in the newborn of other species.

The observations summarized in Figs. 5 and 6 were all made in animals that had not been deprived of food before the measurement of protein synthesis. This observation is critical, because quite clearly growth only occurs as a result of the activation of protein anabolism following the ingestion of nutrients. In fact, it has been shown, in both rats and pigs, that in those tissues that exhibit a marked fall in protein synthesis as development proceeds, much of the difference relates to the response to nutrient intake itself. This factor, we believe, is closely related to another phenomenon associated with growth during the immediate postnatal period.

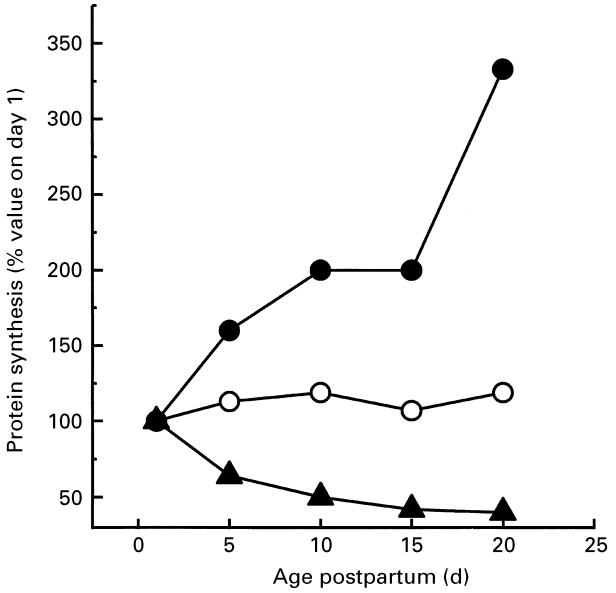

Fig. 6. Changes with age in the fractional rate of synthesis in suckling pigs. (▲), Fast-twitch muscle; (○), liver; (●) small intestine. (Data for fed animals from Davis *et al.* 1991 and Burrin *et al.* 1991, 1992.)

In the discussion of the utilization of milk amino acids, we highlighted the fact that over the whole of the preweaning period the efficiency with which milk amino acids are deposited in body protein is very high. However, the data that we used to support this statement were deliberately assembled for the whole sucking period, and when we examine the efficiency of milk amino acid utilization across the sucking period itself it is clear that the efficiency of amino acid utilization also falls. Critically, this decrease in efficiency is not simply a reflection of the interaction between protein intake, deposition and maintenance requirements, because when the estimated maintenance amino acid needs are taken into account the incremental efficiency of protein utilization in pigs, sheep and rats falls from between 79 and 85 % during the first week of life to between 52 and 68 % by the fourth week.

The fact that the efficiency of amino acid utilization for protein deposition falls in parallel with the fed rate of whole-body protein synthesis, suggests to us that the efficiency of amino acid utilization is linked in some way to the degree to which a given meal can stimulate protein anabolism. For skeletal muscle, but critically not for secretory tissues such as the intestine and liver, this link appears to be present. Data from both rats (Table 7) and pigs (Davis *et al.* 1996) show that the increase in the fractional rate of muscle protein synthesis associated with feeding falls progressively from birth to weaning. Of particular importance is the observation that the increase in total muscle protein synthesis per unit protein intake also falls; i.e. postnatal development is associated with a progressively lower protein synthetic response to the ingestion of a given quantity of protein. This finding suggests that as the animal approaches weaning the protein taken in a given meal becomes progressively greater than the magnitude of the anabolic response that the animal is capable of mounting. The consequence is that increasingly larger quantities of dietary amino acids are catabolized shortly after their absorption.

These observations pose the question of which regulatory factors are responsible not only for the stimulation of protein synthesis by feeding, but also for the apparent fall in this stimulation as development proceeds. In order to gain some

Table 7. The relationship between weight-specific protein intake and the effect of feeding on protein synthesis in the plantaris muscle of sucking and weaned rats (Recalculated from Davis *et al.* 1989, 1991)

		Protein synthesis					
		Fractional rate (% per d)		Absolute rate (mg/d)		Change in protein synthesis (mg)	
Age (d)	Protein intake (g/kg per d)	Fed	Fasted	Fed	Fasted	per kg body wt	per g protein intake
5	33	33	16	0·11	0·05	6·0	0·181
10	30	24	16	0·23	0·15	3·83	0·127
16	28	25	20	0·71	0·57	4·11	0·146
21	22	22	20	1·32	1·21	2·0	0·083

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more insights into this important question of growth regulation, Davis, Wray-Cahen and Beckett (see Wray-Cahen *et al*. 1997) have developed an insulin–glucose–amino acid clamp technique, and have used this technique with 7-d- and 26-d-old pigs to attempt to answer two key questions:

1. under conditions of constant substrate concentrations does insulin stimulate tissue protein synthesis to the same degree as feeding?
2. are changes in the response of tissue protein synthesis to insulin the primary factor responsible for the fall in the metabolic response to feed intake?

In their first experiments the influence of insulin on whole-body amino acid utilization was investigated. The results of this experiment (Fig. 7) revealed a number of interesting observations. First, at insulin concentrations (approximately 30 μU/ml) that were characteristic of the fed state whole-body amino acid disposal approached an asymptotic value which approximated to 65 % of the amino acid intake of the sow-fed piglet. Second, the amino acid utilization of the younger pigs was very sensitive to insulin, with a measurable effect occurring at insulin concentrations below 10 μU/ml. Third, increasing age was associated with the appearance of a distinct threshold (approximately 20 μU/ml) below which there appeared to be no effect of insulin on amino acid utilization. Finally, the insulin concentration for half maximal stimulation of amino acid utilization shifted from 18 μU/ml in the 7-d-old pigs to 45 μU/ml in the 26-d-old animals, and the maximum response was substantially lower in the older group. Taken together these data imply that on a whole-body basis the fall in the efficiency of protein utilization is reflected in changes in both the sensitivity and the responsiveness of protein deposition to insulin.

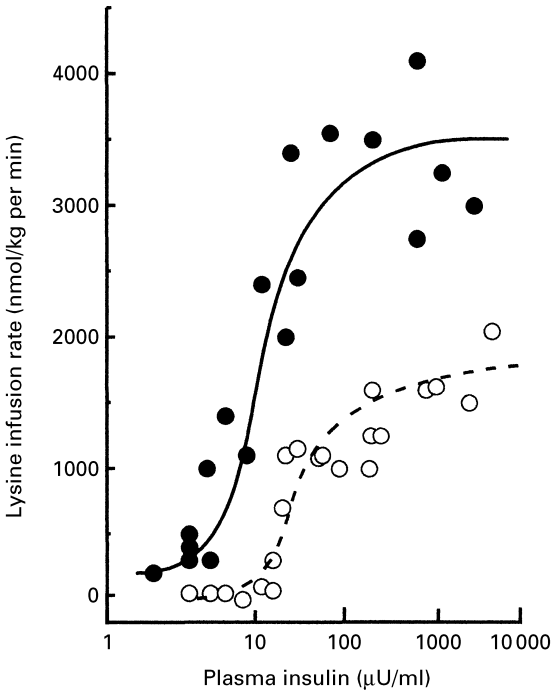

Fig. 7. The effect of insulin on amino acid utilization in 7- (●) and 26 (○)-d-old pigs. (Replotted from Wray-Cahen *et al.* 1997.)

This experiment left unanswered the question of whether the influence of insulin on whole-body amino acid disposal was due to a stimulation of tissue protein synthesis. In fact, when tissue protein synthesis was measured at the end of a 6 h hyperinsulinaemic–euaminoacidaemic clamp (Wray-Cahen *et al*. 1998) it became clear that once again whole-body responses had concealed important differences among the tissues (Table 8). Thus, as had been observed by Garlick *et al*. (1983) in rats, insulin, within the normal range, was capable of fully stimulating muscle protein synthesis to the fed value. However, protein synthesis in the liver, and the intestine (data not shown), was completely unresponsive to insulin. In addition to these tissue differences, Davis' studies (Wray-Cahen *et al*. 1998) on protein synthesis demonstrated two other important phenomena. First, the responsiveness of muscle protein synthesis to insulin fell with age. Second, insulin-stimulated muscle protein synthesis reached a maximum at insulin levels (approximately 10 μU/ml) well below those required to maximize whole-body amino acid disposal. Given that insulin lowered the rate of whole-body degradation (at least as estimated from leucine kinetics), this result implies that the efficient disposal of dietary amino acids requires both a stimulation of protein synthesis and a reduction in protein degradation.

The interaction between amino acids and insulin in regulating tissue protein synthesis in sucking animals is currently under investigation. Table 8 shows data from 7-d-old pigs. These results show that in skeletal muscle both insulin and amino acids can stimulate protein synthesis independently of one another, but that in liver only amino acids are capable of stimulating protein synthesis. Finally, preliminary data suggest that the responsiveness of muscle protein synthesis to amino acids is also much lower in 26-d-old pigs compared with 7-d-old pigs. The picture that emerges, therefore, is that the fall in protein synthesis, and the associated fall in the efficiency of amino acid utilization, appear to be related to the capacity of the tissues to synthesize protein and the magnitude of the response to any anabolic stimulator. This pattern undoubtedly reflects a fall in the ribosome concentration, and studies are under way to investigate whether the ancillary translation factors alter in activity with age.

Table 8. Effect of feeding, insulin and amino acids on the fractional rate of tissue protein synthesis (%/d) in 7-d-old pigs. Data of Davis *et al.* 1996 and TA Davis, D Wray-Cahen and H Nguyen, unpublished results

	Condition					
Tissue	Fast	Insulin (30 μU/ml)	Amino acids (2× fasting)	Insulin + amino acids	Fed	Pooled SD
Muscle	11·9	19·3*	19·9*	21·7*	23·7*	2·8
Liver	64	67	78*	81*	87*	7

*Values were significantly different from the fasting value: $P<0{\cdot}05$.

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Conclusion

Over the last 20 years, large strides have been made in understanding growth and metabolic regulation in the neonate. Nevertheless, a number of critically-important questions remain. Thus, although it is clear that the neonate is capable of carrying out extensive amino acid interconversions, the pathways and the regulatory mechanisms remain poorly characterized. Similarly, although the changes in protein turnover that accompany the transition from birth to weaning are clearly regulated, and presumably genetically and developmentally programmed, the underlying regulatory factors and the mechanisms by which they exert their effects are areas of substantial ignorance. This situation is regrettable, because the nutritional experiences during the neonatal period are of crucial importance to the long-term health of the individual.

Finally, we believe that it is important to emphasize that the neonate presents a potentially powerful tool for understanding broader aspects of the regulation of tissue function and growth regulation. Thus, understanding the protein and amino acid metabolism of the neonate might well enhance our knowledge of the adult, and hence contribute important information of relevance to the nutritional amelioration of metabolic diseases associated with maturity and old age.

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