Optimizing amino acid and protein supply and utilization in the newborn

By ALAN A. JACKSON, *Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO9 3TU*

As has been pointed out already (Casey, 1989) homologous milk appears to promote optimal growth and well-being in the infant, although it is not entirely clear why this should necessarily be so. The difficulty in fully understanding the basis of the relations is particularly well illustrated for protein and amino acids. Within the framework of our general appreciation of nitrogen and protein requirements (Food and Agriculture Organization/World Health Organization/United Nations University, 1985), the N content of human milk is remarkably low, whether expressed in relation to the total energy content, or the amount ingested daily by the infant. It would appear that all the protein in the milk is not digested and absorbed. This may be particularly true for certain classes of proteins such as immunoglobulins, although we do not have any precise, quantitative information on the extent of this malabsorption. On the other hand, about 25% of the N in human milk is not present as protein at all. A portion can be identified as free amino acids, but by far the largest contribution, about 60%, comes from urea-N (Harzer *et al.* 1984). The urea-N has generally been considered to be metabolically unavailable. If this is so, the available protein would appear to be exceptionally low at a time when the demands for infant growth, development and elaboration are at their highest (Fomon & Macy, 1958). With our present concepts of protein and amino acid metabolism it is not possible to rationalize the apparent contradiction between the N requirements for growth and that available from breast milk. In order to make sense of the observed reality, we have to be prepared to consider alternative frameworks of understanding.

In order to usefully approach this problem I have to start from a base of assumptions founded on experimental observations. There are four propositions I think it necessary to accept in order to be able to proceed.

1. The classic approach to the study of N metabolism by the balance technique is able to determine that change has taken place, but not necessarily the mechanisms whereby that change was brought about. The introduction of methods that enable us to study dynamic aspects of protein metabolism (Waterlow, 1967), and the application of stable isotope techniques to their application in vivo have enabled us to advance our understanding significantly (Waterlow, 1984). The simplest model for whole body protein turnover assumes that a single metabolic pool of amino acids acts as the precursor for protein synthesis in the whole body (Mehta, 1989). The model has to be a simplification, but it serves to demonstrate that protein turnover is very intense, and at least four to five times greater than implied by the intake of protein (Picou & Taylor-Roberts, 1969). The stool N is equivalent to 10–15% of the dietary intake. Although it has generally been assumed that this represents malabsorbed N, it is more likely that it is the result of a far more intense metabolic activity in the lower bowel (Jackson, 1986). More recent evidence suggests that any refinement of the model for protein turnover has to accommodate an extensive exchange of protein and N metabolism within the gastrointestinal tract, at least as great as the dietary intake of protein (Fig. 1) (Jackson *et al.* 1987*a*). It is likely that this exchange is not simply a movement of amino acids in two directions across a mucosal barrier, but also represents modulation of the pattern of N compounds by the gastrointestinal microflora. The N in the lower bowel

https://doi.org/10.1079/PNS19890041 Published online by Cambridge University Press

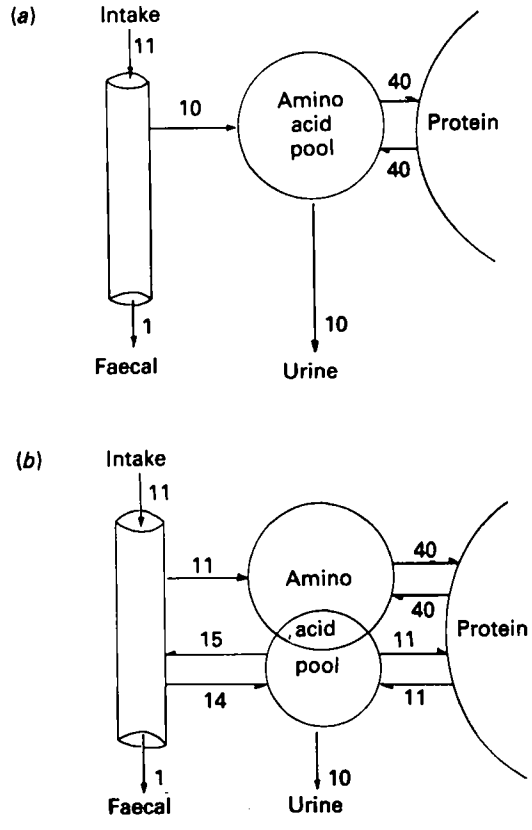

Fig. 1. The simplest model for whole body protein turnover. (*a*) This assumes a single precursor pool of amino acids from which proteins are synthesized and the end-products of metabolism derived. The reality is more complicated, and there is evidence for a more complex interchange of protein and amino acids with the gastrointestinal tract (*b*) which amounts to 14–15 g nitrogen/d (Jackson, 1986). Values are expressed in g N/d.

may be derived in part from food residue, but is also endogenous in origin, from secreted protein, sloughed cells, mucus and urea.

2. The body is able to accommodate a range of dietary protein intakes without apparent adverse effects. The process for adapting to a low protein diet is primarily determined by the rate of synthesis and pattern of excretion of urea (Waterlow, 1968). On moving from a higher to a lower protein intake the rate of urea synthesis is reduced, with a fall in both the activity and the amount of the urea-cycle enzymes (Das & Waterlow, 1974). Furthermore, the disposal of the urea produced is altered, with retention within the body of a greater proportion of the urea, a salvage system that operates primarily at the level of the large intestine (Jackson, 1983). The increased salvage of urea is not simply related to the absolute dietary intake of protein (Picou & Phillips, 1972), but is influenced by a range of factors, importantly the relative demand for protein in synthetic pathways in relation to the dietary supply (Jackson, 1986).

3. In infants there is an absolute demand for a component of the 'non-essential N' in the diet. Snyderman *et al.* (1962) showed that if the dietary protein intake is reduced to a level where N balance and weight gain can no longer be sustained, positive balance can be restored by the addition of non-essential N to the diet in the form of urea or glycine.

This response is not specific for infants and can be shown to be true at all ages. The most effective combination of non-essential N is glycine with either glutamic acid or an ammonium salt (Kies, 1972). Therefore, non-essential N might be limiting for normal growth.

4. The classical perception of essential (indispensable) and non-essential (dispensable) amino acids was based on the presumption that essentiality was a feature conferred by the structure of the carbon skeleton (Rose, 1957). This perception is too narrow (Jackson, 1983; Laidlaw & Kopple, 1987). With the use of ^{15}N labels it is possible to demonstrate that there is specific channelling of amino groups between amino acids (Jackson & Golden, 1980; Jahoor *et al.* 1988). Thus, it is possible to consider the relative availability, and hence the essentiality, of the amino group (Table 1). On this basis only two amino acids can be considered to be absolutely essential, lysine and threonine, and a small number absolutely non-essential. The C skeleton of the non-essential amino acids has to be a readily available metabolic intermediate, e.g. pyruvate, α-ketoglutarate or oxaloacetate, which can be readily aminated in transamination reactions. All the other non-essential amino acids can be shown to be conditionally essential, and are potentially formed from essential amino acids (Table 2) (Laidlaw & Kopple, 1987).

Balance studies

In the human there is relatively little information available on aspects of growth in relation to dietary intake or net balance in the healthy term infant during the first month of life. This in part represents methodological problems, but also is an indication of the

Table 1. *Classification of amino acids with four categories (Jackson, 1983)*

	Carbon skeleton	
Amino group	Essential	Non-essential
Essential	Lysine	Serine
	Threonine	Glycine
		Cysteine
Non-essential	BCAA	Glutamate
	Typtophan	Alanine
	Phenylalanine	Aspartate
	Methionine	

BCAA, branched chain amino acids.

Table 2. *A classification of amino acids in relation to the ease with which they may be synthesized in the body*

Amino acids		
Essential	Conditionally essential	Non-essential
Leucine	Proline	Glutamate
Isoleucine	Histidine	Alanine
Valine	Arginine	Aspartate
Tryptophan	Tyrosine	Glutamine
Phenylalanine	Cysteine	
Methionine	Taurine	
Threonine	Glycine	
Lysine	Serine	

inherent difficulties in carrying out reliable balances without disturbing the intimate relationship between mother and infant. It is exceptionally difficult to obtain reliable indices of breast-milk intake and composition, and the collection of urine and stool without any restraint can only be carried out reliably in a metabolic facility. There is far more information available on preterm infants, which by definition is not a normal population.

The longitudinal studies carried out by Fomon (Fomon & Macy, 1958) on a series of institutionalized infants fed on donor breast milk, either continuously or intermittently up to the age of 6 months, give a strong basis of support for accepting the rate of growth of breast-fed infants as the reference for normality, in relation to N and protein metabolism. The pattern of weight gain and N retention provides no evidence for a specific limitation during this period. The average weight gain of the infants was 31·9 g for a retention of N of 1 g. A retention of 1 g N, if equivalent to 6·25 g protein, could account for the deposition of 31·25 g lean tissue, suggesting that a large proportion of the weight gained could be accounted for by lean tissue deposition. The rate of N retention was related to the rate of weight gain, and hence the age of the children. There was a slowing down with time. The dietary N was utilized with an efficiency equivalent to that in older age groups. Faecal N in seven children was 15·4 (SE 3·7)% of intake, very similar to that measured by Slater (1961), 16·9% in thirteen infants at 1 week of age. There was no evidence for a change in stool N with time, that might suggest a maturation or alteration of gastrointestinal function. It is widely appreciated that the N content of human milk is not synonymous with protein content.

Utilization of non-protein N

For most studies on mature milk it has been found that up to 25% of the N is not associated with the protein fraction. The non-protein N includes a contribution from free amino acids and other compounds, but the largest single component, up to 60%, is urea-N (Harzer *et al.* 1984). It is possible to calculate from the findings of Slater (1961) that at 1 week of age the infants in her study were receiving a N intake of 397 mg/kg per

Table 3. *A theoretical calculation, based on the findings of Slater (1961) and Heine* et al. *(1986), of the utilization of dietary and endogenous urea in a breast-fed infant*

(The urea-nitrogen available for anabolic processes is about 21% of the dietary protein intake)

	N (mg/kg per d)		
	Intake and endogenous production	Excretion	Retention
N intake	397		
Non-protein N intake	90		
Urea-N: Intake	60		
Excreted		36	
Retained			24
Endogenous urea production:	82		
Excreted		41	
Retained			41
Urinary loss of urea-N		77	
Faecal loss of urea-N		1	
Urea-N available for anabolism			65*

*About 400 mg protein/kg per d.

https://doi.org/10.1079/PNS19890041 Published online by Cambridge University Press

Table 4. *The percentage of an oral dose of [^{15}N]urea retained by normal infants in relation to the type of feed*

Feed	Retention of [^{15}N]urea (%)	Reference
Human milk	25–58	Heine *et al.* (1984)
Human milk	17–61	Heine *et al.* (1986)
Milk formula	6–28	Fomon *et al.* (1987)

d, of which 90 mg/kg per d was non-protein N, containing an estimated 60 mg urea-N/kg per d. These same infants had a urinary excretion of urea-N of 77 mg/kg per d (Table 3).

The dietary urea has three possible routes of disposal: stool, urine or incorporation into the body's N pool. We know from isotopic studies that following the ingestion of ^{15}N-labelled urea the losses in stools are very small, up to a maximum of 2%, which is incorporated into bacterial protein (Heine *et al.* 1984, 1986). If the dietary urea was excreted unchanged in urine this would only allow for the endogenous synthesis of 19 mg N/kg per d, or 6% of the dietary intake, a remarkable level of efficiency. Using ^{15}N-labelled urea it has been shown that urea-N might be retained, and although the relative and absolute amounts appear to vary between studies, the findings would suggest that about 40% of an oral dose is retained within the body (Table 4). One may assume that orally ingested urea is handled in a manner similar to that synthesized endogenously, and that labelled urea reliably traces the fate of the urea pool. Hence, it can be estimated that urinary urea is derived in about equal parts from dietary and endogenous sources, with a retention and utilization of the order of 60–70 mg urea-N/kg per d. This would represent a substantial contribution to the N economy of the body, equivalent to 400 mg protein/kg per d (Table 3). Although there are no measurements of urea synthesis rates in young infants on a low protein diet, such as human milk, one would expect that at least 50% of the urea-N produced would be retained by the host and utilized (Doherty *et al.* 1989).

Factors influencing urea utilization

We have spent some time exploring the factors that are likely to influence, or exert a control over the reutilization of urea-N, i.e. energy intake, protein intake, metabolic demand, quality of dietary energy, antibiotic therapy, gastroenteritis. The hydrolysis of urea is a function of the microflora of the lower gastrointestinal tract, and the primary metabolic activity of the microflora is determined by the energy available to them, mainly in the form of non-digestible carbohydrate. In young children recovering from malnutrition the hydrolysis and utilization of urea-N was greater on a diet enriched with maize starch than one enriched with arachis oil (Fig. 2) (Doherty *et al.* 1989). The effect exerted by dietary N is not simply related to the absolute dietary intake, but rather to the intake in relation to the metabolic demand for protein synthesis (Jackson *et al.* 1988). Indeed, we have considered that the switch to increased urea hydrolysis may represent one reasonably sensitive index of the efficiency of a particular dietary protein. A considerable amount of work has been done on the influence of antibiotics and bowel sterilization on urea hydrolysis, in relation to the management of patients with hepatic damage, or patients requiring low protein diets for the management of renal failure. Diarrhoeal disease would be expected to disturb the delicate balance of this ecosystem, and this may be of particular importance in the aetiology of malnutrition and growth failure in childhood (Jackson & Grimble, 1989).

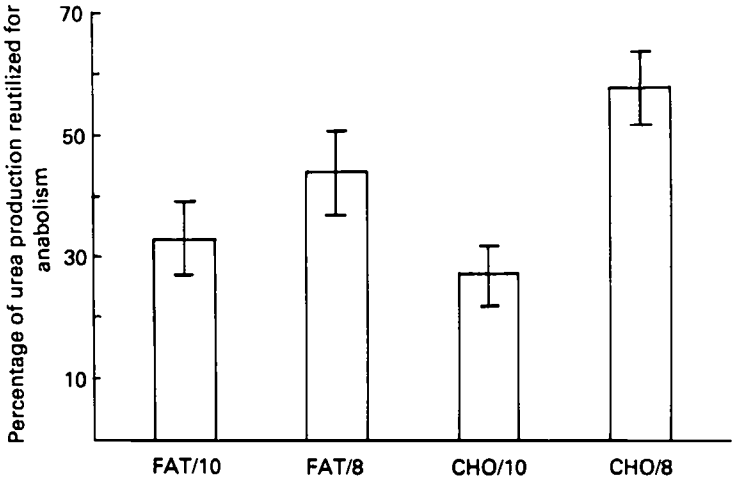

Fig. 2. During rapid catch-up growth in infants recovering from severe undernutrition, the extent to which endogenous urea was made available for anabolic processes was influenced by the dietary intake of protein and energy. Each child received 711 kJ (170 kcal)/kg per d of a formula enriched with either arachis oil (fat) or maize starch (CHO), with protein at either 10 (FAT/10, CHO/10) or 8 (FAT/8, CHO/10)% of gross energy. There was a significant interaction with both the quality of dietary energy and the level of protein. Urea reutilization was enhanced by a low protein, carbohydrate-rich diet (Doherty *et al.* 1989). Values are means with their standard errors represented by vertical bars.

There has been resistance to the idea that N coming from hydrolysed urea might confer a metabolic benefit. In part this reluctance might be attributed to the mistaken assumption that the N would only be made available as ammonia passing up the portal tract. Ammonia presented to the liver in this way is almost invariably converted to urea (Jahoor *et al.* 1988). It is more likely that to be of use the urea-N is fixed by the microflora into protein and amino acids that can then be made potentially available to the host. Although it has been shown that urea-N can be made available in this way as both essential and non-essential amino acids (Tanaka *et al.* 1980), few have considered that this contribution is likely to be of great quantitative significance. However, this belief, based on the assumption that the large bowel is effectively impermeable to amino acids, is probably misplaced. Most of the physiological studies on colonic transport have used a perfused bowel. We have recently demonstrated that the absorptive characteristics of the perfused bowel are very different from the unperfused bowel (Moran *et al.* 1989). Of most importance is the demonstration by Heine *et al.* (1987) that ^{15}N-labelled yeast protein placed in the colon of infants could not be recovered from the effluent, and about 90% was retained within the body, indicating effective absorption and retention of amino acids at the level of the colon.

Glycine: conditionally essential

The available evidence indicates a net output of glycine by the gastrointestinal tract (Elwyn, 1970). The demonstration by Snyderman *et al.* (1962) that it was possible to promote a return of weight gain and N balance by adding glycine to a low protein diet was the first indication that glycine might act as a first limiting nutrient in infants. Our

attention was drawn to glycine when we were carrying out a study using [^{15}N]glycine to measure protein turnover in preterm infants and found that the ^{15}N label did not move to urea in infants on the intakes of human milk containing the lowest level of protein, an observation that has since been confirmed by others (Jackson *et al.* 1981; Catzeflis *et al.* 1985). This observation caused us to speculate that glycine was acting as a conditionally essential amino acid. Yu *et al.* (1985) have demonstrated that in a normal adult man on a low protein diet the endogenous synthesis of glycine may be inadequate to satisfy the normal metabolic demand. Quantitatively the largest demand for glycine is for the synthesis of proteins, including collagen which is particularly rich in glycine—30% of the residues. However, glycine also satisfies a range of other important functions in intermediary metabolism, including the synthesis of nucleotides, porphyrrin and haem, creatinine, bile salts and glutathione.

The demands of both the fetus and infant for glycine seem to be excessive. Widdowson *et al.* (1979) have shown that on a molar basis the accumulation of glycine by the infant is about two to ten times that of any other amino acid. When this is compared with the availability of the amino acid in the milk it is a surprise to find that milk is a particularly poor source of glycine, and can satisfy less than 20% of the demand.

The techniques required to measure *de novo* glycine synthesis in the body are difficult and expensive, and for some time we have been exploring simple non-invasive approaches that might be usefully applied on a wide scale. We have shown that the urinary excretion of 5-oxoproline (5-OP) may provide a useful index of glycine sufficiency (Jackson *et al.* 1987*b*). Under normal circumstances 5-OP is only excreted at low levels in the urine. By creating a drain on the glycine pool by feeding benzoic acid it is possible to provoke an increased excretion of 5-OP in urine. In children recovering from malnutrition, in whom the demands for glycine are high to satisfy the needs for rapid growth, 5-OP excretion is excessive and reverts towards normal when supplementary dietary glycine is given (Persaud *et al.* 1987). During pregnancy, when the fetal demands for glycine exceeds the mother's capacity to form the amino acid, we have found a marked, progressive rise in the excretion of 5-OP as pregnancy progresses (Persaud *et al.* 1989). In the last year we have been following the pattern of excretion of 5-OP in the urine of newborn infants, those born preterm as well as those born at term. In a series of sixty-six preterm infants aged from 25 to 35 weeks post-conceptual age, the urinary excretion of 5-OP, 180 μmol/mmol creatinine, was about twenty times that found in normal adults. At term twenty infants had levels that were lower, 120 μmol/mmol creatinine, but still very much higher than in adults (Persaud & Jackson, unpublished results).

One mechanism whereby xenobiotics are detoxified for excretion is by hepatic conjugation with amino acids such as glycine or cysteine. There are several indications that this process is impaired in the infant compared with the older child or adult. Although this impairment could be accounted for by immaturity of the hepatic enzyme systems responsible for conjugation and excretion, limited availability of glycine has been shown to contribute to a limited ability to clear benzoic acid as hippurate (Vest & Rossier, 1963). Therefore, there are now a number of individual, independent pieces of evidence that would argue strongly in favour of glycine being of limited availability during the early months of life (Snyderman *et al.* 1962; Vest & Rossier, 1963; Widdowson *et al.* 1979; Jackson *et al.* 1981; Persaud *et al.* 1989).

Conclusion

The endogenous synthesis or metabolism of a number of amino acids is modified during the neonatal period. Thus, the enzymes of the trans-sulphuration pathway are

only poorly developed and show very low levels of activity, meaning that cysteine synthesis from methionine is limited (Sturman *et al.* 1970). As a consequence cysteine is conditionally essential during the neonatal period. The requirement for an amino acid is not an absolute value, but has to be related to the metabolic demand for tissue growth and function (Jackson, 1986). This will vary from infant to infant and from time to time. In the face of this varying demand there cannot be an ideal composition for a single formula. Instead nature has contrived a mechanism that is infinitely adaptable by ensuring that the endogenous mechanisms capable of synthesizing amino acids are flexible and capable of responding to a changing pattern of demand with time.

REFERENCES

Casey, C. E. (1989). *Proceedings of the Nutrition Society* **48,** 271–281.

Catzeflis, C., Schutz, Y., Micheli, J.-L., Welsch, C., Arnaud, M. J. & Jequier, E. (1985). *Pediatric Research* **19,** 679–687.

Das, T. K. & Waterlow, J. C. (1974). *British Journal of Nutrition* **32,** 353–373.

Doherty, T., de Benoist, M.-H., Hibbert, J., Persaud, C. & Jackson, A. A. (1989). *Proceedings of the Nutrition Society* **48,** 55A.

Elwyn, D. H. (1970). In *Mammalian Protein Metabolism*, vol. 4, pp. 523–557 [H. N. Munro, editor]. New York: Academic Press.

Food and Agriculture Organization/World Health Organization/United Nations University (1985). *Energy and Protein Requirements: Report of a Joint Expert Consultation. Technical Report Series* no. 724. Geneva: WHO.

Fomon, S. J. & Macy, C. D. (1958). *Pediatrics* **22,** 101–115.

Fomon, S. J. & Matthews, D. E., Bier, D. M., Rogers, R. R., Rebouche, C. J., Edwards, B. B. & Nelson, S. E. (1987). *Journal of Pediatrics* **111,** 221–224.

Harzer, G., Franzke, V. & Bindels, J. G. (1984). *American Journal of Clinical Nutrition* **40,** 303–309.

Heine, W., Tiess, M., Stolpe, H. J. & Wutzke, K. (1984). *Journal of Pediatrics, Gastroenterology and Nutrition* **3,** 709–712.

Heine, W., Tiess, M. & Wutzke, K. D. (1986). *Acta Paediatrica Scandinavica* **75,** 439–443.

Heine, W., Wutzke, K. D., Richter, I., Walther, F. & Plath, C. (1987). *Acta Paediatrica Scandinavica* **76,** 741–744.

Jackson, A. A. (1983). *Lancet* **i,** 1034–1037.

Jackson, A. A. (1986). *Proceedings of the XIIIth International Congress of Nutrition 1985*, pp. 403–409 [T. G. Taylor and N. K. Jenkins, editors]. London: John Libbey & Co.

Jackson, A. A., Badaloo, A. V., Forrester, T., Hibbert, J. M. & Persaud. C. (1987*a*). *British Journal of Nutrition* **58,** 207–214.

Jackson, A. A. & Golden, M. H. N. (1980). *Clinical Science* **58,** 517–522.

Jackson, A. A. & Grimble, R. F. (1989). In *Protein-Calorie Malnutrition: 19th Nestlé Nutrition Workshop* (In the Press).

Jackson, A. A., Landman, J. P., Stevens, M. C. G. & Serjeant, G. R. (1988). *European Journal of Clinical Nutrition* **42,** 491–496.

Jackson, A. A., Persaud. C., Badaloo, A. V. & de Benoist, B. (1987*b*). *Human Nutrition: Clinical Nutrition* **41C,** 263–276.

Jackson, A. A., Shaw, J. C. L., Barber, A. & Golden, M. H. N. (1981). *Pediatric Research* **15,** 1454–1461.

Jahoor, F., Jackson, A. A. & Golden, M. H. N. (1988). *Annals of Nutrition and Metabolism* **32,** 1034–1037.

Kies, C. (1972). *Federation Proceedings* **31,** 1172–1177.

Laidlaw, S. A. & Kopple, J. D. (1987). *American Journal of Clinical Nutrition* **46,** 593–605.

Mehta, A. (1989). *Proceedings of the Nutrition Society* **48,** 257–262.

Moran, B., Persaud, C. & Jackson, A. A. (1989). *Proceedings of the Nutrition Society* **48,** 117A.

Persaud, C., Forrester, T. & Jackson, A. A. (1987). *Proceedings of the Nutrition Society* **46,** 136A.

Persaud, C., McDermott, J., de Benoist, B. & Jackson, A. A. (1989). *British Journal of Obstetrics and Gynaecology* (In the Press).

Picou, D. & Phillips, M. (1972). *American Journal of Clinical Nutrition* **25,** 1261–1266.

Picou, D. & Taylor-Roberts, T. (1969). *Clinical Science* **36,** 283–296.

Rose, W. C. (1957). *Nutrition Abstracts and Reviews* **27**, 631–647.
Slater, J. E. (1961). *British Journal of Nutrition* **15**, 83–97.
Snyderman, S. E., Holt, L. E., Dancis, J., Roitman, E., Boyer, A. & Balis, M. E. (1962). *Journal of Nutrition* **78**, 1–15.
Sturman, J. A., Gaull, G. & Raiha, N. C. R. (1970). *Science* **169**, 74–76.
Tanaka, N., Kubo, K., Shiraki, K., Koishi, H. & Yoshimura, H. (1980). *Journal of Nutritional Science and Vitaminology* **26**, 247–259.
Vest, M. F. & Rossier, R. (1963). *Annals of the New York Academy of Sciences* **111**, 183–198.
Waterlow, J. C. (1967). *Clinical Science* **33**, 507–515.
Waterlow, J. C. (1968). *Lancet* **ii**, 1091–1097.
Waterlow, J. C. (1984). *Quarterly Journal of Experimental Physiology* **69**, 409–438.
Widdowson, E. M., Southgate, D. A. T. & Hey, E. N. (1979). In *Nutrition and Metabolism of the Fetus and Infant*, pp. 169–177 [H. K. A. Visser, editor]. The Hague: Martinus Nijhoff.
Yu, Y. M., Yang, R. D., Matthews, D. E., Wen, Z. M., Burke, J. F., Bier, D. M. & Young, V. R. (1985). *Journal of Nutrition* **115**, 399–410.

Printed in Great Britain