HUMAN AMINO ACID AND PROTEIN REQUIREMENTS: CURRENT DILEMMAS AND UNCERTAINTIES

D. J. MILLWARD, A. A. JACKSON[1], GILLIAN PRICE AND J. P. W. RIVERS

Nutrition Research Unit, London School of Hygiene and Tropical Medicine, 4 St Pancras Way, London NW1 2PE and [1]Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton, SO9 3TU

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INTRODUCTION

Various attempts to estimate protein requirements have been made over the last century, by individuals, by various national committees and by international expert consultations,

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reporting at regular intervals since 1955 (see Munro, 1985). These estimates have varied in both directions with each successive expert consultation. The most recent report (Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU), 1985) considered a great deal of new information before arriving at its recommendations and, given the general advances which have been made in the biological sciences, might be thought to be approaching a high degree of accuracy. This is not believed to be the case. On the contrary, an attempt will be made to show that current understanding of the biological basis of protein requirements is inadequate, and this is why protein and amino acid requirements remain difficult to estimate. The intention in the present paper is to identify what are believed to be outstanding problems with current estimates of requirement, and to broaden the debate by considering whether or not newer biological models for protein and amino acid metabolism could point the way towards solutions to the problems.

CURRENT PROTEIN REQUIREMENT ESTIMATES AND MAJOR ISSUES

The report of FAO/WHO/UNU (1985) does not define or discuss specific requirement models in any detail, but implies a simple model in which the dietary protein requirement is an amount sufficient for the needs of the various components of the body, taking account of the fact that the efficiency of utilization is considerably less than 100%. The needs of the body are defined as maintenance, which is perceived as the replacement of the obligatory nitrogen losses (ONL), with additions for growth, pregnancy, lactation and any nutritional rehabilitation. It is implicitly assumed that the magnitude of the dietary protein requirement, the sum of the biological need and the efficiency of utilization, is an intrinsic and fixed function of individual body-weights within age groups. Thus, for populations, mean values have been defined with estimates of inter-individual variability. These estimates have been transformed into safe allowances, average requirement plus two standard deviations of the estimated variance, assuming that moderate excess of intake is not harmful, and that a recommended dietary allowance should cover the needs of most of the population. Indispensable amino acid (IAA) requirements and an amino acid scoring pattern are defined for all ages, allowing adjustment of the protein intake if the IAA content of the diet is inadequate.

The first question which can be posed about these latest requirement values is: how secure are they? There were in fact two major changes compared with the previous report (Food and Agriculture Organization/World Health Organization (FAO/WHO), 1973).

First, the adult safe protein allowance (0·75 g/kg per d) was increased (by 32%). Whilst there has not been any specific challenge to this new value it is believed that close inspection of the published reports on which it is based reveals a body of data which is markedly disparate, much more so than the current report (FAO/WHO/UNU, 1985) implies, and which by no means inspires confidence in this report's description of adult protein requirements. Whilst the protein requirement values for infants and children were not substantially changed in the report, criticisms have nevertheless been voiced over the recommendations for infants. Thus Beaton & Chery (1988) have suggested that the protein requirement estimates for this group must be overestimates.

Second, the FAO/WHO/UNU (1985) report grasped the nettle of adult IAA requirements, side-stepped in the FAO/WHO (1973) report, accepting that the marked fall with age in IAA requirements must mean that the scoring pattern by which protein quality is assessed for adults will indicate that all diets, even those based on minimally

supplemented cereals, are equally able to satisfy IAA requirements, apart from possible increases to account for low digestibility (Table 1). There is certainly unease about this conclusion. Young & Pellett (1989) have argued that the adult IAA requirement values are in most cases considerably underestimated, going on to propose a new IAA scoring pattern and making a plea to reconvene a United Nations advisory group to consider the problem.

Given that there do seem to be difficulties with accepting current requirement values a further question must be asked: how secure is the methodology employed in their assessment?

Table 1. *Current requirement patterns for critical amino acids in comparison with amino acid composition of practical low-cost diets (mg/g protein)**

	Lysine	Methionine +cystine	Threonine	Tryptophan
Requirement pattern				
Preschool child	58	25	34	11
Schoolchild	44	22	28	9
Adult	16	17	9	5
Diet composition				
Indian diets	34–49	26–36	24–34	6–11
Tunisian diets	33–40	37–38	30–32	11
Brazilian diets	53–56	30–35	37–38	11–12
Guatemalan diets	38–39	28–31	36–37	7–10
Mexican-Indian diets	58	22	52	8
Nigerian diets	39–63	24–34	30–37	11–12

* Table 35 of Food and Agriculture Organization/World Health Organization/United Nations University (1985).

THE METHODOLOGY OF ASSESSMENT OF REQUIREMENTS

In the FAO/WHO/UNU (1985) report the magnitude of the dietary protein requirement was taken as the amount needed for N equilibrium (or to sustain desired growth, pregnancy or lactation), as estimated by N balance studies. The adult IAA requirement values are also based on N balance studies deriving from the FAO/WHO (1973) revision of earlier N balance data (Rose, 1957).

NITROGEN-BALANCE STUDIES AND MAINTENANCE REQUIREMENTS

Short-term balance studies in adults were deemed acceptable if protein was fed at several levels below and above an amount expected to promote N equilibrium (zero balance). The aggregated data provide an estimated mean requirement of 0·63 g highly digestible, good-quality protein/kg. The long-term balances involved studies at a single level of intake (0·57–0·61 g/kg per d, the FAO/WHO (1973) safe level), with one exception at 0·356 g protein/kg per d, an amount equivalent to the ONL. This collection of long-term data was interpreted as establishing that the 1973 *safe level* (0·57 g/kg) was too low, so that with the short-term data a value of 0·605 rounded to 0·6 g/kg per d (96 mg N/kg per d) for the average requirement was settled on.

For older infants and children, a much more limited range of short-term balance studies was considered, but this indicated that the maintenance value was not very different from

that in adults, ranging from 80 to 118 mg N/kg per d. A value of 120 mg N was taken to represent the value for children up to 12 months of age, and intermediate ages (between 1 and 20 years) were then interpolated from this and the adult value (96 mg N/kg).

This mean value implies for adults an overall efficiency of utilization of dietary proteins of 56% in replacing the ONL (54 mg N/kg per d), compared with an assumed efficiency of 77% by the FAO/WHO report (1973). It was said to be higher than the 1973 value because (*a*) earlier studies involved relatively high energy intakes, promoting more positive N balances, (*b*) many of the earlier balance studies did not include enough levels of intake in the region of zero balance, so that the efficiency of utilization was overestimated, and (*c*) the 1973 values allowed 5 mg N/kg for miscellaneous losses, in contrast to the 8 mg/kg assumed by the FAO/WHO/UNU (1985) report.

Whilst there were more balance studies available than previously, the interpretation of the data presented a difficult task. First, there is widespread concern about the validity of balance studies, especially the possibility of systematic overestimation of retention indicated by unrealistic positive balances (Hegsted, 1976). Second, there is difficulty in defining the particular level aimed at of body N (maximum stores, average stores, etc.). This in turn leads on to questions of adaptation and adjustment and arguments that N balance will occur eventually on most intakes after adjustment of body N has occurred. Third, N balance is known to be dependent on other factors such as energy intake, physical activity, stress of any kind and, as discussed later, habitual intakes. Whilst there is discussion of the various factors which influence N balance in the FAO/WHO/UNU (1985) report, no coherent, comprehensive and generally agreed set of criteria for study design was defined or has emerged since. This would have resulted in two specific problems in the preparation of the 1985 report, namely which studies should be selected from the literature as 'representative' studies, and how to decide on an aggregate value.

The problem of selection of 'representative' studies

The first problem is a real one in that inspection of the FAO/WHO/UNU (1985) report shows that by no means all published balance studies were evaluated. It is the case that there is such disparity between the results of different studies that some authors suggest that comparisons between studies cannot be made. As stated in a recent balance study 'because N balances are sensitive to various factors... particularly the level of energy intake and body energy balance, it is not feasible to compare more extensively the present results with those obtained earlier' (Young *et al.* 1984).

The choice of the energy intake at which balance studies should be done is most problematical. There have been several studies of the influence of energy intake on N balance (e.g. Calloway & Spector, 1954), but no generally agreed quantitative relationship has emerged (see Calloway, 1981). The requirement for N equilibrium with egg protein increases markedly as energy intake falls (Kishi *et al.* 1978), so that if a single value is to be chosen judgement must be made about which level of energy intake is appropriate. Energy equilibrium may well be the objective but is not easily measured. Weight maintenance alone is inadequate, since the energy intake for weight maintenance varies with the protein intake.

Recent N-balance studies on Nigerian students (Atinmo *et al.* 1988) demonstrate other poorly understood influences (Fig. 1). The studies were done with intakes ranging from 55 mg N/kg per d to 125 mg N/kg per d (fed for 10 d) and are shown together with the value obtained with a minimum protein diet in a previous study (Atinmo *et al.* 1985). The shape of the response curve varies significantly with the order in which the change of intake occurs. Subjects given a low intake of beef protein (55 mg N/kg per d) achieve a more positive N balance if the low intake is approached with gradual reductions (descending series),

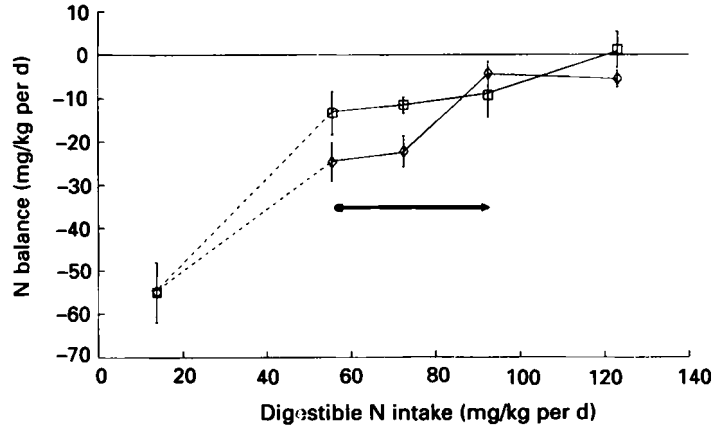

Fig. 1. Influence of experimental design on nitrogen balance response curve. The studies were done with either ascending (◇) or descending (□) changes in the intakes of beef protein (Atinmo *et al.* 1988), and values are shown together with the balance observed with a minimum protein intake in a previous study (Atinmo *et al.* 1985). Data recalculated assuming unmeasured losses of 8 mg N/kg. ↔, Biological values: 0·55 ascending, 0·11 descending.

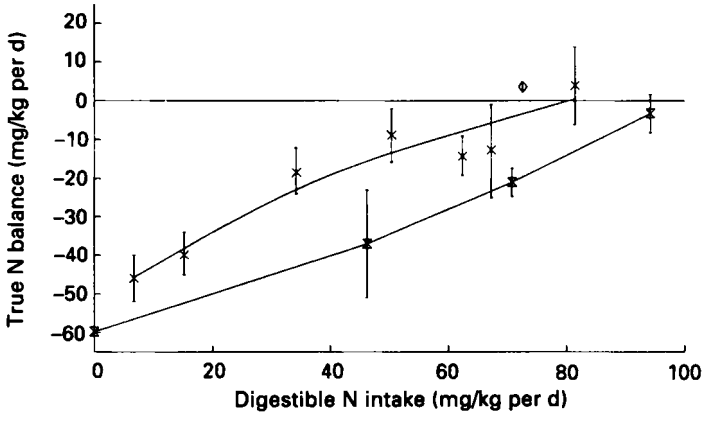

Fig. 2. Unaccountable differences between nitrogen balance trials with egg. Data from Young *et al.* (1973) in USA (×) and Yanez *et al.* (1982) in Chile (⧖). The single value was reported by Nicol & Phillips (1976*a*), in Nigerian farmers (◇). Data recalculated assuming unmeasured losses of 8 mg N/kg.

compared with the more abrupt change from usual intakes to the low intake (ascending series). Why this occurs is not known. Furthermore, whilst a reduction of protein intake below 130 mg N/kg per d induces negative balance, the degree of negative balance does not change substantially down to 55 mg N/kg per d. Such behaviour means that calculations of biological values between intake values of 55 mg N/d and 100 mg N/d result in values of only 11 % for the descending series, compared with 55 % in the ascending group. Clearly, the design of studies does influence the outcome, and this is why most sequential studies in individuals usually involve randomization of the dietary changes. On the basis of achieving balance, these particular studies (carried out with energy intakes of 199 kJ (47·6 kcal)/kg per d) imply an average requirement value of 117 mg N/kg per d (0·73 g protein/kg per d).

An example of the marked differences between studies which can occur for no apparent reason is shown in Fig. 2. One is a series of short-term balance studies with egg in

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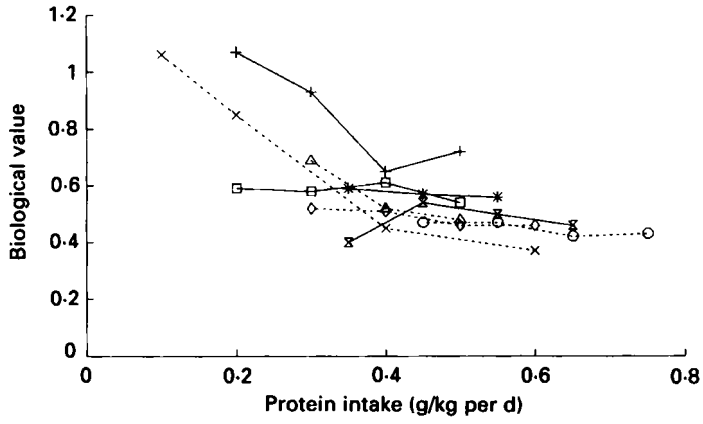

Fig. 3. Biological value of various protein sources determined in adults by nitrogen balance studies. Data from Young *et al.* (1973), egg (a) (+); Huang & Lin (1982), egg (b) (*), mixed diet (b) (○); Young *et al.* (1984), egg (c) (□), soya bean (c) (◇); Inoue *et al.* (1974), gluten (×); Young *et al.* (1975), whole wheat (△); Scrimshaw *et al.* (1983), skim milk (⧖).

Massachusetts Institute of Technology (MIT) students in the USA (Young *et al.* 1973). The other involves young, underprivileged Chilean adults (Yanez *et al.* 1982). Energy intakes were similar, 192–197 kJ (46–47 kcal)/kg per d for most of the MIT students and 207 kJ (49·5 kcal)/kg per d for the Chileans) and diets were randomized in both cases, yet the responses of the two groups are very different. Since the Chilean study involved young, underprivileged adults who weighed less and were possibly habituated to lower protein intakes than the MIT students, the poorer utilization of the egg protein is quite surprising – all the more so if the values reported by Nicol & Phillips (1976*a*) are included for comparison. Their values, widely quoted as evidence of the ability to adapt to low-protein diets (see p. 116), when viewed in the context of the MIT studies, appear unremarkable and within the overall range, but quite different from the Chilean studies. There is no obvious explanation for these disparate results. More importantly there is no explanation why the Chilean study was included as 'representative' in the FAO/WHO/UNU (1985) report, whilst the MIT study, by far the most extensive study in the literature, was not.

The fact that intake *v.* balance curves are complex means that the analysis of individual studies is problematical and can be somewhat arbitrary. Linear regression is used in most cases to define intakes for zero balance (although more complex curves usually describe the data better, e.g. Young *et al.* (1973)). The values computed in this way vary over a wide range (0·49–0·74 g protein/kg per d in the FAO/WHO/UNU (1985) report), and in many studies (e.g. Fig. 1) there is a range of intakes close to maintenance over which there is evidence of what the authors describe as adaptive changes to adjust output to match intake.

These problems with the N-balance technique apply equally to the determination of protein quality, especially biological value, which is central to the debate about the adult IAA requirements. In contrast to studies in laboratory animals – in which it is easy to demonstrate differences in the biological value of proteins in relation to the amino acid content and chemical score – in humans it is extraordinarily difficult.

Whilst differences between protein sources have been reported in N-balance studies in young adults (e.g. biological values of 0·27 for wheat compared with 0·51 for beef (Young *et al.* 1975)), when the calculated biological values of several proteins measured in separate studies are examined together (Fig. 3), the differences between wheat and wheat gluten, and

other proteins and mixed diets are much less apparent. This is because of the lack of reproducibility between studies with the same protein. Within individual studies, inter-individual variability is very marked, with biological values often associated with coefficients of variation (CV) of 15–20% (e.g. Young *et al.* 1975), and even 50% (Young *et al.* 1984). In a study of the effect of lysine supplementation of wheat gluten (Scrimshaw *et al.* 1973), widely quoted as indicating protein quality effects in adults, the magnitude of the response (a 2·9–7·7% fall in urea excretion) was such that with the CV of mean urea excretions ranging from 11 to 36% the response was not statistically significant. The combination of within-study variability and poor reproducibility between trials means that statistical analysis of the data is almost impossible. Rand *et al.* (1981) calculated the size of the experimental groups necessary to provide significant differences in biological value between proteins with the variability observed in the balance trials done at MIT (Young *et al.* 1973). They showed that unless biological value differs by the order of 50%, significant differences cannot be demonstrated without unrealistic numbers of subjects (e.g. twenty-one subjects needed to discriminate between proteins which differ in their biological value by 15% with a beta error of 50%). To reduce the error to a more acceptable 10%, fifty-four subjects would be needed, and such trials are not feasible.

To some extent, then, it might be concluded that the FAO/WHO/UNU (1985) report is correct in arguing that biological value is not an important consideration in adults, since N-balance studies are such a blunt tool that differences cannot be consistently measured. What is clear is that protein utilization in humans is much more dependent on complex extrinsic factors which influence the behaviour of the organism, rather than factors such as amino acid content which determine the intrinsic properties of the protein.

The problem of data aggregation

After the selection and tabulation of representative data the second problem which arises is how to evaluate it and derive a 'requirement' value. The fact that this is by no means straightforward is illustrated by the recent arguments of Beaton & Chery (1988) who, in effect, questioned the derivation of the requirement value for the 3–4-month-old infant after indentifying a logical flaw in the FAO/WHO/UNU (1985) report. Thus, the factorially derived average protein requirement for the 3–4-month-old infant (1·47 g protein/kg per d) was validated by showing it to be very similar to average breast-milk protein intake (1·49 g protein/kg per d) for this age-group. Beaton & Chery (1988) argued that since in breast-fed infants prevalence rates of inadequate protein intakes are assumed to be negligible, this implies the intake to be an indication of the safe level (2 SD above average requirements) rather than an average requirement. They conclude that protein requirements for infants aged 3–4 months must be substantially lower, and were able to construct an alternative lower requirement value. They did this by dispensing with the 50% addition (for day-to-day variability) to the growth component, and by using the median maintenance value from the quoted short-term balance studies in the FAO/WHO/UNU (1985) report (i.e. 100 mg N/kg per d from values which ranged from 80 to 118 mg N/kg per d). This is quite justifiable given that there is no agreed framework or consistency in aggregating data from separate studies. Thus in the 1985 report the derived value of 120 mg N/kg per d was rounded up from the highest value, in marked contrast to the selection of data from the adult balance studies in which an average value was used.

As far as the magnitude of the protein requirements of infants is concerned, whilst the statistical arguments of Beaton & Chery (1988) are indisputable, the problem may partly arise because the comparison between estimated protein requirements, originating from N-balance studies with formula or mixed feeds, and breast-milk intakes in the FAO/WHO/UNU (1985) report, was misplaced (Millward, 1989*a*). Protein intakes of breast-

fed infants would only be relevant to the requirements for weaned children if the utilization of breast milk is similar to that of formula feeds (69–74%). This is the age of most rapid growth, and the N in breast milk is utilized with very high efficiency; an indication of the special properties and qualities of breast milk, which are poorly understood (Jackson, 1989). This higher efficiency of utilization of breast milk would explain the discrepancy, at least in part. Clearly, the extent to which N in breast milk can be utilized becomes a crucial issue in the debate.

As to the general problem of data aggregation, the FAO/WHO/UNU (1985) report is unsatisfactory because of its inconsistency and it seems questionable whether or not either of the adopted approaches (selection of the highest value as with infants, or of a simple average of the zero intercept values from the selected studies) is an adequate aggregate descriptor of the various studies. Such an approach obscures what are obvious complex responses of the organism which, if better understood, should make it possible to construct a more comprehensive model for requirements.

BIOLOGICAL IMPLICATIONS OF NITROGEN-BALANCE DATA

It is clear that the behaviour of the system does not conform to a simple model. There is a major problem, however, because of different interpretations of the significance of the disparate behaviour. In the FAO/WHO/UNU (1985) report it is implied that studies which yield data differing substantially from 'representative' data have involved inappropriate extrinsic factors (energy intake, previous diet, etc.). In contrast it might be argued that the 'non-representative' behaviour indicates the failure of the FAO/WHO/UNU (1985) requirements model to allow for adaptation. What follows is an attempt to examine the observed responses separating 'non-representative', or adaptive, from conventional behaviour.

'NON-REPRESENTATIVE' BEHAVIOUR: ADAPTATION

The question of adaptation to low intakes raises several difficult conceptual problems which are the subject of much debate (see Sukhatme & Margen, 1978; Blaxter & Waterlow, 1985) and are in no way resolved. In the context of the existing model for protein requirements in which dietary protein replaces the ONL with a particular efficiency, adaptation could involve changes in the ONL or in the efficiency of utilization, or both. In fact, there is little evidence to suggest that the magnitude of the ONL varies between comparable age groups from different parts of the world (Uauy *et al.* 1982; FAO/WHO/UNU, 1985). Thus, adaptation will involve changes in the efficiency of utilization. However, even when restricted in this way, the concept of adaptation is still difficult. Thus, Atinmo *et al.* (1988) interpret their findings (Fig. 1) as indicating that adaptive mechanisms are occurring on low protein intakes, even though the consequence of these adaptations is to reduce the apparent biological value of beef in the sub-maintenance range to very low levels.

However, the most frequently quoted and discussed examples of adaptation involve short- and long-term balance studies which indicate increased efficiency of protein utilization. Nicol & Phillips (1976*a*, *b*) reported that in Nigerian low-income farmers egg or rice protein achieved biological values of 93 and 88% respectively at intake levels resulting in apparent N equilibrium (e.g. see Fig. 2). Durkin *et al.* (1981; quoted in FAO/WHO/UNU, 1985) reported studies from Berkeley in which men achieved balance in a statistical sense after 77 d on an intake equal to the ONL (0·36 g egg protein/kg), implying 100% efficiency of utilization.

The evidence for adaptation in these studies is not just the very high efficiency of

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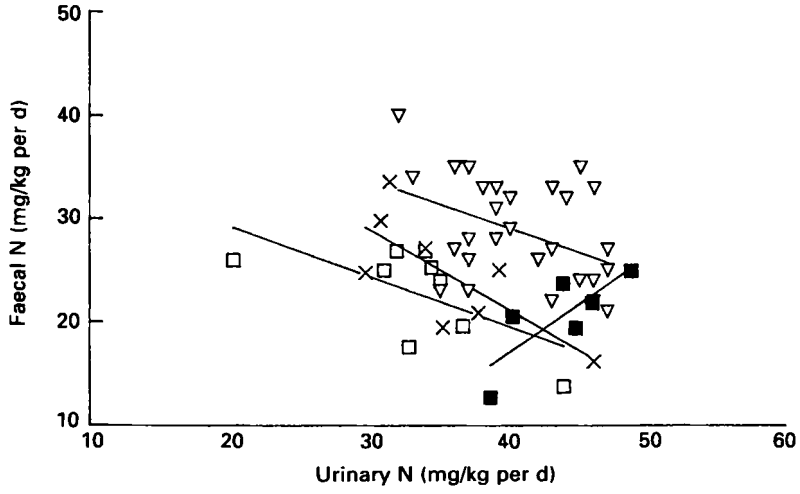

Fig. 4. Correlations between urinary and faecal nitrogen excretion. Inverse correlations are observed in Nigerian farmers consuming a minimum protein diet (MPD) (a) (□), egg (a) (×) or rice (a) (▽) (Nicol & Phillips 1976*a*, *b*). In contrast, in Chilean underprivileged adults, MPD (b) (■) the more usual positive correlation was observed (Yanez *et al.* 1982).

utilization, but additional unique behavioural responses. In the Nigerian studies (Nicol & Phillips, 1976*a*, *b*) this was an inverse correlation between urinary and faecal N excretion (see Fig. 4), not observed when these subjects were fed on a surfeit of protein (Nicol & Phillips, 1976*b*), or in Chilean underprivileged adults fed on a minimum-protein diet (Yanez *et al.* 1982); it has been reported in Indian men (Gopalan & Narasinga Rao, 1966) and in Indian children fed on wheat protein (Reddy, 1971). Adaptation is indicated in these children since there was no response to the lysine supplement in contrast to previous studies (Graham *et al.* 1969).

No explanation for these responses was offered by the authors and, whilst the responses appear rare (they are not observed in Chilean urban poor adults; Yanez *et al.* 1982), there is no reason to question their validity.

The behavioural response observed in the long-term balance studies at Berkeley (Durkin *et al.* 1981) is a non-random pattern in the daily N-balance data (i.e. auto-correlations with exponential decay implying a regulatory mechanism which adjusts daily N balance over a period of several days). The authors argue that the data support the hypothesis of Sukhatme & Margen (1978) that regulatory homoeostatic or adaptive mechanisms exist which adjust output to balance intake and limit extent of any loss (or gain) of body protein. This is an alternative model defining the requirement as a range of intakes, between upper and lower limits, over which equilibrium can occur. In contrast the conventional model is based on an intrinsic requirement which is a fixed function of body-weight.

To date these studies are unique. Rand *et al.* (1985) were unable to identify auto-correlations in any of the long-term balance studies at MIT (Young *et al.* 1973) after correcting for long-term trends. The Berkeley studies (Durkin *et al.* 1981) also differed from the MIT studies because they lacked any identifiable pathophysiology linked to the low intake. At MIT, with intakes of 0·57 g/kg, Garza *et al.* (1977) observed increased serum transaminase levels which they interpreted as pathophysiology.

Why such differences should exist between the studies is not obvious. Jeejeebhoy (1986) argues that comparisons may not be valid since the studies of Durkin *et al.* (1981) were

performed at a lower level of intake, where adaptive mechanisms might be expected to be observed. However, the Sukhatme & Margen (1978) hypothesis predicts adaptive responses (auto-correlations of N balance independent of long-term trends) at the intakes of the MIT studies (Young *et al.* 1973), so that these studies are contrary to such a hypothesis. Nevertheless, it seems that although such studies raise difficult practical problems in terms of decisions about requirement recommendations, they are most important in terms of the search for a more comprehensive model to explain the biological behaviour of the organism.

Adaptive mechanisms: colonic nitrogen metabolism

It might be concluded from the previous discussion that there are several aspects of the behaviour of the organism which need to be explained. First are the responses associated with the high efficiency of protein utilization reported for the Nigerian farmers (Nicol & Phillips, 1976*a*, *b*), in the Berkeley long-term balances (Durkin *et al.* 1981), and possibly in infants fed on breast milk (Millward, 1989*a*). Second is the statistical behaviour of N balance observed at Berkeley but not by Young *et al.* (1973) at MIT. To our knowledge there is as yet no indication of mechanisms for this latter phenomenon. However, the data obtained for Nigerian farmers (Nicol & Phillips, 1976*a*, *b*) shown in Fig. 4 suggest the activity of one potentially important adaptive mechanism.

The high efficiency of protein utilization and the inverse relationship between urinary and faecal N excretion could be linked through urea re-utilization in the lower gut. Increased faecal N loss and associated limitation of urinary N loss suggest urea hydrolysis and incorporation as amino acids into microfloral protein which was then degraded and recycled into the body, increasing conservation of body protein. Although such a behavioural response was deemed unlikely in the FAO/WHO/UNU (1985) report, there is evidence that it can occur.

Gastrointestinal urea hydrolysis is a function of microflora of the lower gastrointestinal tract. Their primary metabolic activity is determined by the energy available to them, mainly as non-digestible carbohydrate. Hence, in young children recovering from malnutrition, urea recycling is greater on a diet enriched with maize starch than with arachis oil (Doherty *et al.* 1989). The extent of recycling varies in a complex way with protein intake since it 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, it has been suggested that the switch to increased urea hydrolysis may represent one reasonably sensitive index of the efficiency of a particular dietary protein. Diarrhoeal disease undoubtedly disturbs the delicate balance of this ecosystem, and may be of particular importance in the aetiology of malnutrition and growth failure in childhood (Jackson & Grimble, 1989).

Resistance to the idea that N coming from hydrolysed urea can confer metabolic benefit originates in part from the mistaken assumption that the N would only be made available as ammonia which would pass via the portal tract to the liver to be converted to urea (Nissim *et al.* 1981; Jahoor *et al.* 1988). However, to be of use in terms of protein conservation, the urea-N would need to be fixed by the microflora into protein and amino acids which 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 IAA and dispensable amino acids (Tanaka *et al.* 1980), few have considered that this contribution is likely to be of great quantitative significance. However, this belief is based on the misplaced assumption that the large bowel is effectively impermeable to amino acids. Most of the physiological studies on colonic transport have used a cleansed bowel which has absorptive characteristics very different from the unprepared 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.

Urea hydrolysis and re-incorporation into amino acids would explain the results of the Nicol & Phillips (1976*a*, *b*) studies by allowing the organism a second chance to make use of dietary protein after amino acids had been oxidized and the N converted to urea. Such a mechanism would also help to account for the extraordinary efficiency with which infants utilize human milk, already referred to. In mature milk, up to 25% of the total N is accounted for by non-protein compounds of which the largest single component, up to 60%, is urea-N (Harzer *et al.* 1984). Isotopic studies show that following the ingestion of ^{15}N-labelled urea, the losses in stool are very small (< 2% as bacterial protein; Heine *et al.* 1984, 1986), with a considerable fraction of N retained. Although the relative and absolute amounts appear to vary between studies, the findings would suggest that about 40% of an oral dose of urea is retained within the body (Jackson *et al.* 1988), demonstrating that urea hydrolysis can represent a substantial contribution to the N economy of the body (Doherty *et al.* 1989).

Whether or not such activities in the lower gut account for the high efficiency of protein utilization in the Berkeley studies of Durkin *et al.* (1981) is not known, since the authors do not report specifically on the relationship between urinary and faecal N excretion. However, they do report, in one subject, increased faecal N with time and significant auto-correlation in N balance but not with urinary N excretion, which they interpret as suggesting the involvement of the gut in some undefined way.

'REPRESENTATIVE' BEHAVIOUR: INADEQUACY OF CURRENT MODELS

The subjects of colonic N metabolism, adaptation and alternative models of the behaviour system are issues about which there is fierce controversy. However, the FAO/WHO/UNU (1985) requirements model does not allow adequate explanation of what is deemed to be 'representative' behaviour.

Low biological value of high-quality proteins

The inefficiency of utilization of high-quality proteins observed in human studies has never, to our knowledge, been satisfactorily explained. Munro (1985) has referred to it as the 'non-linear response', on the grounds that it results from curvature of the N balance *v.* intake plot as equilibrium is approached. However, from what we have already seen this may not be the case. It is apparent that in some cases in adults a poor biological value is observed throughout the sub-maintenance range, as with the studies of Yanez *et al.* (1982) (Fig. 2). Furthermore, the data reported in FAO/WHO/UNU (1985) on short-term balances in young children showed that reference proteins with scores approaching 100% have efficiencies of utilization of only 69–74% (i.e. retention *v.* intake slopes of 0·69–0·74), which must be maintained throughout the sub-maintenance range, given that the ONL predicted from the intakes for maintenance and from these slopes are quite similar to the measured values (see Millward & Rivers, 1988).

It is current practice that when safe protein requirements are corrected for proteins with varying scores, the scores should predict not the absolute utilization of protein but the relative utilization compared with a reference protein. This implies two separate, independent and additive factors influencing protein utilization: a 'primary' inefficiency which always occurs (although to a very variable extent), and another inefficiency reflecting protein quality. Existing models of protein utilization do not allow for this. The 'primary'

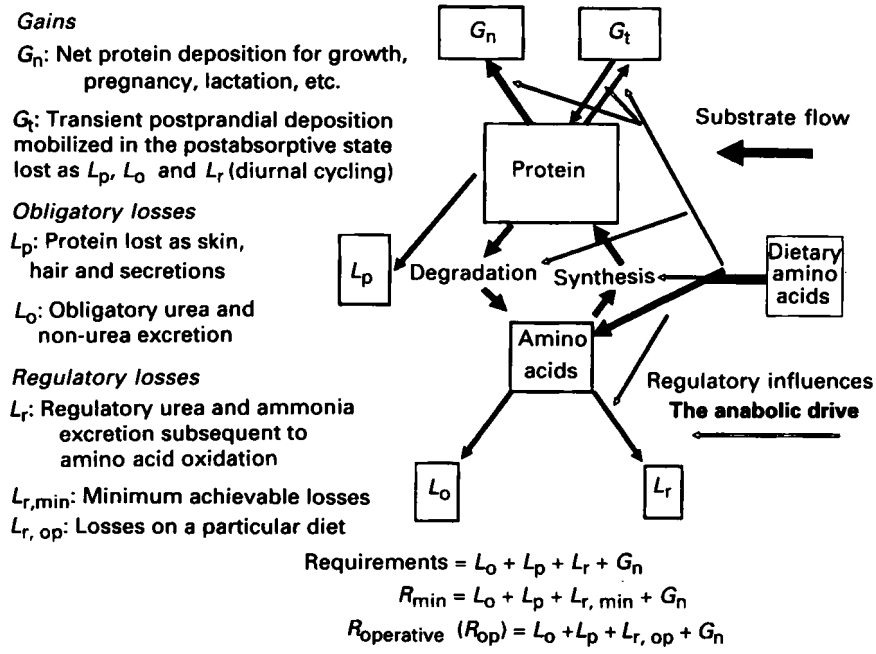

Fig. 5. Model for amino acid requirements. Dietary amino acids are shown as providing substrates for utilization in the processes of protein turnover, growth and diurnal cycling, as well as obligatory and regulatory losses. Obligatory losses are assumed to be mainly intrinsic, a function of the organism. Regulatory losses, growth and diurnal cycling are assumed to be mainly extrinsic, a function of external (mainly dietary) influences. Dietary amino acids also exert a regulatory influence on several of these pathways of utilization, the anabolic drive.

inefficiency of utilization of high-quality proteins means in effect that at equilibrium a large proportion of the amino acid intake is oxidized, serving no apparent purpose (see Millward & Rivers, 1988).

For these reasons Millward & Rivers (1988) have developed a new, more suitable, metabolic model, within which IAA requirements can be better defined and the factors which influence the requirement identified and assessed.

A MODEL FOR AMINO ACID AND PROTEIN REQUIREMENTS

The main novel feature of the model (Fig. 5; see Millward & Rivers, 1988, 1989), is that dietary amino acids are shown as serving two different functions. Dietary amino acids entering the free amino acid pool serve as substrates and are metabolized in a variety of pathways, resulting in either gains or losses by or from the organism. Oxidative losses of amino acids occur during feeding because they are consumed at a rate which is usually in excess of the rate at which net protein synthesis can occur and because the organism does not accumulate IAA, maintaining very small tissue-free IAA pools. One part of the requirement for amino acids will reflect the net extent of these various gains and losses.

Dietary amino acids also exert a regulatory influence on many of the individual pathways involved in their utilization, collectively defined as the anabolic drive. These regulatory influences, primarily aimed at the stimulation of growth but also including stimulation of oxidative losses, may be transient, i.e. exerted by the amino acids for short periods after

feeding, before their oxidation. Their importance in the context of any model for amino acid requirements is that if such regulatory influences require intakes and consequent tissue concentrations of amino acids in excess of the capacity of the identifiable pathways of utilization, then this 'excess' intake should be considered to be part of the requirement for amino acids, even though it is oxidized.

MODEL IMPLICATIONS AND EXPERIMENTAL EVIDENCE

Transient protein gain

The main consequences of the phenomenon (diurnal cycling) are: (*a*) there will be a relationship between transient protein gain (G_t) and regulatory losses (L_r) and hence the amino acid requirement, since the amplitude of diurnal cycling will determine post-absorptive L_r, which in turn will influence the IAA requirements; (*b*) the amplitude of G_t could also influence the pattern of the IAA requirement because G_t is tissue protein containing high concentrations of IAA.

The extent of diurnal cycling is assumed to be under nutritional influence, particularly of dietary protein, and to be related to the rate of protein turnover, and to be, therefore, a target for the anabolic drive.

Regulatory losses

L_r includes all those processes which degrade amino acids and is the determinant of what would currently be judged to be the apparent inefficiency of protein utilization. Because there is close regulation of the pool size of most of the IAA at very low concentrations ($< 0{\cdot}2$ mM for many of them, in marked contrast to the dispensable amino acids), L_r must vary with intake. An example of this regulatory response to intake is provided by the branched-chain α-keto acid dehydrogenase (*EC* 1.2.4.4), which is activated in response to dietary protein (Beggs *et al.* 1986), and which will increase oxidation of all three branched-chain amino acids. This is particularly important in the context of balance studies with purified amino acid mixtures. It would appear to be very difficult for the organism to adapt to low intakes of leucine by reducing oxidative capacity if the diet supplies excess isoleucine and valine. Studies of leucine balance in adults fed on an amino acid mixture modelled on egg with graded reductions of leucine (Meguid *et al.* 1986) indicate that the subjects go into negative leucine (and consequently negative protein) balance at leucine intakes below 62 mg/kg per d (see Millward & Rivers, 1988). This is much higher than the intakes previously reported by Rose (1957) as necessary to maintain N balance when fed with low levels of the other IAA and excess dispensable N.

Millward & Rivers (1988) examined the magnitude of the current adult IAA requirement values by comparing them with calculated values of the obligatory oxidative losses (OOL), the rates at which amino acid liberated from tissue proteins would be oxidized to give rise to the ONL (see Fig. 6). By making this comparison we could identify the rate-limiting amino acid which 'drives' the ONL, i.e. the one released from body protein at a rate exactly equal to its removal in some non-protein biosynthetic pathway (L_o). For this amino acid the OOL could be close to the minimum L_r ($L_{r,min}$). All other non-limiting IAA would be released at rates greater than their L_o values resulting in positive values for L_r. It is apparent from Fig. 6 that the current requirement value for the sulphur amino acids is very similar to the OOL, tending to confirm that this value is close to the minimum requirement value and that the ONL occur at a rate determined by the utilization of S-amino acids. The current requirement values were obtained under dietary conditions (excessive dispensable N and minimum intakes of IAA) where L_r was close to $L_{r,min}$ (Millward & Rivers, 1988). However, when high-quality protein such as egg is fed L_r rises for all amino acids so that

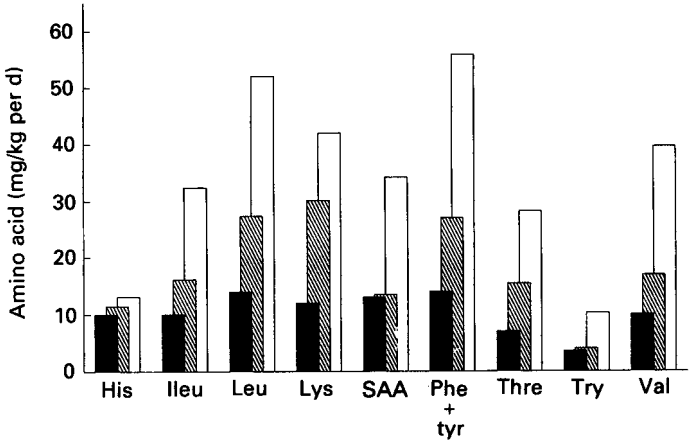

Fig. 6. Current adult requirement values (■), rates of obligatory oxidative loss (▨), and intakes of indispensable amino acids (IAA) provided by current requirement level for egg protein (0·6 g; □). Current requirement values are similar to rates of obligatory oxidative loss for the sulphur amino acids (SAA), but lower for all other amino acids, suggesting that current requirement values are close to the minimum requirement (R_{min}) and that the obligatory losses occur at a rate dictated by the requirement for SAA. The intakes of IAA which satisfy the protein requirement level, R_{op}, shown for egg, are high due to the stimulation of regulatory losses (L_r) by feeding and will vary with the protein. The operative requirement (R_{op}) values which exert sufficient anabolic drive are currently unknown and cannot be predicted. For details of R_{min}, R_{op} and L_r, see Fig. 5.

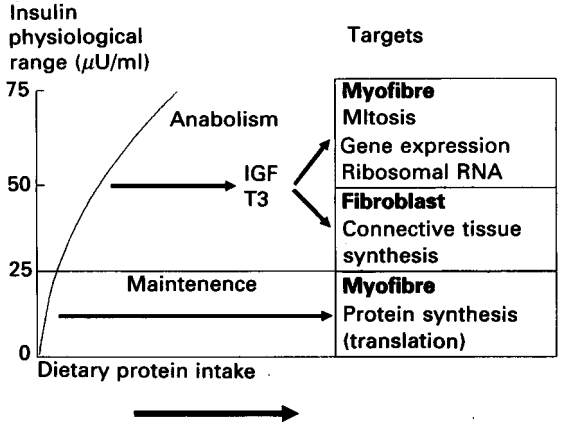

Fig. 7. Potential role of insulin in the anabolic drive. Judging by the relationship between dietary protein intake, plasma insulin, concentrations of other anabolic hormones, and tissue responses (Jepson *et al.* 1988; Millward & Rivers, 1989), participation of insulin in the anabolic drive is concentration-dependent being permissive at low concentrations, and exerting indirect anabolic influence through its induction of other growth factors (insulin-like growth factors and triiodothyronine) at higher concentrations.

the operative requirement (Fig. 5) is higher than that observed on the artificial diets used in the original balance studies from which current requirements are derived.

The potential value of regulatory losses: the anabolic drive

The anabolic drive (Fig. 5) is the regulatory influence of dietary amino acids on protein synthesis, degradation, G_t net protein gain (G_n) and L_r, and includes regulation of growth

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of bone and all other tissues. Some of the hormonal aspects of the anabolic drive of dietary protein on growth and protein metabolism, as observed in animal studies, have recently been reviewed elsewhere (Millward, 1989*b*; Millward & Rivers, 1989). These studies suggest that insulin could have a key role to play in mediating the anabolic drive of dietary protein through its dual role of maintaining protein synthesis in tissues such as muscle and in regulating levels of the other anabolic hormones (Fig. 7). What is most important is that, far from L_r constituting an inefficiency of utilization of dietary protein, such oxidative losses are associated with an important transient regulatory influence exerted by dietary IAA on growth, protein turnover and maintenance of organ function before they are oxidized.

The relationship between the extent of the anabolic drive and L_r is undocumented but, given the known influence of dietary protein on growth (e.g. Jepson *et al.* 1988), it would appear that maximal anabolic drive is associated with relatively high intakes of IAA which will increase postprandial L_r, the rate of protein turnover, the amplitude of diurnal cycling and post-absorptive L_r. This constitutes another reason why oxidative losses should be increased in association with the anabolic drive.

IMPLICATIONS OF THE MODEL FOR PROTEIN REQUIREMENTS

The implications of this model for IAA requirements are twofold. First there is an intrinsic component of the requirement, R_{min}, a value obtainable under artificial conditions when oxidative losses are minimal ($L_{r,min}$), and an extrinsic component of the requirement involving $L_{r,op}$, determined in a complex way in response to both acute and chronic dietary influences. The extent of this complexity is evident by our current failure to explain the wide range of N balance *v.* N intake curves discussed previously. Thus, IAA and hence protein requirements can only be unambiguously defined in terms of R_{min}.

Second, because of the anabolic drive $L_{r,op}$ is associated with the crucial component of the requirement serving a needed regulatory function, rather than constituting just an inefficiency of utilization. An operative requirement of practical value, R_{op}, will only be definable when the value to the organism of the anabolic drive associated with the particular amino acid intake and associated $L_{r,op}$ can be assessed. Hormone levels might constitute acute indicators, with height growth or lean tissue deposition in the longer term.

Studies with both preterm infants (McIntosh *et al.* 1977) and older children (Fomon *et al.* 1977) fed on cow's-milk protein, have shown that markedly increasing protein intakes above usual intakes of breast-fed infants increase longitudinal growth. This suggests that the anabolic drive on bone growth may be inadequate on breast milk or on currently recommended intakes and that higher protein requirements are indicated, an opposite conclusion to Beaton & Chery's (1988) claim that protein requirements for infants are too high.

In adults, if the anabolic drive was extended to maintenance of organ mass and function, more possibilities for appropriate descriptors emerged. Thus, whether the low-protein intakes in the Nigerian farmers (Nicol & Phillips, 1976*a*, *b*) or in the Berkeley (Durkin *et al.* 1981) long-term studies maintained an adequate anabolic drive as well as N balance could only be assessed in terms of functional tests. These were not reported in the Nigerian studies, although the level of body protein at which they were apparently equilibrated was low, as indicated by their marked weight gains and positive N balance when given a large protein supplement (Nicol & Phillips, 1976*b*). In the Berkeley studies (Durkin *et al.* 1981) there were no signs of pathophysiology as was apparent in the MIT studies on a higher intake (Garza *et al.* 1977). All this serves to indicate the need to evaluate better the functional responses to different levels of intake.

SCORING PATTERNS AND THE INDISPENSABLE AMINO ACID REQUIREMENT

AMINO ACID DISPENSABILITY

There are several important aspects of this issue which challenge the adequacy of current concepts. First 'dispensable' N might be limiting for normal growth. Snyderman *et al.* (1962) showed that when protein intake was reduced until N balance and weight gain could no longer be sustained, positive balance was restored by the addition of dispensable N (urea or glycine). The most effective combination of dispensable N is glycine with either glutamic acid or an ammonium salt (Kies, 1972).

Second, the classical perception of dispensability is too narrow (Jackson, 1983; Laidlaw & Kopple, 1987). The division between IAA and dispensable amino acids presumes that dispensability is conferred by the carbon skeleton (Rose, 1957). With ^{15}N it has been shown that there is specific channelling of amino groups between amino acids (Jackson & Golden, 1980; Jahoor *et al.* 1988). Thus, the relative availability and dispensability of the amino group can be considered (Table 2). On this basis, only lysine and threonine can be considered to be absolutely indispensable, and only alanine, glutamate and aspartate, which are formed by transamination from readily available intermediates, dispensable. All other dispensable amino acids are conditionally indispensable, being derived from IAA or exhibiting occasions in which the demand for them exceeds the capacity for their synthesis (Laidlaw & Kopple, 1987). Thus, leucine, isoleucine, valine, tryptophan, phenylalanine, methionine, threonine and lysine are indispensable; glutamine, proline, histidine, arginine, tyrosine, taurine, glycine and serine are conditionally indispensable; and glutamate, alanine and aspartate are dispensable. Two particularly interesting conditionally IAA are glycine and glutamine.

Glycine

As indicated, Snyderman *et al.* (1962) demonstrated that glycine added to a low-protein diet promoted weight gain and N balance and suggested that glycine might act as a first limiting nutrient in infants. Also, during [^{15}N]glycine studies with preterm infants there was virtually no ^{15}N label transferred to urea when the infants were fed on human milk, which contains low levels of protein (Jackson *et al.* 1981; Pencharz *et al.* 1983; Catzeflis *et al.* 1985). This may partly reflect dilution of ^{15}N-labelled urea by any unlabelled dietary urea which is hydrolysed and recycled to urea by the mechanisms discussed previously. Nevertheless, in the fetus and infant, because glycine accounts for 30% of the residues of collagen, and accumulates at about two to ten times the rate of any other amino acid (Widdowson *et al.* 1979), the demand for glycine is excessive. On the basis of such needs, milk is a particularly poor source of glycine, satisfying less than 20% of the demand.

The limited availability of glycine in the developing fetus and in infants and children can be demonstrated by the urinary excretion of 5-oxoproline (5-OP; see Jackson *et al.* 1987). Under normal circumstances 5-OP, which is formed during the γ-glutamyl amino acid transport cycle and which requires glycine to be resynthesized into glutathione, is usually only excreted at low levels in the urine. However, when there is a drain on the glycine pool (for example, by feeding benzoic acid), there is increased urinary excretion. In children recovering from malnutrition, 5-OP excretion is excessive and reverts towards normal when supplementary dietary glycine is given (Persaud *et al.* 1987). There is a marked, progressive rise in the excretion of 5-OP during pregnancy (Persuad *et al.* 1989). 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

Table 2. *Classification of amino acids according to dispensability of carbon skeleton and amino group*

(As the movement of amino groups between amino acids is not free, both the C skeleton and the amino group can confer indispensability, giving rise to a classification with four categories of amino acids (Jackson, 1983))

	C Skeleton	
Amino group	Indispensable	Dispensable
Indispensable	Lysine Threonine	Serine Glycine Cysteine
Dispensable	BCAA Tryptophan Phenylalanine Methionine	Glutamate Alanine Aspartate

BCAA, branched-chain amino acids.

term, twenty infants had levels that were somewhat lower, 120 μmol/mmol creatinine, but still very much higher than in adults (C. Persaud & A. A. Jackson, unpublished results). In addition, limited availability of glycine has been shown to contribute to a limited ability to clear benzoic acid as hippurate (Vest & Rossier, 1963). Taken together, these findings lend strong support to the proposition that glycine is of limited availability during the early months of life and could be a rate-limiting amino acid.

However, glycine also satisfies a range of other important functions in intermediary metabolism, including the synthesis of nucleotides, porphyrin and haem, creatinine, bile salts and glutathione. Yu *et al.* (1985) have demonstrated that in normal adult men on a low-protein diet, the endogenous synthesis of glycine may be inadequate to satisfy the normal metabolic demand.

Glutamine

There are two aspects of glutamine metabolism which are relevant to this discussion. First, there is increasing evidence to suggest that glutamine is an important fuel for replicating cells, and that in patients on parenteral nutrition the ability of the organism to provide glutamine may be limited. Glutamine is a favoured fuel for intestinal epithelial cells (Souba *et al.* 1985) and activated lymphocytes and fibroblasts (Newsholme *et al.* 1985), providing N for purine and pyrimidine synthesis as well as substrate for the Krebs cycle and gluconeogenesis. There is concern that during parenteral nutrition the provision of glutamine to the intestine may be compromised. Second, it appears to have an important regulatory role in protein balance in cells, particularly muscle. There is a striking direct correlation between glutamine concentration and the rate of protein synthesis in rat skeletal muscle in vivo (Jepson *et al.* 1988). Glutamine infusions can reduce the post-operative efflux of amino acids for dog hind limb (Kapadia *et al.* 1985) and can directly stimulate protein synthesis (MacLennon *et al.* 1987) and inhibit protein degradation (MacLennon *et al.* 1988) in muscle.

Muscle glutamine is a major component of the labile N pool which can participate in acute changes in N balance in the whole body. The concentration of glutamine in human skeletal muscle is < 20 mM (e.g. Millward *et al.* 1982), equivalent to more than 15 g N in an adult, and the size of this pool is very sensitive to insults which influence whole-body N

balance, e.g. surgery (Vinnars *et al.* 1975), sepsis (Askanasi *et al.* 1980), fasting and diabetes (Furst *et al.* 1982), exercise (Rennie *et al.* 1981) and glucocorticoids (Muhlbacher *et al.* 1984). Maintenance of the high concentration of glutamine in muscle appears to involve a highly regulated unique transporter (Rennie *et al.* 1986). The extent to which acute changes in muscle glutamine can occur which might influence N balance can be demonstrated from exercise studies (Millward *et al.* 1982; Rennie *et al.* 1981). During a 3·75 h treadmill exercise at 50% V_{max} muscle glutamine concentration fell from 21·6 to 14·3 mM, equivalent to a loss of 5·72 g N during the exercise. Thus, this loss of muscle glutamine could account for the entire negative N balance observed in the study (increased urinary excretion of 4·97 g N).

We would interpret these results as indicating that the combination of its unique regulatory influence on protein metabolism, its metabolic role as a cellular fuel, and as a potential source of ammonia to be drawn on during periods of disturbance of acid–base balance (Souba *et al.* 1985), coupled with the characteristics of the muscle glutamine transporter which regulates the muscle glutamine pool, enable it to serve an important homoeostatic role in the organism, acting as a labile store of N which is mobilized in times of stress. Against this background it would appear sensible to include glutamine in the category of conditionally IAA, at least in the clinical context of total parenteral nutrition (TPN), where there is much activity associated with the development of appropriate therapies aimed at reversing the loss of muscle glutamine during illness. Glutamine is absent from TPN solutions, being unstable, so that efforts are being made to provide it in the form of glutamine-containing dipeptides (Adibi *et al.* 1987).

It would appear, therefore, that any discussion of amino acid requirements should be based on a broader classification of amino acid dispensability than that conventionally adopted at present.

VALIDITY OF CURRENT SCORING PATTERNS

Analysis of the derivation of the current IAA requirements indicates that the age-related fall primarily reflects the different dietary designs of the original balance studies (Millward & Rivers, 1988). Thus, the adult values are derived from studies by Rose (1957) and others, with artificial dietary amino acid mixtures with a low IAA: dispensable N ratio, resulting in low rates of L_r and a low apparent requirement approaching $L_{r,min}$. In contrast, the values for infants, preschool children and to a large extent schoolboys are derived from studies with mixtures containing high levels of IAA with no added non-essential N (see FAO/WHO/UNU, 1985). In each case high values of L_r can be expected with high apparent requirements.

As to their validity, the current adult values are not helpful as the basis for a scoring pattern since such low values of L_r are unlikely to occur on natural diets. Their value is in defining $L_{r,min}$. In contrast, in infants and children it is highly likely that the apparent requirements determined in those studies are closer to the values of $L_{r,op}$ that occur on natural diets. In any case, the actual scoring pattern recommended for use with infants by the FAO/WHO/UNU (1985) report is that based on the composition of milk protein, and the response of the anabolic drive in terms of weight gain to such intakes of IAA and protein is known to be satisfactory. This means that there is less reason to be concerned about the current values. Nevertheless, the question of adequacy in terms of height growth (discussed previously) remains, as do the studies showing that growth can be maintained on relatively low levels of IAA when dispensable N and particularly glycine is adequate (Snyderman *et al.* 1962). Investigation of the relationships between dietary IAA intake, diurnal cycling and the anabolic drive on height growth, would undoubtedly further clarify our understanding of the requirements in children.

Table 3. *Derivation of the proposed new amino acid requirement values (mg/kg) in comparison with current adult values and obligatory oxidative losses*

	Current adult values (FAO/WHO/UNU, 1985)	ONL*	Values reported by Young & Pellet (1989)					
			Calculated values		Corrected values			
			ONL method*	Protein turnover method†	ONL method‡	Protein turnover method‡	^{13}C tracer studies§	New requirement values‖
Histidine	8–12	11·5	—	—	—	—	—	—
Isoleucine	10	16·2	16	17	23	24	—	23
Leucine	14	27·4	27	28	39	39	30–40	(40)
Lysine	12	30·1	30	31	42	43	30	(30)
Methionine + cystine	13	13·5	14	14	16	17	13	(13)
Phenylalanine + tyrosine	14	27	27	28	39	39	—	39
Threonine	7	15·5	15	16	21	22	15	(15)
Tryptophan	3·5	4·0	4	4	6	5·6	—	6
Valine	10	16·9	17	18	24	25	20	(20)

ONL, obligatory nitrogen loss; FAO/WHO/UNU, Food and Agriculture Organization/World Health Organization/United Nations University.

* From Millward & Rivers (1988). Rates calculated on the basis of an ONL of 54 mg N/kg per d, deriving from body protein (amino acid composition as beef). Values should be greater than the minimum requirement (R_{min}; see Fig. 5) for all amino acids except the rate-limiting one, for which values might be similar, as in the case of methionine.

† Values calculated assuming whole-body protein turnover rate in healthy adults is 3–4 g protein/kg per d with 90% re-utilization of amino acids; i.e. oxidation will be 10%, 350 mg protein equal to 56 mg N/kg, similar to the ONL (54 mg N/kg per d). The 90% re-utilization value was calculated originally from the magnitude of the ONL compared with the turnover rate so that it should be the same as the calculated value by the ONL method.

‡ Values adjusted for 70% efficiency of dietary utilization, except for methionine, where more efficient utilization is proposed.

§ Values from metabolic tracer studies.

‖ Proposed revised estimates of mean requirements used to generate scoring pattern; values in parentheses are values from the kinetic tracer studies.

CAN WE DEFINE A BETTER SCORING PATTERN FOR ADULTS?

Young & Pellet (1989) have suggested new requirement values for all ages, excluding infants (Table 3), the values being derived in part from the table of OOL published by Millward & Rivers (1988) and in part from kinetic tracer studies in adults. This new pattern is believed to be conceptually flawed and derived from data inadequate for the purpose.

In the context of the model shown in Fig. 5, the adult IAA requirement pattern of R_{op} will be equal to the sum of amino acids lost as $L_o + L_p + L_r$, by a diet exerting an acceptable anabolic drive. The IAA content of the major component of losses, L_r, cannot be predicted, having a composition varying with the diet ($L_{r,op}$), and comprising an as yet undefined component which exerts the anabolic drive. For these reasons it is clear that the pattern of the maintenance requirement cannot be predicted from first principles. There is no reason why it should in any way reflect the amino acid pattern of body protein, which is by definition the amino acid pattern of the OOL.

All that can be predicted is that for the single rate-limiting IAA, which 'drives' the ONL, whichever it may be, the magnitude of its obligatory requirement ($L_o + L_{r,min}$) may be similar to the magnitude of its OOL. For lysine, for example, the current requirement value

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of 12 mg/kg is perfectly consistent with an OOL of 30 mg/kg, simply indicating the high content of lysine in body proteins and a relatively low metabolic need ($L_o + L_{r,min}$).

Young & Pellett (1989) do not justify or explain the use of the OOL as the basis for their pattern. However, they show the pattern to be similar to a second pattern (Table 3), but this is not surprising since it is derived ultimately from the same values used to generate the OOL.

Some of the values in the new pattern (leucine, lysine, threonine and valine) are derived from kinetic tracer studies which can be criticized both on technical grounds and on the grounds that the dietary design of the tracer studies (purified amino acids based on egg, with reducing amounts of the test amino acid) may have maintained particularly high values of L_r (see Millward & Rivers, 1988). Other minor criticisms of the proposed new values include the use of an arbitrary utilization efficiency factor of 0·7 and calculation of a new scoring pattern from the FAO/WHO/UNU (1985) *mean* adult protein requirement, which are compared with values from the FAO/WHO/UNU (1985) report calculated from the *safe* protein requirements. We believe that these new requirement values are of extremely limited value, and in no way acceptable.

CONCLUSION

In examining both general and specific aspects of shortcomings with the current suggested protein and amino acid requirements, we have tried to show that the reason why protein and amino acid requirements are so difficult to define is insufficient understanding of the behaviour of the system to allow even qualitative, let alone quantitative, description. We have focused on several areas where the interaction between man and dietary protein remains obscure: infants achieving better growth than expected on breast milk and responding to dispensable N, some groups of adults exhibiting responses to low-protein diets indicative of regulatory mechanisms not exhibited by other groups; a generally incoherent body of N-balance data. Out of this examination we have attempted to construct an alternative framework in which the biological basis of protein requirements can be better understood and assessed.

This framework has two components. One is the recognition that our perspective of amino acid dispensability needs to be widened, allowing for circumstances when amino acids like glycine and glutamine, which can be synthesized by the organism, might become limiting or deficient with adverse consequences for the organism. At the same time, the possibility that qualitative adjustments to dietary amino acid supply might be possible by the lower gut should be reconsidered as within the range of normal behaviour. Thus, adaptation could involve qualitative adjustments in 'requirements'.

The other component is a model which allows better description of the metabolic role of dietary amino acids. The outcome of this exercise is not a 'more accurate' description of protein requirements, but rather an identification of what we can and cannot define on the basis of current methodologies.

The implication of protein nutrition influencing both metabolic and functional responses of the organism (the anabolic drive) is that protein requirements need to take account of the levels and qualitative nature of body protein in tissues or stores which influence function. Work capacity or muscle strength is one example. A more suitable definition of protein requirements might be 'amounts considered adequate to establish and maintain sufficient levels of tissue protein in body tissues or organs which would enable adequate function'.

In this context we would suggest that for infants, the important question is not whether the current protein requirement value is too high, as suggested by Beaton & Chery (1988),

but whether or not our current methods of assessing the nutritional adequacy of infant feeds allow us to determine optimum requirements. Faced on the one hand with the apparently 'special properties' of breast milk allowing growth on remarkably low intakes and with evidence for growth limitation by non-specific N, and on the other with evidence that increased protein is needed for maximal height growth, there is urgent need to determine the extent to which the anabolic drive is operating in both breast-fed and older mixed-fed infants. We need to consider whether height growth should be used as an indicator.

As for the extent to which current IAA requirement values for adults are underestimates and the related problem of whether or not adjustments of protein requirements need to be made to take account of score, this cannot yet be resolved because of lack of appropriate information. What are needed are studies with real proteins, or at least balanced mixtures of IAA, with measurements of both amino acid balances by reliable techniques and behavioural responses which would allow definition of the range of values for $L_{r,op}$ and R_{op} which were associated with an acceptable anabolic drive. Given the likelihood that the anabolic drive occurs as a collective response to amino acids, we would predict that an amino acid pattern rather than values for individual amino acids will be the most appropriate descriptor of this component of the IAA requirement. As to what would constitute an acceptable anabolic drive, since in the adult it is defined as the maintenance of organ mass and function, there is obvious scope for inclusion of a wide range of responses as indicators of adequacy, which may be as important as the achievement of balance alone. Both the anabolic drive and diurnal cycling are closely related to rates of protein turnover, so that study of these rates is of clear importance. Only then will the importance of the regulatory oxidative losses to the organism be judged and the magnitude of IAA requirements (R_{op}) be properly assessed.

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