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# Assessment of protein requirements by nitrogen balance

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The earliest estimates of protein requirements, such as those of Smith (1863) and Pavy (1874) who recommended about 125 g per day for the average working man, were based on studies of the diets of individuals or of groups of subjects who were considered to be healthy and leading normal active lives. Such estimates were simply a reflection of the dietary habits of those under study, but helped to establish minimum standards of feeding in institutions. About the turn of the century, similar recommendations ranging from 118 to 150 g were made by Voit (1881), Atwater (1895) and Rubner (1903) (Fig. 1), and these came to be vigorously defended as representing not merely feeding standards but as irreducible minima below which a proper state of health and vigour could not be maintained.

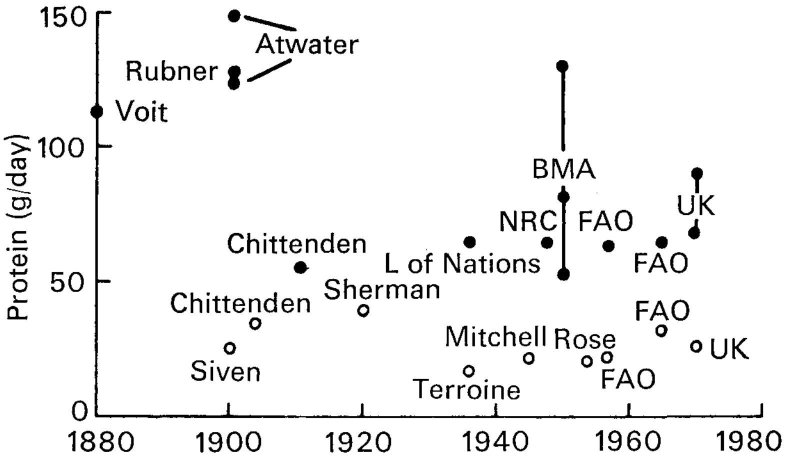

Fig. 1. Recommended dietary protein intakes (●) and estimated minimal requirements of net dietary protein (○) for a 65 kg man. BMA, British Medical Association; L of Nations, League of Nations; NRC, National Research Council; UK, United Kingdom Department of Health and Social Security.

Throughout history there have been repeated claims by individuals that health could in fact be sustained at much lower levels of protein, although many of these claims seem more remarkable to us now for the low energy intakes suggested. Thus Cornaro (1558) described a daily regimen which would probably have afforded him about 49 g of protein and 1220 kcal per day. Baker (1767) reported the claim of the 'Miller of Essex' to have lived for 18 years on no more than 16 oz of flour a day which would have yielded 48 g protein and 1533 kcal. While a combination of advanced age, small physical size and a sedentary existence might have lent some credence to these claims (the calculated basal metabolic rate of an 80-year-old man weighing 40 kg and 5 ft in height is 1100 kcal per day) it was not until 1901 when Sivén demonstrated nitrogen equilibrium upon himself at levels of intake equivalent to between 25 and 30 g of protein per day that such observations became a serious threat to the views held at that time by the majority of nutritionists.

Sivén's experiments were of very short duration (6 days only) and it was left to Chittenden (1905) to carry out long-term experiments over periods of up to 9 months upon himself and a group of his colleagues. His estimates of minimal requirements are equivalent to an average of 36 g of utilizable protein per day (Miller & Payne, 1964), which as Fig. 1 shows is in very good agreement with the latest figures. Somewhat later (1911) Chittenden summarized the results of all his experiments on a total of 108 individuals and suggested that in practice a safe level of dietary protein would be 56 g per day, again in very close agreement with modern recommendations for dietary intakes.

Such views as these were at the time widely regarded as heretical and were much under attack for the most part from a position of ignorance. However, the controversy did for the first time focus attention upon the real problem of establishing proper objective criteria for health and physical performance upon which physiological, as opposed to purely sociological, requirements could be based. Thus Chittenden gave evidence of unimpaired physical and mental performance throughout his lengthy experimental periods. McKay (1912), who was his only critic of any substance, carried out one of the first attempts to relate the nutrient intakes of population groups with vital statistics. McKay contrasted the physical stature, disease rates and life expenctancy of Eurasian as compared with rice-eating Bengali students, to the detriment of the latter. He observed that their diets contained different levels of protein and noted that the intake of the Bengalis (0·94 g/kg body-weight per day) was close to Chittenden's recommendation (0·85 g/kg per day) and attributed their poor general health to this factor. Chittenden's defence was to draw attention to the monotony and general lack of balance of the Bengali diets, a point which would be well taken today but which perhaps seemed somewhat obscure at a time before the discovery of vitamins.

### *Criteria for the establishment of minimum physiological requirements for protein*

As we have seen, attempts to establish minimal needs must in the last analysis be related to the maintenance of some defined state of health of the individual. Chittenden's contribution to the subject has been quite simply the suggestion that a level of protein intake which is just sufficient to balance the minimal rates of inevitable nitrogen losses by various routes from the body will, at the same time, maintain an optimum state of health. There is continuing controversy about this proposition for three reasons:

(a) It is impossible to define a single optimum state of health. This is because the optimum is always likely to be a matter for compromise: the many individually desirable qualities known to be influenced by nutrition are most unlikely to be all brought to their maximum potential be the same level of intake. For example, such qualities as stature and life span are not favoured by the same diet (Miller & Payne, 1968). Again, resistance to malaria is reduced by a high level of protein in the diet (Platt, Dema & Miller, 1960), and for hookworm increased (Orraca-Tetteh, 1964). Thus it is impossible to specify a level of protein that will always give optimal protection against disease.

(b) Evidence about possible effects of levels of intake higher than those needed for N equilibrium is largely negative evidence. Thus it has been shown that high levels of protein prior to injury or exposure to radiation do not influence subsequent recovery rates, nor do they confer resistance to the effects of various stresses (Munro, 1968). There is, however, some evidence, mainly from the results of animal experiments, for detrimental effects of high-protein diets in respect of longevity (Miller & Payne, 1968), and incidence of kidney disease (Bras & Ross, 1966).

(c) There is no agreement about the significance of protein reserves. However, it has been shown recently (Young, Hussein & Scrimshaw, 1968; Gopalan & Rao, 1966) that the magnitude of N reserves is less than 1% of the total body N in both normal and undernourished human subjects.

Thus at the present time the balance of evidence favours the view that minimum physiological requirements should be based upon N balance criteria.

### *The factorial method for calculating net protein requirements*

An adult subject can be maintained in a state of N equilibrium over a wide range of intakes and the problem is to find the minimum dietary level. This can be done in two ways. Firstly by continued balance studies in which the level is maintained as close as possible to the region in which negative balance occurs. Such experiments should be of long duration and should provide evidence of continued maintenance of the health of the subjects. The work of Chittenden (1905, 1911), and latterly Bricker, Shively, Smith, Mitchell & Hamilton (1949), falls into this category. Secondly it can be done by separate evaluation of the magnitude of each of the components of inevitable N loss from the body and subsequent addition of these—the so-called 'factorial method'. Table 1 shows the magnitudes of the loss in urine, faeces and from the skin adopted in the report of the United Kingdom Department of Health and Social Security (1969).

Table 1. *Inevitable losses of nitrogen in relation to basal energy expenditure*

| Component of N loss | Ratio of N loss to BMR (mg N/kcal) |
|---|---|
| Endogenous urinary | 2·0 |
| Metabolic faecal | 0·57 |
| Skin losses | 0·08 |
| Total | 2·65 |

Losses by routes other than those listed are possible. Costa (1960) has suggested that losses of elemental N from the lungs may provide an explanation for long-continued apparent positive balances in men and animals unaccompanied by changes in body-weight. More recently Costa, Ulbrich, Kantor & Holland (1968) have evaluated this effect by maintaining animals and human subjects in N-free atmospheres and measuring the rate of formation of gaseous N. Their estimates show that loss of elemental N could be as much as 10–30% of the daily minimal requirement. Since this has not been confirmed, the values are not included. However, the figure of 2·0 mg N per basal kcal for urinary loss is probably an overestimate. Gopalan & Rao (1966) found in a number of young adults values ranging from 1·3 to 1·65 mg N/kcal.

The N content of the tissue laid down during growth is calculated from the weight increment and the N concentration of the body. Weight increments of children at different ages are derived from an average curve representative of a particular population (Tanner, Whitehouse & Takaishi, 1966). The N concentration in the whole body is taken as 1·83% at birth, increasing to 2·34% at the age of 1 year (Fomon,

1967) and finally increasing to 2·9% for the adult. Fig.2 shows requirements for growth and maintenance. It should be noted that the growth increments for man are small compared with other species, but the extended growth period of man is unique.

The protein content of the products of conception and of the increased weight of the maternal reproductive tissues has been estimated (FAO, 1965) as 950 g, which is equivalent to 0·54 g of N per day. This requirement should be distributed over the entire period of pregnancy because there is evidence (Naismith, 1969) that protein is stored in early pregnancy and subsequently utilized. The average daily milk output has been taken as 850 ml (FAO, 1965) with an average protein content of 1·2% and thus the average N output in the milk is 1·6 g/day. These data have been used to calculate the additional protein requirements during pregnancy and lactation.

An estimate of the variation in requirements between individuals is more difficult to assess but a coefficient of variation of around 10% has been observed for measurements both of basal metabolism and of minimal N requirements for maintenance. In the absence of any further information, it has been assumed that individual requirements are distributed normally and that therefore an allowance of 20% above the mean will provide for the needs of 97% of the population.

Fig. 2 shows the protein requirements at different ages. The maintenance needs are calculated by the factorial method as (basal metabolic rate)×2·65×$\frac{120}{100}$, and increments for growth added where appropriate. The amount of protein calculated as described is the net dietary-protein (NDp) requirement. It can be combined with recommended calorie requirements to show what proportion of the calorie content of diets should be present as utilizable protein (NDpCal%). Table 2 shows these proportions for different physiological groups.

Fig. 2 Protein requirements for maintenance and growth (drawn from data adopted in the United Kingdom Department of Health and Social Security (1969) report, in terms of net dietary protein).

These estimates of protein requirements are low in comparison with those mentioned in the introduction to this paper and may be disquieting to those advocating

Table 2. *Human protein requirements**

| | | Energy requirement (kcal) | Net dietary-protein g | Net dietary-protein Cal%† |
|---|---|---|---|---|
| Children: | Newborn | 400 | 8·0 | 8·0 |
| | 6 months | 760 | 10·0 | 5·3 |
| | 1 year | 1000 | 11·4 | 4·6 |
| Men: | 16 years | 3000 | 35 | 4·6 |
| | Adult | 3200 | 32 | 4·0 |
| | Active | 3600 | 32 | 3·6 |
| | Old | 2100 | 27 | 5·1 |
| Women: | 16 years | 2300 | 28 | 4·8 |
| | Adult | 2300 | 27 | 4·7 |
| | Pregnant | 2400 | 31 | 5·2 |
| | Lactating | 2700 | 39 | 5·8 |

*Estimated from data adopted in the United Kingdom Department of Health and Social Security (1969) Report.

†NDpCal%=NDpJ%.

high-protein diets. However, there is a wealth of evidence in the literature to show that such intakes are adequate in maintaining N balance over short periods, and sufficient to demonstrate that they will also maintain good health over extended periods (see Table 3).

Table 3. *Levels of net dietary protein which have maintained health in man*

| | Subjects | | | NDp | |
|---|---|---|---|---|---|
| Reference | Age | No. | Duration (weeks) | Intake | Requirement† |
| Chittenden (1905) | Adult | 5 | 36 | 36 | 32 |
| Bricker *et al.* (1949) | Adult | 9 | 10 | 19 | 27 |
| Miller & Mumford (1967) | Adult | 2 | 6 | 18 | 32 |
| Miller & Mumford (1967) | Adult | 6 | 4 | 19 | 32 |
| James (1960) | 8 years | 36* | 7 | 16 | 21 |
| Chan & Waterlow (1966) | 1 year | 12* | 3 | 10·7 | 11·4 |

*Growing children.

†See Table 2.

No claim is made that such diets are culturally acceptable or that higher levels of protein are necessarily detrimental; merely that protein intakes above the minimal confer no known benefit other than improved palatability.

### *Assessment of diets*

It is absurd to state requirements in terms that cannot be applied to the design or assessment of actual diets. It is therefore important to be able to measure the amount of utilizable protein that there is in any given diet, and this may be achieved by a simple biological assay or, in the present state of knowledge, slightly less accurately from chemical analysis.

Dealing with the biological method first, diets are constructed as eaten, freeze-dried, and fed to rats for N balance studies to determine the efficiency with which the rat is able to convert the dietary protein into rat carcass protein (net protein utilization, NPU). In diets used for institutions this is relatively easy, but in those parts of the world where malnutrition is rife dietary data are extremely limited. There is no National Food Survey and only limited surveys for villages, usually only during a small part of the year. In such cases one can make an assessment on the basis either of food balance sheets constructed from a consideration of food imports, exports and production, or by an examination of the traditional diet. The latter is not so inaccurate as it may sound since the diet consumed is frequently monotonously the same from day to day.

The biological determination of the efficiency of utilization of protein in diets as eaten required us (Miller & Payne, 1961*a*) to distinguish between two types of NPU, although the method employed for their evaluation is similar. In the academic studies care is taken to ensure that the amino acid composition of the protein under test is the limiting feature and for this the concentration of protein in the diet is kept low, yielding maximal NPU values: these we have called standardized values ($\text{NPU}_{\text{st}}$). In the evaluation of diets as eaten no additions or dilutions are made and efficiency of utilization is a function of the diet as a whole; these we have called operative values ($\text{NPU}_{\text{op}}$). For example, egg protein has an $\text{NPU}_{\text{st}}$ near to 100, that is when fed at 4% in the diet, but no-one eats egg dishes at such a low concentration of protein and their $\text{NPU}_{\text{op}}$ is frequently nearer 60. The calculation of utilizable or NDp from $\text{NPU}_{\text{op}}$ is straightforward:

$$\text{NDp} = \text{P} \times \text{NPU}_{\text{op}},$$

where P is the quantity of protein expressed either as g per day or more usually as a percentage of the total calories of the diet, when the product becomes NDpCal% (N.B. NDpCal% = NDpJ%).

With this technique one can make a comparison of diets containing different concentrations of protein, and also study the effect of adding increasing amounts of a supplement to an indigenous diet. But the greatest advantage is that one can compare the biological assay results directly against standards for human requirements.

Although these biological methods are known to give the best results, the technique is laborious and requires the use of an animal house and skilled technicians. Somebody sitting in the middle of Africa requires an easy method for calculating values from food tables (Miller & Payne, 1961*b*, 1961*c*). Protein contents are easy to calculate, but the efficiency of utilization of that protein is more difficult to assess and depends upon a number of factors:

(i) *The amino acid composition.* There are various techniques available for arriving at a protein score, or measure of protein quality, from a consideration of amino acid analyses. Most techniques involve the comparison of dietary amino acids with a reference pattern of amino acids; although the reference patterns differ from author to author they are consistent when it comes to the sulphur amino acids which are those most likely to limit protein synthesis in human diets (Miller & Donoso, 1965).

(ii) *The percentage of protein in the diet.* As this rises so the efficiency of utilization falls. This fall in protein efficiency is not because the capacity of protein synthesis has been saturated, but because protein is being used for energy purposes. This illustrates the important principle that energy and protein metabolism are interrelated. Equations and nomograms are available for predicting this effect (Miller & Payne, 1961*b*).

(iii) *The energy intake.* If the intake of energy is restricted below a certain level, the efficiency of protein utilization falls because protein is used primarily for energy purposes (Miller & Payne, 1961*c*). If the energy intake is reduced to that required to meet only the needs for basal metabolism, then the efficiency of protein utilization is zero. Table 4 shows the effect on the NPU of an Iranian diet at reduced energy intakes. In our view, most protein malnutrition in the world is due to calorie insufficiency.

Table 4. *Net protein utilization operative of an Iranian diet at different levels of energy intake*

| Energy intake expressed as percentage of basal metabolism | NPUop |
|---|---|
| 430 | 55 |
| 340 | 56 |
| 200 | 45 |
| 160 | 32 |
| 110 | 20 |

(iv) *Other factors.* Other dietary factors influencing the efficiency of N utilization are the levels of vitamins and minerals, but these effects are not important in practical regimens.

Of the four factors, the energy intake is by far the most important and it is impossible to evaluate protein supplements without considering concomitantly energy requirements. However, on the assumption that these are met, the efficiency of utilization of most diets is of the order of 60% (Miller & Payne, 1961*b*) and this raises the daily protein requirement from 32 g NDp for the adult to 53 g dietary protein, a figure close to that given by Chittenden.

### *Protein and the world food problems*

During the next 30 years the world's population will double, and so will mankind's total nutrient requirements. In order to meet this growing demand it is essential to realize that the need is to supply increased amounts of nutrients in much the same proportions as are consumed at present. An examination of staple foods (see Table 5) currently consumed shows that all but three have sufficient protein to meet the needs of man. The three exceptions are cassava, sago and plantain. However, when one examines diets, even those based on cassava, one finds that the staple is never eaten alone, but with sufficient supplements to fill the protein gap (see Table 5), except for the newborn which would receive breast milk. Thus if protein deficiency exists at the present time it is associated with an inadequate intake of food. Such low food intakes imply a concomitant low protein intake but also as mentioned

Table 5. *The utilizable protein content* (NDpCal%*) *of a number of staples and diets based on them*

| Staple | Alone | Diets† |
|---|---|---|
| Sago | 0·3 | — |
| Cassava | 0·9 | 4·0 (7) |
| Plantain | 1·6 | — |
| Yam | 4·6 | 4·6 (2) |
| Maize | 4·7 | 6·2 (3) |
| Rice | 4·9 | 6·4 (9) |
| Sorghum | 4·9 | 8·0 (5) |
| Millet | 5·3 | 11·5 (1) |
| Potato | 5·9 | 9·6 (4) |
| Wheat | 5·9 | 9·2 (7) |

*Net dietary protein calories as % of total calories. (cf. Requirement for adult man=4·0%).

†The figures in parentheses are the numbers of diets assayed.

earlier a lower efficiency of utilization of the protein. Hence the protein deficiency is an indirect result of the energy deficiency and could not be corrected by the addition of extra protein to the diet. This principle has been recognized by WHO who refer to the deficiency syndrome as protein-calorie malnutrition.

A complete scientific evaluation of nutrient requirements does not, however, provide a recipe for a satisfying diet, however economical. We eat food because we like it and it may be that a varied diet is essential for the consumption of enough food. It cannot be denied that most of the diets of the world are monotonous and frequently made up of only a few foods. Also a varied diet is more likely to meet the requirements for vitamins and minerals. In the future it may be that the food technologist could provide cheap appetising foods which are also nutritionally balanced. Unfortunately the only successful enterprise in underdeveloped countries is Coca-cola, which fails to meet the last criterion.

The lowered estimates for protein allowances do not in any way diminish the magnitude of the task of solving the world food problem, but they should be seen as directing our efforts towards the production of more palatable foods of all kinds rather than searching for some apparently easier solution to the problem by increasing the supply of specific nutrients such as protein.

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# Enzymes and the assessment of protein nutrition

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This symposium is concerned with the assessment of protein nutritional status in man. The question we have to examine is whether measurements of enzyme activity are, or may be in the future, of any value for this purpose. There are several concepts which may be useful in considering this problem, such as the nature of enzymes and the regulation of their amount in the body, the effect on activity of a biochemical lesion at the cellular level, and the adaptive changes that reflect an altered balance of metabolic pathways.

Since enzymes are themselves proteins it seems logical to expect that in general a fall in the amount of dietary protein might be reflected in a reduction in the amounts of enzymes in blood or tissues. Furthermore, many enzymes depend for their action on trace metals or vitamins of the B complex (for example, riboflavine, pyridoxine), all of which may be in short supply when protein is scarce. One might hope, therefore, that measurements of enzyme activity would provide a useful tool for assessing

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