

# Protein-Calorie Malnutrition

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# Amino Acid Requirements and Age

*Guillermo Arroyave*

## INTRODUCTION

One objective of this paper is to review the evidence that the essential amino acid requirements of man per kilogram of body weight decrease with age faster than do nitrogen requirements. As a consequence, the quality of proteins for human nutrition differs depending on the age of the subjects consuming them. The second objective is to assess the potential of cereal-legume based diets to ensure adequate protein and amino acid nutrition of preschool age children.

## AMINO ACID REQUIREMENTS AND PROTEIN QUALITY

Protein intake is nutritionally adequate when it satisfies nitrogen and essential amino acid requirements. Nitrogen and amino acid requirements are, therefore, the logical yardsticks of protein quality, and precise knowl-

edge of these requirements is essential. Data regarding amino acid requirements are still incomplete. When these gaps are filled, we shall be able to develop a rational approach to the problem of predicting protein nutritional quality and to the even more complex problem of combating protein malnutrition.

For infants, two different sources of information are available. Holt and Snyderman with their research group (12) have determined the level of intake of each essential individual amino acid that can maintain expected normal growth and nitrogen retention in young infants. They have used synthetic diets made up of a mixture of 18 amino acids in the proportion found in breast milk and have proposed a table of requirements based on the intake per kilogram per day that is adequate for all the subjects.

Fomon and Filer (6) fed infants a variety of infant formulas and determined the minimum intakes of these for adequate growth in all the infants. Then, from the composition of the protein in the formulas, they calculated the amount of each essential amino acid received by the infants. Because the two approaches gave data in relatively good agreement, a composite of the lowest values from the two sets of information were proposed to represent the requirements of infants 0–6 months of age.

The only other children's age group for which there are some data is preadolescent children, reported by Nakagawa *et al.* (14–17). Most of the subjects have been between 10 and 12 years of age. The final figures proposed by these authors are based on experimentally determined lowest intakes required to produce adequate nitrogen balance in all the subjects. They have used mixtures of pure essential amino acids plus glycine as a source of nonessential nitrogen. It is uncertain whether these figures can be considered minimum values. The increments between intakes in the experimental design are large and, therefore, it cannot be assumed that the level giving positive nitrogen balance is the lowest possible. They are, however, the only data available.

Amino acid requirements for adults are available from several sources. The studies by Rose and his group, carried out with young men (18), have utilized a diet of mixtures of pure essential amino acids, with glycine and urea as sources of nonessential nitrogen. Their estimates of requirement are based on the level of intake that results in positive balance. The individual variability is large and the authors have selected the intake of the subject with the highest requirement. The studies in women by several groups of investigators [see Leverton (13)] have used the attainment of nitrogen equilibrium in all subjects as a criterion for the lowest requirement. The different criterion used is obviously a more plausible reason for why higher values are found for men than for women, even when expressed per kilogram of body weight, than is an explanation based only

on sex. Hegsted (11), using the data for women available in the literature, has published figures derived by regression analysis between intake and nitrogen balance, basing the requirements on the point at which the regression line crosses the line of nitrogen equilibrium. These figures have been most widely accepted, although it is suspected that they are underestimates. One general criticism of these studies in adults is that they fail to subtract skin plus miscellaneous nitrogen losses. According to estimates by FAO/WHO (8), these may amount to about 5 mg/kg/day. For this reason, it is likely that Rose's figures are, in fact, more realistic. Rounded figures, in general slightly lower than Rose's and somewhat higher than Hegsted's, have been tabulated by FAO/WHO (8). The results for the three age groups discussed are summarized in Table I.

It is impossible within the scope of this introduction to discuss in detail the several limitations of the data. Several groups of experts have dealt with this problem (8, 9). It must be emphasized that more research is needed to improve or consolidate the available information, particularly in children. The very important preschool group has not been studied.

The studies described permit the calculation of amino acid patterns that, on theoretical grounds, can be considered "ideal." From data on total protein ( $N \times 6.25$ ) requirements for age, estimated as described elsewhere, (8) and those on amino acid requirements for the same age, one can calcu-

TABLE I

Estimated Amino Acid Requirements<sup>a</sup>  
(mg/kg/day)

| Amino acid        | Children |                 |        |
|-------------------|----------|-----------------|--------|
|                   | Infants  | 10-12 years old | Adults |
| Histidine         | 28       | —               | —      |
| Isoleucine        | 70       | 30              | 10     |
| Leucine           | 161      | 45              | 14     |
| Lysine            | 103      | 60              | 12     |
| TSAA <sup>b</sup> | 58       | 27              | 13     |
| TAAA <sup>c</sup> | 125      | 27              | 14     |
| Threonine         | 87       | 35              | 7      |
| Tryptophan        | 17       | 4               | 3.5    |
| Valine            | 93       | 33              | 10     |

<sup>a</sup> From FAO/WHO (8).

<sup>b</sup> TSAA, total sulfur amino acids (methionine + cystine).

<sup>c</sup> TAAA, total aromatic amino acids (phenylalanine + tyrosine).



late the composition in essential amino acids of such "ideal" proteins for the different age groups. These, as estimated by FAO/WHO, are given in Table II.

Despite a general agreement among the data, there are some inconsistencies in the estimates of individual amino acid requirements with age. It is not clear to what extent these inconsistencies result from the different methods used or are caused by true age differences. However, on a biological basis it is difficult to accept, for instance, that the school age child needs a protein more concentrated in lysine than the infant.

One obvious truth is that the requirement of total essential amino acid nitrogen as a percent of total nitrogen is much lower in the adult than in the infant. The data for 10- to 12-year-old children suggest that the drop is slow up to at least this age, but we must await more information on the age groups intermediate to infancy and adulthood to draw definite conclusions.

**TABLE II**

**Suggested Patterns of Amino Acid Requirements (mg/gm protein)<sup>a</sup>**

| Amino acid  | Infant <sup>b</sup> | School child <sup>b</sup><br>(10-12 years old) | Adult <sup>b</sup> |
|-------------|---------------------|--|--------------------|
| Histidine   | 14                  | —  | —                  |
| Isoleucine  | 35                  | 37   | 18                 |
| Leucine     | 80                  | 56   | 25                 |
| Lysine      | 52                  | 75   | 22                 |
| TSAA        | 29                  | 34   | 24                 |
| TAAA        | 63                  | 34   | 25                 |
| Threonine   | 44                  | 44   | 13                 |
| Tryptophan  | 8.5                 | 4.6  | 6.5                |
| Valine      | 47                  | 41   | 18                 |
| Total       |                     |  |                    |
| + Histidine | 373                 | —  | —                  |
| — Histidine | 359                 | 326  | 152                |

<sup>a</sup> From FAO/WHO (8).

<sup>b</sup> Calculated on the basis of the following protein requirement figures: infant 2.0 (0-3 months) gm/kg/day; child 0.8 gm/kg/day; adult 0.55 gm/kg/day.

The use of amino acid patterns specific for different age groups is a much more logical approach to judging proteins for their nutritional quality than is the use of a single pattern. Single patterns heretofore proposed have been made to fit the requirements for amino acids of the preschool age child and they underestimate the quality of a given protein for the adult.

We shall attempt to demonstrate here that, when proteins are properly scored against age-specific amino acid requirement patterns, their nutritional value is age dependent; that is, for example, a particular protein may prove very inadequate for the infant and still be very adequate for the adult.

The nutritional value of a protein can be defined as the extent to which it satisfies the requirements of an individual for each essential amino acid when ingested in sufficient quantity to satisfy his nitrogen requirement. This concept can be represented by the following equation.

$$\text{Protein quality index} = \frac{\text{Requirement of protein (N} \times 6.25) \text{ for age}}{\text{Amount of test protein to satisfy requirement of most limiting amino acid of subjects of the same age}} \times 100$$

**Table III**

**Scoring of Cow's Milk Protein against Amino Acid and Protein Requirements of Infants 3-6 Months Old**

| Amino acid | Amino acids in 1.85 gm of "ideal" protein <sup>a</sup> (mg) | Amino acid composition of milk protein (mg/gm) | Intake of milk protein to satisfy amino acid requirement of 3-6 month-old infant (gm/kg/day) |
|------------|---|--|--|
| Isoleucine | 65  | 47   | 1.38   |
| Leucine    | 148   | 95   | 1.56   |
| Lysine     | 96  | 78   | 1.23   |
| TSAA       | 54  | 34   | 1.59   |
| TAAA       | 116   | 101  | 1.15   |
| Threonine  | 81  | 44   | 1.84   |
| Tryptophan | 15.7  | 14.2   | 1.10   |
| Valine     | 87  | 58   | 1.50   |
| Histidine  | 26  | 27   | 0.96   |

$$\text{Protein quality index} = \frac{1.85}{1.84} = 100\%$$

<sup>a</sup> Theoretical protein containing essential amino acids in adequate proportion and concentration to satisfy the infant requirements.

In light of this relationship, let us examine cow's milk and corn protein for adults and for infants. Tables III–VI give the basic calculations. All the data on amino acid composition of foods used throughout this work are from FAO (7). To construct the column of requirements (first column of figures) the following procedure has been followed for each age group.

Protein requirement (gm/kg/day)

× Amino acids in the amino acid requirement pattern (mg/gm)

The amino acid requirement patterns for infants and adults are those given in Table II. The protein requirements are from FAO/WHO (8), as follows.

|                    |                |
|--------------------|----------------|
| Infant 3–6 months  | 1.85 gm/kg/day |
| Adult (both sexes) | 0.55 gm/kg/day |

A summary of the protein quality index obtained for each protein in both age groups is given in Table VII. It is clear that the figures obtained are age dependent.

**TABLE IV**

**Scoring of Corn Protein against Amino Acid and Protein Requirements of Infants 3–6 Months Old**

| Amino acid | Amino acids in 1.85 gm of "ideal" protein <sup>a</sup> (mg) | Amino acid composition of corn protein (mg/gm) | Intake of corn protein to satisfy amino acid requirements at 3–6 months (gm/kg/day) |
|------------|---|--|---|
| Isoleucine | 65  | 37   | 1.76  |
| Leucine    | 148   | 125  | 1.18  |
| Lysine     | 96  | 27   | 3.55  |
| TSAA       | 54  | 35   | 1.54  |
| TAAA       | 116   | 87   | 1.33  |
| Threonine  | 81  | 36   | 2.25  |
| Tryptophan | 15.7  | 6.1  | 2.57  |
| Valine     | 87  | 48   | 1.81  |
| Histidine  | 26  | 27   | 0.96  |

$$\text{Protein quality index} = \frac{1.85}{3.55} = 52\%$$

<sup>a</sup> Theoretical protein containing essential amino acids in adequate proportion and concentration to satisfy the infant requirements.

TABLE V

## Scoring Cow's Milk Protein against Amino Acid and Protein Requirements of Adults

| Amino acid | Amino acids in 0.55 gm of "ideal" protein <sup>a</sup> (mg) | Amino acid composition of milk protein (mg/gm) | Intake of milk protein to satisfy amino acid requirements of adults (gm/kg/day) |
|------------|---|--|---|
| Isoleucine | 9.9   | 47   | 0.21  |
| Leucine    | 13.8  | 95   | 0.15  |
| Lysine     | 12.1  | 78   | 0.16  |
| TSAA       | 13.2  | 34   | 0.39  |
| TAAA       | 13.8  | 101  | 0.14  |
| Threonine  | 7.2   | 44   | 0.16  |
| Tryptophan | 3.6   | 14.2   | 0.25  |
| Valine     | 9.9   | 58   | 0.17  |

$$\text{Protein quality index} = \frac{0.55}{0.39} = 141\%$$

<sup>a</sup> Theoretical protein containing essential amino acids in adequate proportion and concentration to satisfy the adult requirement.

TABLE VI

## Scoring Corn Protein against Amino Acid and Protein Requirements of Adults

| Amino acid | Amino acids in 0.55 gm of "ideal" protein <sup>a</sup> (mg) | Amino acid composition of corn protein (mg/gm) | Intake of corn protein to satisfy amino acid requirements of adults (gm/kg/day) |
|------------|---|--|---|
| Isoleucine | 9.9   | 37   | 0.27  |
| Leucine    | 13.8  | 125  | 0.11  |
| Lysine     | 12.1  | 27   | 0.45  |
| TSAA       | 13.2  | 35   | 0.38  |
| TAAA       | 13.8  | 87   | 0.16  |
| Threonine  | 7.2   | 36   | 0.20  |
| Tryptophan | 3.6   | 6.1  | 0.58  |
| Valine     | 9.9   | 48   | 0.21  |

$$\text{Protein quality index} = \frac{0.55}{0.58} = 95\%$$

<sup>a</sup> Theoretical protein containing essential amino acids in adequate proportion and concentration to satisfy the adult requirement.

TABLE VII

**Protein Quality Index of Cow's  
Milk and Corn Protein in  
Relation to Age**

|        | Cow's milk | Corn |
|--------|------------|------|
| Infant | 100        | 52   |
| Adult  | 141        | 95   |

## PRACTICAL APPLICATIONS

The second part of this paper deals with the practical application of the concept treated above to the problem of protein-calorie malnutrition (PCM), which affects so many children in the developing countries. In fact, the term "protein-calorie malnutrition" has been substituted for "protein malnutrition" because of evidence from dietary surveys (4, 5, 10) that calories are also limiting, and sometimes more so, than proteins per se.

More evidence is accumulating that increasing the intake of the cereal-legume based diets, which are presently consumed by rural children where PCM is highly prevalent, results in marked nutritional improvement. This throws doubt on the validity of programs that supplement these types of diets with concentrated protein sources alone. Increased consumption of diets based on foods already in use may be the preferred solution to the problem. Although this concept does not necessarily apply to all situations, for instance, to the extremely low-protein cassava-plantain diets, there are still millions of children who at preschool age depend on cereal-legume based diets and for whom proof of this hypothesis may be extremely valuable.

In order to contribute information on this question, we have attempted to assess the potential of corn-bean diets to meet the protein and amino acid requirements of preschool children.

## Theoretical Approach

Our approach has consisted of scoring the proteins from corn-bean mixtures against the amino acid requirements of the preschool child. As mentioned above, there are no experimentally determined amino acid requirement figures for the preschool age child. However, FAO/WHO have proposed an amino acid pattern (milligrams per gram protein) that can be assumed adequate for the preschool child. In other words, a protein with

a particular concentration and proportions of essential amino acids can be considered "ideal" for this age group. Such a pattern is given in Table VIII.

**TABLE VIII**  
**Provisional Amino Acid**  
**Scoring Pattern<sup>a</sup>**

| Amino acid | Protein (mg/gm) |
|------------|-----------------|
| Isoleucine | 40              |
| Leucine    | 70              |
| Lysine     | 55              |
| TSAA       | 35              |
| TAAA       | 60              |
| Threonine  | 40              |
| Tryptophan | 10              |
| Valine     | 50              |
| Total      | 360             |

<sup>a</sup> FAO/WHO (8).

**TABLE IX**

**Scoring Cow's Milk Protein against Protein Requirements of Preschool Children (2 Years Old) and Their Estimated Amino Acid Requirements Based on FAO/WHO, 1973 Provisional Pattern**

| Amino acid | Amino acids in 0.90 gm of "ideal" protein <sup>a</sup> average requirement (mg) | Amino acids in 1.20 gm of protein pattern average + 30 % (mg) | Amino acid composition of milk protein (mg/gm) | Intake of milk protein to satisfy: |                            |
|------------|---|---|--|------------------------------------|----------------------------|
|            |   |   |  | Average requirement (gm/kg/day)    | Average + 30 % (gm/kg/day) |
| Isoleucine | 36  | 48  | 53   | 0.68                               | 0.91                       |
| Leucine    | 63  | 84  | 99   | 0.64                               | 0.85                       |
| Lysine     | 50  | 66  | 73   | 0.76                               | 0.90                       |
| TSAA       | 32  | 42  | 35   | 0.90                               | 1.20                       |
| TAAA       | 54  | 72  | 98   | 0.55                               | 0.73                       |
| Threonine  | 36  | 48  | 42   | 0.86                               | 1.14                       |
| Tryptophan | 9   | 12  | 14.2   | 0.63                               | 0.84                       |
| Valine     | 45  | 60  | 64   | 0.70                               | 0.94                       |

$$\text{Protein quality index} = \frac{0.90}{0.90} = 100 \%$$

<sup>a</sup> Theoretical protein containing essential amino acids in adequate proportion and concentration to satisfy the requirements of children 2 years of age.

Using this pattern we have estimated the protein quality index of cow's milk protein and of a corn-bean protein mixture containing 76% protein from corn and 24% protein from beans. We have used this ratio of corn to beans because it is the ratio actually consumed by the preschool child in the rural, low-socioeconomic populations of Guatemala (3). As is discussed below, there is evidence that this ratio is not nutritionally the best. The results of these theoretical estimates are presented in Tables IX and X. The second and third columns in each table correspond to the average amino acid requirements and the average  $\pm$  30% (2 S.D.) for 2-year-old children (8). The latter values are the amounts of each amino acid that would satisfy the requirements of 95% of the children of this age group. It can be seen that cow's milk protein has a protein quality index of 100% because the amount of it that must be ingested to fill the requirement of the most limiting amino acid (TSAA) is the same as the amount needed to fill the protein requirement. In contrast, the corn-bean protein mixture (76:24) has a theoretical utilization value (protein quality index) of 69%, because 1.33 gm/kg/day are needed to satisfy the requirement of the

**TABLE X**

**Scoring a Corn-Bean (74:26) Protein Mixture against Protein Requirements of Preschool Children (2 Years Old) and Their Estimated Amino Acid Requirements Based on FAO/WHO 1973 Provisional Pattern**

| Amino acid | Amino acids in 0.90 gm "ideal" protein, average requirement (mg) | Amino acids in 1.20 gm of protein pattern, average $\pm$ 30% (mg) | Amino acid composition of corn-bean protein mixture (76:24) (mg/gm) | Intake of corn-bean protein mixture to satisfy: |                               |
|------------|--|---|---|---|-------------------------------|
|            |  |   |   | Average requirement (gm/kg/day)                 | Average $\pm$ 30% (gm/kg/day) |
| Isoleucine | 36   | 48  | 38  | 0.95  | 1.26                          |
| Leucine    | 63   | 84  | 114   | 0.55  | 0.74                          |
| Lysine     | 50   | 66  | 38  | 1.33  | 1.74                          |
| TSAA       | 32   | 42  | 31  | 1.03  | 1.35                          |
| TAAA       | 54   | 72  | 85  | 0.64  | 0.85                          |
| Threonine  | 36   | 48  | 37  | 0.97  | 1.30                          |
| Tryptophan | 9  | 12  | 7   | 1.28  | 1.71                          |
| Valine     | 45   | 60  | 48  | 0.94  | 1.25                          |

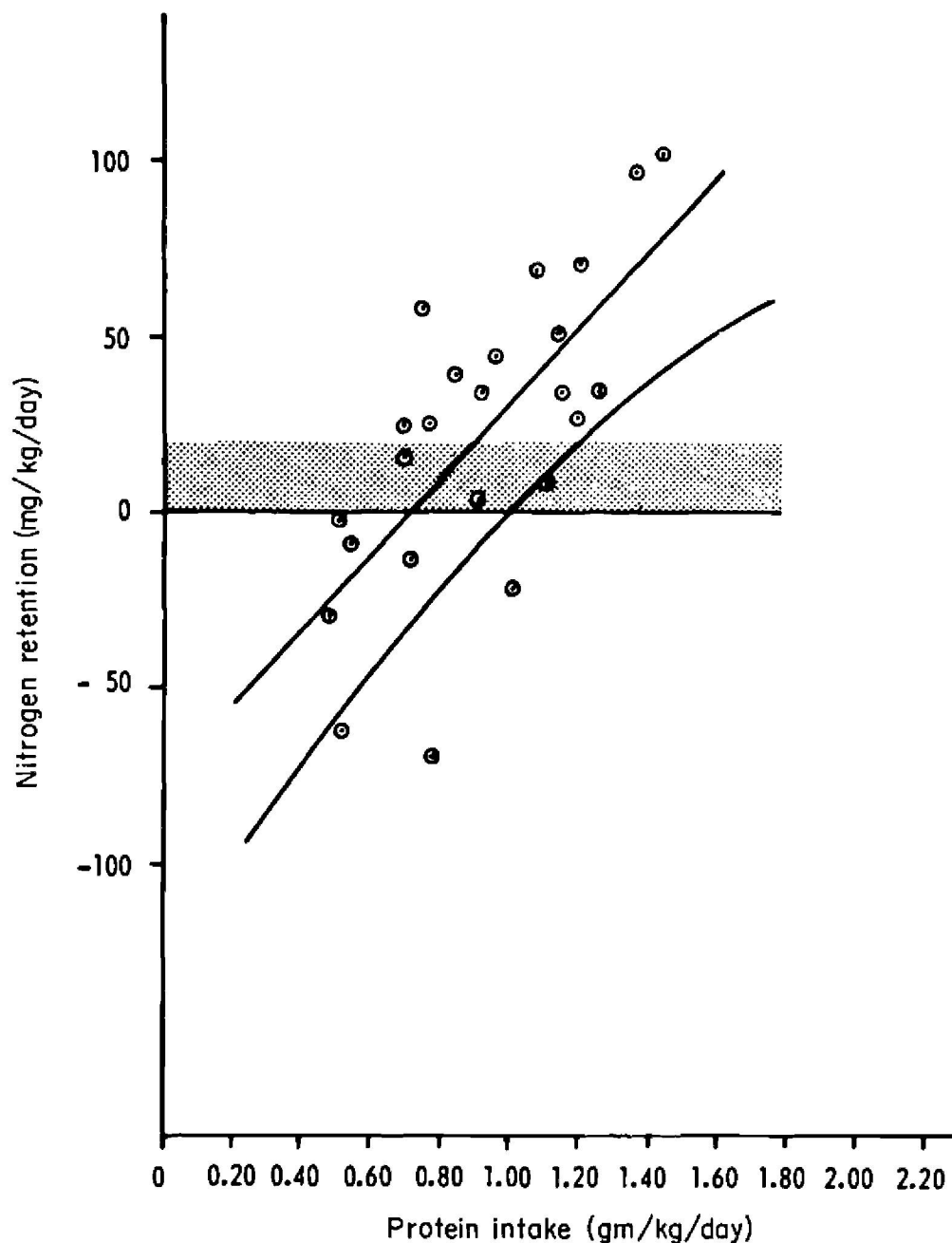
$$\text{Protein quality index} = \frac{0.90}{1.33} = 69\%$$

\* Theoretical protein containing essential amino acids in adequate proportion and concentration to satisfy the requirements of children 2 years of age.

most limiting amino acid (lysine) compared to a protein ( $N \times 6.25$ ) requirement of only 0.90 gm/kg/day of "ideal" protein. To meet the upper limit of the requirement for the most limiting amino acid (lysine), 1.74 gm/kg/day of corn-bean and 6:24 protein are needed.

### Experimental Approach

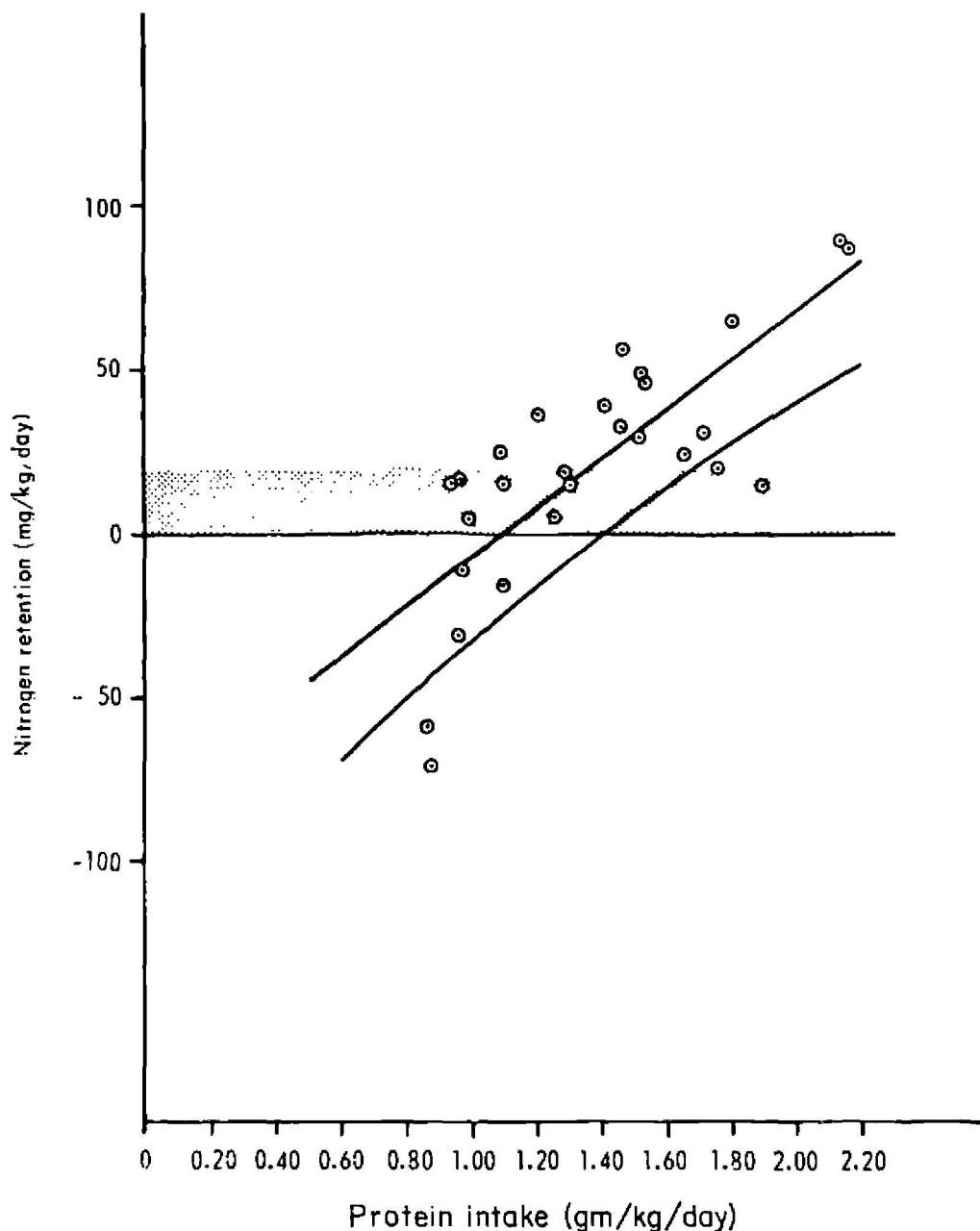
We have had the opportunity to test these figures experimentally in preschool children and to determine whether the theoretical predictions hold.



**Fig. 1** Protein requirements of children of preschool age. (Corn-bean 75:24 protein mixtures.) The upper limit of the shaded area is adequate retention of nitrogen between 1 and 2 years of age. The straight line is the regression line:  $r = 0.705$ ;  $Y = 20.7$ ,  $X = 0.906$ . The curved line is the 95% confidence limits for the predicted mean values. The values on the ordinate have been corrected for estimated skin losses.



The diet containing this corn-bean mixture (87% corn + 13% beans; 76% corn protein + 24% bean protein) was prepared in the form of a gruel and filled in with calories in order to feed the children the desired amount of protein per kilogram per day in 100 cal/kg/day. Fat contributed 20% of the calories and a multivitamin-mineral supplement was given. The results are graphically represented in Figs. 1 and 2. The regression line indicates that the value of  $x$  for the value of 20.7 mg N per kilogram per day retention (estimated adequate for this age) is 0.906 gm/kg/day for milk protein and 1.38 gm/kg/day for corn-bean 76:24 protein. The line for the 95% confidence limits crosses the 20.7-mg retention line at



**Fig. 2** Protein requirements of children of preschool age. (Cow's milk protein.) The upper limit of the shaded area is adequate retention of nitrogen between 1 and 2 years of age. The straight line is the regression line:  $r = 0.775$ ;  $Y = 20.7$ ,  $X = 1.38$ . The curved line is the 95% confidence limits for the predicted mean values. The values on the ordinate have been corrected for estimated skin losses.

TABLE XI

**Contribution of 1.20 Grams of Cow's Milk Protein and of 1.70 Grams of Corn-Bean 76:24 Protein Mixture to the Theoretical Amino Acid Requirements of the 2-Year-Old Child**

| Amino acid | Requirement <sup>a</sup><br>(mg/kg/day) | Amino acids in<br>1.20 gm of cow's<br>milk protein<br>(mg) | Amino acids in<br>1.70 gm of corn-bean<br>76:24 protein<br>mixture<br>(mg) |
|------------|---|--|--|
| Isoleucine | 48                                      | 64   | 65   |
| Leucine    | 84                                      | 119  | 194  |
| Lysine     | 66                                      | 88   | 65   |
| TSAA       | 42                                      | 42   | 53   |
| TAAA       | 72                                      | 118  | 144  |
| Threonine  | 48                                      | 50   | 63   |
| Tryptophan | 12                                      | 17   | 12   |
| Valine     | 60                                      | 77   | 82   |

<sup>a</sup> Based on the amounts of amino acids in 1.20 gm of a protein with the "provisional" amino acid pattern of FAO/WHO (8).

1.20 gm/kg/day for milk and at 1.70 gm/kg/day for corn-bean protein. The agreement with the theoretically predicted values (0.90 and 1.20 gm for milk protein; 1.33 and 1.74 gm for corn-bean 76:24 protein) is remarkably good. Tables XI and XII illustrate the reason for this agreement in a different form.

### Interpretation and Significance

The diet was well accepted with no sign of intolerance and was easily consumed by the children at all levels of protein tested.

The results indicate that it is feasible to feed children of this age (around 2-3 years old) with such a cereal-legume mixture. The mixture (not the diet) contains 9.6% protein and its cost is around 0.05 United States dollars a pound. Children weighing 10 kg should consume  $10 \times 1.70 = 17.0$  gm/day of protein at a cost of 1.9 cents a day.

The next question is: Why this ratio of corn and beans? As mentioned above, the ratio of 76% corn protein to 24% bean protein (87% corn + 13% beans) was selected for our studies because thorough diets surveys have shown this to be the proportion in which the rural Guatemalan populations actually consume these foods. However, rat experiments indicate that mixtures in which approximately half of the protein is contrib-

TABLE XII

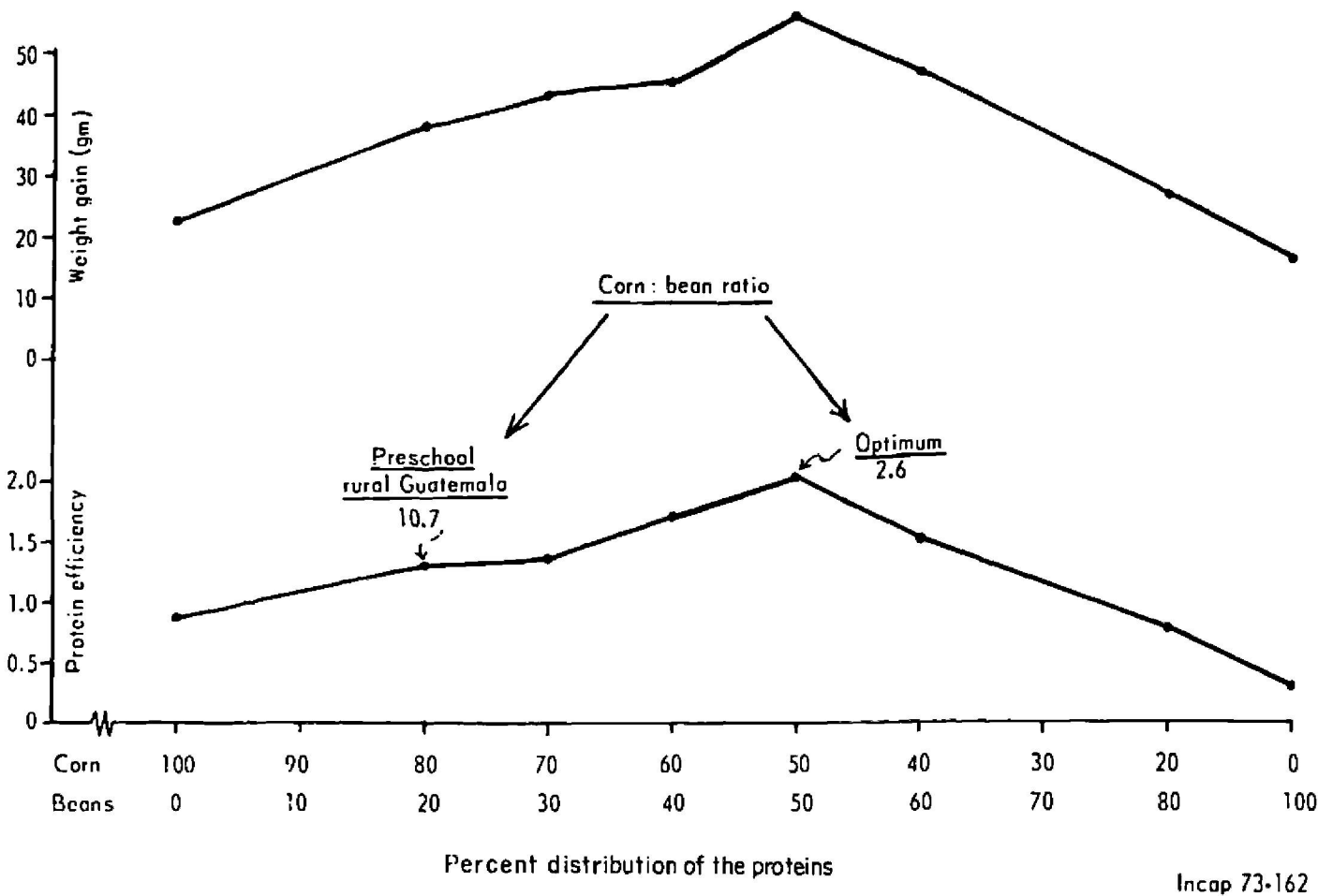
Contribution of Several Levels of Intake of Corn-Bean 76:24 Protein Mixture to the Amino Acid Requirements of Preschool (2-Year-Old) Children

| Amino acid | Average requirement + 30 % <sup>a</sup> amino acid in 1.20 gm of "ideal" protein (mg/kg/day) | Amino acid content and percent of requirement |       |                      |       |                      |       |                      |       |
|------------|--|---|-------|----------------------|-------|----------------------|-------|----------------------|-------|
|            |  | 1.20 gm                                       |       | 1.38 gm <sup>b</sup> |       | 1.70 gm <sup>b</sup> |       | 1.74 gm <sup>c</sup> |       |
|            |  | mg  | %     | mg                   | %     | mg                   | %     | mg                   | %     |
| Isoleucine | 48   | 45.6  | 95.0  | 52.4                 | 109.2 | 64.6                 | 134.6 | 66.1                 | 137.7 |
| Leucine    | 84   | 136.8   | 162.8 | 157.3                | 187.3 | 193.8                | 230.7 | 198.3                | 236.8 |
| Lysine     | 66   | 45.6  | 69.1  | 52.4                 | 79.4  | 64.6                 | 93.9  | 66.1                 | 100.2 |
| TSAA       | 42   | 37.2  | 88.6  | 142.7                | 101.7 | 52.7                 | 125.5 | 53.9                 | 128.3 |
| TAAA       | 72   | 102.0   | 141.7 | 117.3                | 162.9 | 144.5                | 200.7 | 147.9                | 205.4 |
| Threonine  | 48   | 44.4  | 92.5  | 51.0                 | 106.2 | 62.9                 | 131.0 | 64.3                 | 134.0 |
| Tryptophan | 12   | 8.5   | 71.0  | 9.8                  | 81.6  | 12.1                 | 100.8 | 12.4                 | 102.9 |
| Valine     | 60   | 57.6  | 96.0  | 66.2                 | 110.3 | 81.6                 | 136.0 | 83.5                 | 139.2 |

<sup>a</sup> Individual variability on the basis of protein requirement (8).

<sup>b</sup> Value of  $x$  at which the regression line and the line of 95 % confidence limit cross the  $y$  value of 20.7 mg/kg/day nitrogen retention (See Fig. 2.)

<sup>c</sup> Theoretical adequate intake on the basis of lysine (most limiting).



**Fig. 3** Protein quality of different combinations of corn and beans. Data from reference (1).

uted by corn and half by beans give the highest protein quality, as illustrated in Fig. 3, from the work of Bressani *et al.* (1). Furthermore, given free choice between two feed dishes, one containing corn and the other beans, rats select a ratio of about 78% corn and 22% beans, which provides just about 60% of the protein from corn and 40% from beans, much richer in bean protein than the diet actually consumed by the rural Guatemalan populations (2).

Based on these considerations we have tested a corn-bean mixture, as the sole source of protein for 2-year-old children, at a ratio that provides 60% of the protein from corn and 40% from beans, at a level of intake of 1.75 gm/kg/day and 100 cal/kg/day with 20% of the calories from fat. As in previous experiments, the requirements of all other essential nutrients were met by adding vitamins and minerals. The data are still incompletely analyzed but we can already report that under the conditions of this trial the four children responded very well, maintaining throughout 3 months a normal growth curve, and accepting the diet with good appetite. Table XIII shows how such a diet is adequate to meet the essential amino acid requirements of these children even at a lower level of intake (1.57 gm/kg/day). Furthermore, when corn and beans were offered separately

TABLE XIII

Contribution of Several Levels of Intake of Corn-Bean 60:40 Protein Mixture to the Amino Acid Requirements of Preschool (2-Year-Old) Children

| Amino acid | Average requirement + 30 % <sup>a</sup> amino acid in 1.20 gm of "ideal" protein (mg/kg/day) | Amino acid content and percent of requirement |       |                      |       |                      |       |
|------------|--|---|-------|----------------------|-------|----------------------|-------|
|            |  | 1.20 gm                                       |       | 1.57 gm <sup>b</sup> |       | 1.75 gm <sup>c</sup> |       |
|            |  | mg  | %     | mg                   | %     | mg                   | %     |
| Isoleucine | 48   | 46.8  | 97.5  | 61.2                 | 127.5 | 68.2                 | 142.0 |
| Leucine    | 84   | 127.2   | 151.4 | 166.4                | 198.0 | 185.5                | 217.3 |
| Lysine     | 66   | 54.0  | 81.8  | 70.6                 | 106.9 | 78.7                 | 119.2 |
| TSAA       | 42   | 33.6  | 80.0  | 43.9                 | 104.5 | 49.0                 | 116.6 |
| TAAA       | 72   | 99.6  | 138.3 | 130.3                | 180.9 | 145.2                | 201.7 |
| Threonine  | 48   | 45.6  | 95.0  | 59.6                 | 124.1 | 66.5                 | 138.5 |
| Tryptophan | 12   | 9.1   | 75.8  | 11.9                 | 99.4  | 13.3                 | 110.9 |
| Valine     | 60   | 57.6  | 96.0  | 75.3                 | 125.5 | 84.0                 | 140.0 |

<sup>a</sup> Individual variability on the basis of protein requirement (8).  
<sup>b</sup> Theoretical adequate intake on the basis of tryptophan (most limiting).  
<sup>c</sup> Actual amount fed to children in experiment (as described in text).

to these children, in order to allow them to select a free-choice ratio of these foods *ad libitum*, they chose a caloric intake of not less than 90 cal/kg/day in five mealtimes a day, which was composed of a mixture higher in beans than the rural area population of Guatemala gets (about 50:50 of protein from each source) and which is higher in protein quality.

These results pose the interesting question of why the rural, low-socio-economic Guatemalan families choose a nutritionally less favorable ratio of corn and beans. My view is that the main reason for this is economic limitation. Beans cost, in Guatemala, between three and four times more than corn, and because the majority of these people are extremely poor, they satisfy their energy needs with the cheapest source of calories.

The children in our study have been fed rations distributed in five meals a day containing 20% of calories from fat. This nutritional plan minimizes the problem of excessive bulk (low caloric density) that may be encountered with diets composed mainly of cereals and legumes. Low caloric density may prevent children from consuming enough quantity to satisfy their caloric requirement. It would be more difficult to feed all the food of this type in only two meals a day. Caloric density, number of meals, or both deserve most careful consideration and both are presently being studied in our laboratories.

To conclude I should like to reemphasize the general significance that

our results may have, not only in terms of nutrition but also in terms of economics. They strongly suggest that utilizing the diets and foods already available in our communities in a more rational way as an approach to preventing protein-calorie malnutrition is quite feasible. This may mean, in fact, that the countries facing this problem do not have to depend on the so-called "high-protein sources." These are for the most part "foreign" not only in origin (they have to be imported), but also from the point of view of cultural food patterns.

As have other researches into the problem of PCM, moreover, the results suggest that the main nutritional problem facing the populations of developing countries is not so much one of quantity and quality of protein per se, but one of the quantity of food they already consume. This situation is obviously being worsened by exaggerated metabolic losses of nutrients caused by diarrhea and infectious diseases in general.

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# Discussion

*(Arroyave paper)*

**Dr. Scrimshaw:** There are five reasons why these very meticulously conducted balance studies of Dr. Arroyave's may not apply to the field.

1. On the metabolic ward, the food has been supplied in five approximately equal meals a day, whereas the pattern of two to three meals a day is more common in the villages.

2. The caloric density of the experimental diets has been increased by adding fat, with the intention of ensuring that calories do not limit the utilization of the protein consumed. Most families in Guatemala, however, have little or no free fat to add to their diets because fat is costly.

3. The duration of the study periods is relatively short and the child has been fully replete at the start of each such period.

4. The proportion of corn and beans fed has been based on the average proportions found in dietary surveys, but many village children consume diets with a smaller proportion of beans relative to corn than the average and would be at greater risk of insufficient protein.

5. Children in the villages have frequent infections that significantly increases protein needs above those of children studied under the protected conditions of the metabolic ward.

Under village conditions, there is a particularly high frequency of respiratory and diarrheal disease during the early preschool years as well as of the common communicable diseases of childhood (7). With each episode, the child is not only depleted by an associated decrease in food intake and by a decreased absorption

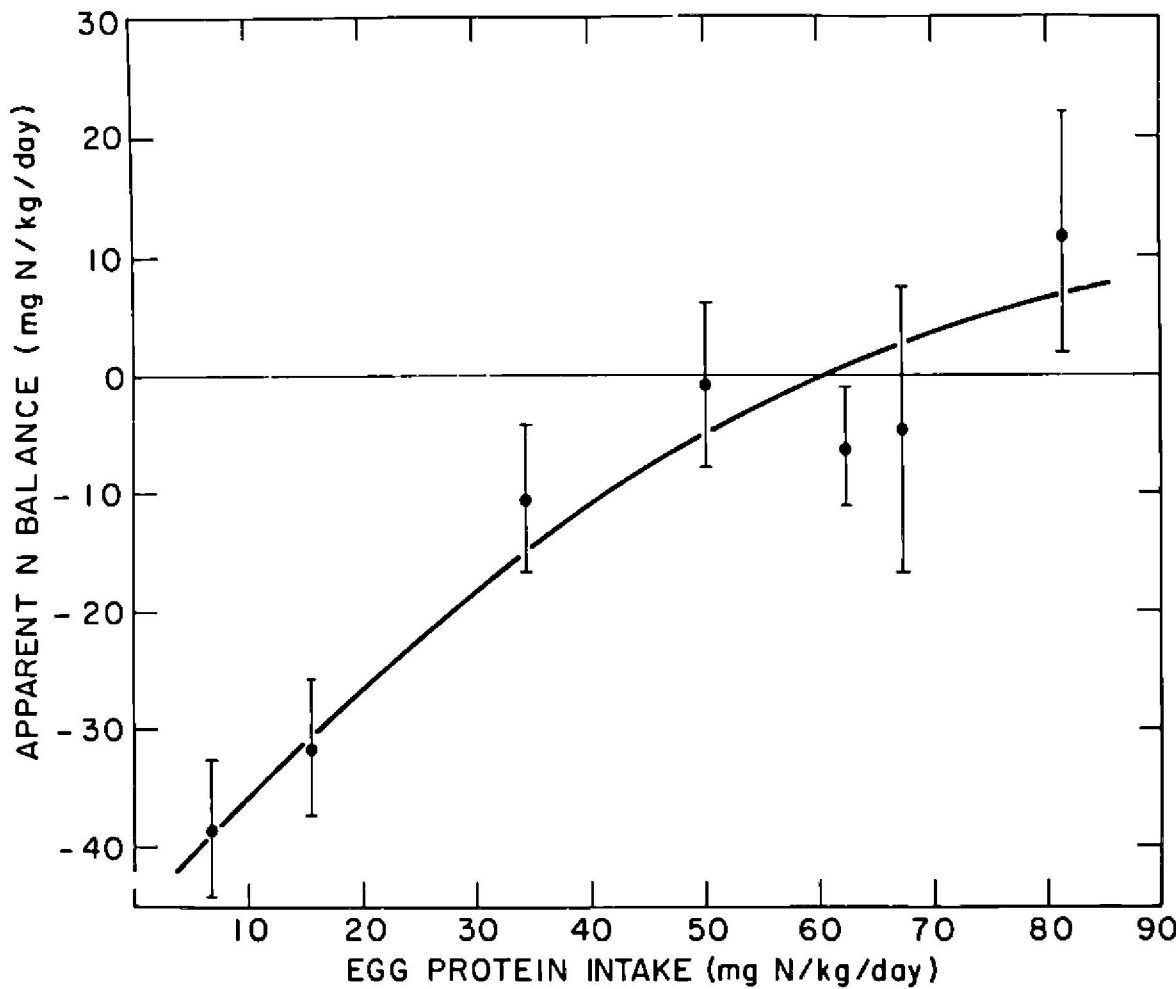


if the gastrointestinal tract is involved, but also by direct metabolic loss in the urine of nitrogen and a number of other essential nutrients as a result of the stress response.

It has been assumed, without sufficient critical evaluation, that the efficiency of dietary nitrogen utilization is constant for protein intakes at and below the minimum maintenance requirement in the adult human or up to a level approaching maximal growth in the child. This assumption is crucial in relation to the comparisons of biological value (BV). However, the recent findings by Calloway and Margen (3) and by Inoue *et al.* (6) raise serious doubts about whether the efficiency of dietary N utilization is constant within the submaintenance range of protein intake. Similarly, studies conducted in our laboratory provide data on the dietary N utilization in young men given egg protein at levels either approximating or well below their maintenance N requirement. A graphic summary of our findings is shown in Fig. 1.

Similar conclusions can be drawn from studies with children, as shown in Fig. 2, taken from the data of Bressani and Viteri (2).

How are maximum values for protein utilization as now conventionally obtained related to the corresponding values at levels sufficient to satisfy protein needs? Relevant evidence has been reviewed at the 1971 Joint FAO/WHO Expert Committee on Protein-Calorie Requirements (4), whose report is in press. The available data



**Fig. 1** Relationship between apparent N balance and intake of egg protein within the submaintenance range of nitrogen intake in young men. The regression line is described by the equation  $y = -0.005X^2 + 1.051X - 45.3$ ; where  $y$  = N balance and  $x$  = N intake, expressed as mg N per kilogram body weight per day. Vertical bars indicate  $\pm\sigma$  for each point.

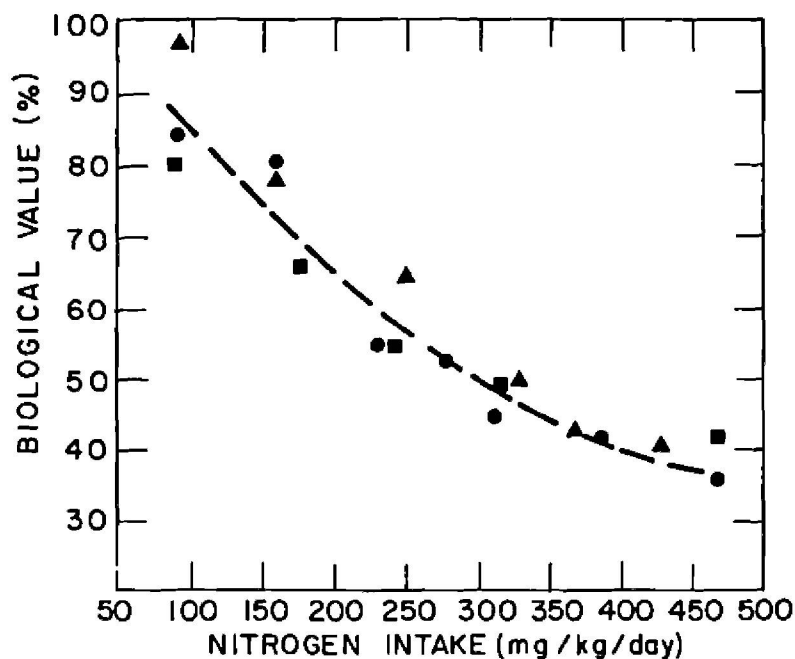


Fig. 2 Relationship between N intake and BV of three protein sources in children. (▲), Whole egg; (●), milk; (■) Incaparina (formula number 9). Reprinted from reference (2) with permission of the Institute of Food Technologists.

suggest that about 30% more protein is required to maintain nitrogen equilibrium than can be predicted from either factorial losses or the NPU of proteins tested in the conventional way. This figure is derived from studies with proteins of high biological value, particularly milk and egg, although it presumably also applies to a mixed diet of relatively high protein value. If one is concerned, however, with the ability of certain relatively poor quality proteins, such as wheat gluten, to meet protein requirements, there is considerable evidence that the figure must be much higher than 30%. In other words, considerably more of most vegetable proteins is required to maintain nitrogen balance than is predicted from their protein value relative to milk and egg at the usual test level (8). The conclusion, as both Hegsted (5) and Bressani and Viteri (2) have pointed out and as has been proposed earlier by Allison (1), is that it is necessary to evaluate proteins at more than one level of intake. One can then use differences in the ratio of the slopes of the resulting regression lines to compare proteins of widely different qualities and to predict the amount of a given protein that is required for nitrogen equilibrium (9).

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**Dr. John F. Mueller:** Dr. Arroyave, would you comment on the question of niacin adequacy in relationship to tryptophan intake by children in the field? I assume that your subjects on the ward were receiving adequate amounts of niacin. What is the situation in the villages?

**Dr. Waterlow:** What is the pattern of essential amino acids required at different ages for maintenance and for growth? The maintenance requirements should depend on the extent to which essential amino acids are oxidized or recycled and this may differ for different amino acids; but during growth there is a concomitant synthesis of new tissue that may have a different amino acid composition than "old tissue." It seems to me, therefore, that there should be a different pattern of essential amino acids in the two conditions but, as far as I can make out, this has never been established.

**Dr. Arroyave:** I am in agreement with most of the points that Dr. Scrimshaw has made. It will be of interest to see what may happen if we can provide the villages of Guatemala with corn and beans at the proper ratio and at equal price. The introduction of fat into the diet is critical because of the caloric deficit and the high bulk but this requires economic subsidy and nutrition education. It is my belief, however, that Guatemalan children eat more than two meals a day and that certainly we can educate them to eat at least three times a day. It is, of course, a nonlimiting variable in the experiments done on the ward. In the villages, corn is a staple food. One of the limiting amino acids is tryptophan but fortunately for our population the intake of niacin is sufficiently high to prevent pellagra. We have assumed that the limited amount of tryptophan goes primarily for protein synthesis. Additional work, of course, is needed to answer this question.

With regard to Dr. Waterlow's concern about different patterns of essential amino acids at different ages, our data indicate there are no important differences in the patterns. The differences are, as he noted, in the ratio of essential amino acids and total nitrogen. It is likely that the new tissue arising during growth has the same average amino acid composition as old tissue and, therefore, no distinction is possible between the patterns required for maintenance and growth.