5-1977

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Arthur John Wittwer
Utah State University

Recommended Citation

Wittwer, Arthur John, "The Influence of Various Factors on Nitrogen Balance and Protein Quality Measured in Adult Human Beings" (1977). *All Graduate Theses and Dissertations*. 5206.
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THE INFLUENCE OF VARIOUS FACTORS ON NITROGEN BALANCE AND PROTEIN QUALITY MEASURED IN ADULT HUMAN BEINGS

by

Arthur John Wittwer

A thesis submitted in partial fulfillment
of the requirements for the degree
of
MASTER OF SCIENCE
in
Biochemistry

UTAH STATE UNIVERSITY
Logan, Utah
1977

ACKNOWLEDGMENTS

Dr. R. Gaurth Hansen has provided encouragement and advise throughout the course of this study. I am deeply grateful to him, not only for this specific help, but also for the general academic guidance he has offered me, both as an undergraduate and as a graduate student. The members of Dr. Hansen's laboratory group, both past and present, have provided both pleasant and meaningful association. The opportunity to work with individuals like Jim Hulse, Rick and Marlys Turnquist, Ann Sorenson and Bonnie Wyse, has been the most profitable experience of my education thus far.

I would like to thank Dr. D. M. Hegsted of the Department of Nutrition, Harvard University School of Public Health, for his critical commentary on my thesis problem via correspondence. I would also like to thank Dr. Thomas M. Farley, Dr. Arthur W. Mahoney and Dr. Grant Gill Smith, members of my committee, and Dr. Thomas F. Emery of the Department of Chemistry and Biochemistry, for the continual interest and concern they have shown for my work and for my academic progress in general. Dr. Rex L. Hurst and Dr. David White of the Department of Applied Statistics and Computer Science provided much-needed counsel concerning statistical procedure.

Two important groups contributed materially to this study. The Division of Consumer Studies, Bureau of Foods, Food and Drug Administration provided the financial support (Contract No. 223-74-2101) and innumerable researchers in the field of human protein nutrition provided the raw data.

I am very thankful to family members for their encouragement, assistance and patience during the writing and final preparation of this thesis. To my wife, Mary Sue, and my daughter, Kathleen, I express a special love and appreciation. Their love, their support, and their confidence in me, make any success which I might enjoy truly worthwhile.

Arthur John Wittwer

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ABSTRACT

The Influence of Various Factors on Nitrogen Balance and Protein Quality Measured in Adult Human Beings

by

Arthur John Wittwer, Master of Science

Utah State University, 1976

Major Professor: Dr. R. Gaurth Hansen
Department: Chemistry and Biochemistry

The effect of nitrogen intake, nitrogen source, calorie intake, body weight, adaptation time, research group and sex on the nitrogen balance of human adults was investigated. Data from studies reported in the literature were combined and analyzed statistically by multiple regression techniques. Analyses were made separately for six sources of nitrogen: egg, beef, rice, corn, wheat and wheat gluten.

Nitrogen intake, caloric intake and body weight exerted significant effects on nitrogen balance (5% level of confidence) for six, three and two of the six nitrogen sources, respectively. Other variables were not significant at the 5% level. Although differences were not significant (5% level), the correlation between nitrogen intake and nitrogen balance was greatest for four of the six nitrogen sources when data were expressed as grams per square meter of body surface area (g/m^2) as opposed to when they were expressed per kilogram body weight or per kilogram raised to the 0.73 power.

Curvilinear relationships between intake and balance in the submaintenance range of intakes were evident for all protein sources except corn. The regression lines for several protein sources tended to converge at both lower and higher levels of intake. At levels of nitrogen intake below 1 g/m², protein appeared to be utilized with near 100% efficiency, regardless of source. At levels of intake above 4.4 g/m², all nitrogen sources except wheat gluten gave nitrogen balances which did not differ significantly (5% level). In general, caloric intake exerted a positive but diminishing effect on nitrogen balance when nitrogen intake was held constant and caloric intake increased from maintenance to excessive levels. The mean amount of egg nitrogen required to achieve zero nitrogen balance decreased from 3.1 g/m² to 2.2 g/m² as caloric intake increased from 1475 kilocalories per square meter of body surface area (kcal/m²) to 1760 kcal/m².

The findings are discussed in terms of present energy and protein requirements, the traditional concepts of the biological value of proteins, and the prediction of protein quality from amino acid composition.

142 pages

INTRODUCTION

Most of the nitrogen in living systems is present in protein molecules or free amino acids. Other nitrogen-containing substances typically account for only 5 to 15% of the total nitrogen in the body or in the diet (Bigwood, 1972; Munro, 1964a). For this reason, nitrogen (N) intake and N excretion are looked upon as measures of the flow of protein into and out of the body. Nitrogen balance--N excretion minus N intake--is used as an indicator of protein balance. Negative N balance means the body is experiencing a net loss of protein, while N balances of zero or positive are compatable with maintenance of body protein or growth, respectively. If two protein sources are fed at equal levels of intake, the one giving the most favorable nitrogen balance response will be the one which is of highest quality, that is, used most efficiently.

These are, of course, simplifications. They are such useful simplifications, however, that conclusions regarding protein requirements and efficiency of protein utilization are based largely upon measurements of nitrogen balance. This was recognized by the Joint Food and Agriculture Organization-World Health Organization (FAO/WHO) Ad Hoc Expert Committee (FAO/WHO, 1973) who chose to express protein requirements in terms of crude protein, derived by multiplying N content by the factor 6.25 grams of protein per gram of N. Hence, "protein" requirements established by the Committee were actually nitrogen requirements. Such an equivalence of protein and nitrogen will be used in the present study. "Protein" will refer to N multiplied by 6.25.

There are a significant number of studies in the recent literature which report the nitrogen balance of adults fed diets of controlled protein content. These studies represent a large investment of time, resources and patience on the part of both the researcher and the subjects. Low protein test diets of a highly purified nature are monotonous and sometimes unpleasant, while the collection and analysis of feces and urine for nitrogen can be described, at best, as tedious.

Economics would dictate that as much information be gleaned from these studies as possible. For example, caloric intake has been shown to significantly affect nitrogen balance status. Yet researchers frequently report caloric intake for their experimental subjects without drawing any conclusions as to the effect caloric intake might have had on the nitrogen balances they obtained. A similar situation exists for body weight and height. Some researchers use these variables (by expressing measurements per kilogram body weight or per square meter of body surface area) to help explain experimental variation and others do not. In either case the values are reported and await analysis.

This thesis is part of a larger effort to relate biological data on protein utilization in adult human beings with chemical data on the amino acid composition of proteins. Key in this process is the definition of a suitable biological measure of protein quality or utilization efficiency which could be calculated from nitrogen balance data. In this process, nitrogen balance must be corrected for the various factors--other than the amino acid content of the protein in question--which influence it. It is the aim of this thesis to investigate the quantitative effect of factors such as caloric intake, nitrogen intake, body weight and size on nitrogen balance in adult subjects. Conclusions will be drawn about the feasability of correcting for these effects and deriving a standardized measure of protein quality from nitrogen balance measurements. It is hoped that this will stimulate further investigation into both the metabolic mechanisms responsible for these effects and the influence of amino acid composition on protein quality.

LITERATURE REVIEW

Differences in Protein Quality

It has long been recognized that some proteins are of greater nutritional value than others. Munro (1964c) has provided a concise review of the development of this concept. In the early 1800's a French physiologist, Francois Magendie, was the first to recognize that nitrogen-containing substances are essential in the diet. He also noted that gelatin was for some reason inferior to other sources of nitrogen. Thus, dogs not only died when fed exclusively sugar or olive oil, but could also not be maintained on a diet of pure gelatin. Diets of only cheese or eggs resulted in very weak but at least live animals. In the mid-nineteeth century, other researchers, among them Liebig and Voit, reported the nutritional difference of gelatin from other proteins.

The development of the nitrogen balance technique during the nineteenth century marked the beginning of quantitative measures of protein quality. In 1909 Thomas defined a method for the determination of the biological value of proteins based on efficiency of absorbed nitrogen utilization. This method was later refined by Mitchell (1924) who began extensive measures of biological value in rats.

Biological value (BV) is defined as the percentage of absorbed dietary nitrogen (N) which is retained and utilized by the body. If nitrogen balance (B) is defined as:

$$B = I - (U + F)$$

where I is N intake and U and F are nitrogen in urine and feces, then

$$BV = \frac{B + (E_u + E_f) \times 100}{I - F + E_f} = \frac{\text{retained N} \times 100}{\text{absorbed N}} \qquad (1)$$

where E_u and E_f are the obligatory or endogenous losses of N in the urine and feces measured when a N-free diet is fed. Measurement of endogenous nitrogen losses is important to correct the apparent absorption (I - F) and the apparent retention (B) for nitrogen originating with the body and not with the test protein. For the proteins Thomas tested using himself as a subject, a wide variation in biological value was observed.

Net Protein Utilization (NPU) is a measure of protein quality based on nitrogen balance first described by Miller and Bender (1955). It is defined as the proportion of ingested nitrogen which is retained by the body:

$$NPU = \frac{B + (E_u + E_f) \times 100}{I} = \frac{\text{retained N} \times 100}{\text{ingested N}} \qquad (2)$$

NPU is equal to BV multiplied by the coefficient of true digestibility (D). In other words, if both sides of equation (1) are multiplied by the quotient absorbed N/ingested N, equation (2) results:

$$\begin{aligned} BV \times D &= \frac{B + (E_u + E_f) \times 100}{I - F + E_f} \times I - \frac{F + E_f}{I} \\ &= \frac{B + (E_u + E_f) \times 100}{I} \\ &= NPU \end{aligned}$$

where

$$D = \frac{I - F + E_f}{I} = \frac{\text{absorbed N}}{\text{ingested N}}$$

Growth implies a sustained positive nitrogen balance. The magnitude of this balance and therefore the amount of growth is another measure of the efficiency of nitrogen utilization. A quantitative measure of growth-related protein quality was described by Osborne, Mendel and Ferry (1919). Their method, termed the protein efficiency ratio (PER), was defined as the gain in body weight of young test animals divided by the quantity of protein consumed:

$$\text{PER} = \frac{\text{grams gain in body weight}}{\text{grams protein consumed}}$$

Because of the relatively slow weight gain of even human infants, measurements of PER values have been confined primarily to young weanling rats.

Table 1 lists the biological values of several protein sources for both man and the weanling rat. PER measurements made in young weanling rats are included for comparison. In the preparation of Table 1 it was noted that BVs measured in human subjects showed a marked dependence upon protein level (Young, 1975). The BVs given in Table 1 are therefore grouped according to the N intake, rounded to the nearest gram, at which they were determined.

There are many interesting aspects of the data in Table 1, some of which will warrant further discussion. At present, however, it is sufficient to point out that differences in protein quality are evident. If one examines the BVs measured in human subjects at an intake of about 4 grams of N, at which the differences between different proteins appear to be maximized, one finds that they are roughly comparable to those measured in the young rat. Additionally, the PERs tend to correlate with the BVs.

Table 1. Biological values and protein efficiency ratios for selected protein sources

	BV for human adults at nitrogen intakes (g/day) of:					BV and PER for young weanling rats[a]:	
Protein source	2g	3g	4g	5g	6g	BV	PER
Whole egg	107[b] 94[f] 91[h]	97[c] 93[b]	96[d] 92[e]	65[b] 65[g]	89[e] 72[b]	93.7	3.92
Beef	79[i]	84[c]	70[i]	67[f] 65[i]	61[i]	74.3	2.30
Milk		79[j]	74[k] 62[f]	52[j]		84.5	3.09
Casein		92[l]	69[f]	68[f]		79.7	2.86
Peanut		83[c]		56[f]		54.5	1.65
Soybean		81[c]	80[e] 65[k]			72.8	2.32
Whole wheat			65[i]	48[i]	45[i] 28[j]	64.7	1.53
Wheat gluten	85[m]		45[m]	42[f]	37[m]	58.2	0.51

[a] FAO, 1970. These are average values for a protein level of about 10%.

[b] Young et al., 1973

[c] Murlin, Edwards and Hawley, 1944

[d] Young et al., 1971

[e] Murlin et al., 1946b

[f] Hawley et al., 1948

[g] Sumner and Murlin, 1938

[h] Bricker and Smith, 1951

[i] Young et al., 1975

[j] Martin and Robison, 1922

[k] Bricker, Mitchell and Kinsman, 1945

[l] Mueller and Cox, 1947

[m] Inoue et al., 1974

Since the first descriptions of BV, NPU and PER, many researchers have added refinements, discussed the importance of standardized conditions and have described other indices of protein quality based on nitrogen balance, growth, or other criteria (McLaughlan and Campbell, 1969; Allison, 1964; Hartog et al., 1972). Whatever method is employed to measure protein quality, however, the picture is similar to that presented in Table 1: there are almost as many differences in protein quality as there are different sources of protein.

Chemical Methods for the Prediction of Protein Quality

The first experiments relating protein quality with chemical data took place in the early 20th century when it became possible to partially analyze proteins for their amino acid content (Munro, 1964c). The condition of young rats fed zein (a protein completely lacking in tryptophan) was improved by the addition of tryptophan (Willcock and Hopkins, 1906). If tryptophan and lysine were added, normal growth was sustained (Osborne and Mendel, 1914).

In the years that followed, other proteins were investigated. It was similarly found that for most proteins the addition of one specific amino acid would improve the growth or nitrogen retention of young rats. Other amino acids had no such effect. The reason for this limiting-supplementary nature of certain amino acids became clear with the classification by Rose and coworkers of amino acids as dispensible or indispensible in the diet of the rat (Rose, Oesterling and Womack, 1948) and of man (Rose, Haines and Warner, 1954).

An indispensible or essential amino acid cannot be synthesized either rapidly enough or at all to make up for its shortage in a protein source. It was reasoned, therefore, that nitrogen retention is effectively limited by the essential amino acid in shortest supply in relation to its requirement.

Chemical scoring methods

In 1946, Mitchell and Block compared the results of amino acid analyses with biological data on nitrogen utilization in the growing rat. In 16 out of 20 cases "the largest percentage deficits in essential amino acids for the various food proteins [were in harmony with] the amino acids limiting the growth-promoting values of the proteins for the rat." (Mitchell and Block, 1946, p. 604) When they compared the greatest percentage deficits with the rat biological values of twenty-three proteins, the correlation was -0.861.

The determination of the greatest percentage deficit in an essential amino acid in relation to a reference amino acid pattern forms the basis for the calculation of a protein's chemical score. Block and Mitchell (1946) defined chemical score as the smallest percent sufficiency in amino acid content when compared to a protein taken as the standard, i.e., as the greatest percentage deficit subtracted from 100. Chemical score may, therefore, be defined as the minimum of all possible percentages (A_i/S_i) x100 where A_i is the test protein content of essential amino acid i and S_i is the standard protein or pattern content of essential amino acid i. Because of sparing effect of cysteine and tyrosine on the methionine and phenylalanine requirements, respectively, the contents of total sulfur containing amino acids (methionine and cysteine) and total aromatic amino acids (phenylalanine and tyrosine) in the test protein as compared with the standard are generally used in forming the A_i/S_i ratios.

There have been several suggestions to improve the basic concept of Mitchell and Block. Oser (1951) showed that the geometric mean of all percentage sufficiencies of essential amino acids (the essential amino acid (EAA) index) also correlated well with the same biological values used by Mitchell and Block, with a correlation coefficient of 0.85. The EAA index may be defined

$$\text{EAA index} = 100 \sqrt[n]{\frac{A_1}{S_1} \times \frac{A_2}{S_2} \times \frac{A_3}{S_3} \times \ldots \times \frac{A_n}{S_n}}$$

where n is the number of essential amino acids. Armstrong and Mitchell (1955) suggested that the A_i/S_i ratios be calculated using total sulfur-containing and total aromatic amino acid contents with a protein being credited with no more cysteine or tyrosine than were present in the standard protein or pattern. The original Oser method combined the methionine and cysteine contents, but did not include tyrosine with phenylalanine.

Although the EAA index has been criticized by several authors on theoretical grounds (Payne, 1972; McLaughlan and Campbell, 1969), it was shown by Mitchell (1954) to correlate better with biological values measured in growing rats, pigs and dogs, than chemical score. In addition, Bender (1975) suggests that the EAA index might preferentially be used to predict the supplementary effects which result when proteins are mixed.

In the calculation of both chemical score and the EAA index, excesses of essential amino acids in the test protein relative to the reference amino acid pattern were not considered of importance. An A_i/S_i ratio greater than 1.00 was set equal to 1.00. Korpaczy, Lindner and Varga (1961) hypothesized that excesses of essential amino acids might cause a detrimental effect on BV. They modified the EAA index calculation such that if the ratio of a test protein to pattern amino acid was greater than one, the reciprocal of the ratio was used in calculating the geometric mean. Also included in their formula was an allowance for differences in non-essential nitrogen content of the test and pattern proteins. Their formula may be represented as follows:

$$BV = 75 \sqrt[n]{R_1 \times R_2 \times R_3 \times \ldots \times R_n} + 25 \left[1 - \frac{A_{NE} - P_{NE}}{P_{NE}} \right]$$

where R_i is equal to A_i/P_i if this ratio is less than or equal to one and is equal to P_i/A_i, otherwise; n is the number of essential amino acids; and A_{NE} and P_{NE} are the amounts of non-essential amino acids in the test and pattern proteins, respectively. The authors do not present any evidence that their method is superior to that of Oser (1951) or the even simpler chemical score.

The 1963 joint FAO/WHO Expert Group on Protein Requirements (FAO/WHO, 1965) proposed the calculation of a chemical score based on the most limiting ratio of each essential amino acid to the total essential amino acids (A/E ratio) in a protein. The A/E ratios of whole egg protein were used as reference. Such scores, however, have been shown to correlate poorly with biological value (Payne, 1972; Cresta et al., 1971).

An important variable in determination of chemical score is the reference amino acid pattern used. Mitchell and Block (1946) suggested using the amino acid pattern of whole egg because of its high digestibility and utilization for both rodent and human nutrition. The Joint FAO Expert Group on Protein Requirements (FAO, 1957) developed a pattern based on human amino acid requirements. Because of criticism that the suggested pattern contained excesses of tryptophan and the sulfur-containing amino acids, the 1963 Expert Committee (FAO/WHO, 1965) re-evaluated the pattern. Lowering the levels of the amino acids in question made the pattern similar to that in egg or human milk. The Committee therefore suggested the use of either of these proteins as standards. The 1971 Joint FAO/WHO *Ad Hoc* Expert Committee on Energy and Protein Requirements (FAO/WHO, 1973) recommended an amino acid scoring pattern based on the amino acid requirements of children. They emphasized, however, that the resultant chemical score should be compared with the ratio NPU-of-test-protein/NPU-of-egg-or-milk, rather than the absolute NPU.

A minority of those in the 1971 Expert Committee disagree with the procedure which was adopted. They felt that chemical score should predict the absolute NPU rather than the NPU/NPU-of-egg-or-milk ratio and that the scoring pattern should be so adjusted. This approach has been explored by Payne (1972). By examining seventeen protein sources, seven of which were limiting in lysine and the remainder in the sulfur-containing amino acids, the levels of these amino acids in the scoring pattern which gave the best prediction of measured NPU were calculated. Although by this procedure the absolute deviation of predicted from measured NPU was decreased (8 percent as compared to 15 percent when calculations are based on the FAO/WHO (1965) chemical scoring pattern), the correlation between predicted and measured values remained unchanged (0.935 as compared to 0.930).

Cresta et al. (1971) studied the relationship between chemical score and BV for 87, NPU for 65, and PER for 187 different food samples. The scoring pattern used exerted an important effect on the correlations observed. While scores calculated on the basis of egg protein patterns correlated the best with NPU and PER, a milk protein pattern chemical score correlated best with BV. The best correlations between chemical score and BV, NPU and PER were 0.673, 0.827 and 0.726, respectively.

Multiple regression methods

It has been suggested that the levels of all the essential or of all essential and non-essential amino acids are important factors in the prediction of protein quality. The EAA index of Oser (1951) and its modification by Korpaczy, Lindner and Varga (1961) are examples of prediction formulas which have attempted to take this into account.

Cresta et al. (1971) undertook a unique approach to the prediction of protein quality. They carried out multiple regression analyses of biological measures of protein quality with essential amino acid content. Taking data subsequently to be published by the FAO (1970) they attempted to predict the biological value of 87, the NPU of 65, and the PER of 187 protein sources. The prediction equation they used was of the form

$$Y = m_1X_1 + m_2X_2 + \ldots m_nX_n + b$$

where Y is the predicted value for the biological measure in question, X_i is the level of amino acid i, m_i is the coefficient describing the effect of X_i on Y, b is some constant, and n is the number of amino acids included in the analysis. A correlation of 0.71 between measured and calculated BV was reported. This compared with a correlation of 0.67 between measured BV and the best chemical score prediction using milk as the reference protein. Cresta et al. concluded that it would be necessary to make fresh biological studies and estimate amino acids in the same samples to increase this correlation.

Hansen and Eggum (1973) conducted a multiple regression analysis similar to those of Cresta et al. (1971), using data in which both rat BV measurements and amino acid analyses had been performed on identical samples of 221 different feeds. A correlation coefficient of 0.74 was found between measured and predicted BV. When samples were divided into seven food groupings and "dummy" variables were included in the regression model to correct for the effect of feed stuff groupings on BV, the correlation increased to 0.85. The researchers explained this increase to be due to a reduction in the effects of amino acids imbalance, antagonism, and availability, which were assumed to be the same within a given food grouping for purposes of the model.

Solubility-related methods

There have been other attempts to define protein quality in terms of chemical measurements. Almquist, Stokstad and Halbrook (1935) developed a protein quality index based on a chemical determination of solubility and digestibility. Their index was defined:

$$\text{Protein quality index} = A - (B + 0.6C) + 0.4D$$

where A is the percent protein precipitated by copper salts, B is the percent protein not digested with pepsin, C is the percent protein soluble in hot water, and D is the percent protein precipitated by phosphotungstic acid. The index was shown to give satisfactory correlations with PER values in rats for animal protein concentrates by these researchers and others. Evans and St. John (1945) showed that the same index gave a correlation of 0.946 with the PERs of vegetable protein concentrates if the products were not overcooked. For heat-treated soybean oil meal concentrates, a correlation of 0.928 was found between PER and the percent protein soluble in 0.2% potassium hydroxide solution (defined as the "glutelin" fraction). Although technically much simpler to determine than a measure based on amino acid composition, the index of Amquist, Stokstad and Halbrook (1935) has the disadvantage of being correlated differently with animal proteins than with vegetable proteins, even though the correlation within each group is high (Evans and St. John, 1945).

More recently, Bajaj et al. (1971) have shown that the PER of 21 different strains of peas was highly correlated with the content of water-soluble albumin protein. The correlation coefficient was 0.949. The authors presented evidence that this correlation was due to a higher biological value of the albumin fraction compared to the globulin fraction. This, in turn, seemed to be due to higher amounts of lysine, methionine, cysteine, and tryptophan in the albumin protein.

Factors Which Affect Nitrogen Balance

The nitrogen balance status of adult human subjects is commonly assessed by subtracting the measured nitrogen loss in the urine and feces from the amount of nitrogen ingested. One must realize, however, that there are so-called minor routes of nitrogen loss from the body. These include epithelial renewal, hair and nail growth, menstruation, seminal ejaculation, flatus, nasal discharges, blood loss from trivial wounds, and saliva loss due to tooth brushing, or otherwise. The magnitude of these losses has been estimated and reviewed (Hegsted, 1964; Irwin and Hegsted, 1971b; Calloway, Odell and Margen, 1971; Waterlow,

1969) and estimates range from 0.5 to 1.0 grams of nitrogen per day or from 7 to 14 mg of N per kg body weight for a 70 kg man. Nitrogen balance measurements reported in the literature generally ignore these "minor" losses. For present purposes "nitrogen balance" will be understood to be nitrogen intake minus loss of nitrogen in the urine and feces. For a normal adult human subject a positive nitrogen balance of from 0.5 to 1.0 gram N would probably represent true nitrogen equilibrium. Consequently, it must be remembered that "zero nitrogen balance" or "nitrogen equilibrium" as used in this thesis and as commonly reported actually represents a negative nitrogen balance status of the body.

Nitrogen intake

Nitrogen intake is obviously a key factor in determining nitrogen balance. When a nitrogen-free diet is fed, nitrogen output in the urine decreases rapidly until a near constant value is reached after six to ten days. (Scrimshaw et al., 1972). Similar experiments with rats indicate that the elimination of fecal nitrogen follows a similar pattern (Peret and Jacquot, 1972). From a review of the information available at the time, the 1971 WHO/FAO Joint Expert Committee on Protein and Energy Requirements (WHO/FAO, 1973) suggested that this obligatory loss amounted to a total of 1.8 mg N per basal kcal, 49 mg N per kg body weight (mg/kg) or 3.4 grams N per day for a seventy kilogram man. Three recent measurements of nitrogen excretion at very low nitrogen intakes indicate that at zero intake, nitrogen loss in urine and feces would be slightly lower--46 mg/kg for adult male human subjects (Scrimshaw et al. 1972; Huang, Chong and Rand, 1972; Inoue et al. 1974).

Nitrogen balance increases with increasing N intake until a maintenance intake level is reached. Early work with rats and some studies with human subjects seemed to suggest that this relationship was linear, that is, as the intake level was increased from zero, each increment in intake resulted in a proportional increase in N balance. Recent studies and re-examination of these early conclusions, however, have supported a curvilinear interpretation of the nitrogen balance-nitrogen intake relationship.

Before reviewing this evidence, however, the significance of the balance-intake relationship in relation to the BV and NPU of a protein should be made clear. This will be done by consideration of an example from the literature.

Bricker, Mitchell and Kinsman (1945) studied the response of nine adults to varying amounts of nitrogen from five different sources. Linear equations relating N balance (Y) and N intake (X) of the form $Y = -a + bX$ were fit to the data obtained from each protein. The results are given in Figure 1. The authors pointed out that the slope of each regression line could be interpreted as the NPU of the protein in question. A simple rearrangement of the regression equation gave the expression $b = (Y + a)/X$. This latter expression is equivalent to equation (2), where a is equal to the sum of the urinary and fecal N losses at zero N intake, E_u and E_f, respectively. In general, if one draws a line connecting any point on a graph of N intake versus N balance with the point on the N balance axis indicating the N balance at zero intake, the slope of this line is equivalent to the NPU measured at that point. In the special case where the balance-intake relationship is a straight line intersecting the N balance axis at the point of endogenous N loss,

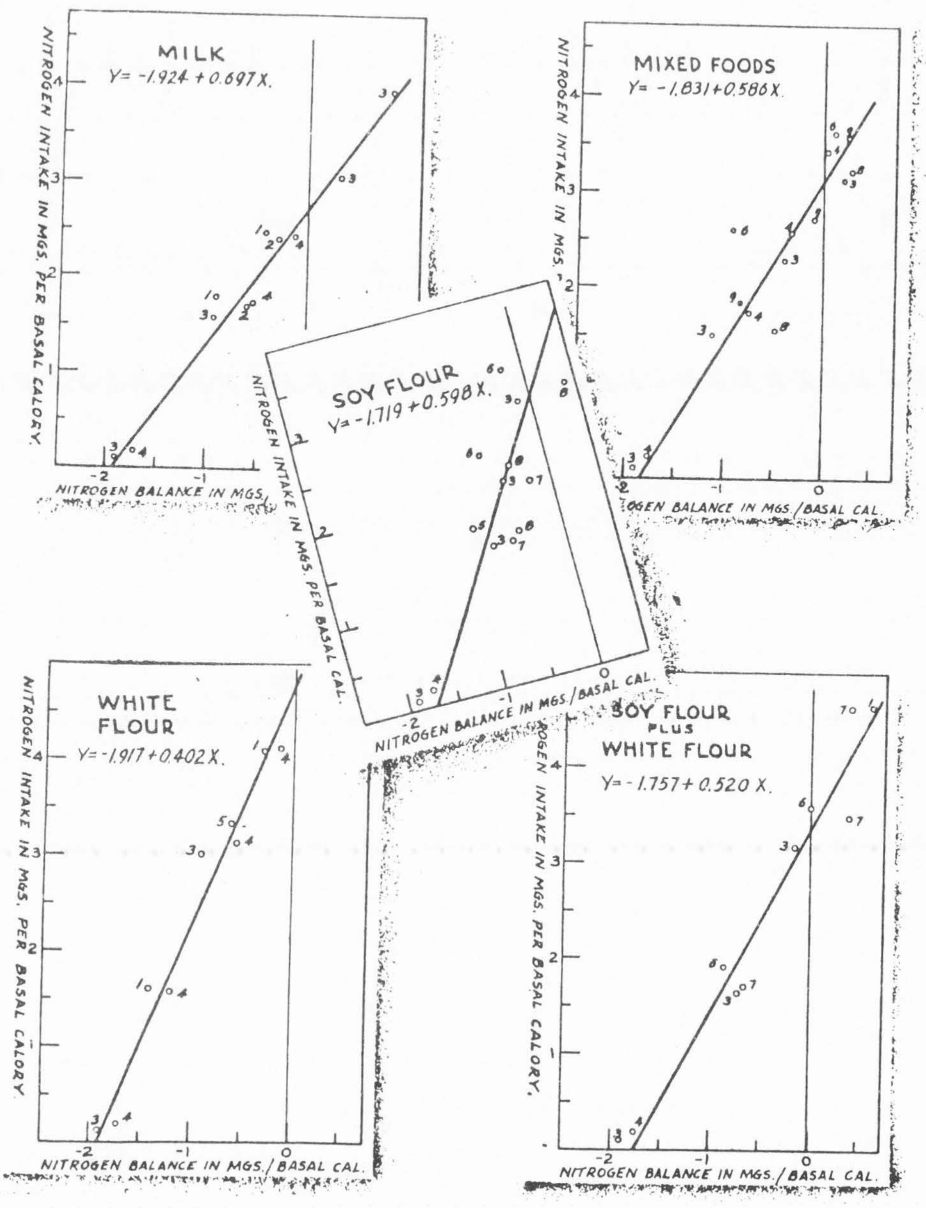


Figure 1. The linear regression of nitrogen balance with nitrogen intake for several diets (Bricker, Mitchell and Kinsman, 1945). The numbers adjacent to each point refer to the subjects producing the raw data. The lines indicate linear regression equations determined by the method of least squares.

NPU, and therefore BV (assuming that the coefficient of true digestibility does not change), will be constant for the points falling on the regression line. If the balance-intake relationship is curved, or if it is a straight line intersecting the N balance axis above the point of measured endogenous N loss, BV or NPU will decrease as the intake of N increases.

Inspection of the data of Bricker, Mitchell and Kinsman (1945) (Figure 1) suggests that for milk and white flour the linear relationship is better than for the other sources. A closer examination of the relationships for soy flour and mixed foods might indicate significant curvature.

The data of Table 1 indicate that BV tends to decrease as nitrogen intake increases from 2 to 6 grams. This implies a curvilinear relationship between intake and balance.

Recently Young (1975) discussed difficulties relating to straight line interpretations of the data of Hegsted et al. (1946) and Bricker et al. (1949). Concerning the latter study, Young (1975, p. 349) concluded that "only 3 of 9 subjects studied clearly showed a linear relationship between level of dietary protein consumption and N balance."

If very low intake levels are not included when the regression of balance with intake is made, y-intercept values are typically less negative than the expected N excretion at zero N intake. As previously described, and as has been noted by Inoue et al. (1974), this implies a decrease in BV as nitrogen intake increases. Regressions of N balance with N intake yielding predicted N balances at zero intake higher than the expected -46 to -49 mg/kg have been reported by Young et al. (1975) for beef (-37 mg/kg) and wheat (-29 mg/kg) proteins, and by Inoue, Fujita and Niiyama (1973) for egg (-37 mg/kg) and rice (-32 mg/kg) proteins. In the latter study the authors suggested that below N intake levels of 40 mg/kg a sharp curvature in the regression line might be present.

A quadratic relationship between balance and intake has been proposed. Miller and Payne (1964) suggested that a quadratic equation relating balance and intake could be useful in interpreting rat data. Young et al. (1973) found that a quadratic curve (Figure 2) explained a submaintenance-to-maintenance range of data for egg protein significantly better than a straight line.

Data supporting a non-linear, non-quadratic relationship between balance and intake have been presented by Inoue et al. (1974). They found (Figure 3) that the relationship between N-balance and intake of wheat gluten could be described satisfactorily by a straight line in the submaintenance range of nitrogen intakes down to about 30 mg/kg. The slope of this regression line was very gentle (0.129) and the y-intercept (-26 mg/kg) was much higher than the actual measured balance at zero intake (-46 mg/kg). Nitrogen balance was also studied at nitrogen intakes of 15 mg/kg and 2 mg/kg. For the regression line to pass through the measured points a dramatic curvature was required. A similar view of the intake vs. balance relationship was taken by Hegsted and Juliano (1974). These authors stated (p. 772): "It is now clear that for most proteins, perhaps all proteins, the dose-response line at low intakes is curvilinear ... ." The general dose-response lines which they gave as examples are almost identical to the response to wheat gluten measured by Inoue et al. (1974).

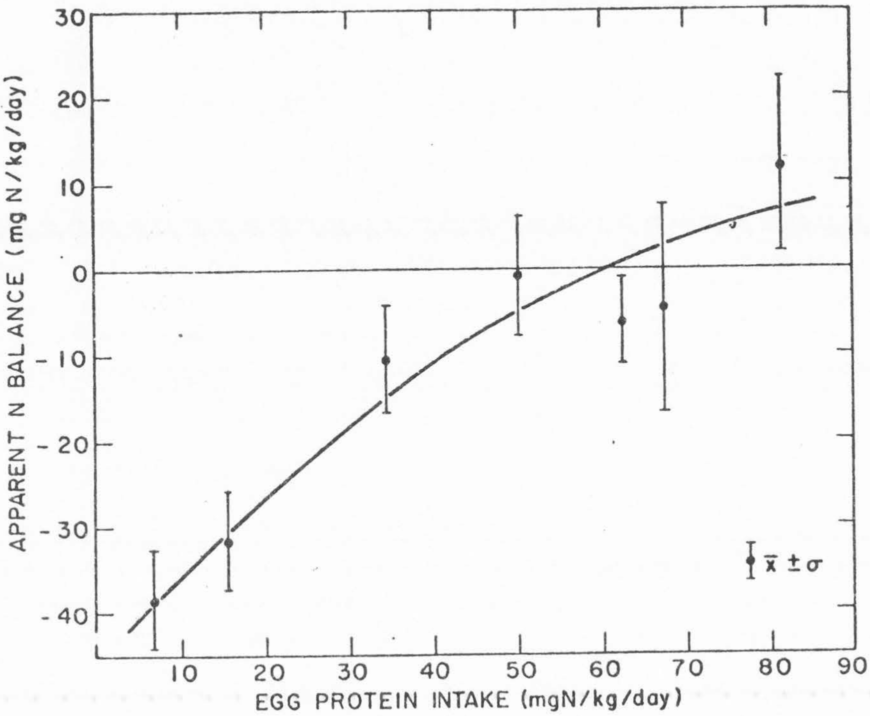


Figure 2. The quadratic relationship between nitrogen intake and nitrogen balance for egg protein (Young et al., 1973). The curve is drawn from the quadratic regression equation. The points indicate means and standard deviations of the raw data.

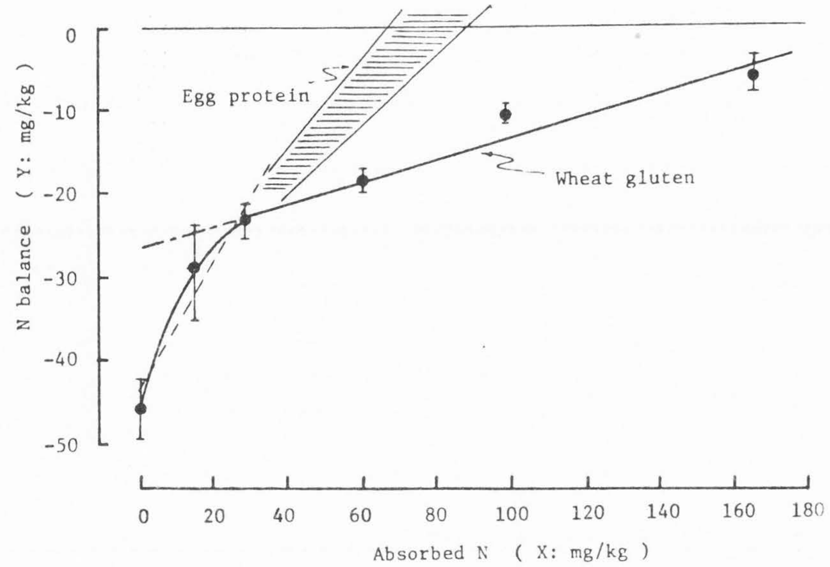


Figure 3. The curvilinear relationship between nitrogen intake and nitrogen balance for wheat gluten (Inoue et al., 1974). The points indicate means and standard deviations of the raw data. Included are egg protein relationships from an earlier study (Inoue, Fujita and Niiyama, 1973).

Caloric intake

The effect of caloric intake on the nitrogen balance of adult human subjects has been reviewed in detail by Munro (1951, 1964b). From the data available, Munro (1964b) estimated that N balance improved about 2 mg for every kilocalorie of energy added to basal diets of normal protein content.

Extensive data on the interactions of energy and protein intakes as they effect N balance are provided by the review of Calloway and Spector (1954). Emphasis was placed on studies involving sub-optimal caloric and nitrogen intakes. It may be calculated from their summary of data (Figure 4) that when a protein-free diet is fed, N balance improved by about 8 mg per kilocalorie (mg/kcal) as caloric intake increased from 0 to 450 kcal. Further increases in energy intake increased N balance until a plateau was reached at about 700 kcal. Increasing the intake above 700 kcal had no effect on N balance. With a low nitrogen intake of 6 to 7 grams, N balance improved by about 4 mg/kcal as caloric intake increased from 700 to 1800 kcal. At N intake levels of from 8 to 12 grams, increases in N balance of about 1.4 mg/kcal were obtained as caloric intake increased from 1600 to 3350 kcal. This compares with Munro's figure of 2 mg/kcal.

Calloway (1975) reviewed some of the more recent literature pertinent to the effect of added energy on N balance. She concluded (p. 921) that "N balance is improved to a greater extent by added energy intake when (a) the diet contains more generous amounts of protein, (b) the protein is of better quality, and (c) the initial energy intake is low." The responses cited by Calloway (1975) ranged from about 1 to 2 mg N

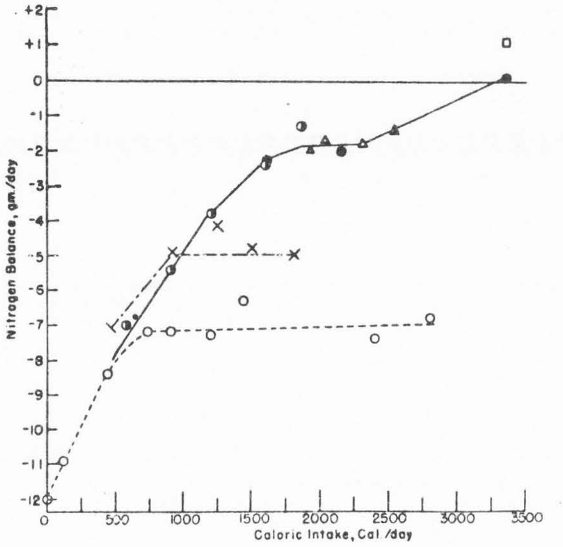


Figure 4. Nitrogen balance at various levels of caloric intake (Calloway and Spector, 1954). Nitrogen intake is indicated: ○ Protein-free, ╲ 1.0-1.9 grams, ✕ 2.4-5.0 grams, ◐ 5.4-7.7 grams, ● 8.1-9.7 grams, △ 10.4-11.7 grams, ▲ 12.4 grams, □ 15.4 grams.

balance improvement per kcal of additional energy. These figures are once again in agreement with the conclusions of earlier researchers.

An additional--and logically consistent--effect of additional calories is to decrease the protein requirement (Inoue, Fujita and Niiyama, 1973). Calloway (1975) commented on this effect and suggested that calorie level and body weight status of the subjects (either increasing, constant, or decreasing) were important in fixing the level at which N balance was achieved.

Few studies have been made with the object of determining the actual mathematical relationship between energy intake and N balance. Rao et al. (1975) demonstrated that urinary nitrogen output decreased steadily as energy intake was increased in five increments from about 40 to 50 kcal/kg. Munro (1964b) discussed studies in which a similar linear effect of calories on N balance was noted. The theoretical considerations of Miller and Payne (1963) and the literature reviewed by Munro (1964b) predicted a linear improvement in N balance with increasing caloric intake. A steep slope of the regression line was expected in the region where energy was the limiting factor while a gentle slope at higher caloric intakes was predicted where nitrogen intake or the animal's capacity to utilize nitrogen became limiting. The data summarized by Calloway and Spector (1954) similarly suggest a decreasing effect of energy on nitrogen balance as the energy intake increases.

Amino acid pattern

It was concluded as early as 1915 that the nutritional value of a protein depended upon the proportions of the various indispensable amino acids it contained (Osborne and Mendel, 1915). This difference in protein quality manifests itself as a difference in nitrogen balance when equal levels of nitrogen are fed. That is, the closer the pattern of amino acids absorbed by the body matches the pattern needed for protein synthesis, the more efficiently the nitrogen will be utilized. More efficient use of ingested nitrogen results in an improved N balance status.

For purposes of review, the effects of amino acid pattern on N balance may be divided into three classes: Those due to a limiting amino acid, those due to amino acid imbalances, antagonisms, and toxicities, and those due to the addition to, or isonitrogenous replacement or "dilution" of, the N source with non-essential amino acids, urea or ammonia (termed non-specific nitrogen).

The chemical score concept assumes that nitrogen utilization is limited by the essential amino acid in shortest supply relative to its need. The extent to which this amino acid is limiting determines the extent of N utilization and the magnitude of N balance at any given intake level. This principle would seem operable in human subjects if chemical scores correlated well with BV or NPU measured in humans or if the addition of a limiting amino acid to a deficient diet improved N balance.

Although the relationship of chemical score and nitrogen utilization has been demonstrated exhaustively with data gathered on young growing rats (Mitchell and Block, 1946; Block and Mitchell, 1946; Rao, Norton and Johnson, 1964; Morrison and Rao; 1966, Mitchell, 1954; Payne, 1972; Cresta et al. 1971) studies specifically relating protein quality in human subjects to chemical score data are limited. Block and Mitchell (1946) compiled data on BV measured in human subjects and found that although some correspondence to chemical score seemed present, the overall correlation was much poorer than that experienced with rat BV. Mitchell (1954), however, found a correlation of 0.915 between biological values of several proteins for the growing rat and for man. As a result it was suggested that the BV of proteins for adult man may be satisfactorily predicted from chemical score indices. Other researchers have provided evidence of good correlations between biological determinations of protein quality in both the rat and the human being (Jacquot and Peret, 1972). Although these studies imply that chemical scores might correlate with BV measured in human subjects, the possibility has yet to be evaluated extensively.

Improvement of N balance upon supplementation with a limiting amino acid has been observed in human subjects. Bressani, Elias and Brenes (1972), and Jansen(1974) reviewed some of these effects. They cited studies which indicated that N balance improved when the following supplements were made: wheat diets supplemented with lysine, corn diets with lysine and tryptophan; rice diets with lysine, threonine, and methionine; and oat diets with thronine. Vaghefi, Makdani and Mickelsen (1974) disagreed that lysine supplementation of wheat products resulted in improved N balance. They concluded that the responses observed by other researchers were due to test periods too short in length or to diets limiting in calories. It is evident, however, that there is at least a short-term response to limiting-amino-acid supplementation.

The effects of amino acid toxicities, imbalances, and antagonisms on the growth of young rats have been extensively reviewed by Harper (1964) and Harper, Benevenga and Wohlhueter (1970). In general, these three terms refer to growth depressions caused by the addition of certain non-limiting essential amino acids to the diet of the rat. When normal growth can be restored by supplementation with the limiting amino acid, the growth depression is said to be caused by an amino acid imbalance. Antagonisms refer to similar growth depressions which are alleviated by the addition of an amino acid which is not normally limiting. For example, excessive leucine causes growth depressions reversed by isoleucine and valine supplementation of diets not normally limiting in isoleucine and valine. Harper (1964, p. 129) defines toxicities as "the effects of excessive intakes of individual amino acids that do not fit the above categories ... ."

From the data reviewed, Harper, Benevenga and Wohlhueter (1970) concluded that the decreased growth rate observed in the case of amino acid imbalance or antagonism was due primarily to a lowered food intake, rather than any decreased efficiency of nitrogen utilization. In studies where the N intake of animals consuming imbalanced diets and control diets was equalized, the efficiency of utilization was not depressed and was even observed to be improved by the imbalance or antagonism. A similar view has been expressed by Payne (1972).

Because of the biochemical interactions of leucine, isoleucine, and valine, it is tempting to hypothesize that part of the antagonistic effects of added leucine may be due to decreased efficiency of N utilization. The oxidative decarbonxylation of α-ketoisovalerate, α-keto-β-methylvalerate, and α-ketoisocaproate, the deamination products of valine, isoleucine, and leucine, respectively, is catalyzed by the same enzyme (Lehninger, 1975). Harper, Benevenga and Wohlhueter (1970) reviewed evidence that excess leucine induces higher activities of this enzyme. Thus, in the leucine-adapted rat eating inadequate quantities of protein, isoleucine and valine might become growth-limiting and cause the inefficient use of dietary nitrogen in general. In a study of nitrogen balance in animals fed equal amounts of high leucine and control diets, however, no difference was found in N utilization (Harper, 1964).

Surprisingly, some researchers have used the data reviewed by Harper and associates to suggest that amino acid imbalances and antagonisms might effect N balance and N utilization in studies involving human subjects (Kakade, 1974; Fisher et al., 1963). Studies with adult human beings are done almost invariably by comparing responses at equal levels of nitrogen intake which are "force-fed." That is, contrary to studies with rats fed diets ad libitum, appetite plays no part in determining N intake. In spite of this there is evidence that amino acid disproportions can significantly effect the utilization of nitrogen by human beings.

An example of amino acid imbalance and antagonism was presented by Young (1975). He noted the large difference between the valine requirement determined by Young et al. (1972)--14 to 16 mg/kg--and that determined by Fisher, Brush and Grimminger (1971)--less than 4.5 mg/kg--and pointed out that the former was measured in the presence of levels of other essential amino acids which were two to three times higher than those used when the latter was determined. Young suggested that the higher levels of leucine and isoleucine in the former study may have depressed the utilization of valine and caused a higher requirement to be observed. Fisher, Brush and Grimminger have shown in other requirement studies that valine is not unique in this regard. The levels of lysine and tryptophan (1969) and of leucine and methionine (1971) required for zero N balance were all less when measured at low intakes of essential and total nitrogen than when measured at higher levels (Irwin and Hegsted, 1971a).

Suggestions of amino acid antagonism between leucine, isoleucine, and valine have been reported by other researchers. Kies and Fox (1972) conducted studies with human adults fed 4.0 grams of whole wheat nitrogen and whole wheat nitrogen supplemented with leucine to a level commonly found in corn. They found that N retention with the leucine-supplemented diet was significantly less than with the unsupplemented control diet. A supplement of lysine improved N balance in both the control and leucine-supplemented diets while tryptophan had no effect. Fox, Fry and Tolmann (1964) showed that when one of the branched-chain amino acids was omitted or severely restricted, N balance was more favorable if low--rather than high--levels of the other two were consumed. Kolski et al. (1969) presented evidence, however, that high levels of leucine or valine relative to an amount of isoleucine (1.38 grams) likely to be found in a 6 gram intake level of corn nitrogen did not significantly affect the N retention of subjects fed a flour-based diet supplemented with essential amino acids. It is possible that the greater nitrogen intake in this study as compared with the study of Kies and Fox (1972) (6 grams as compared to 4 grams) accounts for the different responses to leucine supplementation. It has been shown in rats that amino acid imbalances and antagonisms are less severe at higher levels of N intake (Harper, 1964).

Further evidence for imbalances among amino acids has been reviewed by Irwin and Hegsted (1971b). They reported the conclusion of Clark et al. (1966) that the dietary level of one amino acid helps to determine the most advantageous level of others. This conclusion was based on studies where nitrogen balance was measured in adults who consumed wheat-based diets supplemented with different amounts of purified essential amino acids. It was found that the addition of either tryptophan or isoleucine improved retention, but that the effect was not additive.

Kies and Fox (1970b) observed that the second limiting amino acid for corn protein was tryptophan if lysine-supplemented corn N was fed at a level of 3 grams with a total N intake of 4 grams. When total N intake was increased to 12 grams by the addition of urea, methionine became the second limiting amino acid. From this they inferred that the human requirement for certain essential amino acids is influenced by the level of total nitrogen intake.

Hundley et al. (1957) observed a varying response of four subjects to the supplementation of rice diets providing 4 or 5 grams of nitrogen with 0.62 gram lysine and 0.38 gram threonine or with 6 grams of a mixture of essential amino acids. Highly significant negative responses in the N balance of two subjects were interpreted as evidence for an amino acid imbalance. Chen, Fox and Kies (1967) studied the supplementation of diets providing from 2.5 to 5.0 grams of nitrogen from rice and variable amounts of glycine and diammonium citrate to raise the total N intake to 13 grams. Supplementation with 0.11 gram lysine and 0.10 gram threonine or with 1.2 grams of an essential amino acid mixture resulted in improved nitrogen balances for all six subjects. The smaller amino acid supplements and the larger amount of total dietary nitrogen in the latter study may explain why no evidence of an amino acid imbalance was observed.

It is evident that some complex interrelationships exist between essential amino acids which affect N utilization. As concluded by Young (1975), more work in this area is needed to uncover the quantitative relationships involved and their effect on human nutrition.

Studies on the effect of the addition to or dilution of dietary protein by non-specific nitrogen (NSN) have been reviewed by Irwin and Hegsted (1971b) as they relate to protein requirements and are commented upon by Young (1975) as they relate to protein quality. In general, the nitrogen of some proteins may be isonitrogenously replaced or "diluted" with NSN without changing the N balance, or equivalently (assuming no change in digestibility) the urinary N output. In addition, some proteins when fed at submaintenance levels can be made sufficient for the attainment of N balance by the addition of NSN.

Kofranyi and Jekat (1964) studied 3 human subjects and found that egg protein could be diluted 60% without changing N retention. Calculations from an earlier paper (Kofranyi and Mueller-Wecker, 1961), however, show that a 50% replacement of egg protein with NSN caused the total N requirement to increase by about 1 gram in two subjects and by 2.5 grams in another. These latter results are more in agreement with Scrimshaw et al. (1966) who found that 30% (6 subjects), 40% (4 subjects) or 50% (1 subject) dilutions of egg protein did not significantly change N utilization. Other studies show that milk protein could be diluted 20% (Kofranyi and Jekat, 1964) or 20 to 25% (Scrimshaw et al. 1969), beef protein 25% (Huang et al. 1966) and tuna protein not at all (Kofranyi and Jekat, 1964).

The addition of NSN to proteins has showed that some proteins contain sufficient essential amino acids to promote N balance, but insufficient total nitrogen. Thus Kies, Fox and Chen (1972) showed that addition of 4 and 8 grams of NSN to diets each supplying 4 grams N from milk, corn, wheat or rice greatly improved N retention. Supplementation of a diet containing 6 grams N from cornmeal with 2, 4, or 6 grams NSN caused successive increases in N retention (Kies, Williams and Fox, 1965b; Kies, Fox and Williams, 1967). In addition, Kies, Williams and Fox (1965a) showed that when 6 grams of N from cornmeal was fed, N retention was increased similarly by the addition of 2 grams of N from any of several essential amino acids or from NSN. They concluded that NSN was the first limiting nitrogenous factor in corn protein for N retention in adult men.

There is no agreement in the literature on the response of adults consuming high quality proteins or synthetic mixtures of amino acids patterned after requirement levels or the levels in egg protein to different levels of NSN supplementation. For example, Kies, Shortridge and Reynolds (1965) reviewed the available literature and cited three studies in which no improvement in N balance was observed when constant amounts of essential amino acids were fed and the total nitrogen varied from 3.5 to 12 grams. Subjects were in approximate N equilibrium at both levels. They also cited four studies in which subjects showed better nitrogen retention with medium or high intakes of nitrogen (8 to 12 grams) than with lower intakes (4 to 6 grams) and one study conducted on older men which indicated that NSN supplementation decreased nitrogen retention.

Kies, Shortridge and Reynolds (1965) were able to show that N balance increased as NSN supplementation raised total N intake from 4 to 8 grams when essential amino acids were provided by a synthetic mixture simulating 20 grams of egg protein. Similar results were obtained by Kies and Fox (1973a) during a later study. A diet in which essential amino acids were provided by a mixture patterned after the minimal requirements established by Rose was supplemented with from 1.77 to 10.77 grams of NSN. N balance increased consistantly at each increment.

Other factors

A multitude of other factors may affect the nitrogen balance of adult human beings. These factors have been reviewed recently by Irwin and Hegsted (1971b) as they relate to protein requirements and by Scrimshaw and Young (1972) as they relate to N balance measurements in humans. In general, nitrogen balance measurements can be affected by experimental conditions and by the physical or mental state of the subjects.

Variations in experimental design and techniques can exert a significant influence on measured N retention. Such factors as the level of fluid intake, the environmental temperature, the number of meals into which the protein to be studied is divided, the length of time allowed for adaptation to the experimental diet, the randomization of test periods for different N sources fed consecutively, and the accuracy to which N intake and output is measured were all mentioned by Scrimshaw and Young (1972). In addition, Irwin and Hegsted (1971b) pointed out that balance studies on subjects restricted in activity by bed rest or immobilization show a greater urinary N excretion than during moderate activity. An increase in physical activity above the normal level also seems to increase urinary excretion, but the data on this point are conflicting. The suggestion of Cuthbertson, McGirr and

Munro (1937) that variable findings are largely due to the energy intake of the subjects has been supported by studies reported by Kofranyi (1972) and was discussed by Munro (1964a). Munro concluded (p. 26) that "failure to provide energy to cover the work cost in experimental studies may account for part of the extra N excretion observed, through a change in the subject's energy balance."

Increased physical activity could also affect N balance indirectly by increasing the sweat losses of nitrogen through the skin (Munro, 1964a; Scrimshaw ans Young, 1972). Gontzea, Sutzescu and Dumitrache (1974) suggested from both their own studies and a review of others that the omission of sweat loss from usual N balance considerations has cast doubt upon the significance of previous research on the relationship between muscular activity and nitrogen balance.

It is well known that deficiencies of vitamins and minerals will adversely effect nitrogen utilization by impeding growth and development, (Payne, 1972; Munro, 1964a). There is some evidence that supplementation with vitamins of the B complex improves nitrogen balance. It has been shown that supplementary niacin reversed the detrimental effect of leucine on the nitrogen balance of adult subjects fed a wheat-based diet (Kies and Fox, 1972) and improved the nitrogen balance of subjects fed a corn diet (Kies and Fox, 1969). Studies with rats have shown increased nitrogen utilization upon supplementation with vitamins of the B complex (Munro, 1964a) and vitamin B_6 seems to aid rats in the utilization of the D-isomers of some essential amino acids as well as improving nitrogen retention in general (Bressani, Elias and Brenes, 1972).

The physical and mental condition of the subjects studied has been shown to affect N balance. Irwin and Hegsted (1971b) listed various forms of stress which have been shown to increase nitrogen excretion. These include infection, injury (whether accidental or surgical), nervous tension, and disruption of normal living patterns. Scrimshaw and Young (1972) similarly commented on the effect of infection, even of a seemingly mild degree, and of anxiety and fear on urinary N excretion.

The relation of body size to N retention is not agreed upon in the literature. Measurements of obligatory nitrogen losses have commonly been expressed per kg body weight, per basal kcal, or per m^2 surface area (Irwin and Hegsted, 1971b), and are commonly assumed to correlate better with basal caloric expenditure or surface area than with body weight because of the work done by Brody (1945) and Smuts (1935). Calloway and Margen (1971), however, found no significant correlation between obligatory endogenous urinary nitrogen, body weight and basal metabolism. They admitted that this may have been due to the relatively narrow range of body sizes of their subjects, but expressed the opinion (p. 213) that "urinary nitrogen and basal metabolic rate both simply vary about 10 to 15% around a uniform mean value in healthy male adults, and not necessarily in relation to each." In a study of 83 young men, Scrimshaw et al. (1972) found significant correlations of 0.56, 0.35 and 0.60 between body weight and obligatory urinary, fecal and total nitrogen excretion, respectively. Basal metabolic rate (BMR) gave somewhat lower correlations of 0.49, 0.26 and 0.52 with these three latter variables and the correlation with fecal nitrogen excretion, although not significant at the 1% level, was significant at the 5%

level. A similar study of 50 male Chinese university students (Huang, Chong and Rand, 1972) gave correlations of 0.56, 0.06 and 0.51 between body weight and 0.62, 0.29 and 0.65 between BMR and obligatory urinary, fecal and total nitrogen excretion, respectively. The coefficient of 0.06 was not significant at the 5% level. It is difficult to conclude from this information whether it is better to express data in terms of body weight or BMR. Although significant correlations were found, they accounted for little of the variation among individuals. Scrimshaw et al. (1972) reported that variation in body weight accounted for about 30% of the variation in urinary N excretion. If body weight was taken into account, and obligatory urinary N expressed on a body weight basis, the coefficient of variation was reduced from 18 to 15%.

The relationship between N balance and intake may be defined by expressing the variables as grams of N per individual, kilogram, basal kcal, m^2 surface area, or some other measure. For example, Clark and co-workers have usually expressed balance and intake simply as grams of N per individual, as have Kies, Fox, and associates (Clark et al., 1974, Kies, Fox and Chen, 1972). The MIT group of Scrimshaw and Young have commonly expressed these measures per kg body weight (Young et al., 1973). Hegsted et al. (1946) presented evidence of an increased correlation of balance and intake when both were expressed per square meter of body surface area. They concluded (p. 281) that "the nitrogen requirement is more closely related to surface area (basal caloric expenditure) than to body weight." Similarly, Bricker, Mitchell and Kinsman (1945, p. 273) stated that by expressing nitrogen balance and intake "in milligrams per basal calorie, it is possible to pool the data for all subjects regardless of body size." These results have been reproduced previously (Figure 1). Although a good correlation is apparent in several cases, the authors do not offer any evidence that the correlations were maximized by this means of presentation.

Calloway and Margen (1971) reported that the minimum nitrogen requirement of 9 adults for egg protein was not significantly correlated with age, weight, height, lean body mass, urinary creatinine, total endogenous nitrogen excretion, basal metabolic expenditure, or caloric intake for constant body weight. The authors admitted, however, that their subjects represented a narrow range of body weights. Kofranyi (1972, p. 43) reported that expressing the requirement of 27 subjects for egg protein in terms of body weight "does not correspond exactly to the physiological reality, but all other parameters, such as body size, body surface, total potassium content, were proved to be less useful than body weight, on account of greater deviations." The Joint FAO/WHO Expert Committee (FAO/WHO, 1973) chose to express protein requirements in terms of mg of nitrogen per kg body weight within any one age or sex group, but calculated each group requirement in terms of basal metabolic rate.

METHODOLOGY

Sources of Data

A literature search was conducted for nitrogen balance studies performed on young adult human subjects. It was found that almost all articles of interest were in journals available at the Utah State University Merrill Library. Those not available were generally published in Polish or Russian journals, in the appropriate language, and would have presented translation problems even if they were locally available. If, however, articles were referenced by studies at hand and appeared to contain pertinent data, an effort was made to obtain these by interlibrary loan procedures.

Acceptance Criteria for Data

Data were included in the statistical analyses if the following conditions were met:

1. The experimental subjects were normal, young adult human beings. Subjects were considered "young" if they were between 18 and 39 years of age. They were considered "normal" if they were not sick, injured or malnourished, and were moderately active, i.e., allowed to continue "normal activities," or subjected to "inforced exercise" if confined. Studies which involved a significant change in activity for the participants or which were conducted exclusively on overweight subjects were not included.

2. The experimental design minimized the effect of adaptation or adjustment to the diets. Several investigators have found that a protein-free period at the start of a nitrogen balance experiment helps to minimize the adaptation period required when low nitrogen, test protein diets are subsequently fed (Scrimshaw and Young, 1972, Kies and Fox, 1972). Under these conditions Scrimshaw and Young (1972) suggested that a 5-day adaptation period could allow the accurate estimation of N balance during the next 5 days. When no N-free period was included at the start, adaptation periods of 10 days or longer were required before the nitrogen balance became reasonably constant (Kofranyi and Mueller-Wecker, 1960; Vaghefi, Makdani and Mickelsen, 1974). As the subject became adapted to a low N regime, however, little or no adaptation time was required, especially if the change in protein quality was not great between periods (Scrimshaw and Young, 1972).

   In light of this information studies were accepted if (a) a non-protein period followed by an adjustment period of at least 5 days, or (b) an adjustment period only of at least 10 days, preceeded experimental periods of at least 5 days in length during which nitrogen balance was measured.

3. The N balance were not measured in diets which were limiting in calories. It has been shown experimentally (Calloway and Spector, 1954; Munro, 1951, 1964b) and proposed theoretically (Miller and Payne, 1963) that entirely different relationships exist between N intake and N balance under conditions where the subjects' energy needs are not met.

To determine the sufficiency of caloric intake, weight maintenance was taken as a measure of energy balance. Studies were accepted if, during the balance periods, body weights were constant, increasing, or if they were decreasing at a rate less than or equal to that implied by the nitrogen balance. For the latter purpose, a value of 30 grams of N per kg lean body mass (Garza, Scrimshaw and Young, 1976) was used to calculate the weight change which would be expected.

4. The data were complete enough for purposes of the analysis. Studies were rejected if the body weight, nitrogen intake, nitrogen balance or diet composition were not reported.

As these acceptance criteria were applied to the studies in the literature, it became evident that relatively few studies conducted previous to 1960 qualified. This was primarily due to a lack of complete data--body weights of the subjects were frequently not reported (Watts et al., 1959a, 1959b; Hundley et al., 1957; Cremer et al., 1951; Esselbaugh et al., 1952; Murlin et al., 1946; Sumner and Murlin, 1938; for example). Rather than include a few--and perhaps non-representative--studies from the earlier literature, it was decided to restrict acceptable data to that deriving from studies published since 1960.

Extraction and Initial Treatment of Data

The weight, nitrogen intake and nitrogen balance of adult subjects were taken from studies which met the criteria previously described. Nitrogen balance was defined as nitrogen intake minus nitrogen excretion in the urine and feces. No allowance for miscellaneous nitrogen losses were made at this stage of data treatment. If caloric intake was not reported, values of 46 kcal/kg for men and 40 kcal/kg for women were assumed (FAO/WHO, 1973). Although higher than the requirements suggested by the National Academy of Sciences--National Research Council (NAS/NRC, 1974) these figures are in accord with intake levels which are common in nitrogen balance studies (Calloway, 1975). If height of the subjects was not reported, this was determined from body weight through the use of standard tables (Davidson, Passmore and Brock, 1972). In the case of the study of Inoue et al. (1974), height was calculated from weight using the formula:

$$\text{height in cm} = 0.4900 \times \text{weight in kg} + 137.4$$

This equation was derived by means of a least square regression of height with weight, using data reported for similar subjects (Inoue, Fujita and Niiyama, 1973). Body surface area in square meters was calculated from height and weight using the formula of DuBois and DuBois (DuBois, 1968).

In addition to body size, nitrogen intake and caloric intake, the effect of adaptation time, sex and research group were studied. A measure of adaptation to low-nitrogen, experimental diets was derived by totaling the number of consecutive days the experimental subject had been consuming such a diet at the end of the test period in which nitrogen balance was measured. For example, if a study consisted of a 2-day N-free diet followed by a 5-day adaptation period and 4 consecutive 5-day periods in which nitrogen balance was measured, the adaptation times would be 12, 17, 22, and 27 days, respectively, for the nitrogen balances measured in each period.

Five major research groups were identified and data were coded to indicate which research group had produced them. The research groups were (1) Calloway, Margen and coworkers, (2) Clark and coworkers, (3) Inoue and coworkers, (4) Kies , Fox and coworkers, and (5) Scrimshaw, Young and coworkers.

Prior to analysis, data were divided into groups according to nitrogen source. This allowed regression analyses to be performed in which protein type was not an independent variable. Two nitrogen sources were considered equivalent if at least 70% of the nitrogen in the diet was supplied by the source in question. Sufficient data were available on six protein sources to allow analyses to be done, namely, egg, beef, white milled rice, cornmeal, whole wheat and wheat gluten. These six data groups are reproduced in Appendix A.

Some studies used as sources did not report data for individual subjects, but instead reported the mean nitrogen balance response to a dietary treatment of several subjects. The use of mean rather than individual observations introduced a potential source of error into the analyses which were performed. As data were extracted from the source studies, nitrogen intakes, nitrogen balances and caloric intakes were all expressed as grams of nitrogen per individual or as kcal per individual for consistency. In the course of the statistical analyses, these variables were then re-expressed per kg body weight, per kg body weight raised to 0.73 power ($kg^{0.73}$), and per square meter of body surface area. Although the recalculation process involved was mathematically simple, it was not strictly correct for means. That is, one cannot divide the average nitrogen intake by the average body weight and expect to obtain the same average nitrogen intake per kg body weight value one would obtain by dividing each individual intake level by each body weight and averaging the resultant quotients. The discrepancy between the two averages depends on the distribution of values around each mean and the degree of correlation between the two variables. The same situation would exist whenever mean values are expressed in one set of units (i.e. per kg) and the analysis requires that they be expressed differently (i.e. per person or per kg$^{0.73}$).

The extent of the error in the recalculation procedure introduced through the use of means was estimated. For several representative studies, mean intakes and balances were calculated either by dividing mean intake or balance by mean weight (scheme I) or by dividing each individual intake or balance by each individual weight and then averaging (scheme II). It was found (Table 2) that the differences between

Table 2. Effect of two calculation methods on the resultant average N intakes and N balances.

N intake (mg/kg)		N balance (mg/kg)		Reference
Scheme I[a]	Scheme II[b]	Scheme I	Scheme II	
63.5	64.2	-4.0	-4.2	Kies and Fox, 1971
116.4	117.6	9.8	9.6	
76.3	77.8	-10.4	-9.7	Kies and Fox, 1970a
88.9	89.2	7.2	7.2	Clark et al., 1975
42.9	42.9	-6.0	-6.4	Young et al., 1971
80.2	81.3	-3.6	-3.0	Calloway, 1975
109.7	111.1	4.7	5.0	

[a] mean N intake or balance was divided by the mean body weight

[b] each individual N intake or balance was divided by each individual body weight and the resultant quotients were averaged

the two schemes were neither large nor consistent. It was concluded that the error introduced by the use of means in the place of individual observations was negligible.

Statistical Analyses

Linear statistical models

A general linear statistical model (Mendenhall, 1968) was used to evaluate the significance of different sources of variation in nitrogen balance determinations. This type of statistical model has the general form

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + \ldots + b_k x_k + e$$

where y is the dependent variable (nitrogen balance for present purposes) and $x_1, x_2, \ldots, x_k$ are independent variables which are either continuous variables (such as nitrogen intake, caloric intake, or days of adaptation) or are "dummy" variables representing non-continuous categorical variables (such as research group or sex). The parameters $b_0, b_1, b_2, \ldots, b_k$ represent unknown quantities which measure the effect of each independent variable upon the dependent variable. The error component, e, represents the "left-over" variation in the dependent variable, nitrogen balance, which is unexplained by the independent variables included in the model.

An in-depth discussion of the merits of linear statistical models is not necessary. It should be mentioned, however, that the mixing of continuous and "dummy" variables in such a model allows a simultaneous analysis of covariance and variance as one carries out the multiple regression analysis and determines the parameters $b_0, b_1, b_2, \ldots, b_k$, by the method of least squares.

To computer program packages adapted for use with the Burroughs B6700 computer facility at Utah State University were used to evaluate the statistical models. Both the Statistical Program Package (Hurst, ca. 1971) and the Statistical Package for the Social Sciences (Nie et al., 1975) produce statistically identical analyses and were used interchangeably. Correlation coefficients and other basic statistics were also calculated by means of these program packages.

Tests of significance

Tests of significance for correlation coefficients were done according to standard procedures (Spiegel, 1961). Tests of significance involving the linear statistical models were conducted as described by Mendenhall (1968). When a model was subjected to regression analysis the statistical program calculated the amount of variation in nitrogen balance which would become unexplained if each variable in turn, were removed from the model. This amount of variation (the "mean square" for each variable) was then divided by the unexplained or residual variation (the "mean square error") to give F-ratios. The F-ratios, when compared to a standard table of the F-distribution, indicated the significance of each variable in explaining the variation in nitrogen balance.

Certain problems were cuased if means of several sets of observations were used as data for a regression analysis when the single observations were not reported in the literature. Since a mean represents more than one observation, it should contribute proportionally more than a single observation in determining the nature of the regression equation. A common solution is to assign a "weight" to the mean which is equal to the number of observations it represents. This is equivalent to treating the mean as a single observation and repeating it as data for the regression analysis a number of times equal to the number of observations. The regression equation which results from such an analysis is comparable to that which would result if the individual observations were available. This weighting procedure was used in the present study whenever means were included as data.

The simple replication of the mean in the weighting process, however, does not reproduce the actual variability of the values making up the mean. This results in a mean square error or a residual or unexplained variation which is smaller than it should be, F-ratios larger than they should be and statistical tests of significance which are too liberal. Of the several procedures available to rectify this situation, the simplest is to eliminate the means from the data when statistical tests of significance were desired. This method was used for the analysis of the data for egg, beef and whole wheat protein in several studies. When statistical tests of significance were required for regression analyses in which both single observations and replicated means were used as data, the mean square error was estimated from identical analyses in which the means were not included. If only means were used as data for an analysis--as was the case with the data on wheat gluten--the mean square error was either recalculated using the standard deviation of the individual nitrogen balance measurements as reported in the original literature, or, if this was not possible, the mean square error was assumed equal to the mean square error of the same regression analysis conducted with the data on corn or whole wheat protein.

RESULTS AND DISCUSSION

Preliminary Analysis: the Effect of Adaptation, Sex and Research Group on Nitrogen Balance

The data reproduced in Appendix A were analyzed to determine the effect of adaptation to low nitrogen diets, sex and research team on the nitrogen balance of adults. To correct for the influence of nitrogen intake, caloric intake and body weight on nitrogen balance, these variables were included in the statistical model as covariates. Nitrogen intake and balance were expressed in grams, caloric intake in kcal, body weight in kg, and adaptation in days. "Dummy" variables were included in the model for the effects of sex and research group. Data reported as means were excluded from the data on egg, beef and wheat proteins before these data groups were subjected to analysis.

Table 3 summarizes the results of the six multiple regression analyses performed. Nitrogen intake explained a consistantly significant proportion of the variation in nitrogen balance. Nitrogen intake was significant at the 1% confidence level in four cases and at the 5% level in the remaining two. Caloric intake was a significant source of variation at the 1% level in three cases and at the 25% level in one other. Body weight was significant in two analyses at the 5% level and in two more analyses at the 25% level. The effect of adaptation was significant for two of the six nitrogen sources at the 25% level. An effect of research team upon nitrogen balance was not evident in three out of the four cases which could be examined. There were, however, differences which were significant at the 10% level between

Table 3. Regression analysis of nitrogen balance response to six nitrogen sources

	Egg				Beef		
Source	df[a]	MS[a]	F ratio	Source	df	MS	F ratio
N intake	1	1.056	4.96*	N intake	1	4.36F	15.25**
kcal intake	1	1.539	7.22**	kcal intake	1	0.037	<1
body weight	1	1.333	6.26*	body weight	1	0.072	<1
adaptation	1	0.431	2.02[b]	adaptation	1	0.001	<1
DRG 1,2,3&5[c]	3	0.730	3.43[d]	DRG 4&5[c]	1	0.064	<1
error	67	0.213		error	21	0.286	
total	74	0.350		total	26	0.544	

	Rice				Corn		
Source	df	MS	F ratio	Source	df	MS	F ratio
N intake	1	5.579	42.17**	N intake	1	18.560	54.45**
kcal intake	1	1.298	9.81**	kcal intake	1	0.025	<1
body weight	1	0.224	1.70[b]	body weight	1	1.722	5.05*
adaptation	1	0.363	2.74[b]	adaptation	1	0.063	<1
DRG 2,3&4[c]	2	0.117	<1	error	45	0.341	
error	40	0.132		total	49	0.763	
total	46	0.393					

	Wheat				Wheat Gluten		
Source	df	MS	F ratio	Source	df	MS	F ratio
N intake	1	1.659	4.86*	N intake	1	6.119	17.89**
kcal intake	1	0.522	1.53[b]	kcal intake	1	3.446	10.08**
body weight	1	0.639	1.87[b]	body weight	1	0.247	<1
adaptation	1	0.125	<1	adaptation	1	0.091	<1
sex	1	0.554	1.62[b]	DRG 3&5[c]	1	0.189	<1
error	47	0.341		error	43	0.342[e]	
total	52	0.451		total	48	0.535	

** significant at the 1% level

* significant at the 5% level

[a] df = degrees of freedom, MS = mean square

[b] significant at the 25% level

[c] DRG = difference in research groups:
1 = Calloway, Margen and co-workers
2 = Clark and co-workers
3 = Inoue and co-workers
4 = Kies, Fox and co-workers
5 = Scrimshaw, Young and co-workers

[d] significant at the 10% level

[e] recalculated using the standard deviations reported by Scrimshaw, Taylor and Young (1973)

the four reserach groups contributing the data on egg nitrogen. A sex difference in nitrogen balance response could only be investigated in one analysis, and in this case it was significant at the 25% level. This cannot be regarded as sufficient evidence either for or against a true difference between the sexes.

The fact that variables were significant in some analyses but insignificant in others was at first disturbing. When the range of values was considered that each variable assumes for each protein source (Table 4) an explanation became apparent. The significance of a variable may not be detected if its range is not great enough. Thus, in three of the four cases when adaptation was not sinificant (corn, wheat and wheat gluten) the range of this variable was from one-half to one-third as great as in the cases when this variable was significant (egg and rice). Similarly, the two instances when body weight was not significant (beef and wheat gluten) were also the two instances when the range of this variable was the least. This type of reasoning may also explain why caloric intake failed to be significant for beef and corn proteins.

The large ranges of body weight and caloric intake for wheat protein, however, are inconsistent with their low level of significance. For these data energy intakes were not reported and had to be calculated on the basis of body weight. Consequently, body weight and caloric intake were highly correlated. When two such variables are included as independent variables in a multiple regression analysis the effect is to reduce the significance of both variables even though they each may be highly significant if included alone (Wonnacott and Wonnacott, 1969). A separate analysis showed that if either body weight or

Table 4. Ranges for each variable.

Data group		N intake (g)	kcal intake	body weight (kg)	adaptation (days)
		Ranges for each variable[a]			
Egg[b]	minimum	2.48	2236	48.7	7
	maximum	8.44	4045	93.0	77
	difference[c]	5.96	1809	44.3	70
Beef[b]	minimum	3.93	2918	63.6	12
	maximum	8.80	4004	86.3	55
	difference	4.87	1086	22.7	43
Rice	minimum	2.73	2293	48.3	17
	maximum	8.31	3760	85.5	59
	difference	5.58	1467	37.2	42
Corn	minimum	4.00	2636	61.2	18
	maximum	8.70	3877	91.4	33
	difference	4.70	1241	30.2	15
Wheat[b]	minimum	4.80	2001	50.0	13
	maximum	6.80	4463	97.0	28
	difference	2.00	2462	47.0	15
Wheat gluten	minimum	1.58	2643	54.6	7
	maximum	9.45	3811	74.2	31
	difference	7.87	1168	19.6	24

[a] ranges are for the data given in Appendix A

[b] excluding any data reported as means

[c] maximum minus minimum

caloric intake were deleted from the model, the remaining variable was significant at the 5% level.

It is possible to gain some idea as to the quantitative effects of caloric intake and adaptation time on nitrogen balance by an examination of the coefficients determined for these variables when they were significant. The coefficients of caloric intake for egg, rice and wheat gluten, with their 95% confidence interval estimates indicated that N balance would increase with increasing energy intake at an average rate of 0.50±0.37, 0.64±0.41, and 0.94±0.60 mg per kcal, respectively, over the range of caloric intakes given in Table 4. These estimates are within the lower range of values reported and reviewed by other investigators (Munro, 1951, 1964b; Calloway, 1975).

The coefficients of adaptation time for egg and rice with their 95% confidence interval estimates are 7.0±9.9 and 11.3±13.8 mg of nitrogen per day. This suggests that at a fixed low level of nitrogen intake, N balance would tend to increase as the subject becomes more and more adapted over the range of days indicated in Table 4.

The above estimates may be compared with the findings of others. The data of Horwitt et al. (1956) yield average increases in nitrogen balance of 8 mg per day from 2 to 16 weeks of adaptation and 4 mg per day from 16 to 36 weeks, with the increase during the latter period being primarily between weeks 16 and 24. Schwartz and Merlis (1948) reported urinary nitrogen figures for one hypertensive patient from which may be calculated that N balance increased an average of 19 mg per day between days 30 and 90 on the experimental diet. Clark et al. (1962) performed a regression analysis of N balance with time for subjects adapted to diets for from 6 to 30 days. Average increases in nitrogen balance ranged from 11 to 22 mg per day. An average increase in N balance of 5 mg per day may be calculated from the 45 day study of Kunerth and Pittman (1939). No increase, however, was observed in a similar study in which the protein level was more than doubled (from about 4 grams of N to about 11 grams of N) (Pittman and Kunerth, 1939). Bricker et al. (1949) concluded that N balance had not changed significantly over a 10 week period in subjects fed a minimum protein diet (5 grams of N). However, their subjects were consuming low N diets (averaging 2.5 grams of N per day) for 41 days prior to the commencement of the 10 week study.

The variability of the foregoing data may be predicted from the large 95% confidence interval estimates of the response to adaptation measured in the present study. These indicate that the response might be expected to lie between -2.9 and 16.9 mg per day for egg protein and between -2.5 and 25.1 mg per day for rice. Although it is reasonably certain that nitrogen utilization tends to improve as subjects become adapted to low nitrogen regimes, the magnitude of this improvement is uncertain.

Differences between the four research groups contributing data on egg protein utilization were significant at the 10% confidence level. Comparison tests showed that when other factors included in the analysis were held constant, the nitrogen balances measured by research groups 1 (Calloway and Margen) and 2 (Clark) were significantly higher at the 5% level than nitrogen balances measured by groups 3 (Inoue) and 5 (Scrimshaw and Young). This amounted to an average difference of about 0.5 grams of nitrogen between nitrogen balances measured by the former and the latter research teams. There was no significant difference between group 1 (Calloway and Margen) and group 2 (Clark) or between group 3 (Inoue) and group 5 (Scrimshaw).

These results would be more meaningful if they were supported by the results of other analyses. However, no difference significant at even the 25% level was evident between research groups 2 (Clark), 3 (Inoue) and 4 (Kies and Fox), who had contributed the data on rice protein. On the basis of the egg protein analysis a significant difference would have been expected between the research groups of Clark and Inoue. It is, of course, possible that differences between these groups depends, in part, upon the source of nitrogen fed. The nitrogen balance of subjects fed egg protein may be more sensitive to differences in experimental technique or conditions than is the nitrogen balance of subjects fed rice.

The Influence of Body Weight and Size on Nitrogen Balance

Body weight exerted a significant influence upon the nitrogen balance of subjects consuming egg, rice, corn or wheat protein (Table 3). In the case of wheat protein, body weight increased in significance when a variable with which it was highly correlated--caloric intake--was deleted from the regression analysis. The lack of a significant influence of weight on N balance for beef protein and wheat gluten was probably due to the narrow range of weights in the data analyzed (Table 4).

It was of practical importance to determine the units in which nitrogen balance and nitrogen intake should be expressed in order to minimize the effect of body weight and size. This was done by expressing nitrogen intake and nitrogen balance as grams, grams per kilogram body weight (grams per kg), grams per kilogram body weight raised to the 0.73 power (grams per $kg^{0.73}$ or per kilogram metabolic body size), or grams per square meter of body surface area (grams per m^2) and calculating the correlation between them. The data of Appendix A were used for this purpose. Means were deleted from the egg, beef and wheat data groups.

Results of the correlation study are given in Table 5. For three of the six protein sources, the highest correlation between N balance and N intake was observed when both meausres were expressed as grams per m^2. If the possibility of expressing these measures in grams was dismissed, four of the six showed highest correlations when expressed as grams per m^2.

Table 5. Correlation coefficients of nitrogen intake and nitrogen balance[a].

	N intake and N balance expressed as			
Data group	grams	grams per kg	grams per $kg^{0.73}$	grams per m^2
Egg	0.4776	0.5275	0.5315	0.5381
Beef	0.7454	0.6715	0.6995	0.6913
Rice	0.7626	0.6116	0.6825	0.6892
Corn	0.7224	0.7464	0.7622	0.7668
Wheat	0.3066[b]	0.4315	0.4581	0.5081
Wheat Gluten[c]	0.5874	0.8198	0.7778	0.7562

[a] All correlation coefficients are significant at the 1% level with one exception[b].

[b] significant at the 5% level

[c] tests of significance not performed

Tests of significance showed that all correlations for egg, beef, rice, corn and with one exception for wheat were significant at the 1% level. There were no significant differences, however, between correlations at the 5% level within a given data group. For example, the increase in correlation coefficient for the wheat data group when data was expressed in grams as compared to when data was expressed as grams per m^2 (an increase from 0.3066 to 0.5081), was significant at about the 10% level. Other increases in correlation coefficient were generally less, and were of a lesser significance also. Tests of significance were not made for the correlations involving the wheat gluten data because these correlations were based on means rather than single observations.

Although there were no highly significant statistical differences between the possible ways of expressing nitrogen balance and nitrogen intake, a general trend toward higher correlations as values were expressed per m^2 surface area or per metabolic body size ($kg^{0.73}$) was evident. This supports the conclusions of early researchers (Brody, 1945; Smuts, 1935) that protein requirements and endogenous nitrogen losses are similar to the basal metabolism rates in that they are best expressed in relation to surface area or body weight raised to some fractional power. The present study, however, must be regarded as inconclusive as to which method of expressing nitrogen balance and nitrogen intake minimizes the effect of body weight and body size on them. More data are necessary to confirm the apparent trend in Table 5.

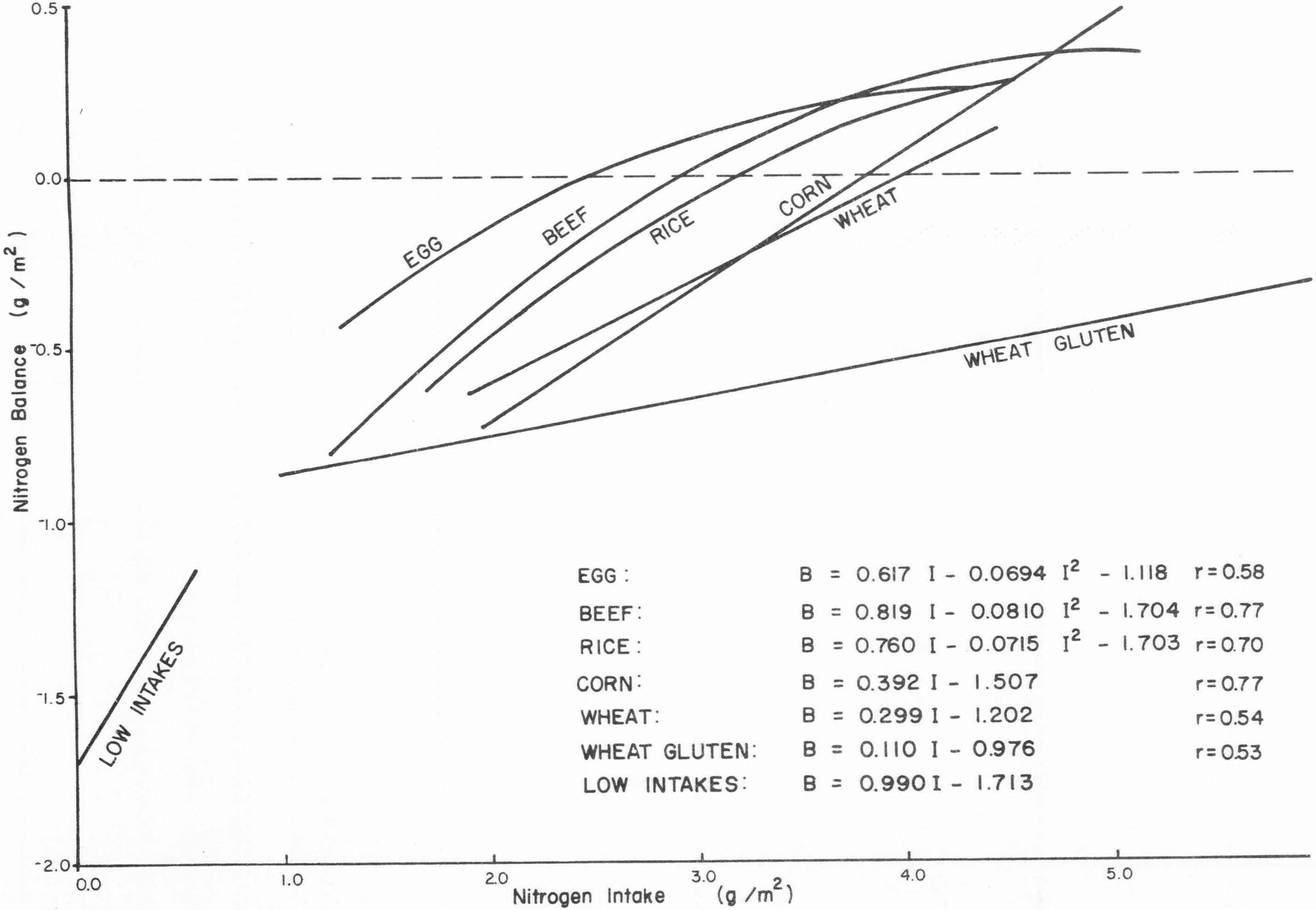
The Effect of Nitrogen Intake and Nitrogen Source on Nitrogen Balance

Linear or quadratic equations relating nitrogen balance and nitrogen intake were fit to the data available for each protein source (Appendix A). In accordance with the effect of body weight and size on the correlation between intake and balance discussed previously, both N intake and N balance were expressed as grams per square meter of body surface area (g/m^2). The regression analyses of the data for egg and beef protein indicated that the nitrogen intake squared term was significant at the 5% and 1% level, respectively. For rice protein the nitrogen intake squared term exhibited a borderline significance at the 20% level. The F-ratios associated with the nitrogen intake squared terms for corn, wheat and wheat gluten protein were all much less than 1.00 and it could be safely concluded that no significant curvature in the regression lines was implied by the data. It was decided that quadratic regressions would be used to summarize the data for egg and beef protein while linear regressions would be used for corn, wheat and wheat gluten. Rather than imply that the response to rice protein was similar to the response to corn, wheat and wheat gluten, and showed no curvature whatsoever, a quadratic regression was used to summarize these data. It must be remembered, however, that there is a 20% probability that the true relationship is linear and that the curvature implied by the present analysis is due to chance.

Figure 5 shows the regression lines obtained for nitrogen balance with intake for the proteins studied. Regression lines were plotted only for the range of intake values which were actually used in their derivation. Included in Figure 5 is a regression of N balance with N intake for very low intake levels of various nitrogen sources. These data are recorded in Appendix B and were drawn from studies on the endogenous nitrogen excretion of young adult male subjects.

A word should be said about the statistical significance of the regression lines. The standard error of the predicted mean nitrogen balance response to corn and rice protein, for example, varied from about 0.04 g/m^2 at the middle of the regression lines to about 0.10 g/m^2 at the extremes of either end. The standard deviation of an individual predicted nitrogen balance in these cases varied from about 0.20 g/m^2 at the mid-regions to 0.29 g/m^2 at the extremes. It should be remembered that the variability of the individual data points is always greater than the variability of the mean.

Figure 5. The relationships between nitrogen intake and nitrogen balance for six nitrogen sources in the submaintenance-to-maintenance intake range. Curves are drawn from the regression equations where B is nitrogen balance (g/m^2) and I is nitrogen intake (g/m^2). Included is a regression of nitrogen balance with nitrogen intake for very low intake levels of various nitrogen sources. (See Figure 6.) The 95% confidence limits of the mean nitrogen balance at various intake levels vary from about ±0.8 g/m^2 in the mid-regions of the regression lines to about ±1.5-±2.5 at the extremes of either end. (See Appendix C.) The correlation coefficient, r, is indicated after each regression equation.



EGG: $B = 0.617\ I - 0.0694\ I^2 - 1.118$ $r = 0.58$

BEEF: $B = 0.819\ I - 0.0810\ I^2 - 1.704$ $r = 0.77$

RICE: $B = 0.760\ I - 0.0715\ I^2 - 1.703$ $r = 0.70$

CORN: $B = 0.392\ I - 1.507$ $r = 0.77$

WHEAT: $B = 0.299\ I - 1.202$ $r = 0.54$

WHEAT GLUTEN: $B = 0.110\ I - 0.976$ $r = 0.53$

LOW INTAKES: $B = 0.990\ I - 1.713$

Further calculations showed that the mean nitrogen balances expected for corn and rice were statistically different in the nitrogen intake range of 2.0 to 3.8 g/m^2 with 95% confidence. The least significant difference between the rice and corn regression lines with 95% confidence varied from 0.21 g/m^2 at an intake level of 2.0 g/m^2 to a minimum of 0.12 g/m^2 at an intake of 3.5 g/m^2 . At an intake level of 3.8 g/m^2 the least significant difference increased to 0.13 g/m^2, which was approximately the distance between the two lines at this point.

Because of the inclusion of means in the data for egg, beef, wheat and wheat gluten, the significance of these regressions could only be estimated. When these estimates were made it was found that the standard errors were similar to those calculated for corn and rice. The 95% confidence limits on the mean nitrogen balance at several levels of nitrogen intake for each of the six nitrogen sources are given in Appendix C. They range from about ±0.6 to ±0.9 g/m^2 in the mid-region of the regression lines to about ±1.5 to ±2.5 g/m^2 at the extremes of either end. It is evident from a consideration of this information (Appendix C, Figure 10) that the mean nitrogen balance responses to beef and rice protein are not significantly different over the range of nitrogen intakes studied. The same conclusion holds for corn and wheat. Different mean responses are evident between egg and beef at nitrogen intakes ranging from 1.3 to 2.6 g/m^2 and between rice and corn or rice and wheat at intakes of 2.4 to 3.7 g/m^2 .

It is interesting to note that the quadratic equations relating intake to balance have maxima at N balance levels which agree closely with the estimates of nitrogen loss via the so-called minor routes (skin, hair, flatus, saliva, etc.). The maxima for egg, beef, and rice come at nitrogen balances of 0.25, 0.37 and 0.32 g/m^2, respectively, or 0.46, 0.68 and 0.59 gram per 70 kg individual of average height (172 cm). These later values compare with the estimates of from 0.5 to 1.0 gram of N per day miscellaneous loss suggested by many researchers (Hegsted, 1964; Irwin and Hegsted, 1971a; Calloway and Margen, 1971; Waterlow, 1969).

Although the present results suggest that the N balance response to egg, beef and rice proteins approaches a constant positive balance as higher levels of nitrogen are consumed, this conclusion has been disputed. Hegsted (1976) compiled data on the nitrogen balances of a wide variety of human subjects consuming high levels of nitrogen from various sources. He concluded that the published data indicated consistant retentions amounting to about 20% of the nitrogen intake above 5 grams per day. Nitrogen balances showed no tendency to level off at higher intakes. Hegsted pointed out that such retentions are not only unexpected, but are completely impossible in non-growing adults. The nature or the reality of these increasingly positive nitrogen balances at high intake levels remains controversial.

An examination of Figure 5 may be made in light of the dietary requirements for protein established by the 1971 FAO/WHO Expert Committee (FAO/WHO, 1973), the National Academy of Sciences--National Research Council (NAS/NRC) (NAS/NRC, 1974) and the Food and Drug Administration (FDA) (FDA, 1972). Table 6 summarizes these requirements as compared with amounts of protein (nitrogen multiplied by 6.25) predicted by the present study which are compatible with zero nitrogen balance or with a positive nitrogen balance of 0.20 g/m^2. All protein amounts and requirements have been calculated for a 70 kg, 172 cm adult.

Table 6. Protein requirements compared to estimates suggested by Figure 5

	Requirement (grams of protein)[a]					
	Present study					
Nitrogen source	for zero balance	for 0.2 g/m^2 balance	for 0.2 g/m^2 balance + 30%	FAO/WHO[b] (1973)	NAS/NRC[c] (1974)	FDA[d] (1972)
Egg	29 (27-32)[e]	41 (36-)	53	40	56	45
Beef	34 (31-37)	41 (37-)	53	40		
Rice	37 (33-40)	46 (41-)	60	60		65
Corn	44 (41-47)	50 (47-55)	65	81		
Wheat	46 (42-54)	54 (48-65)	70	75		

[a] Calculated for a 70 kg, 172 cm adult male

[b] Calculated by adjusting the safe level of egg or milk protein for protein quality by using chemical scores according to the procedure suggested (FAO/WHO, 1973).

[c] The single requirement is based on an average protein utilization efficiency of 75%.

[d] The FDA requirement for protein is 45 grams if the PER of the protein is greater than or equal to the PER of casein (2.50). Otherwise, the FDA requirement is 65 grams.

[e] The 95% confidence limits on the mean requirement are given in parentheses. For egg, beef and rice, the upper limits could not be calculated on the requirement for 0.2 g/m^2 nitrogen balance because of curvature in the regression line.

To appreciate the significance of the comparisons offered by Table 6, it should be remembered that the FAO/WHO and NAS/NRC standards were derived by increasing the estimated mean requirement 30% to allow for individual variation. Additionally, the FAO/WHO, NAS/NRC and FDA requirements include allowances for the loss of nitrogen via "miscellaneous" routes. The magnitude of this minor loss of nitrogen was assumed to be 5 mg of N per kg by the FAO/WHO committee and 0.30 grams of N by the NAS/NRC. Hence the requirements suggested by these groups should be compared to requirements estimated by the present study for a positive nitrogen balance of 0.2 g/m^2. This would represent an allowance of nitrogen loss via minor routes equal to 0.37 grams of N or 5.2 mg of N kg for a 70 kg, 172 cm individual.

For these reasons, the protein requirements given in Table 6 for a 0.2 g/m^2 nitrogen balance were increased by 30% and included as an additional column. Comparison of these latter values with the FAO/WHO and FDA requirements reveals that although there is fairly good agreement among the requirements for rice, corn and wheat, the requirements estimated by the present study for egg and beef are significantly higher than those recommended by the FAO/WHO committee or the FDA. On the other hand the NAS/NRC requirement is sufficient for higher quality proteins but is too low for rice, corn and wheat.

These results are supported by the recent study of Garza, Scrimshaw and Young (1976) who concluded that a significant proportion of the population may require excess caloric intakes to maintain zero nitrogen balance when fed egg protein at the level suggested by the FAO/WHO Expert Committee. Other researchers have reported requirements for egg protein which are in agreement with that determined in the present study and have similarly found that energy intakes in excess of that required for maintenance were necessary to achieve nitrogen equilibrium at lower levels of protein intake (Inoue, Fujita and Niiyama, 1973; Calloway, 1975).

Consideration of Table 6 and Figure 5 would lead one to conclude that the minimum protein requirement depends heavily upon the magnitude of the so-called minor nitrogen losses. The egg, beef and rice regression lines indicate that the additional nitrogen required to increase nitrogen balance for 0.0 to 0.2 g/m^2 is used at a very low efficiency. A small increase or decrease in the estimated positive nitrogen balance required for true nitrogen equilibrium would consequently exert a relatively large effect on the amount of nitrogen needed to achieve that balance. This is apparently the reason why the present study indicates an egg protein requirement higher than the FAO/WHO estimate. The FAO/WHO requirement was calculated assuming a 77% utilization of nitrogen at the requirement level of intake. According to the results of the present study, egg nitrogen is used 68% efficiently for the replacement of obligatory nitrogen loss at the point of zero nitrogen balance, but only 48% efficiently at a positive N balance of 0.2 g/m^2 . The rapid decrease in utilization between these two points contributed to the higher nitrogen requirement.

Figure 5 and Table 6 also indicate a trend for nitrogen source to become a less important factor in determining nitrogen requirement as one assumes larger and larger amounts of nitrogen loss via minor routes. The difference between the requirements for egg and wheat protein was 17 grams when no minor losses were considered, but dropped to 13 grams when a nitrogen loss of 0.2 g/m^2 was assumed. It will be remembered that many estimates of minor losses of nitrogen are higher than 0.2 g/m^2 and fall in the range of 0.5 to 1.0 grams or 0.27 to 0.54 g/m^2. This would suggest that there are only small and perhaps insignificant differences between most dietary proteins in terms of their true maintenance requirements. Unfortunately, there are fairly large 95% confidence limits on the regression lines of Figure 5 at maintenance levels of nitrogen intake (Appendix C, Figure 10). Any conclusion, therefore, regarding the regression lines at this level of intake must be considered tentative. More nitrogen balance studies and better estimates of miscellaneous nitrogen losses are required if protein requirements are to be more closely bracketed and significant differences between nitrogen sources established.

Figure 5 indicates that several of the regression lines tend to converge at higher (4.0 - 5.0 g/m^2) and lower (1.0 - 2.0 g/m^2) levels of intake. The convergence at higher levels is to be expected as levels of intake are supplied which are compatible with the maintenance requirements for increasing numbers of proteins. In this regard, Figure 5 (see also Appendix C, Figure 10) suggests that at N intakes above 4.4 g/m^2 (7.3 grams N or 46 grams protein per 70 kg, 172 cm individual) all the proteins except wheat gluten give mean nitrogen balances which are nearly equal and which are not statistically different at the 5% level of confidence.

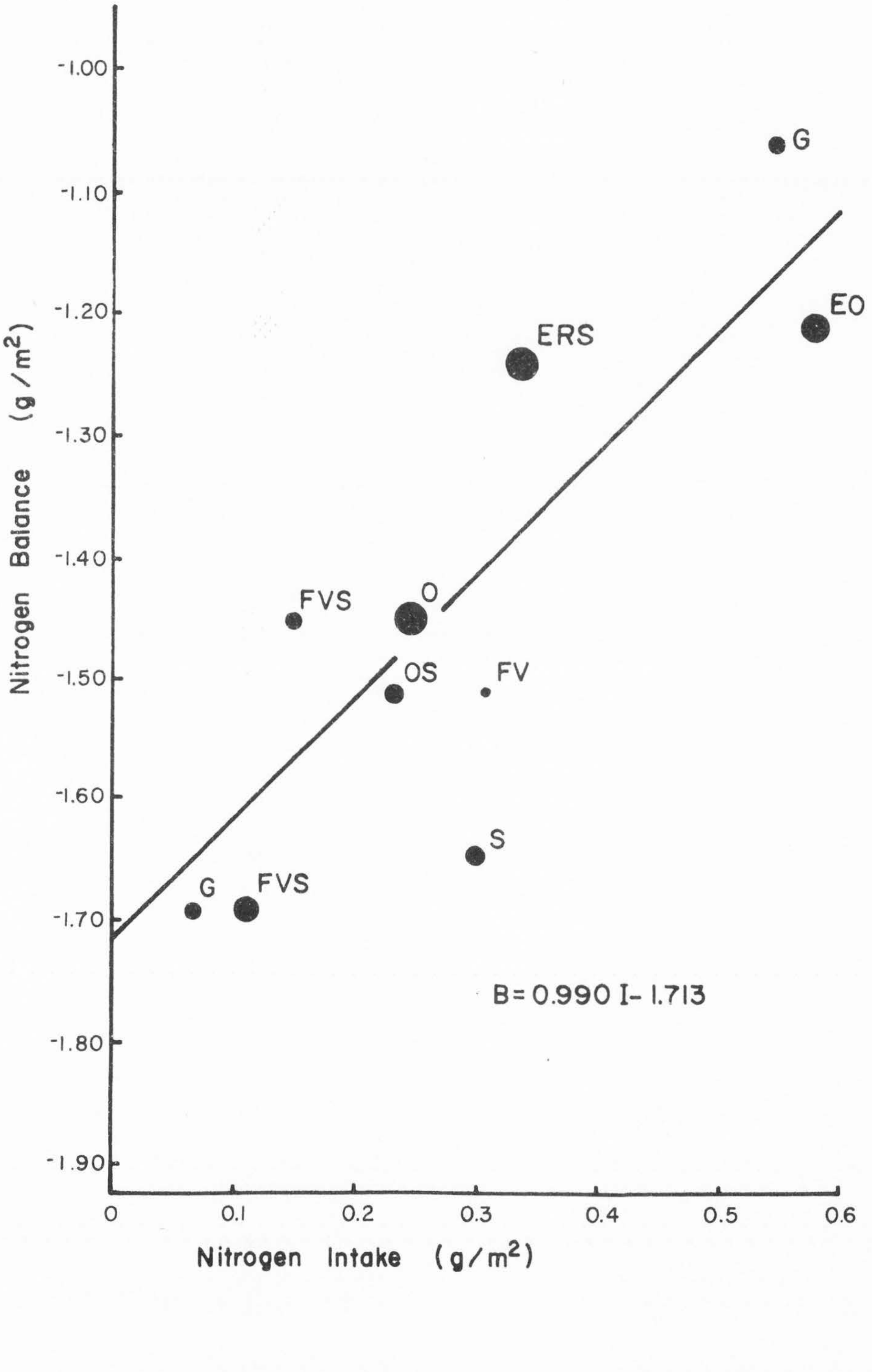
The convergence of the beef, rice, wheat and wheat gluten regression lines in Figure 5 at nitrogen intakes of 1.0 to 2.0 g/m^2 is not easily explained. A similar convergence of egg and wheat gluten regression lines has been reported by Young (1975) and Inoue et al. (1974). In the latter study, Inoue and co-workers found that the urinary N output of young men receiving 26 mg N/kg (about 0.9 g N/m^2) from egg or wheat gluten was essentially identical.

Unfortunately, there is a dearth of information on nitrogen balance response at intake levels between 0.5 and 1.5 g/m^2, especially for the proteins from rice, corn and wheat. This makes extrapolation of the regression lines into this region uncertain without additional data. Intake levels of 0.5 g/m^2 and lower have been used in conjunction with determinations of endogenous nitrogen loss. These data (Appendix B) are interesting because they indicate an equality of nitrogen balance response, regardless of nitrogen source (Figure 6). Indeed, during the measurement of endogenous nitrogen loss, it has been common practice to consider that small amounts of nitrogen are utilized 100% efficiently. The slope of the regression line in Figure 6 is 0.990, indicating nearly complete utilization of ingested nitrogen.

Returning to consideration of Figure 5, it seems reasonable to expect that most, if not all, of the regression lines plotted should coincide with the "low intakes" regression line if extended. This necessitates curvatures in the balance versus intake regression line for wheat gluten and wheat in the range of 0.5 to 1.0 g N/m^2 intake. No great curvature is necessitated in the corn line, however, For rice, beef and egg, the single fitting of a quadratic curve (as was done by Young et al. (1973) for egg protein) over the entire range of intake levels might be adequate.

The mechanisms responsible for the efficient use of dietary nitrogen at intake levels below about 1 g/m^2 deserve further exploration. Wheat and wheat gluten have been shown to be limiting in lysine for nitrogen balance purposes in human adults (Scrimshaw, Taylor and

Figure 6. The relationship between nitrogen intake and nitrogen balance for very low intake levels of various nitrogen sources. The regression line is drawn from the equation given where B is nitrogen balance (g/m^2) and I is nitrogen intake (g/m^2). The letters by each point indicate the nitrogen source(s): E, egg, FV, low-nitrogen fruits and vegetables; G, wheat gluten; O, oatmeal; R, rice; S, wheat or corn starch. Values plotted are means of from 4 to 50 observations. The size of each point is proportional to the number of observations.


-1.00
-1.10
-1.20
-1.30
-1.40
-1.50
-1.60
-1.70
-1.80
-1.90
Nitrogen Balance (g/m²)
0
0.1
0.2
0.3
0.4
0.5
0.6
Nitrogen Intake (g/m²)
G
EO
ERS
FVS
O
OS
FV
S
G
FVS
B = 0.990 I - 1.713

Young, 1973; Rice et al., 1970; Kies and Fox, 1970a). There is increasing evidence that the nature of the nitrogen balance response is influenced drastically by the identity of the essential amino acid which is limiting. Bender (1965) reported that the NPU measured in young rats of a lysine-free diet was about 40. The NPUs of diets lacking tryptophan, threonine, histidine, phenylalanine, leucine, or isoleucine were about 20 while valine or methionine-cysteine free diets had NPUs of zero. The findings of Said and Hegsted (1970) and Ashida and Yoshida (1975) are similar. They found that adult rats responded quite differently to diets lacking in one essential amino acid than they did to a protein-free diet. Table 7 gives the negative nitrogen balances observed, expressed as percentages of the negative nitrogen balance when no protein was fed. It will be noted that if lysine was completely removed from the diet of an adult rat, the negative nitrogen balance was only 28% of 15% of what might be expected.

Table 7. Response of adult rats to diets missing different essential amino acids as compared to the response to a protein-free diet

Amino acid missing	Said and Hegsted (1970)	Ashida and Yoshida (1975)
lysine	28	15
leucine	30	29
histidine	46	16
phenylalanine[a]	54	26
valine	59	70
tryptophan	66	37
isoleucine	75	68
threonine	86	58
methionine[b]	93	81
all (protein-free)	100	100

[a] in the absence of tyrosine

[b] in the absence of cysteine

The data are scanty, but it is likely that similar conclusions may be drawn about nitrogen balance in human adults. Said and Hegsted (1970) concluded from the studies of Leverton et al. (1956) and Jones, Baumann and Reynolds (1956), that a lysine-free diet induced a less negative nitrogen balance in adult subjects than did a tryptophan-free diet. Similar conclusions can be made from Rose's classic studies on amino acid essentially. The elimination of lysine (Rose, Haines and Warner, 1954) resulted in balances of -1.72 and -1.87 grams of nitrogen in two subjects, while the elimination of isoleucine (Rose, Haines and Warner, 1951) caused much more negative balances of -3.79 and -3.90 grams of nitrogen in two other subjects. These may be compared with an expected negative balance of about -3.4 grams of nitrogen for subjects consuming a protein-free diet (FAO/WHO, 1973).

It seems reasonable to assume that whatever mechanisms operate to decrease N wastage in lysine-free diets, those same mechanisms might also be operating at very low intakes of proteins which are limiting in lysine. It will be noted that of the sources of nitrogen indicated in Figure 6, wheat gluten, oats, starch (whether corn or wheat), and rice have all either been demonstrated biologically to be first limiting in lysine (Bressani, Elias and Brenes, 1972; Jansen, 1974) or it can be calculated that they are most limiting in lysine when compared to egg (FAO, 1970; Orr and Watt, 1957). This fact may explain why it is justifiable to assume complete utilization of protein at low nitrogen intakes during measurement of endogenous nitrogen losses. It is quite possible that most of these low protein intakes are limiting in lysine.

There are several mechanisms whereby a shortage of lysine or of some other essential amino acid might be tolerated at low levels of nitrogen intake. (1) The amino acid pattern of the ingested protein may not be important at very low intake levels because of the large amount of endogenous gastrointestinal tract nitrogen absorbed along with it. (2) Limited lynthesis of the essential amino acid might occur, or (3) Catabolism of the limiting amino acid might be slowed or halted altogether at low intake levels. These three possibilities will be discussed.

A significant amount of protein is secreted into the alimentary tract by the body, only to be later digested and re-absorbed. Munro (1969) has estimated that for a 70 kg man this amounts to a turnover of 67 grams of protein or 10.7 grams of nitrogen (about 5.8 g/m^2) daily. Others have suggested even higher amounts (Costa, 1971). It is evident that 1 or 2 grams of dietary nitrogen will not influence greatly the overall pattern of amino acids absorbed by the body. This theory was expressed by Nasset (1956) as quoted by Gitler (1964, p. 52): " ... the supplementary value of the digestive tract amino acids may enable the body temporarily to extract considerable nutritive value from a protein which over the long run is inadequate for growth or maintenance." Gitler (1964) pointed out, however, that dietary and endogenous protein are not digested at the same rate. Protein secretions into the digestive tract, which are mostly digestive enzymes and sloughed-off intestinal mucosa, are by necessity resistant to digestion. Since supplements to diets limiting in essential amino acids may not be effective if they are delayed (Geiger, 1948), this delay may be a critical factor which limits the supplementary ability of the endogenous protein. It is also difficult to explain the variety of response to omissions of different essential amino acids from the diet (Table 6) by endogenous supplementation alone.

Synthesis of some of the essential amino acids at sub-optimal rates could explain the apparent unimportance of amino acid pattern at low levels of nitrogen intake. Numerous studies with ^{14}C-labeled carbohydrate or acetate, however, have failed to show the incorporation of label into the essential amino acid carbon skeletons isolated from many mammalian species (Munro, 1969). Said, Hegsted and Hayes (1974), recently concluded that there was no reason to suspect that essential amino acids could be synthesized in the body and cited the basic isotopic work of Seele (1952) as reference. There is evidence that a small amount of threonine may be synthesized in mammalian tissues (Chung et al., 1966; Berkersky and Daivd 1968), and this might explain the small improvement in nitrogen balance of rats fed threonine-free as opposed to protein-free diets (Table 6). However, synthesis of lysine or leucine, amino acids with which much larger improvements were associated, is not supported by any experimental evidence.

There are important metabolic adaptations which take place as the level of nitrogen in the diet decreases. Young et al. (1973) have commented that these changes result in a more efficient utilization of endogenous amino acids. Thus, activities of enzymes associated with the urea cycle decrease (Schimke, 1970), albumin is degraded at a slower rate (Waterlow, 1969), and amino acids are recycled more efficiently (Stephen and Waterlow, 1966). In addition, there is significant evidence that the metabolic pathways for the degradation of some essential amino acids show adaptation in relation to intake (Munro, 1970).

Both Young (1975) and Said, Hegsted and Hayes (1974), have suggested that lysine and perhaps other essential amino acids are conserved at low intake levels. In this regard, the findings of McFarlane and Holt (1969a, 1969b) are particularly interesting. They reported that the oxidative degradation of leucine and phenylalanine in rats fed a low nitrogen diet decreased whereas the oxidation of glutamate and alanine remained the same. The authors were able to correlate the lowered leucine oxidation with a loss of leucine transaminase activity. Similar studies have shown a depression of lysine catabolism in animals fed a lysine-free diet (Yamashita and Ashida, 1969) and of valine catabolism in protein-depleted animals (Reeds, 1974).

Additional evidence for the conservation of lysine relative to other amino acids was discussed by Said and Hegsted (1970). Yang, Tilton and Ryland (1968), showed that a delayed supplement of lysine to a lysine-free diet promoted tissue regeneration, whereas a delayed supplement of tryptophan to a tryptophan-free diet did not. Finally, inhibition of tumor growth due to amino acid deficiencies was found to vary according to which amino acid was deficient (Sugimura et al., 1959).

Whatever mehcanism or mechanisms, inclusive or exclusive of the three discussed, are actually involved in the efficient use of small amounts of protein the implication is that many differences in protein quality--and particularly those due to differences in lysine content--will be difficult or even impossible to recognize, in terms of nitrogen balance, at low levels of nitrogen intake. The apparently equal nitrogen balance responses to 1.2 g/m^2 of beef and wheat gluten nitrogen (Figure 5) do not, by any means, indicate that wheat gluten is equivalent to beef nutritionally. Rather, it is the intake of nitrogen required for maintenance which is important. This is obviously quite different for the two protein sources.

The traditional concept of biological value and net protein utilization is that these measures are constant in the sub-maintenance to maintenance range of nitrogen intakes. Mathematically, if BV or NPU are to remain constant, the relationship between nitrogen intake and nitrogen balance must be a straight line which intersects the nitrogen balance axis at the level of endogenous nitrogen loss. It is apparent from Figure 5 that none of the protein sources investigated--with the possible exception of corn--conform to the traditional requirements.

The compilation of data in Table 1 is interesting in this light. Biological value tends to decrease in magnitude as the level of nitrogen at which it is determined increases. Table 1 also indicates a convergence of BV at levels of nitrogen intake of 2 or 3 grams (about 1.1 or 1.6 g/m^2). This is consistent with the convergence of the regression lines in Figure 5 at these levels of intake.

Several researchers have noted the deviation from linearity of nitrogen balance versus intake regressions and some have commented upon the theories of BV and NPU in this light (Said and Hegsted, 1970; Young et al., 1973; Inoue, Fujita and Niiyama, 1973; Inoue et al., 1974; Said, Hegsted and Hayes, 1974; Young, 1975, Young et al., 1975, Hegsted and Juliano, 1975). The general conclusion of these authors is that the theory behind BV and NPU is incorrect. The relationship between nitrogen balance and intake is not a straight line and efficiency of utilization is not constant. Nitrogen balance measurements made at low, submaintenance nitrogen intakes are made under conditions of abnormally efficient nitrogen metabolism. If this efficiency of utilization is extrapolated to maintenance nitrogen intakes, nitrogen requirements are underestimated--especially if the nitrogen source is limiting in an amino acid for which there exists conversative mechanisms, as is the case with lysine-limited wheat or wheat gluten.

These conclusions hold not only for the lower quality proteins but for higher ones as well. Egg protein, long considered to have a BV or NPU in the range 90 to 100, has an NPU at a requirement level of nitrogen intake in the range 60 to 70 (Calloway and Margen, 1971; Inoue, Fujita and Niiyama, 1973; Young et al., 1973). Only at levels of nitrogen about one half the requirement level does the NPU reach the expected range (Young et al., 1973). Similar variations have been found for the NPU and BV of beef protein (Young et al., 1975).

The present analysis supports these findings. Table 8 gives mean NPUs for the six nitrogen sources studied at several submaintenance levels of intake. These were calculated from the formula NPU = (B - E) /I, where B is nitrogen balance calculated from the regression equations given in Figure 5, E is the estimated nitrogen balance at zero nitrogen intake calculated from the "low intakes" regression equation given in Figure 5, and I is nitrogen intake. NPU may be defined in this context as the change in nitrogen balance (B - E) as nitrogen intake increases from zero to I, divided by the change in nitrogen intake (I - 0, or I). Decreases in NPU are evident for all proteins as the level of nitrogen intake increases. The NPU of corn appears to be affected to a lesser extent by nitrogen intake than the other nitrogen sources investigated.

The regression line for corn protein in Figure 5 seems to break up a seemingly consistent pattern exhibited by the other proteins.

Table 8. Predicted NPU for six protein sources at several sub-maintenance levels of nitrogen intake

Nitrogen source	Level of nitrogen intake (g/m^2)					
	1.5	2.0	2.5	3.0	3.5	4.0
Egg	91	78	68	-	-	-
Beef	70	66	62	58	-	-
Rice	66	62	59	55	-	-
Corn	53	49	47	46	45	44
Wheat	64	55	50	47	44	43
Wheat gluten	60	48	40	36	32	29

First, it crosses the wheat regression line and does so with slope which is statistically different at the 10% level. Second, if extrapolated it would intersect the N balance axis at a point much closer to the estimated endogenous nitrogen loss of 1.71 g/m^2 than would the regression lines for wheat or wheat gluten.

The anomalous behavior of the nitrogen balance response to corn protein might be rationalized by a consideration of amino acid patterns. Corn has been shown to be first limiting in lysine for human nutrition, but is closely second-limiting in tryptophan and third limiting in isoleucine, (Bressani, Elias and Brenes, 1972; Jansen, 1974). Some studies have, in fact, shown that lysine and tryptophan are equally limiting. Scrimshaw et al. (1958) and Bressani et al. (1958) found that lysine and tryptophan added individually to the diets of young children fed corn protein were of no benefit. When they were added in combination, however, a significant increase in nitrogen balance resulted which was further improved by addition of isoleucine. On the other hand, wheat protein has consistently been shown to be limiting in lysine and there is no closely second-limiting amino acid as there is in corn (Jansen, 1974; Bressani et al., 1972). It will be noted from Table 6 that deletion of tryptophan or isoleucine from rat diets resulted in a much more negative nitrogen balance than did the elimination of lysine. It might be expected therefore, that corn would be less efficiently utilized at low intake levels than wheat protein because of the partially-limiting character of tryptophan. In addition, the high leucine content of corn could be the cause of an amino acid imbalance or antagonism as the nitrogen intake decreases. Supplementation of a wheat-based diet containing 4 grams of nitrogen with leucine to a level commonly found in corn protein was shown to adversely effect nitrogen balance (Kies and Fox, 1972). At a nitrogen intake of 6 grams, no adverse effect of leucine on N balance could be detected in a comparable study (Kolski et al., 1969). The combined effects of high leucine content and almost equally limiting amounts of lysine and tryptophan give corn an amino acid pattern which may not be as effectively utilized at low levels of intake as that of wheat.

In this light, one might be tempted to explain other deviations from a regular pattern in Figure 5 in terms of differences in amino acid pattern. For example, egg protein has an abundance of essential amino acids in relation to total nitrogen as evidenced by dilution studies (Scrimshaw et al., 1966; Kofranyi and Jekat, 1964). It might be hypothesized that the increasingly higher N balance observed for egg in relation to beef or rice as nitrogen intake decreases may be due to this fact. Unfortunately, since the standard errors of the egg, beef, and rice regression lines are also increasing in this intake range (Appendix C, Figure 10) it is uncertain whether this difference is real. Similarly, it must be remembered that there is a 5 to 10% chance that the corn and wheat regression lines actually have the same slope. Any conclusions made about the nature of the nitrogen balance versus intake relationship must be tempered by such statistical realities.

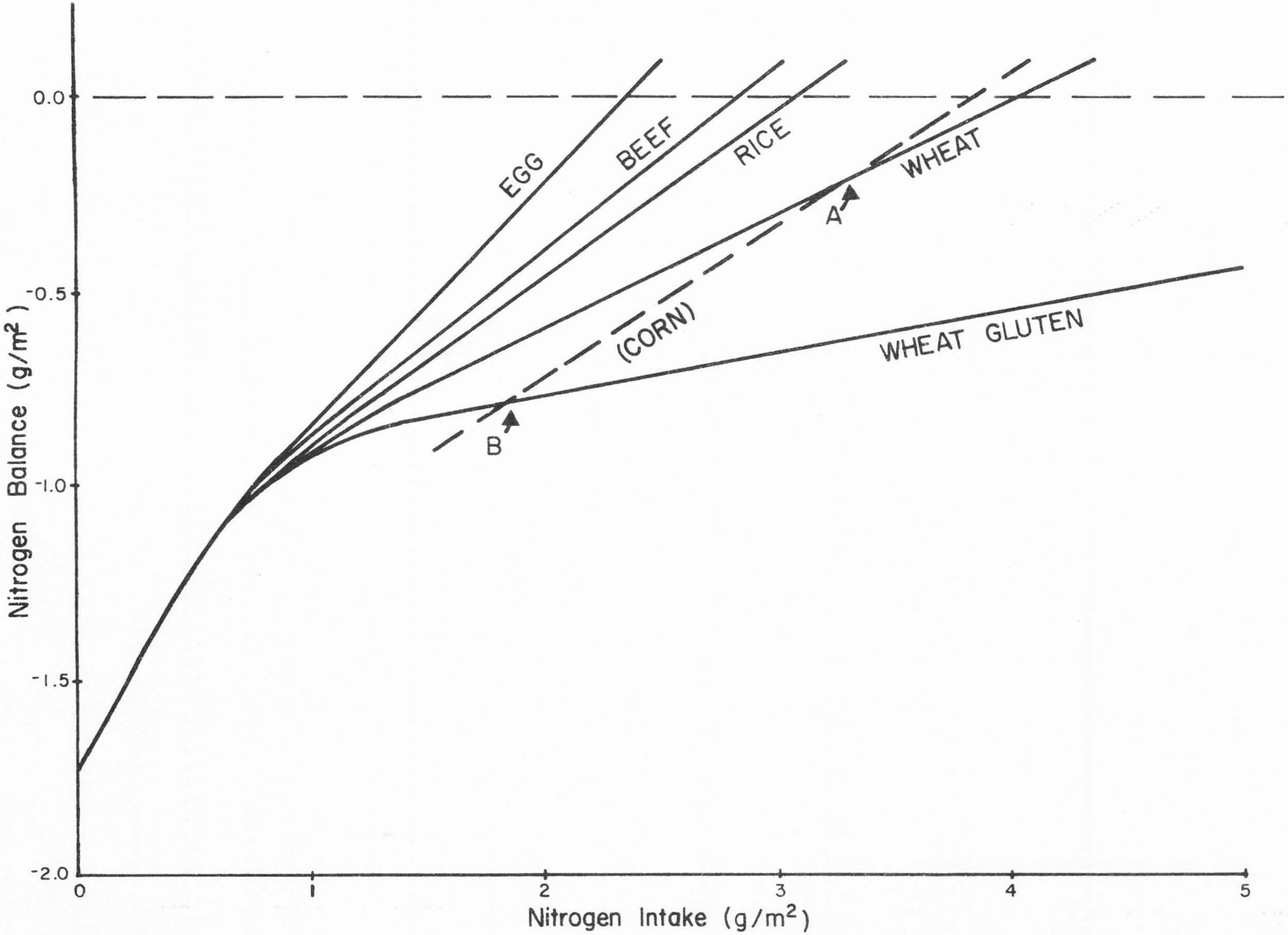
If the seemingly anomalous behavior of corn as compared with beef, rice, wheat and wheat gluten can be shown through subsequent research to be real rather than artifacts of experimental procedure or analysis, this would introduce yet another complication into the traditional ideas about biological value.

The traditional concept of biological value includes the idea that an accurate measurement of BV can be used to predict the maintenance requirement of a protein. The requirement of a protein with a BV of 50 should be twice whatever the endogenous loss of nitrogen is, the requirement of a protein with a BV of 75 should be 33% more than the endogenous loss, and so on. It is apparent that this simplistic concept is generally incorrect if the BV is measured at a submaintenance level of intake. Because of the curvilinear nature of the intake-balance response curve one cannot assume that the efficiency of nitrogen utilization will be as high at a maintenance level of intake as it was at a sub-maintenance level.

A curvilinear relationship between intake and balance, however, could be dealt with if this relationship were mathematically similar for all kinds of protein. One would then obtain a family of curves or lines representing the relationship between intake and balance for a continuum of protein qualities and maintenance requirements. But if the general relationship between balance and intake is not the same for different protein sources, we are faced with a very difficult situation with regards to interpreting isolated nitrogen balances or biological values.

These concepts are illustrated in Figure 7. A hypothetical, mathematically similar relationship between balance and intake is shown for egg, beef, rice, wheat and wheat gluten. These relationships have been drawn to represent the actual data as close as possible (Figure 5). If one imagines a continuum of lines in the spaces between the lines which are plotted, it is apparent that each nitrogen balance-nitrogen intake combination within the triangular area bounded by the

Figure 7. A hypothetical series of relationships between nitrogen intake and nitrogen balance for egg, beef, rice, wheat and wheat gluten, based on Figure 5. The heavy dashed line indicates the regression of nitrogen balance with nitrogen intake for corn protein (Figure 5) and has been extended so as to intersect the curve for wheat gluten. Points A and B are discussed in the text.



Nitrogen Balance (g/m²)
0.0
-0.5
-1.0
-1.5
-2.0
0
1
2
3
4
5
Nitrogen Intake (g/m²)
EGG
BEEF
RICE
WHEAT
(CORN)
WHEAT GLUTEN
A
B

egg and wheat gluten lines could be associated with one and only one estimate of intake required for zero balance. Theoretically, one could determine the average maintenance requirement for almost any protein by measuring the average nitrogen balance at a single nitrogen intake of, say, 2 g/m^2.

Since NPU is essentially the slope of a line connecting a point on the intake-balance regression curve with the point at which the curve intersects the balance axis, the requirement for zero balance could similarly be determined if one knew the NPU (for the BV and the coefficient of true digestibility) and the nitrogen intake level at which it was determined.

Now assume one attempted to determine the maintenance requirement of corn protein by this procedure. The heavy dashed line in Figure 7 represents the relationship between intake and balance for corn protein derived from the present study. Assuming this relationship is correct, if one measured the N balance at an intake level of about 1.8 g/m^2 the N balance-N intake combination obtained (indicated by letter B in Figure 7), would indicate that corn had the same protein quality as wheat gluten. A nitrogen balance determination giving the results indicated by the letter A in Figure 7 would lead the investigator to conclude that corn was of the same quality as wheat. According to the hypothetical relationships assumed between balance and intake it would be impossible to predict the intake required for zero balance by making a single measurement. Multiple measurements at different levels of intake would need to be made and a regression analysis conducted, from which the requirement could be directly determined. No matter what relationship is proposed to hold between balance and intake, this same situation would arise.

It is possible that this difficulty could be overcome by determining the relationship between balance and intake for a large number of proteins. More insight could then be gained into the relationship between amino acid pattern and nitrogen balance response. If the nature of the balance-intake relationship could be predicted from amino acid content, some progress could then be made in relating biological value or nitrogen balance to protein quality.

The Effect of Caloric Intake on Nitrogen Balance

A highly significant linear effect of energy upon nitrogen balance was indicated by the preliminary regression analyses (Table 3) performed with data on egg, rice, and wheat gluten proteins. To further define the quantitative nature of this effect, caloric intake variables were added to the quadradic and linear regression equations determined previously (Figure 5). Three variables were tested for significance: caloric intake, caloric intake squared and caloric intake multiplied by nitrogen intake. The latter variable was chosen to test the hypothesis that the effect of caloric intake on nitrogen balance depends on the level of nitrogen in the diet (Calloway, 1975). For consistency, caloric intakes were expressed as kilocalories per square meter of body surface area (kcal/m^2).

Table 9 gives the regression equations relating nitrogen balance, nitrogen intake, and caloric intake which were obtained. The values of the regression coefficients (b_0, b_1, b_2, ..., b_5) and of the multiple correlations coefficient (r) are those which were obtained after the nonsignificant variables were deleted from the general equation. The variables remaining were significant or estimated to be significant at confidence levels ranging from 25 to 0.1%. There was no significant effect of caloric intake on nitrogen balance for the wheat protein data. This was probably because energy intakes were not reported in a large number of the studies and had to be estimated from body weight.

Much can be learned by considering the signs of the regression coefficients in Table 9. The coefficient of caloric intake, b_3, is invariably positive, indicating that nitrogen balance tends to increase

Table 9. Regression of nitrogen balance with nitrogen and caloric intake

General Equation: $B = b_1 I + b_2 I^2 + b_3 C + b_4 C^2 + b_5 C I + b_0$

where B = nitrogen balance (g/m^2)

I = nitrogen intake (g/m^2)

C = caloric intake ($kcal/m^2$)

Nitrogen source	Values of regression coefficients						multiple correlation coefficient (r)
	b_1	b_2	$b_3 \times 10^3$	$b_4 \times 10^6$	$b_5 \times 10^4$	b_0	
egg	0.716***	-0.0816*	5.65**	-1.49**	NS[a]	-6.52	0.666
beef	0.846***	-0.0862**	0.757[b]	NS	NS	-3.04	0.779
rice	0.344***	NS	5.41[b]	-1.37[b]	NS	-6.38	0.750
corn	1.607*	NS	11.58[c]	-2.81[b]	-7.33[c]	-12.93	0.802
wheat gluten[d]	0.812[c]	NS	21.85*	-5.68*	-4.01[b]	-21.68	0.697

*** significant at the 0.1% level

** significant at the 1% level

* significant at the 5% level

[a] NS = not significant at the 25% level

[b] significant at the 25% level

[c] significant at the 10% level

[d] the mean square error for this regression was assumed to be equal to the mean square error for the corn protein regression. The tests of significance are based upon this assumption.

as caloric intake increases. The sign of b_4, the coefficient of caloric intake squared, is negative. This indicates that the positive effect of caloric intake on nitrogen balance tends to diminish as caloric intake increases. The coefficient of caloric intake multiplied by nitrogen intake, b_5, takes on a negative value. Apparently, the favorable effect on nitrogen balance of increasing the caloric intake is dependent in these cases upon the level of nitrogen intake. The prediction is that the higher the nitrogen intake, the less will be the increase in nitrogen balance per additional kilocalorie.

To illustrate these findings, Figure 8 shows the predicted relationships between nitrogen balance and nitrogen intake at several levels of caloric intake for egg and wheat gluten. The relationships for corn, rice and beef are not included. The regression equation given in Table 9 for corn yields a graph analogous to that for wheat gluten. The equations for rice and beef protein give relationships similar to egg.

Each regression line was calculated from the equations given in Table 9 by holding caloric intake constant at the specified value and varying the nitrogen intake. Caloric levels were chosen which were representative of the range present in the raw data. Regression lines were likewise extended only as far as the range of raw data indicated they were valid.

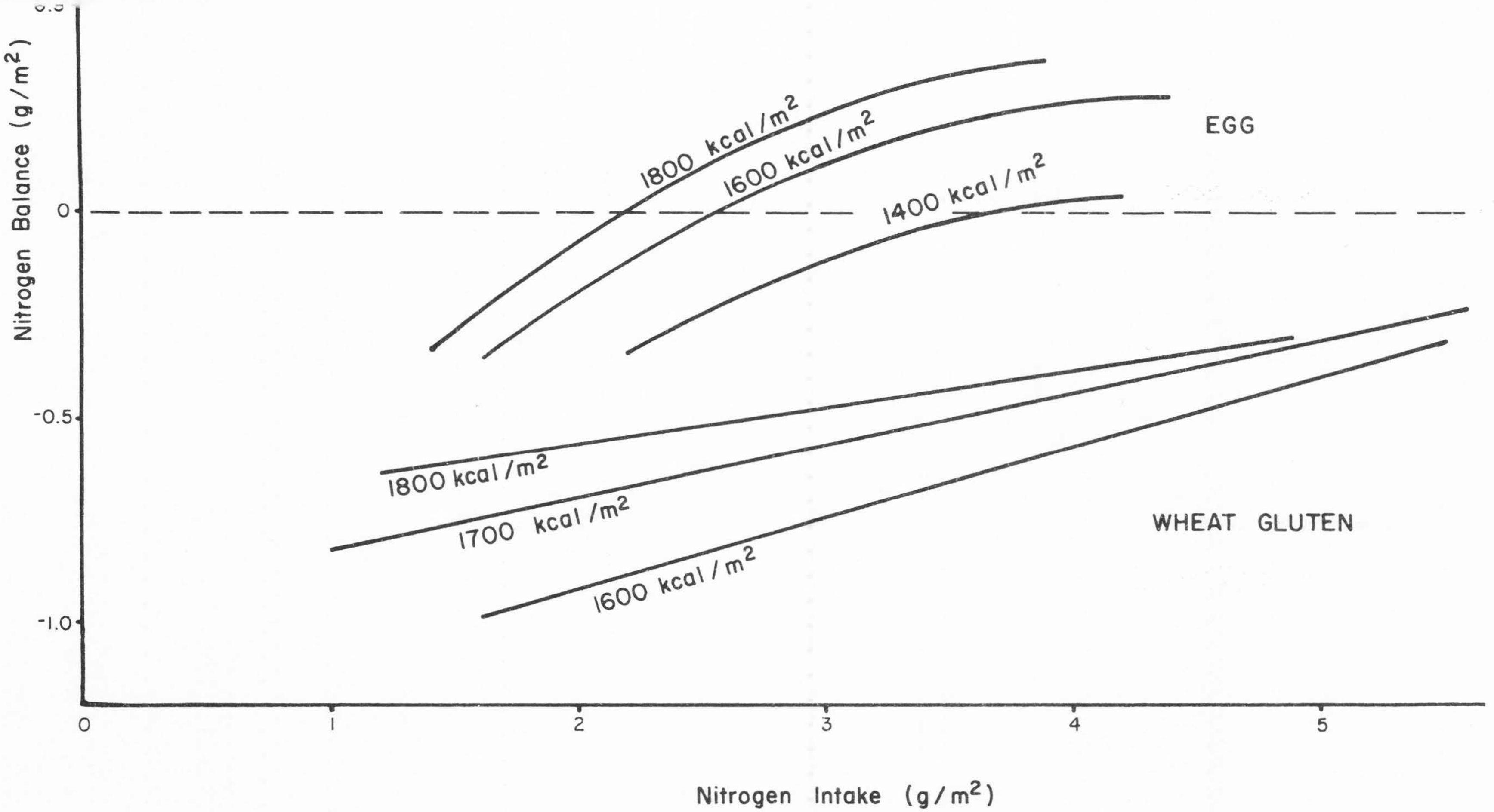
The regression lines for egg protein and for wheat gluten indicate clearly that at a given level of nitrogen intake, nitrogen balance tends to increase with an increase in caloric level. The lines are shifted progressively upward as the caloric level increases. In addition, the effect of the negative coefficient of the caloric intake squared term can be seen in both sets of curves. For egg, the 1600 kcal/m^2 line is closer to the 1800 kcal/m^2 line than it is to the 1400 kcal/m^2 line. While the effect of the 200 kcal/m^2 increase between 1400 and 1600 kcal/m^2 is an increase of about 0.22 g/m^2 in the nitrogen balance, the increase in nitrogen balance for the next 200 kcal/m^2 increase is only about half as great. The wheat gluten lines yield similar conclusions.

In the case of wheat gluten the effect of the negative coefficient of caloric intake multiplied by nitrogen intake can be witnessed. The three lines become indistinguishable at higher intake levels, showing the dependence of the magnitude of the nitrogen balance increase per kcal on the level of nitrogen intake.

The co-significance of caloric intake and caloric intake squared in explaining variation in nitrogen balance is consistent with the results and conclusions of other researchers. Visual inspection of the data compiled by Calloway and Spector (1954) (Figure 4) indicates a curvilinear relationship between caloric intake and nitrogen balance which is suggestive of the quadratic effects observed in the present analyses. Munro (1964b) and Miller and Payne (1963) have suggested that the relationship between caloric intake and nitrogen balance is biphasic. It was predicted that nitrogen balance should increase rapidly with caloric intake when the caloric intake is below the maintenance requirement. Above the maintenance requirement a more gradual increase was predicted. Such a biphasic relationship, if correct, would be interpreted as quadratic under the conditions of the present analysis.

Consideration of Figure 8 indicates that intakes of 1400 and 1600 kcal/m^2 are inadequate in terms of maximum nitrogen balance at low

Figure 8. The relationships between nitrogen intake and nitrogen balance for egg and wheat gluten proteins at several levels of caloric intake. Curves were drawn from the regression equations given in Table 9.


Nitrogen Balance (g/m^2)
0
-0.5
-1.0
0
1
2
3
4
5
Nitrogen Intake (g/m^2)
1800 kcal/m^2
1600 kcal/m^2
1400 kcal/m^2
EGG
1800 kcal/m^2
1700 kcal/m^2
1600 kcal/m^2
WHEAT GLUTEN

nitrogen intake levels. The recommended daily allowance of energy established by the NAS/NRC for a 23-50 year old, 70 kg, 172 cm male is 2700 kcal (NAS/NRC, 1974) or 1475 kcal per m^2 of surface area. It is apparent that the increases in nitrogen balance indicated in Figure 8 are taking place over a range of caloric intakes normally considered adequate or even excessive for American males. The FAO/WHO energy requirement for moderately active men (FAO/WHO, 1973) assumes a higher activity level and suggests that 46 kcal per kg body weight (1760 kcal/m^2 for the same 70 kg, 172 cm reference individual) are sufficient for the maintenance of a constant body composition. By this energy standard the increases in nitrogen balance discussed above are occurring over a range of submaintenance to maintenance caloric intakes. It is likely that the actual energy needs of the subjects contributing the data used in the present study are close to the NAS/NRC recommendation, as the acceptance criteria were designed to exclude studies where submaintenance caloric levels were fed.

By setting nitrogen balance equal to zero in the equation for egg protein (Table 9), the levels of nitrogen and caloric intake compatible with zero nitrogen balance can be predicted. Figure 9 compares these predicted levels with values determined experimentally and reported in the literature. The good correlation between measured values and the prediction curve is to be expected since both were generally derived from the same data (see Appendix A).

The FAO/WHO and NAS/NRC energy requirements of 1760 kcal/m^2 and 1475 kcal/m^2, respectively, are indicated on the ordinate of Figure 9. The predicted amount of egg nitrogen to achieve zero nitrogen balance at the 1475 kcal/m^2 level is 3.06 g/m^2 or 35 grams of egg protein per 70 kg, 172 cm individual. At the FAO/WHO energy requirement an intake of 25 grams would be required. While increases in caloric intake above the FAO/WHO standard are apparently ineffective in reducing the nitrogen requirement, intake levels below the standard result in a rapid increase in the requirement.

A protein requirement is impractical and unrealistic if it requires the consumption of more calories than are needed for constant weight. It is not known, however, if the present results--derived from data obtained under conditions of maintenance or excessive caloric intake--are applicable to populations which consume greater amounts of energy because of increased activity. The present study shows that if energy intake is increased above the requirement level, an improvement in nitrogen balance and a decrease in protein requirement results. It is uncertain that the same favorable effect of increasing caloric intake would be witnessed if the energy requirement was increased concomitantly. The relationships between energy intake, energy expenditure, nitrogen intake and nitrogen balance deserve further study.

If the partial derivatives of nitrogen balance with respect to caloric intake are taken of the equations listed in Table 7, equations are obtained describing the effect of caloric and nitrogen intake on the change in nitrogen balance per added kcal. These relationships are:

egg: $$\frac{\partial B}{\partial C} = -.00298\ C + 5.65$$

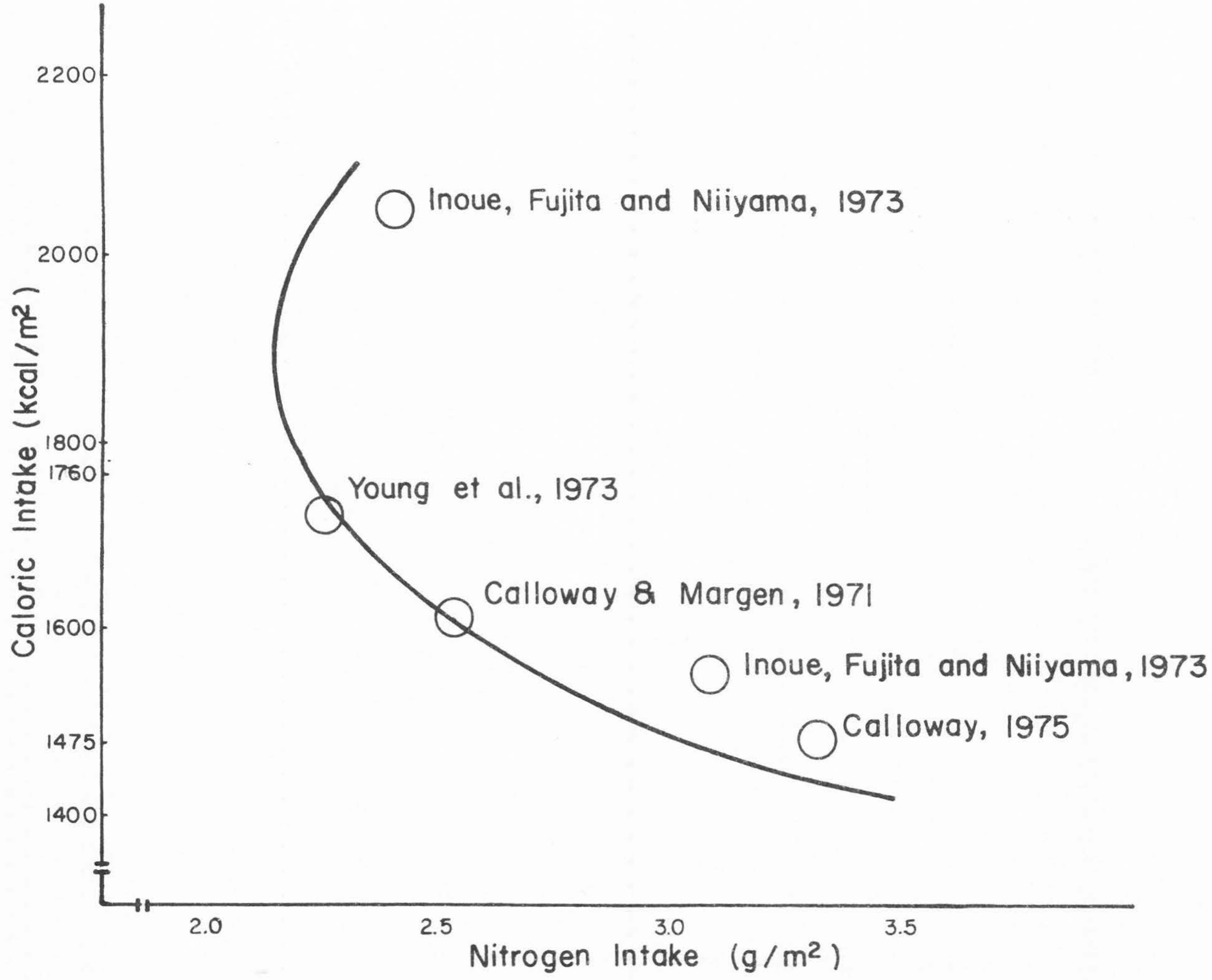
beef: $$\frac{\partial B}{\partial C} = 0.757$$

rice: $$\frac{\partial B}{\partial C} = -.00273\ C + 5.41$$

corn: $$\frac{\partial B}{\partial C} = -.00561\ C - 0.733\ I + 11.58$$

wheat gluten: $$\frac{\partial B}{\partial C} = -.01136\ C - 0.401\ I + 21.85$$

Figure 9. The relationship between caloric intake and the level of egg protein nitrogen compatible with zero nitrogen balance. The curve was calculated from the equation given in Table 9 by setting nitrogen balance to zero and solving for nitrogen intake at several levels of caloric intake. The circles indicate egg protein requirements for zero balance from the literature. It should be emphasized that the curve was not obtained from a quadratic regression analysis of the plotted points; rather, the curve was calculated from a regression equation derived from the data of nine separate studies representing 147 nitrogen balance measurements. Indicated on the ordinate are the NAS/NRC (1974) and FAO/WHO (1973) energy standards for men (2700 kcal and 46 kcal/kg, respectively, or 1475 kcal/m^2 and 1760 kcal/m^2, respectively, for a 70 kg, 172 cm individual).


Inoue, Fujita and Niiyama, 1973
Young et al., 1973
Calloway & Margen, 1971
Inoue, Fujita and Niiyama, 1973
Calloway, 1975
2200
2000
1800
1760
1600
1475
1400
Caloric Intake (kcal/m²)
2.0
2.5
3.0
3.5
Nitrogen Intake (g/m²)

where B, C, and I are as defined in Table 7 except that B is expressed in mg of nitrogen per m^2 surface area. This allows the change in nitrogen balance per unit change in caloric intake, $\partial B/\partial C$, to be expressed in the more common units of mg of nitrogen per kcal (mg/kcal).

The above equations reveal that caloric intake has a negative effect on the increase in nitrogen balance per additional kcal in the case of egg, rice, corn, and wheat gluten. No such trend was significant for beef protein, perhaps due to the narrow range of caloric intakes (1550 to 1850 kcal/m^2 as compared to 1250 to 2150 kcal/m^2 for egg, for example) in the data analyzed. The equations for corn and wheat gluten indicate an additional negative effect of nitrogen intake on the increase in nitrogen balance per kcal.

It will be recalled that calculations based on the study of Calloway and Spector (1954) showed that with a protein-free diet, N balance improved by about 8 mg/kcal as caloric intake was increased from 0 to 450 kcal. By setting caloric intake (C) and nitrogen intake (I) to zero in the above equations it can be seen that N balance improvements of 5.65, 5.41, 11.58 or 21.85 mg/kcal are expected upon the addition of energy to a starvation diet. Although extrapolations of this sort are usually not justifiable, it is gratifying to see the close correspondence between the first three estimates and the 8 mg/kcal figure.

Table 10 gives the mg/kcal increases in nitrogen balance which can be expected upon the addition of energy to diets supplying caloric intakes equivalent to the NAS/NRC and WHO/FAO energy requirements for men. In general these values are in harmony with previously published estimates of 0.70 to 2.85 mg/kcal (Calloway, 1975) or Munro's figure of

Table 10. Predicted effect of energy intake on nitrogen balance at two levels of energy intake

Protein	N balance change per kcal (mg/kcal)	
	1475 kcal/m^2	1760 kcal/m^2
Egg	1.25	0.41
Beef	0.76	0.76
Rice	1.38	0.61
Corn	1.84[a], 0.37[b]	0.24[a], <0[b]
Wheat gluten	4.29[a], 3.49[b]	1.05[a], 0.25[b]

[a] assuming nitrogen intakes of 2 g/m^2

[b] assuming nitrogen intakes of 4 g/m^2

2 mg/kcal (Munro, 1964b). Once again it should be noted that the N balance change per additional kcal is substantially less at the 1760 kcal/m^2 intake level (the FAO/WHO energy requirement) than at 1475 kcal/m^2 (the NAS/NRC requirement). The value for corn at 1760 kcal/m^2 and 4 g N/m^2 was negative, indicating that the predicted N balance change per additional kcal became zero at some caloric level between the two examined in Table 8.

From a limited review of studies on human subjects, Calloway (1975) concluded that N balance was improved to a greater extent by added energy if (1) nitrogen intake was high, (2) the nitrogen source was of high quality, or (3) the energy intake was low. The present study indicates strong support for the validity of condition (3). Condition (1) is not supported however. There is even some evidence in the case of corn and wheat gluten that higher nitrogen intakes decrease the effect of added energy on nitrogen balance. Condition (2) is supported in part by the results of the present study. At high enough nitrogen intakes, the effect of caloric intake on N balance in the case of corn and wheat gluten will be zero. Other than this, however, the effect of calories seems relatively independent of nitrogen source. It is evident that the interaction of nitrogen intake and protein quality with caloric intake in terms of their effect on nitrogen balance status requires further study.

Several researchers have recently pointed out that although a deficit in calories is religiously guarded against in nitrogen balance experiments, the opposite effect of excess calories is rarely considered (Garza, Scrimshaw and Young, 1976; Calloway, 1975). The common procedure is to feed enough energy to maintain or slightly increase the body weight of the subjects. This is done even when the subjects are in negative nitrogen balance. Obviously, a person in energy equilibrium but in negative nitrogen balance should be losing weight. Using a figure of 30 grams of N per kg lean body mass, and 7000 kcal for the energy value of 1 kg of body fat (Garza, Scrimshaw and Young, 1976) it can be calculated that it would take 230 extra kcal of energy daily to produce the body fat necessary to equal the loss of lean body tissue represented by a nitrogen balance of -1 gram. Assuming a 1 mg/kcal improvement in nitrogen balance, this extra energy would result in a nitrogen balance 23% higher than it would have been if it were measured at energy equilibrium.

It can be safely concluded that most nitrogen balance studies have been conducted at a level of calories above that required for energy equilibrium. The present study confirms that this will result in nitrogen balances which are too high and which are not representative of the values which would be attained under maintenance conditions.

SUMMARY AND CONCLUSIONS

The analyses described on the preceding pages provide evidence that the relationships between the nitrogen balance of adults and nitrogen intake, caloric intake, and other factors are complex and sometimes hard to define. The results may be summarized as follows:

1. Nitrogen intake, caloric intake and body weight were significant sources of variation in nitrogen balance at the 5% level of confidence for six, three and two of the six nitrogen sources investigated, respectively (Table 3). At the 25% level, nitrogen intake, caloric intake, body weight, adaptation time and research group were significant sources of variation for six, four, four, two and one of the six nitrogen sources, respectively (Table 3).

2. The correlation between N intake and N balance (Table 5) was generally improved by expressing data as grams per square meter of body surface area as opposed to expressing them per kilogram body weight or per kilogram body weight raised to the 0.73 power.

3. The relationship between N intake and N balance as N intake increased from zero to maintenance levels (Figure 5) was curvilinear with the possible exception of when corn was the source of nitrogen. Sharp bends in the regression lines were required for whole wheat and wheat gluten, while more gentle curvatures were sufficient to describe the relationships for egg, beef and rice. The regression lines for several of the proteins tended to converge at higher or lower intake levels (Figure 5 and Appendix C, Figure 10). At very low levels of N intake, proteins appeared to be utilized with near 100% efficiency,

regardless of source (Figure 6). Protein requirements for maintenance predicted from the regression lines (Table 6) were generally comparable to those suggested by the FAO/WHO (1973), the NAS/NRC (1974) and the FDA (1972). In the case of egg and beef, however, the present study predicted higher requirements than those set by the FAO/WHO or the FDA. This was due to a marked decrease in the efficiency of utilization of these proteins as the maintenance level of intake was approached. It was suggested that there may exist only small and perhaps insignificant differences between most common nitrogen sources in terms of their true maintenance requirements. The large amount of error in the regression lines at the higher levels of nitrogen intake which were studied (see Appendix C, especially Figure 10), however, made any such conclusion tenative. The corn regression line (Figure 5) exhibited anomalous behavior by crossing the wheat regression line with a slope which was significantly greater at the 10% level. This anomaly, if real, could be related to the high amount of leucine and the equal-limiting amounts of lysine and tryptophan in corn protein.

4. Caloric intake, in general, exerted a positive but diminishing effect on nitrogen balance with nitrogen intake held constant (Figure 8 and Table 9). In other words, the nitrogen balance increase per kilocalorie dropped steadily as caloric intake increased. While substantial increases in nitrogen balance resulted from increases in caloric level above the American energy standard for men of 2700 kcal (NAS/NRC, 1974), there was little effect predicted when intakes were increased above the FAO/WHO energy standard of 46 kcal per kg of 3220 kcal for a 70 kg man. Similar conclusions were made about the effect of energy intake on the intake of egg protein required for zero nitrogen balance (Figure 9).

The results of this study suggest that our understanding of certain aspects of protein utilization in human adults might profit from further studies. A relatively simple study, and one which would contribute greatly to our present knowledge would be a comparative investigation of the nitrogen intake-nitrogen balance response curve for several low-quality proteins differing in limiting essential amino acids. Particularly interesting would be a comparison of the nitrogen balance response to whole wheat and corn proteins. The present study predicts (see, for example, Figure 7) that dose-response curves of vastly differing natures would be obtained. Similar experiments could be performed with synthetic mixtures of amino acids limiting or partially limiting in amino acids of interest and the balance-intake relationships compared.

A second area of possible research is suggested by the dramatic effect of energy intake on the amount of egg protein required for zero nitrogen balance (Figure 9). While the present study indicates a sharp decrease in the nitrogen requirement when caloric intake is increased above the level required for maintenance, it is uncertain whether the nitrogen requirement would be similarly affected if caloric intake and caloric expenditure were increased concomitantly. Since high activity levels and limited protein resources tend to be common in underdeveloped parts of the world, the energy-activity-protein relationship deserves further exploration.

Some final comments and observations should be made concerning the prediction of protein quality from the amino acid composition of proteins. It was hoped that the present study would result in the development of some procedure whereby nitrogen balance could be used to derive a measure of protein quality free of the influences of nitrogen intake, caloric intake and as many other variables as possible. Data from the large number of studies reported in the literature where the nitrogen balance of human subjects was measured could then be used to relate protein quality to amino acid composition--perhaps by means of a multiple regression model similar to that used by Cresta et al. (1971) or Hansen and Eggum (1973). It is now evident, however, that there may be serious problems in defining and standardizing a measure of protein quality so that it represents something meaningful, let alone trying to relate such a measure to amino acid pattern.

For purposes of illustration, let us assume that the nitrogen intake required to achieve zero nitrogen balance is chosen as a standardized protein quality measure. This, of course, is only one of many which could be chosen, but the discussion which is to follow would apply equally well to any such measure.

It was previously discussed (see text, pages 78 to 81) how the N requirement for zero balance cannot be predicted from a single N balance determination or from a measurement at biological value if the general relationship between intake and balance, is not the same for all proteins. If the nature of the intake-balance dose-response curve exhibits variations dependent upon the amino acid pattern of the protein--and it appears that it may in the case of corn as compared to wheat, wheat gluten, rice and beef--(see Figures 5 and 7) we are faced with a perplexing circumstance. The goal is to relate amino acid composition to protein quality, measured for purposes of this illustration by the N requirement for zero balance. In order to determine the N requirement for zero balance from a single N balance measurement it is necessary to know the nature of the dose-response curve. But if the nature of the dose-response curve can only be determined by consideration of the amino acid pattern of the protein, we are put in the position of needing to know a relationship which would perhaps be as useful as the one we are trying to discover. In other words, to find out what we would need to know (the nature of the dose-response curve) would require knowledge quite similar to what we hope to find out (how to predict protein quality from amino acid composition).

To effectively use data from the existing nitrogen balance literature to help to establish relationships between amino acid composition and protein quality will require preliminary investigations into the influence of amino acid pattern on the nature of the nitrogen intake-nitrogen balance, dose-response curve. The relationship between nitrogen intake and nitrogen balance is critical to the correct interpretation of isolated measurements of nitrogen balance or of biological value. In deed, the nature of this relationship will determine whether such measurements are useful or whether they should be abandoned as hopelessly misleading.

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APPENDIXES

Appendix A

Data Used in the Analyses

The data given on the following pages (Tables 11 through 16) were taken directly from computer dumps of the data files used as input to the statistical programs. When the literature source reported means instead of the results for each experimental subject, the means were repeated in the data file--and consequently are repeated in the following listings--a number of times equal to the number of single observations from which the means were calculated. All other data in the Tables represent results for single individuals.

In two cases (Huang et al., 1966 and Scrimshaw et al., 1966) nitrogen balances were calculated from nitrogen intake and urinary nitrogen loss by assuming 0.97 digestibility (FAO/WHO, 1973) and an obligatory fecal nitrogen loss of 9 mg per kg body weight (Young et al., 1973).

The column headings in Tables 11 through 16 have the following meanings:

R Research group code:

1 = Calloway, Margen and co-workers.

2 = Clark and co-workers.

3 = Inoue and co-workers.

4 = Kies, Fox and co-workers.

5 = Scrimshaw, Young and co-workers.

DA Adaptation time in days.

SX Sex code:

1 = Male.

2 = Female

WT Body weight in kilograms.

HT Height in centimeters.

KCAL Caloric intake in kilocalories per day.

N IN Nitrogen intake in grams per day.

N BAL Nitrogen balance in grams per day.

Appendix B

Data on Very Low Nitrogen Intakes

Table 17 gives the raw data from which Figure 6 of the text was derived. Note that nitrogen intake and nitrogen balance are expressed as mg per kg body weight.

Appendix C

Confidence Limits on the Regression Lines

Tables 18 through 23 give the 95% confidence interval estimates for the mean nitrogen balance predicted by the regression equations of Figure 5 for six nitrogen sources at several levels of intake. Confidence limits were calculated for the wheat gluten regression line by assuming that the mean square error was equal to the mean square error of the wheat regression. Figure 10 summarizes the confidence interval estimates for the six protein sources.

Table 11. Egg Protein Data

R	DA	SX	WT	HT	KCAL	N IN	N BAL	REFERENCE
5	15	1	69.0	176	3174	2.36	-0.72	
5	15	1	69.0	176	3174	2.36	-0.72	Young et al., 1973
5	15	1	69.0	176	3174	2.36	-0.72	
5	15	1	69.0	176	3174	2.36	-0.72	
5	15	1	69.0	176	3174	2.36	-0.72	
5	15	1	69.0	176	3174	2.36	-0.72	
5	15	1	69.0	176	3174	2.36	-0.72	
5	15	1	69.0	176	3174	2.36	-0.72	
5	15	1	69.0	176	3174	2.36	-0.72	
5	15	1	69.0	176	3174	2.36	-0.72	
5	15	1	69.0	176	3174	2.36	-0.72	
5	15	1	69.0	176	3174	3.48	-0.06	
5	15	1	69.0	176	3174	3.48	-0.06	
5	15	1	69.0	176	3174	3.48	-0.06	
5	15	1	69.0	176	3174	3.48	-0.06	
5	15	1	69.0	176	3174	3.48	-0.06	
5	15	1	69.0	176	3174	3.48	-0.06	
5	15	1	69.0	176	3174	3.48	-0.06	
5	15	1	69.0	176	3174	3.48	-0.06	
5	15	1	69.0	176	3174	3.48	-0.06	
5	15	1	69.0	176	3174	3.48	-0.06	
5	15	1	69.0	176	3174	3.48	-0.06	
5	15	1	71.0	174	3337	4.78	-0.33	
5	15	1	71.0	174	3337	4.78	-0.33	
5	15	1	71.0	174	3337	4.78	-0.33	
5	15	1	71.0	174	3337	4.78	-0.33	
5	15	1	71.0	174	3337	4.78	-0.33	
5	15	1	71.0	174	3337	4.78	-0.33	
5	15	1	71.0	174	3337	4.78	-0.33	
5	15	1	71.0	174	3337	4.78	-0.33	
5	15	1	69.0	176	3174	5.62	0.82	
5	15	1	69.0	176	3174	5.62	0.82	
5	15	1	69.0	176	3174	5.62	0.82	
5	15	1	69.0	176	3174	5.62	0.82	
5	15	1	69.0	176	3174	5.62	0.82	
5	15	1	69.0	176	3174	5.62	0.82	
5	15	1	69.0	176	3174	5.62	0.82	
5	15	1	69.0	176	3174	5.62	0.82	
5	15	1	69.0	176	3174	5.62	0.82	
5	15	1	69.0	176	3174	5.62	0.82	
5	15	1	69.0	176	3174	5.62	0.82	
1	27	1	70.8	179	3069	3.50	-0.63	Calloway and Margen, 1971
1	27	1	70.8	179	3069	3.50	-0.63	
1	27	1	70.8	179	3069	3.50	-0.63	
1	27	1	70.8	179	3069	3.50	-0.63	
1	23	1	70.8	179	3069	4.00	-0.23	
1	23	1	70.8	179	3069	4.00	-0.23	
1	23	1	70.8	179	3069	4.00	-0.23	
1	23	1	70.8	179	3069	4.00	-0.23	
1	23	1	70.8	179	3069	4.00	-0.23	

Table 11. Continued

R	DA	SX	WT	HT	KCAL	N IN	N BAL	REFERENCE
1	23	1	70.8	179	3069	4.00	-0.23	
1	48	1	70.8	179	3069	5.00	0.52	
1	48	1	70.8	179	3069	5.00	0.52	
1	48	1	70.8	179	3069	5.00	0.52	
1	48	1	70.8	179	3069	5.00	0.52	
1	48	1	70.8	179	3069	5.00	0.52	
1	48	1	70.8	179	3069	5.00	0.52	
1	59	1	70.8	179	3069	5.90	0.83	
1	59	1	70.8	179	3069	5.90	0.83	
1	59	1	70.8	179	3069	5.90	0.83	
1	59	1	70.8	179	3069	5.90	0.83	
1	59	1	70.8	179	3069	5.90	0.83	
1	59	1	70.8	179	3069	7.00	0.57	
1	59	1	70.8	179	3069	7.00	0.57	
1	59	1	70.8	179	3069	7.00	0.57	
5	16	1	65.1	174	2930	4.32	-0.35	Scrimshaw et al., 1966
5	15	1	68.6	174	2880	3.94	-0.09	
5	15	1	67.4	174	2897	4.02	-0.35	
5	12	1	81.3	174	2962	4.97	-0.20	
5	7	1	75.1	174	2650	4.88	-0.35	
5	12	1	68.2	174	2742	4.31	-0.73	
5	12	1	70.5	174	2349	4.31	-1.10	
1	15	1	80.4	189	3015	6.16	-1.21	Calloway, 1975
1	15	1	79.6	180	2698	5.54	0.06	
1	15	1	55.5	172	2448	5.10	0.28	
1	15	1	81.2	182	2826	5.92	-0.35	
1	15	1	64.6	180	2842	5.88	0.09	
1	15	1	67.8	182	2848	5.82	-0.40	
1	27	1	80.4	189	3015	8.44	-0.18	
1	27	1	79.6	180	2698	7.58	0.42	
1	27	1	55.5	172	2448	6.91	0.40	
1	27	1	81.2	182	2826	8.12	0.47	
1	27	1	64.6	180	2842	8.03	0.73	
1	27	1	67.8	182	2848	7.98	0.16	
1	27	1	80.4	189	3015	8.17	-0.54	
1	27	1	79.6	180	2698	5.47	0.08	
1	27	1	55.5	172	2448	5.01	0.07	
1	27	1	81.2	182	2826	6.85	0.13	
1	27	1	64.6	180	2842	5.81	0.51	
1	27	1	67.8	182	2848	6.72	0.48	
1	39	1	80.4	189	3465	8.22	-0.10	
1	15	1	79.6	180	3104	5.51	0.58	
1	15	1	55.5	172	2814	5.05	0.88	
1	15	1	81.2	182	3248	6.90	0.79	
1	39	1	64.6	180	3269	5.86	0.97	
1	39	1	67.8	182	3275	6.77	0.41	
2	18	1	71.1	178	3450	6.28	0.24	Clark et al., 1975
2	18	1	77.6	177	3200	6.23	0.27	
2	32	1	65.9	166	3000	6.28	-0.03	
2	32	1	68.3	175	3300	6.23	0.39	

Table 11. Continued

R	DA	SX	WT	HT	KCAL	N IN	N BAL	REFERENCE
2	40	1	71.4	178	3550	6.23	1.07	
2	40	1	70.7	179	3200	6.23	-0.13	
2	24	1	72.6	180	3600	6.28	1.36	
2	24	1	64.5	175	3720	6.23	0.88	
5	40	1	61.8	170	3180	2.64	-1.21	Young et al., 1971
5	40	1	63.1	171	3155	2.67	-0.41	
5	40	1	77.2	177	3665	3.38	-0.24	
5	40	1	73.6	181	3350	3.14	-0.14	
5	40	1	93.0	200	3680	3.95	-0.22	
5	40	1	77.2	177	3250	3.31	-0.34	
5	40	1	74.7	182	3400	3.19	-0.39	
5	40	1	83.6	182	3550	3.62	-0.66	
3	21	1	54.4	161	2502	2.70	-0.64	Inoue, Fujita and Niiyama, 1973
3	21	1	51.5	165	2421	2.58	-0.93	
3	21	1	57.6	168	2534	3.64	-0.24	
3	21	1	56.4	165	2594	3.57	-1.06	
3	21	1	56.2	157	2529	3.96	-0.18	
3	21	1	54.6	162	2566	4.01	-0.94	
3	21	1	53.4	166	2563	4.01	-0.21	
3	21	1	58.5	158	2691	5.07	-0.26	
3	21	1	51.2	166	2458	4.46	-0.14	
3	21	1	54.9	166	2306	5.24	0.17	
3	21	1	50.3	166	2414	4.96	0.41	
3	21	1	56.9	158	3243	2.52	-0.53	
3	21	1	54.9	161	3294	2.48	-0.60	
3	21	1	62.0	173	3658	3.68	-0.03	
3	21	1	64.8	167	3758	3.86	-0.40	
3	21	1	48.7	158	2873	3.84	-0.08	
3	21	1	69.5	174	3823	5.52	0.44	
5	24	1	80.9	180	3479	7.38	-0.58	Garza, Scrimshaw and Young, 1976
5	24	1	55.9	172	2516	5.10	-0.17	
5	24	1	65.3	170	3004	5.96	0.33	
5	24	1	68.7	190	3023	6.27	-1.28	
5	51	1	80.9	180	3641	7.38	-0.11	
5	51	1	55.9	172	2236	5.10	-0.70	
5	59	1	65.3	170	3134	5.96	0.33	
5	56	1	68.7	190	3298	6.27	-0.48	
5	72	1	80.9	180	4045	7.38	1.18	
5	72	1	55.9	172	2795	5.10	0.45	
5	77	1	68.7	190	3641	6.27	1.20	
5	15	1	71.5	176	2746	4.40	-0.51	Young and Scrimshaw, 1968
5	15	1	71.5	176	2746	4.40	-0.51	
5	15	1	71.5	176	2746	4.40	-0.51	
5	15	1	71.5	176	2746	4.40	-0.51	
5	15	1	71.5	176	2746	4.40	-0.51	
5	15	1	71.5	176	2746	4.40	-0.51	
5	15	1	71.5	176	2746	4.40	-0.51	

Table 12. Beef Protein Data

R	DA	SX	WT	HT	KCAL	N IN	N BAL	REFERENCE
5	12	1	66.3	173	2918	4.10	-1.26	
5	12	1	65.1	171	3190	4.02	-0.03	Huang et al., 1966
5	12	1	83.4	198	4004	5.12	-1.40	
5	12	1	76.3	188	3510	4.64	-0.51	
5	16	1	62.2	163	2924	3.93	-0.34	
5	16	1	69.5	178	3267	4.39	-0.61	
5	16	1	74.9	186	3446	4.64	-0.28	
5	16	1	75.9	187	3264	4.74	-0.17	
5	16	1	73.1	183	3144	4.56	-0.19	
4	22	1	68.9	170	3170	4.80	-0.44	Kies and Fox, 1973b
4	22	1	68.9	170	3170	4.80	-0.44	
4	22	1	68.9	170	3170	4.80	-0.44	
4	22	1	68.9	170	3170	4.80	-0.44	
4	22	2	68.9	170	2757	4.80	-0.44	
4	22	2	68.9	170	2757	4.80	-0.44	
4	22	2	68.9	170	2757	4.80	-0.44	
4	22	2	68.9	170	2757	4.80	-0.44	
4	24	1	81.7	191	3759	4.80	0.06	Kies and Fox, 1971
4	55	1	81.7	191	3759	8.80	1.24	
4	24	1	77.2	185	3552	4.80	-0.71	
4	55	1	77.2	185	3552	8.80	0.13	
4	24	1	66.7	169	3069	4.80	-0.67	
4	55	1	66.7	169	3069	8.80	0.61	
4	24	1	86.3	197	3970	4.80	-0.52	
4	55	1	86.3	197	3970	8.80	0.16	
4	55	1	71.3	176	3280	4.80	0.21	
4	24	1	71.3	176	3280	8.80	0.74	
4	55	1	82.6	192	3800	4.80	0.04	
4	24	1	82.6	192	3800	8.80	1.87	
4	55	1	63.6	165	2926	4.80	-0.63	
4	24	1	63.6	165	2926	8.80	0.11	
4	55	1	69.5	174	3198	4.80	-0.46	
4	24	1	69.5	174	3198	8.80	0.62	
4	55	1	81.7	191	3759	4.80	0.01	
4	24	1	81.7	191	3759	8.80	1.20	
5	16	1	72.3	178	3283	2.35	-1.48	Young et al., 1975
5	16	1	72.3	178	3283	2.35	-1.48	
5	16	1	72.3	178	3283	2.35	-1.48	
5	16	1	72.3	178	3283	2.35	-1.48	
5	16	1	72.3	178	3283	2.35	-1.48	
5	16	1	72.3	178	3283	2.35	-1.48	
5	16	1	72.3	178	3283	2.35	-1.48	
5	16	1	74.5	179	3353	3.61	-0.95	
5	16	1	74.5	179	3353	3.61	-0.95	
5	16	1	74.5	179	3353	3.61	-0.95	
5	16	1	74.5	179	3353	3.61	-0.95	
5	16	1	74.5	179	3353	3.61	-0.95	
5	16	1	74.5	179	3353	3.61	-0.95	
5	16	1	72.3	178	3283	4.71	-0.24	
5	16	1	72.3	178	3283	4.71	-0.24	

Table 12. Continued

R	DA	SX	WT	HT	KCAL	N IN	N BAL	REFERENCE
5	16	1	72.3	178	3283	4.71	-0.24	
5	16	1	72.3	178	3283	4.71	-0.24	
5	16	1	72.3	178	3283	4.71	-0.24	
5	16	1	72.3	178	3283	4.71	-0.24	
5	16	1	72.3	178	3283	4.71	-0.24	
5	16	1	72.3	178	3283	5.91	0.30	
5	16	1	72.3	178	3283	5.91	0.30	
5	16	1	72.3	178	3283	5.91	0.30	
5	16	1	72.3	178	3283	5.91	0.30	
5	16	1	72.3	178	3283	5.91	0.30	
5	16	1	72.3	178	3283	5.91	0.30	
5	16	1	72.3	178	3283	5.91	0.30	

Table 13. Rice Protein Data

R	DA	SX	WT	HT	KCAL	N IN	N BAL	REFERENCE
2	59	1	84.5	191	3700	6.31	1.05	
2	59	1	74.4	171	3100	6.31	0.11	Lee et al., 1971
2	59	1	75.3	168	3150	6.31	0.19	
2	20	1	68.1	184	3050	6.31	-0.06	
2	20	1	74.2	172	3200	6.31	-0.19	
2	20	1	77.7	187	3200	6.31	-0.17	
2	34	1	84.5	191	3700	8.31	1.80	
2	34	1	75.3	168	3150	8.31	0.35	
2	45	1	68.1	184	3050	8.31	0.75	
2	45	1	74.2	172	3200	8.31	1.45	
2	45	1	77.7	187	3200	8.31	1.02	
2	39	1	85.5	186	3760	6.72	0.31	Clark, Howe and Lee, 1971
2	39	2	48.3	168	2570	6.72	0.38	
2	52	1	63.8	175	3010	6.72	0.54	
2	52	1	70.5	176	2900	6.72	0.53	
2	52	1	78.6	186	3650	6.72	-0.19	
2	39	1	72.4	178	3450	6.72	-0.10	
4	18	1	65.9	165	3031	6.60	-0.50	Chen, Fox and Kies, 1967
4	18	1	74.1	173	3409	6.60	0.29	
4	18	1	67.5	173	3105	6.60	-0.02	
4	18	2	62.3	157	2492	6.60	0.32	
4	18	2	78.2	183	3128	6.60	-0.14	
4	18	1	74.1	188	3409	6.60	-0.03	
3	21	1	56.9	170	2504	2.82	-1.09	Inoue, Fujita and Niiyama, 1973
3	21	1	55.0	172	2585	2.82	-1.08	
3	17	1	53.9	165	2587	3.60	-1.16	
3	21	1	59.3	167	2787	4.09	-0.71	
3	21	1	54.6	162	2293	3.84	-0.53	
3	25	1	58.1	168	2789	4.95	-0.12	
3	21	1	64.2	175	2953	5.51	-0.51	
3	21	1	57.9	158	2548	5.47	-0.38	
3	21	1	52.2	167	2349	5.47	0.11	
3	21	1	52.2	169	2401	5.48	-0.63	
3	21	1	66.9	164	2676	7.88	0.47	
3	21	1	56.6	157	2434	6.75	-0.09	
3	21	1	53.5	165	2408	6.41	-0.02	
3	21	1	56.5	163	2430	6.86	-0.37	
3	21	1	56.1	165	3254	2.86	-1.03	
3	21	1	53.5	162	2943	2.73	-0.50	
3	21	1	53.2	160	3086	3.10	-0.79	
3	21	1	53.9	161	3126	3.84	-0.28	
3	21	1	58.2	158	3434	4.15	-0.24	
3	18	1	51.9	167	2958	4.40	0.02	
3	18	1	66.3	176	3580	5.70	0.84	
3	21	1	59.7	175	3343	5.15	0.06	
3	21	1	52.6	161	3051	4.66	0.17	
3	21	1	54.1	169	3138	5.22	0.05	

Table 14. Corn Protein Data

R	DA	SX	WT	HT	KCAL	N IN	N BAL	REFERENCE
4	18	1	67.0	173	3216	6.54	0.11	
4	18	1	63.0	170	3402	6.51	-0.20	Chen et al., 1966
4	18	2	67.0	160	2680	4.98	-0.53	
4	28	1	77.0	183	3157	4.00	-1.81	Kies and Fox, 1970b
4	28	1	84.0	175	3108	4.00	-0.67	
4	28	1	81.0	183	3159	4.00	-1.52	
4	28	1	68.0	183	3196	4.00	-1.33	
4	28	1	75.0	173	3075	4.00	-0.38	
4	28	1	72.0	185	3024	4.00	-1.62	
4	28	1	81.0	178	3078	4.00	-2.86	
4	23	1	90.7	185	3719	4.70	-1.98	Kies, Williams and Fox, 1965a
4	23	1	90.7	185	3719	6.70	-1.74	
4	23	1	90.7	185	3719	8.70	0.00	
4	23	1	73.9	180	3289	4.70	-0.45	
4	23	1	73.9	180	3289	6.70	-0.10	
4	23	1	73.9	180	3289	8.70	0.48	
4	23	1	61.2	171	3060	4.70	-0.66	
4	23	1	61.2	171	3060	6.70	-0.28	
4	23	1	61.2	171	3060	8.70	0.24	
4	23	1	72.6	175	3013	4.70	-1.43	
4	23	1	72.6	175	3013	6.70	0.09	
4	23	1	72.6	175	3013	8.70	0.45	
4	23	1	81.6	185	3280	4.70	-0.10	
4	23	1	81.6	185	3280	6.70	0.14	
4	23	1	81.6	185	3280	8.70	1.34	
4	23	1	88.4	191	3014	4.70	-2.21	
4	23	1	88.4	191	3014	6.70	-1.45	
4	23	1	88.4	191	3014	8.70	0.00	
4	23	1	65.8	185	3290	4.70	-0.60	
4	23	1	65.8	185	3290	6.70	-0.14	
4	23	1	65.8	185	3290	8.70	0.14	
4	23	1	84.4	183	3283	4.70	-0.50	
4	23	1	84.4	183	3283	6.70	-0.05	
4	23	1	84.4	183	3283	8.70	0.09	
4	23	1	65.8	175	3283	4.70	-1.26	
4	23	1	65.8	175	3283	6.70	-0.95	
4	23	1	65.8	175	3283	8.70	0.83	
4	23	1	65.9	179	2636	4.70	-1.14	
4	23	1	65.9	179	2636	6.70	-0.21	
4	23	1	65.9	179	2636	8.70	1.47	
4	33	1	81.8	180	3780	6.67	-0.25	Kies, Williams and Fox, 1965b
4	33	1	67.3	173	3119	6.67	-0.04	
4	33	1	67.3	173	3119	6.67	-0.29	
4	33	1	91.4	185	3815	6.67	-0.70	
4	33	1	74.5	178	3447	6.67	0.47	
4	33	1	77.3	180	3580	6.67	-0.76	
4	33	1	72.3	183	3354	6.67	0.41	
4	33	1	70.4	180	3264	6.67	0.18	
4	33	1	71.4	179	3313	6.67	-0.87	
4	33	1	90.4	180	3877	6.67	-0.54	

Table 15. Wheat Protein Data

R	DA	SX	WT	HT	KCAL	N IN	N BAL	REFERENCE
5	16	1	73.2	175	3155	3.57	-1.02	
5	16	1	73.2	175	3155	3.57	-1.02	Young et al., 1975
5	16	1	73.2	175	3155	3.57	-1.02	
5	16	1	73.2	175	3155	3.57	-1.02	
5	16	1	73.2	175	3155	3.57	-1.02	
5	16	1	73.2	175	3155	3.57	-1.02	
5	16	1	73.2	175	3155	3.57	-1.02	
5	16	1	73.2	175	3155	3.57	-1.02	
5	16	1	73.2	175	3155	4.79	-1.02	
5	16	1	73.2	175	3155	4.79	-1.02	
5	16	1	73.2	175	3155	4.79	-1.02	
5	16	1	73.2	175	3155	4.79	-1.02	
5	16	1	73.2	175	3155	4.79	-1.02	
5	16	1	73.2	175	3155	4.79	-1.02	
5	16	1	73.2	175	3155	4.79	-1.02	
5	16	1	73.2	175	3155	4.79	-1.02	
5	16	1	73.2	175	3155	6.00	-0.45	
5	16	1	73.2	175	3155	6.00	-0.45	
5	16	1	73.2	175	3155	6.00	-0.45	
5	16	1	73.2	175	3155	6.00	-0.45	
5	16	1	73.2	175	3155	6.00	-0.45	
5	16	1	73.2	175	3155	6.00	-0.45	
5	16	1	73.2	175	3155	6.00	-0.45	
5	16	1	73.2	175	3155	6.00	-0.45	
5	16	1	73.2	174	3214	7.84	0.04	
5	16	1	73.2	174	3214	7.84	0.04	
5	16	1	73.2	174	3214	7.84	0.04	
5	16	1	73.2	174	3214	7.84	0.04	
5	16	1	73.2	174	3214	7.84	0.04	
5	16	1	73.2	174	3214	7.84	0.04	
5	16	1	73.2	174	3214	7.84	0.04	
4	23	1	88.0	184	4049	4.80	-2.34	Kies and Fox, 1972
4	23	1	89.0	176	4095	4.80	-0.43	
4	23	1	69.0	170	3175	4.83	-0.29	
4	23	1	86.0	178	3957	4.80	-0.31	
4	23	1	77.0	185	3543	4.80	-0.84	
4	23	2	50.0	159	2001	4.80	0.85	
4	23	2	74.0	173	2961	4.80	-1.05	
4	23	1	84.0	185	3865	4.80	-0.84	
4	23	1	95.0	170	4371	4.80	-1.52	
4	23	1	85.0	183	3911	4.80	-1.37	
4	23	1	78.0	187	3589	4.80	-0.69	
4	23	1	66.0	178	3037	4.80	-1.52	
4	23	1	76.0	180	3497	4.80	-0.69	
4	23	1	68.0	168	3129	4.80	-0.40	
4	23	1	76.0	176	3497	4.80	-0.44	
4	23	1	75.0	170	3451	4.80	-0.40	
4	23	1	76.0	179	3497	4.80	-1.05	
4	23	2	54.0	170	2161	4.80	-1.69	
4	23	2	52.0	155	2081	4.80	-0.31	

Table 15. Continued

R	DA	SX	WT	HT	KCAL	N IN	N BAL	REFERENCE
4	23	2	66.0	168	2641	4.80	-0.56	
4	23	2	61.0	164	2441	4.80	-0.85	
4	23	2	61.0	173	2441	4.80	-0.90	
4	23	2	57.0	163	2281	4.80	0.05	
4	23	2	66.0	169	2641	4.80	-0.15	
4	23	2	66.0	168	2641	4.80	-1.05	
4	23	2	65.0	173	2601	4.80	-1.36	
4	23	1	93.0	185	4279	4.80	-2.71	
4	23	1	78.0	185	3589	5.00	-1.34	Kies and Fox, 1970a
4	23	2	56.0	162	2241	5.00	0.73	
4	23	2	68.0	171	2721	5.00	-1.26	
4	23	2	54.0	160	2161	5.00	-0.81	
4	23	1	67.0	175	3083	5.00	-0.90	
4	23	1	67.0	173	3083	5.00	-0.55	
4	23	1	69.0	173	3175	5.00	-1.42	
4	23	1	62.0	170	2853	5.00	-0.58	
4	23	1	53.0	162	2439	5.00	0.14	
4	23	1	81.0	185	3727	5.00	-0.80	
4	18	1	97.0	187	4463	4.80	-0.92	Kies and Fox, 1970c
4	28	1	97.0	187	4463	6.80	-0.52	
4	13	2	58.0	164	2321	4.80	-0.17	
4	33	2	58.0	164	2321	6.80	0.57	
4	18	2	51.0	161	2041	4.80	-0.14	
4	28	2	51.0	161	2041	6.80	0.38	
4	13	2	68.0	168	2721	4.80	-0.59	
4	33	2	68.0	168	2721	6.80	-0.03	
4	33	1	60.0	180	2761	4.80	-1.38	
4	13	1	60.0	180	2761	6.80	-0.76	
4	33	2	54.0	168	2161	4.80	-0.29	
4	13	2	54.0	168	2161	6.80	-0.31	
4	28	1	90.0	192	4141	4.80	-0.52	
4	18	1	90.0	192	4141	6.80	-0.48	
4	28	1	74.0	176	3405	4.80	-0.48	
4	18	1	74.0	176	3405	6.80	-0.44	

Table 16. Wheat Gluten Data

R	DA	SX	WT	HT	KCAL	N IN	N BAL	REFERENCE
3	14	1	56.6	165	2746	1.58	-1.31	
3	14	1	56.6	165	2746	1.58	-1.31	Inoue et al., 1974
3	14	1	56.6	165	2746	1.58	-1.31	
3	14	1	56.6	165	2746	1.58	-1.31	
3	14	1	56.6	165	2746	1.58	-1.31	
3	14	1	56.6	165	2746	1.58	-1.31	
3	14	1	56.6	165	2746	1.58	-1.31	
3	14	1	56.6	165	2746	1.58	-1.31	
3	14	1	56.6	165	2746	1.58	-1.31	
3	14	1	56.6	165	2746	1.58	-1.31	
3	14	1	58.5	166	2838	3.51	-1.08	
3	14	1	58.5	166	2838	3.51	-1.08	
3	14	1	58.5	166	2838	3.51	-1.08	
3	14	1	63.7	169	3090	6.37	-0.65	
3	14	1	63.7	169	3090	6.37	-0.65	
3	14	1	63.7	169	3090	6.37	-0.65	
3	7	1	54.6	164	2643	9.45	-0.32	
3	7	1	54.6	164	2643	9.45	-0.32	
3	7	1	54.6	164	2643	9.45	-0.32	
3	7	1	54.6	164	2643	9.45	-0.32	
3	7	1	54.6	164	2643	9.45	-0.32	
5	15	1	71.5	181	3025	3.09	-2.02	Scrimshaw, Taylor and Young, 1973
5	15	1	71.5	181	3025	3.09	-2.02	
5	15	1	71.5	181	3025	3.09	-2.02	
5	15	1	71.5	181	3025	3.09	-2.02	
5	15	1	71.5	181	3025	3.09	-2.02	
5	15	1	71.5	181	3025	3.09	-2.02	
5	15	1	71.5	181	3811	3.09	-1.29	
5	15	1	71.5	181	3811	3.09	-1.29	
5	15	1	71.5	181	3811	3.09	-1.29	
5	15	1	71.5	181	3811	3.09	-1.29	
5	15	1	71.5	181	3811	3.09	-1.29	
5	15	1	71.5	181	3811	3.09	-1.29	
5	31	1	74.2	177	3562	8.67	-0.72	
5	31	1	74.2	177	3562	8.67	-0.72	
5	31	1	74.2	177	3562	8.67	-0.72	
5	31	1	74.2	177	3562	8.67	-0.72	
5	31	1	74.2	177	3562	8.67	-0.72	
5	31	1	74.2	177	3562	8.67	-0.72	
5	31	1	74.2	177	3562	8.67	-0.72	
5	31	1	74.2	177	3562	8.67	-0.72	
5	31	1	74.2	177	2850	8.67	-1.40	
5	31	1	74.2	177	2850	8.67	-1.40	
5	31	1	74.2	177	2850	8.67	-1.40	
5	31	1	74.2	177	2850	8.67	-1.40	
5	31	1	74.2	177	2850	8.67	-1.40	
5	31	1	74.2	177	2850	8.67	-1.40	
5	31	1	74.2	177	2850	8.67	-1.40	
5	31	1	74.2	177	2850	8.67	-1.40	

Table 17. Nitrogen balance at very low levels of nitrogen intake

	Average				
Number of observations	weight (kg)	height (cm)	N intake (mg/kg)	N balance (mg/kg)	Reference
9	63.3	168	2.0	-44.0	Inoue et al., 1974
9	62.2	168	15.0	-29.0	
50	54.9	167	10.0[a]	-36.5	Huang, Chong and Rand, 1972
39	74.0	180	15.2	-31.6	Young et al., 1973 and Scrimshaw et al., 1972
44	71.0	178	6.7	-38.4	
7	71.9	177	4.0	-38.1[b]	Hawley et al., 1948
4	79.6	188	8.0	-38.9	Mueller and Cox, 1947
22	65.5	172	3.0	-45.7[c]	Murlin et al., 1946a
13	70.8	179	8.0	-44.0	Calloway and Margen, 1971
11	71.3	176	6.0	-39.6	Young and Scrimshaw 1968

[a] Assumed. The authors stated (p. 1606) that "daily total N intake did not exceed 10 mg/kilogram body weight."

[b] Average of all three no-protein periods for 7 men.

[c] Fourth-day excretion of nitrogen.

Table 18. 95% confidence limits--egg protein

N intake (g/m^2)	One-half confidence interval width (g/m^2)	Predicted mean N balance[a] (g/m^2)	95% Confidence interval for mean N balance	
			lower limit (g/m^2)	upper limit (g/m^2)
1.200	0.140	-0.477	-0.618	-0.337
1.400	0.107	-0.390	-0.497	-0.283
1.600	0.083	-0.308	-0.391	-0.225
1.800	0.067	-0.232	-0.299	-0.165
2.000	0.060	-0.161	-0.221	-0.101
2.200	0.059	-0.096	-0.155	-0.037
2.400	0.060	-0.037	-0.097	0.024
2.600	0.061	0.017	-0.043	0.078
2.800	0.060	0.066	0.006	0.126
3.000	0.059	0.109	0.050	0.168
3.200	0.059	0.146	0.087	0.205
3.400	0.065	0.178	0.113	0.243
3.600	0.079	0.204	0.125	0.283
3.800	0.102	0.225	0.123	0.327
4.000	0.134	0.240	0.107	0.374
4.200	0.172	0.250	0.077	0.422
4.400	0.218	0.254	0.036	0.472

[a] According to the equation given in Figure 5.

Table 19. 95% confidence limits--beef protein

N intake (g/m²)	One-half confidence interval width (g/m²)	Predicted mean N balance[a] (g/m²)	95% Confidence interval for mean N balance	
			lower limit (g/m²)	upper limit (g/m²)
1.200	0.186	-0.838	-1.024	-0.652
1.400	0.149	-0.716	-0.865	-0.566
1.600	0.119	-0.601	-0.720	-0.481
1.800	0.097	-0.492	-0.589	-0.395
2.000	0.082	-0.390	-0.472	-0.307
2.200	0.077	-0.294	-0.371	-0.217
2.400	0.078	-0.205	-0.282	-0.127
2.600	0.082	-0.122	-0.204	-0.039
2.800	0.088	-0.045	-0.133	0.042
3.000	0.093	0.025	-0.068	0.117
3.200	0.097	0.088	-0.009	0.185
3.400	0.101	0.145	0.044	0.246
3.600	0.105	0.195	0.090	0.301
3.800	0.111	0.239	0.128	0.350
4.000	0.120	0.277	0.157	0.396
4.200	0.133	0.308	0.175	0.440
4.400	0.151	0.332	0.181	0.483
4.600	0.175	0.350	0.175	0.525
4.800	0.205	0.362	0.156	0.567
5.000	0.242	0.367	0.125	0.608

[a] According to the equation given in Figure 5.

Table 20. 95% confidence limits--rice protein

N intake (g/m^2)	One-half confidence interval width (g/m^2)	Predicted mean N balance[a] (g/m^2)	95% Confidence interval for mean N balance	
			lower limit (g/m^2)	upper limit (g/m^2)
1.800	0.206	-0.567	-0.773	-0.361
2.000	0.163	-0.469	-0.633	-0.306
2.200	0.133	-0.378	-0.510	-0.245
2.400	0.114	-0.291	-0.406	-0.177
2.600	0.106	-0.211	-0.317	-0.105
2.800	0.103	-0.136	-0.239	-0.033
3.000	0.102	-0.067	-0.169	0.034
3.200	0.099	-0.004	-0.103	0.095
3.400	0.095	0.054	-0.041	0.149
3.600	0.091	0.106	0.014	0.197
3.800	0.092	0.152	0.060	0.244
4.000	0.103	0.192	0.089	0.295
4.200	0.129	0.227	0.098	0.356
4.400	0.168	0.256	0.087	0.424
4.600	0.220	0.279	0.059	0.499

[a] According to the equation given in Figure 5.

Table 21. 95% confidence limits--corn protein

N intake (g/m^2)	One-half confidence interval width (g/m^2)	Predicted mean N balance[a] (g/m^2)	95% Confidence interval for mean N balance: lower limit (g/m^2)	upper limit (g/m^2)
2.000	0.144	-0.724	-0.868	-0.580
2.200	0.129	-0.646	-0.775	-0.517
2.400	0.115	-0.567	-0.682	-0.453
2.600	0.102	-0.489	-0.591	-0.387
2.800	0.092	-0.411	-0.503	-0.319
3.000	0.085	-0.332	-0.417	-0.248
3.200	0.081	-0.254	-0.335	-0.173
3.400	0.082	-0.176	-0.258	-0.094
3.600	0.087	-0.097	-0.185	-0.010
3.800	0.096	-0.019	-0.115	0.077
4.000	0.108	0.059	-0.048	0.167
4.200	0.121	0.138	0.017	0.259
4.400	0.136	0.216	0.080	0.352
4.600	0.151	0.294	0.143	0.446
4.800	0.168	0.373	0.205	0.540
5.000	0.185	0.451	0.266	0.636

[a] According to the equation given in Figure 5.

Table 22. 95% confidence limits--wheat protein

N intake (g/m^2)	One-half confidence interval width (g/m^2)	Predicted mean N balance[a] (g/m^2)	95% Confidence interval for mean N balance lower limit (g/m^2)	 upper limit (g/m^2)
1.800	0.130	-0.664	-0.795	-0.534
2.000	0.113	-0.605	-0.718	-0.492
2.200	0.097	-0.545	-0.642	-0.448
2.400	0.084	-0.485	-0.569	-0.402
2.600	0.073	-0.425	-0.499	-0.352
2.800	0.068	-0.366	-0.434	-0.297
3.000	0.069	-0.306	-0.375	-0.237
3.200	0.076	-0.246	-0.323	-0.170
3.400	0.088	-0.187	-0.274	-0.099
3.600	0.102	-0.127	-0.229	-0.025
3.800	0.119	-0.067	-0.186	0.051
4.000	0.136	-0.007	-0.144	0.129
4.200	0.155	0.052	-0.102	0.207
4.400	0.174	0.112	-0.062	0.286
4.600	0.193	0.172	-0.021	0.365

[a] According to the equation given in Figure 5.

Table 23. 95% confidence limits--wheat gluten

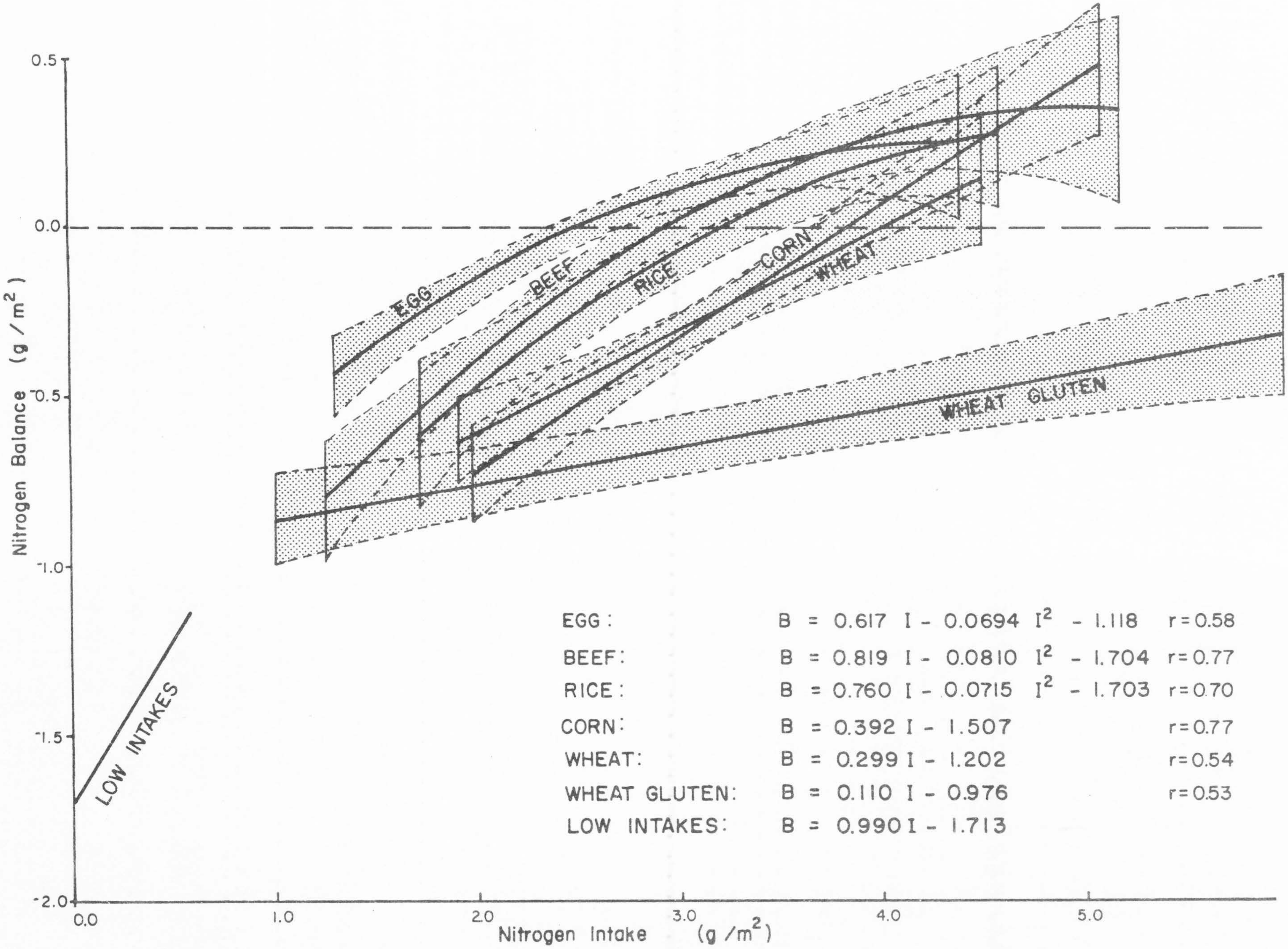
N intake (g/m^2)	One-half confidence interval width (g/m^2)	Predicted mean N balance[a] (g/m^2)	95% Confidence interval for mean N balance	
			lower limit (g/m^2)	upper limit (g/m^2)
1.000	0.138	-0.866	-1.003	-0.728
1.200	0.130	-0.844	-0.974	-0.714
1.400	0.123	-0.822	-0.945	-0.699
1.600	0.116	-0.800	-0.916	-0.684
1.800	0.110	-0.778	-0.888	-0.669
2.000	0.104	-0.756	-0.860	-0.652
2.200	0.099	-0.734	-0.834	-0.635
2.400	0.095	-0.712	-0.808	-0.617
2.600	0.092	-0.691	-0.783	-0.598
2.800	0.090	-0.669	-0.759	-0.578
3.000	0.089	-0.647	-0.736	-0.557
3.200	0.090	-0.625	-0.715	-0.535
3.400	0.091	-0.603	-0.694	-0.512
3.600	0.094	-0.581	-0.675	-0.487
3.800	0.097	-0.559	-0.656	-0.462
4.000	0.102	-0.537	-0.639	-0.435
4.200	0.107	-0.515	-0.622	-0.408
4.400	0.113	-0.493	-0.607	-0.380
4.600	0.120	-0.471	-0.591	-0.351
4.800	0.127	-0.449	-0.576	-0.322
5.000	0.134	-0.427	-0.562	-0.293

Table 23 (continued)

N intake (g/m^2)	One-half confidence interval width (g/m^2)	Predicted mean N balance[a] (g/m^2)	95% Confidence interval for mean N balance: lower limit (g/m^2)	95% Confidence interval for mean N balance: upper limit (g/m^2)
5.200	0.142	-0.406	-0.548	-0.263
5.400	0.150	-0.384	-0.534	-0.233
5.600	0.159	-0.362	-0.520	-0.203
5.800	0.167	-0.340	-0.507	-0.173
6.000	0.176	-0.318	-0.494	-0.142

[a] According to the equation given in Figure 5.

Figure 10. 95% confidence interval estimates for the regression lines of Figure 5. The upper and lower limits on the predicted mean nitrogen balance are shown by light dashed lines above and below each regression line. The area between confidence limits for a given regression line is shaded for clarity. Data were taken from Tables 18 through 23.


0.5
0.0
-0.5
-1.0
-1.5
-2.0
Nitrogen Balance (g / m^2)
0.0
1.0
2.0
3.0
4.0
5.0
Nitrogen Intake (g /m^2)
EGG
BEEF
RICE
CORN
WHEAT
WHEAT GLUTEN
LOW INTAKES
EGG : B = 0.617 I - 0.0694 I^2 - 1.118 r = 0.58
BEEF: B = 0.819 I - 0.0810 I^2 - 1.704 r = 0.77
RICE: B = 0.760 I - 0.0715 I^2 - 1.703 r = 0.70
CORN: B = 0.392 I - 1.507 r = 0.77
WHEAT: B = 0.299 I - 1.202 r = 0.54
WHEAT GLUTEN: B = 0.110 I - 0.976 r = 0.53
LOW INTAKES: B = 0.990 I - 1.713

VITA

Arthur John Wittwer

Candidate for the Degree of

Master of Science

Thesis: The Influence of Various Factors on Nitrogen Balance and Protein Quality Measured in Adult Human Beings

Major Field: Biochemistry

Biographical Information:

Personal Data: Born in Lansing, Michigan, December 28, 1950, son of Sylvan Harold and Maurine Cottle Wittwer; married Mary Susan Keller April 3, 1974; one child--Mary Kathleen.

Education: Attended Liberty Hyde Bailey Elementary School and East Lansing Junior High School in East Lansing, Michigan; graduated from East Lansing High School in 1969; received the Bachelor of Science degree from Utah State University, with a major in chemistry, in 1975; completed requirements for the Master of Science degree in biochemistry at Utah State University in 1976.

Professional Experience: 1974 to present, research assistant, Department of Chemistry and Biochemistry, Utah State University; 1975-76 part-time teaching assistant, Department of Chemistry and Biochemistry, Utah State University; 1969-70, 1972-74, laboratory assistant, Department of Chemistry and Biochemistry, Utah State University.

Awards and Honors: Michigan Mathematics Prize Competition finalist in 1969; awarded Academic Science Scholarship in 1973; elected to membership in the Honor Society of Phi Kappa Phi in 1975; graduated magna cum laude from Utah State University in 1975; named a Harold Stirling Vanderbilt Scholar for study in biochemistry by Vanderbilt University in 1976.