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McCollum and Directions in the Evaluation of Protein Quality

A. E. Harper

Investigations of differences in the nutritive value of food and feed proteins by McCollum and his associates prior to 1915 led to the discovery of the fat-soluble vitamins. Their research provided basic nutritional information that was required for the subsequent development of reliable bioassays for evaluation of protein quality. The principles of the classical bioassays for the nutritional evaluation of proteins were established by the mid-1920s through the research of Thomas and Mitchell on the biological value method for measuring efficiency of nitrogen retention and of Osborne and Mendel on the protein efficiency ratio method based on measuring efficiency of growth. The concept of the limiting amino acid as the major determinant of protein quality was recognized by these early investigators as was the fact that protein quality measurements were essentially measures of the effectiveness of proteins in meeting amino acid needs. Only after Rose and his associates had discovered the nutritional essentiality of threonine, the last of the indispensable amino acids to be identified, in 1935, and rapid methods for analysis of amino acids were developed in the late 1930s and early 1940s, did it become possible to estimate the nutritive value of proteins from knowledge of their amino acid composition. The chemical score procedure proposed by Block and Mitchell in 1946 represented the first effort to predict the nutritive value of proteins from knowledge of their amino acid composition. Since then research on protein evaluation has progressed in two directions: one has consisted of efforts to improve the basic bioassays; the other has consisted of efforts to improve amino acid scoring procedures. Many modifications of the nitrogen balance and animal growth assays have been proposed. Most of these involve the use of a slope-ratio assay of some type to improve the reproducibility and increase the sensitivity of the classical bioassays. Although any of the bioassays can be used successfully to rank proteins in order of nutritive value and to detect deterioration of protein quality during processing, none of them can be used to predict the supplementary or complementary value of proteins in mixed diets. Also, none of them permits accurate prediction of the amount of protein required to meet animal or human requirements. Modifications of the chemical score procedure have been proposed mainly by the Food and Agriculture and World Health Organizations and the Food and Nutrition Board of the National Academy of Sciences/National Research Council. These organizations recommended that an amino acid scoring pattern based on human requirements for amino acids be substituted for the amino acid pattern of whole egg proteins used as the standard for comparison in the chemical score procedure. Results of a limited number of experimental trials indicate that the tentative amino acid scoring patterns can be used to predict quite well the amount of a mixture of proteins needed to meet human requirements. The major problem with the amino acid scoring procedure is that it does not take into account low biological availability of amino acids, particularly from some heat-processed proteins. McCollum discussed this problem in the 1930s. Carpenter has since developed a chemical method for determining unavailable lysine. Major directions for future research are (1) to assess more adequately the reliability of the amino acid scoring procedure for predicting the amounts of mixtures of proteins needed to meet human requirements and (2) to develop reliable methods for measuring the biological availability of the amino acids that are most likely to be limiting in the diets of human beings and domestic monogastric animals.

I am honored to participate in this symposium commemorating the 100th anniversary of the birth of E. V. McCollum. It is just 70 years since McCollum participated with Hart, Steenbock, and Humphrey in a series of studies of the nutritive value of individual feedstuffs initiated by Babcock at the University of Wisconsin.[1] This provided the impetus for much of McCollum's subsequent research on essential nutrients in foods and feeds. Initially he was concerned with protein metabolism and, between 1910 and 1913 he and his associates published eight papers on protein utilization.[2–9]

It is not usually realized that the investigations of McCollum and Davis with diets composed of purified food components,[10,11] which led to the discovery of fat-soluble vitamins,[12–14] were an outgrowth of McCollum's earlier investigations of differences in the nutritive value of food and feed proteins. It is also worthy of note that discoveries made by McCollum and Davis through their use of what they called the "biological method of analysis" of foods and feeds[15] provided basic nutritional information that was critical for successful bioassays for evaluation of protein quality. The essence of the technique they developed was to measure the growth responses of rats fed single foods, either alone or supplemented with inorganic salts, protein, fat-soluble or water-soluble substances, or combinations of these. This enabled them to identify the nature of the nutritional deficiencies in the various products. They demonstrated that the protein-free milk which Osborne and Mendel (see ref 16, p 276 and ref 17, p 20) found was needed in the diet before differences in the nutritive value of proteins could be clearly demonstrated was a source of essential water-soluble nutrients. Subsequently, McCollum and his associates[18] struck off in the direction of identification of unknown growth factors while Osborne and Mendel[19] directed their attention mainly to nutritional studies of proteins and amino acids.

Evidence that food and feed proteins were not nutritionally equivalent had begun to accrue in the 1850s before McCollum was born; but the prestige of Liebig, who accepted Boussingault's view that the nutritive value of a protein could be estimated from its nitrogen content alone, was so great that little attention was paid to observations to the contrary. Rubner is credited with the first clear statement in 1897 that proteins differed in nutritive value.[20]

Departments of Nutritional Sciences and Biochemistry, University of Wisconsin—Madison, Madison, Wisconsin 53706.

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During the 19th century, 12 amino acids had been identified, and it had been shown that proteins differed considerably in their content of these amino acids.[21] McCollum emphasizes in his history of nutrition (see ref 15, p 60) that knowledge of differences in the amino acid composition of protein "forced upon the attention of investigators, after about 1900, the fact that the problems of protein nutrition were actually problems concerning the kinds and amounts of individual amino acids derived from the digestion of food proteins".

In 1907, Osborne recognized that wheat proteins were disproportionately low in lysine and raised a question as to the nutritional significance of such differences (see ref 20, p 250). The observation of Willcock and Hopkins[22] in 1906, that the lives of mice fed a diet in which the tryptophan-deficient protein, zein, was the sole source of protein were prolonged if they were provided with a supplement of tryptophan, was a preliminary answer to Osborne's question. At least one amino acid was shown by this work to be an essential nutrient for at least one mammalian species. In 1909 Osborne and Mendel (see ref 20, p 250) initiated their classic investigations of the nutritive value of proteins. They knew from chemical analyses that wheat proteins were disproportionately low in lysine and that casein was disproportionately low in cystine. Within a short time they demonstrated through amino acid supplementation studies with these proteins the dietary essentiality of lysine and of sulfur-containing amino acids for the growth of rats. Differences in the nutritive value of several proteins were soon found from direct experimental observations to be associated with differences in amino acid composition of the proteins.[20,21]

Osborne, Mendel, and Ferry[23] described in 1919 "a method of expressing numerically the growth-promoting value of proteins". It consisted of measuring the gain in weight of growing rats fed different levels of protein and expressing the results as grams of weight gained per gram of protein consumed. The highest value obtained for a given protein was taken as the protein efficiency ratio (PER). This method provided a relative measure of the efficiency of utilization of different food and feed proteins and, with some modifications, is still a standard method for estimating protein quality.

Earlier, Thomas[24] had used the nitrogen balance procedure as the basis for another method of assessing the nutritive value of proteins. In this method, measurements of nitrogen intake and urinary and fecal nitrogen losses were used to calculate the percent of ingested nitrogen absorbed and the percentage of absorbed nitrogen retained. The numerical value for the percentage of absorbed nitrogen retained was termed the "biological value". The method had the advantage of being applicable to human subjects. McCollum[25] in 1914 applied this method in a study on growing pigs. He compared the efficiency of utilization of proteins of milk and cereal grains and showed the supplementary value of milk. Subsequently, in 1924 this method was modified by Mitchell,[26] who proposed strict standardization of conditions as a means of improving the reliability and reproducibility of the assay. The Thomas–Mitchell method remains another standard procedure for estimating the "biological value" of proteins, especially with human subjects.

In 1929, McCollum and Shukers (see ref 17, p 126) proposed that efficiency of utilization of dietary proteins could be determined directly in animals from measurements of carcass nitrogen accumulation. The method they proposed consisted of measuring total carcass nitrogen content of a representative group of rats on the day a trial was started. Other comparable groups were then fed for 28 days or more on diets containing various test proteins after which their total carcass nitrogen content was measured. From the nitrogen intake for the period and from the difference between the initial and final nitrogen content of the animals, the percentage of ingested nitrogen retained could be calculated directly. This was a measure of net protein utilization. It was equivalent to the product of the Thomas–Mitchell biological value and the coefficient of digestibility.

The principles of assays for the nutritional evaluation of proteins were thus established by the early 1920s.

It is important to recognize that the early investigators of the nutritive value of proteins, Osborne and Mendel, McCollum, and Mitchell, recognized clearly that their various biological methods were indirect measures of the effectiveness of different proteins in meeting the amino acid needs of the test organism. In 1923 Mendel (see ref 20, p 250) stated "When the absolute intake (of a protein) is small, the 'law of the minimum' may come into play to limit the efficiency (of utilization) of the whole (protein) because of the relative shortage of some essential amino acid. Conversely, when an animal ingests a very large quantity of some protein poor in an essential unit, the absolute amount of the latter thereby available may suffice" to meet the nutritional needs. McCollum (see ref 20, p 250) stated at this same time that investigations between 1910 and 1920 on plant proteins "leave no room for doubt that all the amino acids necessary for the nutrition of an animal are contained in the proteins found in each of these foods. Certain of these are, however, present in such limited amounts as to restrict the extent to which the remaining ones, which are more abundant, can be utilized".

Proteins could not be evaluated on the basis of their amino acid content at this time, however, for two reasons. First, all of the amino acids that were nutritionally indispensable had not been identified, even though by 1903 all but two of the amino acids commonly found in food proteins had been discovered. Second, analysis of proteins for amino acids was an arduous and time-consuming task so that information about the amino acid composition of proteins accumulated only slowly.

The first of these problems was solved by the discovery of threonine, the last of the amino acids to be established as essential for growth, by McCoy, Meyer, and Rose[27] in 1935. By 1938 all of the amino acids that were essential nutrients had been distinguished from those that were not.[28] The second problem was solved by the development in the late 1930s of the microbiological method and in the 1940s of the column chromatographic method of analysis for amino acids (see ref 29 and 30 for references). Proteins could be analyzed for their amino acid content in a short time by these methods. By 1946 Block and Bolling[29] had compiled extensive tables of the amino acid composition of food and feed proteins based on chemical and microbiological methods. Analyses for many proteins based on chromatographic methods were published subsequently.[30] These developments opened up the possibility of evaluating food and feed proteins from knowledge of their content of the amino acids that were nutritionally indispensable.

In 1946 Block and Mitchell[20] proposed a method for the chemical evaluation of protein quality based on comparison of the amino acid composition of food and feed proteins with that of whole egg. They used the amino acid composition of whole egg, a protein known to be of high nutritive value, as the standard for comparison because at

Table I. Comparison of Whole Wheat and Whole Egg Proteins[a]

	% whole egg	% whole wheat	% deficit
histidine	2.1	2.1	0
tyrosine	4.5	4.4	2
phenylalanine	6.3	5.7	10
tryptophan	1.5	1.2	20
leucine	9.2	6.8	26
threonine	4.9	3.3	33
arginine	6.4	4.2	34
methionine and cystine	6.5	4.3	34
valine	7.3	4.5	38
isoleucine	8.0	3.6	55
lysine	7.2	2.7	63[a]

[a] Amino acid in greatest deficit is lysine. Chemical score of wheat proteins = 2.7/7.2 × 100 = 37.

that time few quantitative estimates of amino acid requirements, even for the rat, had been made. The method as elaborated by Block and Mitchell was termed the chemical score procedure. It consisted of expressing the quantity of the amino acid in a test protein which was in greatest deficit in relation to the quantity in whole egg proteins as a percent of the amount in whole egg (Table I). High correlations, but by no means perfect, between chemical score values for proteins and various biological measures of protein quality were demonstrated by Block and Mitchell.[20]

This represented achievement of the objective of the early investigators of the nutritive value of proteins, i.e., to assess the adequacy of proteins on the basis of their content of the amino acids that were essential nutrients.

The chemical score method had shortcomings. Whole egg proteins are uniquely high in sulfur-containing amino acids and tryptophan. When they are fed to most mammals in a quantity that is just sufficient to meet the need for nitrogen, they provide amounts of sulfur-containing amino acids and tryptophan in excess of those required. The chemical score procedure does not take into account differences in the digestibility of proteins or in the biological availability of amino acids. This latter shortcoming has probably been the main reason for the method not being more widely adopted.

This brings us to the question of the purpose of nutritional evaluation of proteins. The major objective of protein evaluation is to permit prediction of the amount of a food protein or mixture of food proteins needed to meet amino acid requirements for growth or maintenance. A second objective is to permit the ranking of proteins according to their potential nutritive value and to permit detection of changes that may occur in that potential during storage, processing, or preparation of foods. These objectives must be kept in mind in examining directions in evaluation of protein quality.

During the period since the development of the chemical score concept, two directions in evaluation of protein quality are evident. One, there has been extensive research by many investigators in an effort to improve and refine the original biological assays based on measurements of growth or nitrogen retention in experimental animals. Much of the impetus for this research has been the need for a reliable index of the comparative nutritive value of proteins for regulatory actions and for assessing effects of processing on protein quality. This research has been summarized—in extenso—so I shall not discuss it in detail.

In 1945 Allison[31] introduced the concept of the nitrogen balance index, essentially a slope-ratio assay, which was based on measurements of N balance of subjects consuming different levels of protein. Comparison of the slopes of plots of N balance vs. N intake for different dietary proteins gave a measure of the nutritive quality of each protein relative to one of high quality. The use of several levels of protein eliminated the need to feed one group of subjects a protein-free diet in order to determine endogenous and metabolic nitrogen losses.

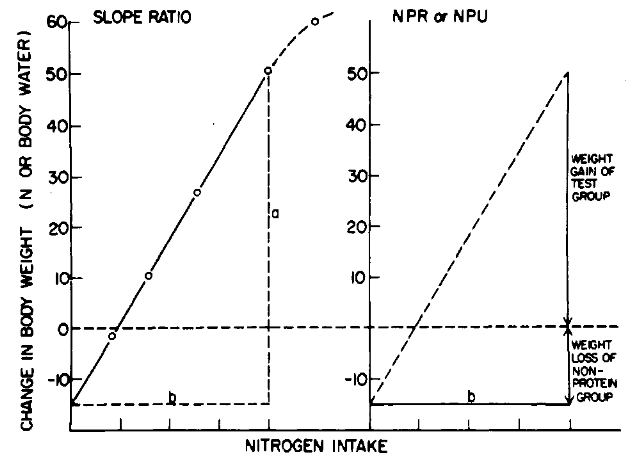

Figure 1. Diagrammatic representation of the principles underlying the slope-ratio, net protein ratio (NPR), and net protein utilization (NPU) assays for protein quality. In the NPU assay, body nitrogen or body water is measured directly rather than body weight (after McLaughlan[36]).

Most of the emphasis since then has been on improving the various methods based on growth. The net protein utilization (NPU) method developed by Bender and Miller[32] in 1953, based on the principle of carcass analysis as proposed originally by McCollum, represents basically a growth method in which measurement of carcass nitrogen accumulation was considered to give a more accurate estimate of N retention than simple weight gain. The use of the H_2O content of the carcass, which was shown to be directly related to nitrogen content, provided a further simplification.[33]

Subsequently, Bender and Doehl[34] proposed an improvement of the original PER method of Osborne and Mendel by including in the calculation the weight loss of a group of rats fed a protein-free diet. This permitted evaluation of proteins for their ability to support maintenance as well as growth.

Since 1965, Hegsted and associates have published considerable information on a slope-ratio assay for protein quality.[35] In this, the slope of the line relating protein intake to changes in body weight or body water content measured at several levels of protein intake provides a measure of protein quality. The slope for a test protein compared to that for a standard protein such as egg or lactalbumin provides a measure of relative nutritive value, or relative protein value.

McLaughlan and associates have examined various of these methods in considerable detail.[36] They have concluded that assays based on the net protein ratio principle, in which the slope of a line linking the value for the weight change of a group of rats fed a protein-free diet with that of a group fed 8–10% of a test protein, provide as satisfactory an assessment of protein quality as any other method. This is illustrated in Figure 1. It is cheaper and more convenient than assays calling for multiple-dose levels of protein.

Many of the problems with these bioassays were recognized by Osborne and Mendel, McCollum, and Mitchell. Results are influenced by the level of protein in the diet

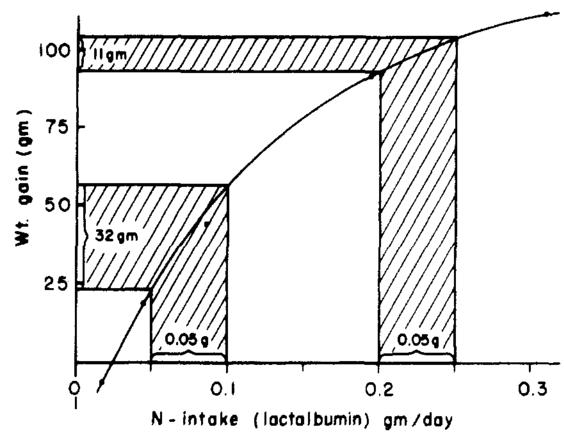

Figure 2. Growth response curve of rats fed graded amounts of lactalbumin (based on results of Hegsted and Ching[50]).

and the age of the animals; PER gives no credit for maintenance so underestimates the value of the low quality proteins; the results obtained with the PER method are not directly proportional to the quality of the protein; the nature of the slope at the low end of the range in all of the slope-ratio assays as shown by Bender and by Hegsted depends upon which amino acid is limiting in the protein.[37] Nevertheless, despite all of these shortcomings, the various methods tend to rank proteins in the same order. The slope assays tend to be less variable and more reproducible than PER.

Any of these methods can be used to assess damage to a protein during processing or preparation of a product and all can provide relative values for ranking proteins for regulatory purposes. None of them can be used to provide an accurate estimate of the amount of protein needed to meet amino acid requirements and, more importantly, none can provide information about the supplementary value of a protein when it is used as only one component of a complex diet.

The major problem with such methods is that the response per increment of protein consumed is not constant but follows, as with biological responses generally, the law of diminishing returns (Figure 2). Hence, the efficiency of utilization of proteins or amino acids in the range in which efficiency of protein utilization is measured—inadequate levels—is higher than it is at the requirement level. Thus, they underestimate the amount of protein required to meet amino acid needs. Efficiency of utilization of proteins of the highest quality when consumed by adults at the requirement level is only 65–70%.[38]

The other direction in evaluation of protein quality, since the development of the chemical score procedure, represents an effort to devise a method for protein evaluation that will permit prediction of the effectiveness of food proteins in meeting amino acid needs of people. The critical information needed to accomplish this objective was obtained during the period between 1949 and 1957. During that time, Rose and associates made the first quantitative determinations of the amino acid requirements of men; Leverton and associates made similar quantitative determinations of the amino acid requirements of women; Holt and Snyderman and associates determined the amino acid requirements of infants consuming crystalline amino acid diets in which the level of each amino acid in turn could be adjusted (see ref 39 for references).

The accuracy of these estimates of amino acid requirements has been questioned. Yet the individual values fall, with very few exceptions, within the range predicted, assuming that requirements are distributed normally and that the coefficient of variation is 15%. They are, therefore, probably as reliable as estimates of many other essential nutrient requirements.[40,41]

Table II. Amino Acid Scoring Patterns

	mg/g of protein	
	NAS/ NRC	FAO/ WHO
histidine	17	
isoleucine	42	40
leucine	70	70
lysine	51	55
methionine and cystine	26	35
phenylalanine and tyrosine	73	60
threonine	35	40
tryptophan	11	10
valine	48	50

Following publication of the estimates of amino acid requirements of men by Rose, a task force assembled by FAO proposed that an amino acid scoring pattern based on the amino acid requirements of adult men might serve as an appropriate standard for application of the chemical score procedure in evaluating the quality of proteins for human nutrition.[42] The tentative pattern tended to underestimate the nutritive value of proteins that were limiting in sulfur-containing amino acids or tryptophan. It was not very satisfactory but did stimulate research.

Howard and associates,[43] for example, proposed that the amino acid requirements of an animal (estimated from information about the amino acid requirements together with information about the amino acid content of high-quality proteins) would provide an appropriate standard for assessing the adequacy of proteins in meeting amino acid needs. In a study with rats they used the ratio of the amount of the limiting amino acid in the protein to the amount that was required to calculate the amount of a protein that could be considered as complete. This was essentially an application of the amino acid scoring procedure. It permitted calculation of the supplementary value of different proteins. Howard et al. were able to predict the nutritive value of several combinations of proteins using the method. Their observations seem, however, to have been neglected.

More recently there have been two major efforts to develop amino acid scoring patterns that would be suitable for evaluating the nutritive quality of proteins for human subjects. One was by an expert committee assembled by FAO/WHO;[38] the other was by a committee of the NAS/NRC, Food and Nutrition Board.[39] Both committees reviewed all of the available information on human requirements for amino acids. Using this information together with information about total nitrogen requirements, they calculated an amino acid scoring pattern expressed as milligrams of amino acid per gram of protein to be used as a standard for evaluating food proteins. The patterns proposed by the two groups are similar but not identical (Table II). The similarities are not surprising as there was overlap in the membership of the two committees and information accumulated by the NRC committee was available at the time the FAO/WHO committee met.

It was the judgment of these committees that a scoring pattern should be based on estimates of the amino acid requirements of the older infant or young child rather than on adult requirements. The pattern was developed from two sources—amino acid requirements determined by Holt and Snyderman and from the amino acid composition of human or cow's milk formulas together with information about protein intakes of infants fed such formulas by

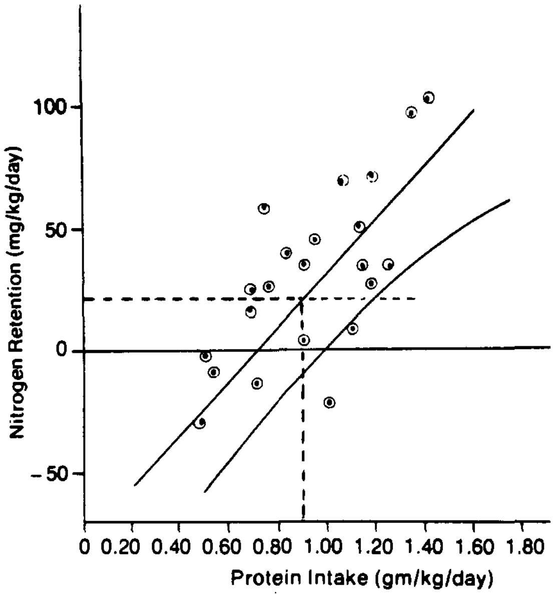

Figure 3. Nitrogen retention of children, 2 years old, fed graded levels of protein from cow's milk (after Arroyave[45]).

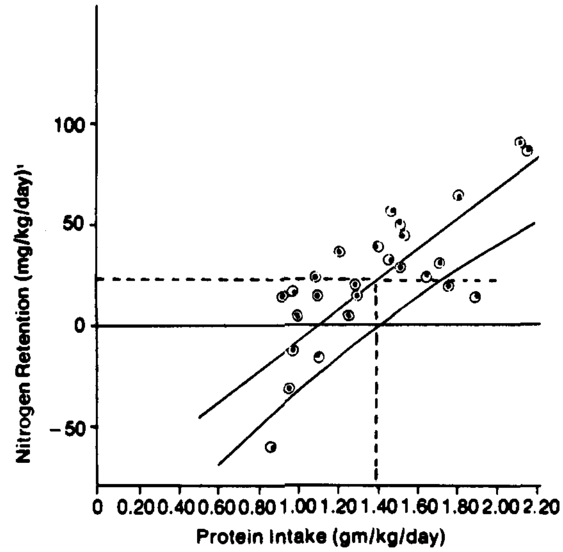

Figure 4. Nitrogen retention of children, 2 years old, fed graded levels of protein from a mixture of corn and beans (after Arroyave[45]).

Foman and associates.[38,39]

Pellet tested the FAO/WHO scoring pattern for its ability to predict the limiting amino acids in mixtures of proteins for the weanling rat. He found it to be superior to other standards for this purpose.[44]

During part of the time when these amino acid scoring patterns were being developed, I was in Guatemala where Dr. Arroyave and I, who were members of both committees, debated the problems of developing amino acid scoring standards at some length. We considered the type of testing that was required to assess the reliability of predictions made with them and proposed a study with children. He recalled that they had recently done studies of utilization of milk protein and a corn-bean mixture by young children, so we calculated, using the scoring patterns, the amount of each of these proteins that would be needed to meet the amino acid requirements of children of that age. Subsequently he compared the results of the trials with these predictions.[45] The plots of the results (Figures 3 and 4) indicated that with very few exceptions the children who consumed less than the predicted amounts of milk protein or the corn-bean mixture failed to grow satisfactorily, whereas those who consumed the predicted quantities or more grew well.

Later, Torun and Vitere[46] performed similar studies with somewhat older children, using soybean preparations which are limiting in sulfur-containing amino acids. They found that the children grew and retained nitrogen adequately with protein intakes that provided somewhat less than the FAO/WHO amino acid scoring value of 35 mg of total sulfur-containing amino acids/g of protein but somewhat more than the NAS/NRC value of 26 mg/g. Their results indicated that the sulfur-containing amino acid requirement of these children was close to that proposed by the NAS/NRC committee.[39]

These observations suggest that the current amino acid scoring patterns are appropriate for evaluating highly digestible proteins for human subjects. As the amino acid requirements per gram gm of protein of older age groups are less than those for infants and young children, the pattern should be satisfactory for them, too. Nevertheless, more tests are needed to establish that the standard amino acid scoring pattern can be used to predict human protein requirements accurately in order to establish its reliability for evaluating food proteins.

One of the major shortcomings of the amino acid scoring method that remains to be solved is that the method does not take low digestibility of proteins or unavailability of specific amino acids into account. In the 1930s McCollum et al.[17] discussed the effects of heat processing on proteins, pointing out that heat treatment improved the quality of some legume proteins but caused deterioration of the quality of cereal grain and other proteins. Since then the problem has been studied extensively. Carpenter, who developed a method for unavailable lysine based on the fluorodinitrobenzene reaction, has discussed this subject.[47]

There is no problem in using a figure for overall digestibility as a correction factor for the amino acid score. There is, however, great difficulty in correcting for unusual unavailability of specific amino acids other than lysine. The question that arises is how significant is this type of unavailability in the proteins of the food supply. Many of the products that are most extensively heat processed represent minor dietary sources of protein. With such a large proportion of the protein in the U.S. food supply from animal products and relatively slightly processed vegetable products, this is unlikely to be a serious problem.

The predictability of the scoring procedure noted earlier for lysine-deficient products[43] and the predictability of protein requirements from the scoring patterns in the studies of Arroyave[45] and of Torun and Vitere[46] would suggest that for many diets the problem is not serious. Uribe[48] in our laboratory used the amino acid composition of rat milk as a standard for calculating the proportion of utilizable protein in a series of food mixtures composed of cereal grains and milk proteins to give a wide range of combinations differing in protein quality. Prediction of growth from N intake was poor (Figure 5), but after correction for utilizable protein using the scoring procedure, prediction of performance was amazingly good (Figure 6).

CONCLUSIONS

In my view, future directions in the assessment of protein quality should focus on assessing the adequacy of proteins for meeting amino acid requirements. It may be necessary to assess diets for only three or four amino acids—lysine, total sulfur-containing amino acids, tryptophan, and possibly threonine—as was suggested by Campbell, McLaughlan, and associates[49] some years ago. If the requirements for these are met, it is most unlikely

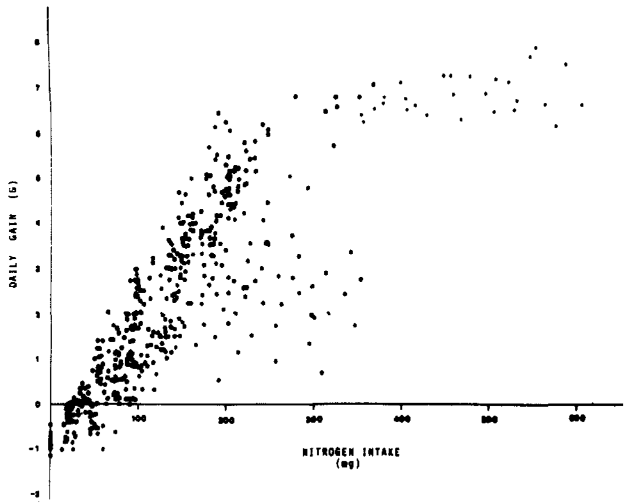

Figure 5. Weight gains of individual rats fed graded levels of protein from a variety of foods, food components, and food mixtures (after Uribe et al.[48]).

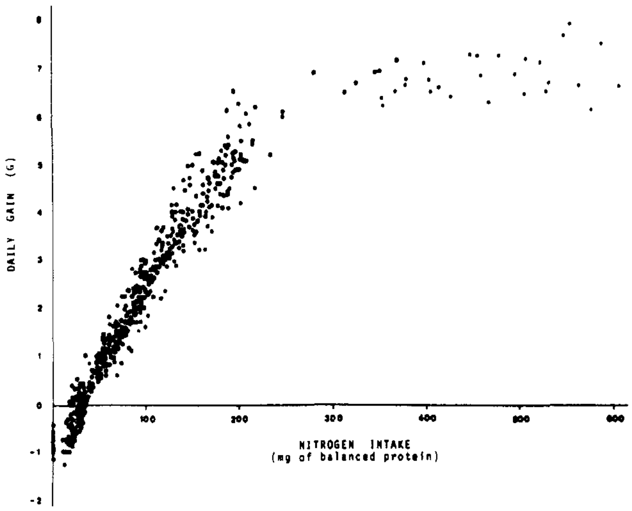

Figure 6. Weight gains of individual rats from Figure 5 plotted as a function of intake of nitrogen from balanced protein, estimated by using an amino acid scoring procedure (after Uribe et al.[48]).

that the requirements for other amino acids will not be met also.

There seems to be hesitation about moving in this direction. There have been questions about the reliability of amino acid requirements and about the significance of low biological availability of amino acids. I am satisfied that estimates of amino acid requirements are no less reliable than those for several other nutrient requirements and are probably better than those for most minerals.

The problem of low availability is no greater than it is for iron, trace minerals, and folate, yet we have no hesitation in making recommendations for dietary allowances for these.

It is important to note that when actual amino acid requirements are used as the basis for the scoring pattern, the fall in efficiency of utilization of amino acids as intake approaches the requirement value is already taken into account. Also, the only feasible way of assessing the supplementary or complementary value of proteins in mixtures is by use of a scoring pattern. This procedure has been used for years by the animal production industry.

McLaughlan[36] has suggested that we do not need measures of protein quality if we can estimate amino acid requirements and the available amino acid content of proteins with reasonable accuracy. Pellett (see ref 36 for a discussion) has stated that prediction of the quality of proteins from amino acid requirement values and knowledge of the amino acid composition of proteins is ready for use despite its shortcomings. In my judgment, it is past time that we applied the amino acid scoring method generally for estimating the ability of foodstuffs to provide amino acids in amounts that will meet requirements and that more effort be directed toward assessing the significance of the problem of unavailability of amino acids.

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Bioavailability: A Factor in Protein Quality

Constance Kies

While the amino acid proportionality pattern of a protein is probably the most important determinant of protein quality, bioavailability of these constituent amino acids consitutes the second most important variable. The degree to which the constituent amino acids of a food protein are actually available to the body is determined by such factors as protein configuration, amino acid bonding, other constituents of the diet, and the physiological condition of the gastrointestinal tract of the individual involved.

The most important determinant of the nutritional quality of a protein is its amino acid composition as compared to the amino acid requirements of the organism consuming it. If protein quality were, in fact, merely a function of amino acid proportionality patterns, scores based on chemical analyses of amino acid composition of food products would give easy, exact predictions of protein quality based on performance in living organisms (WHO/FAO, 1973; National Academy of Sciences, 1978; Block and Mitchell, 1946). Unfortunately, this is not the case (Holmes, 1965). Part of the problem is associated with the obtainment of fast, accurate information on the amino acid composition of the proteins in food products. In spite of remarkable advances in methodologies and instrumentation, analyses of the important essential amino acids methionine and tryptophan still present difficulties.

The other side of the ratio, quantitative requirements of the essential amino acids, presents even more difficulties, at least when the problem of protein quality in human nutrition is addressed. In spite of efforts of such pioneering scientists as Rose et al. (1955), Leverton (1959), Swendseid et al. (1956), Nakagawa et al. (1964), and Holt et al. (1960) as reviewed by Irwin and Hegsted (1971) on amino acid requirements of human men, women, and infants, questions exist not only on quantitative requirements but also even on the essentiality of such amino acids as histidine.

Even with these admitted difficulties, the correlation between prediction and performance is quite good, particularly at extreme ends of the curve. In other words, prediction for poor performance of proteins devoid or nearly devoid of an essential amino acid is excellent. Similarly, the prediction of good performance for proteins containing all essential amino acids according to idealized patterns is also excellent. However, fine-line predictions of intermediate quality are less accurate. Surprises are not uncommon. Improvement of amino acid proportionality patterns by fortification or by genetic selection as in development of high-lysine cereals does not always result in expected improvements in protein quality. Food processing results in changes in protein quality which cannot be explained by obvious alterations in amino acid constituents.

Deviations between prediction of protein quality based on amino acid content/amino acid requirement ratios and actual protein quality based on performance in living organisms assuming accurate determination of both of these factors would seem to be due to variations in the utilization of the amino acids comprising different proteins. More simply, the required essential amino acids may be there and may be there in ideal amounts, but the efficiency to which they may be used constitutes another whole series of problems. In part, many of these factors may be sub-

Department of Human Nutrition and Food Service Management, University of Nebraska, Lincoln, Nebraska 68583.

