Evaluation of Protein in Foods for Regulatory Purposes

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The determination of the protein efficiency ratio of individual foods fed at a 10% protein level in a standardized 4-week rat growth assay has been found to be the most generally applicable procedure for evaluating protein quality. Based on the determination of three amino acids, lysine, methionine, and cystine, a simplified chemical score was found to correlate well with PER data on a series of 43 foods. The method was rapid and reproducible. To evaluate the protein contribution of different foods for regulatory purposes, a protein rating was developed based on both the quantity and quality of protein in a reasonable daily intake of food. This procedure was applied to a variety of foods and appeared to classify them in a logical manner.

RECENT RESEARCH on the role of protein in nutrition has prompted commercial emphasis on this constituent. This emphasis on protein has been applied to a wide variety of foods, some of which are recognized as important sources of protein and others which are not. The Food and Drug Laboratories in Canada have been concerned about claims made for the protein content of some foods and have tried to determine which are justified and which are misleading or likely to create an erroneous impression regarding the value of the food.

Allison (*2*) has stated, "The prime purpose of a dietary protein is to provide a pattern of amino acids appropriate for the synthesis of tissue proteins and for other metabolic functions." The chief problem, therefore, was to evaluate the pattern of amino acids in a food. Methods for protein evaluation have been detailed by Allison (*1, 3*), Grau and Carroll (*13*), Bender (*6*), and others and will not be reviewed here. Although there are many methods available, no definite recommendation has been made as to which is the most suitable or satisfactory for determining the value of proteins. Obviously, if any progress were to be made in the regulatory aspects of the problem, certain more or less arbitrary decisions regarding a method had to be made. The majority of workers in the field seemed to favor the determination of the protein efficiency ratio (PER) for routine purposes.

General Considerations

Some of the implications of different approaches to the evaluation of proteins were considered in this study. Mitchell (*23*), Hart (*16*), and others have pointed out that, because of the supplementary action of amino acids, the classification of proteins individually is of relatively little significance in assessing the protein value of diets. It could be argued, therefore, that the protein contribution of a food should be evaluated only in terms of the complete diet with which it is fed. As Canadians (and Americans) consume, on the average, about 100 grams of protein daily, more than half of which is animal protein, it would be difficult to distinguish between the effect of a good or a poor quality supplement to such a diet. For example, there would be little difference between a supplement of 20 grams of protein as meat or as white bread. Under these conditions, quality of protein would be relatively unimportant and the only factor to be considered would be the amount of protein. Because most individuals, however, consume almost 50% more protein than they require, additional amounts would have little or no value. It might be argued, therefore, that it would be misleading in advertising material to stress the importance of the protein contribution of a food. If one follows this argument to its conclusion it would appear that, for the average individual or the great majority of people, all claims for the importance of and need for protein would be misleading and any statement regarding protein quality would have little if any meaning.

This position is a rather extreme one and, although tenable from one aspect, lacks complete justification. It ignores the vast amount of study on the protein quality of individual foods and the view that protein quality is important. Furthermore, foods are sold individually and advertisements are based on the individual food. The application of average consumption figures to individual cases is undoubtedly open to question. If the diet of some people is not fully adequate in protein, it is useful to add significant amounts of good quality protein to their diet. In these circumstances, it would not be misleading to encourage the use of protein and it would seem desirable to improve existing sources of protein. This stand would imply that, at least for regulatory purposes, it is important to know the value of individual protein foods but to realize, also, that this value may change, and usually increases, when the food is consumed as part of a mixed diet. This is the approach taken in this laboratory. This paper describes experiments with a rat growth method (*10, 24*), the development of a simplified chemical score for use as a rapid screening procedure (*21*), and a protein rating for the classification of foods into categories according to their protein contribution (*9*) for regulatory purposes.

Determination of Protein Efficiency Ratios

Table I presents the details of the method which has been recommended (*10*) for the evaluation of protein quality. The protein to be tested is added at the expense of cornstarch to a level of 10% and the fat content is maintained also at 10%. Possibly the chief innovation is the use of casein as a reference standard and the correction of all PER values to 2.5 for casein. With this exception, the method is very similar to that suggested recently by Derse (*11*) and now being tested collaboratively by the A.O.A.C.

Results obtained by this procedure are affected by several factors. The importance of the age of the animal at the time it is put on test is shown (Figure 1) and marked differences occurred

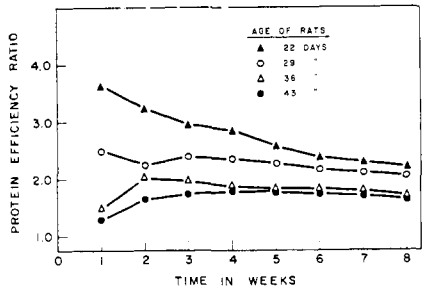

Figure 1. Effect of age of rat when put on test on PER values of casein

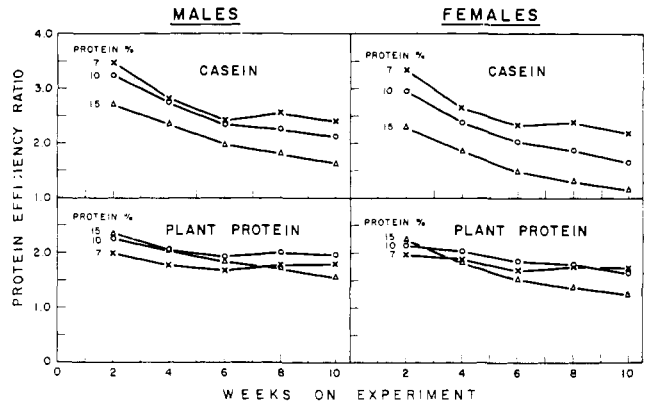

Figure 2. Effect of sex of rat, level of protein, and time on test on PER values of two proteins

Table I. Recommended Method for P.E.R. Assay

Animals, weanling males, 20–23 days old

Diet	%
Cornstarch	80
Corn oil	10 (maximum)
Cellulose	5
Salt U.S.P. XIV	4
Vitamins	1
Protein (N × 6.25)	10 (at expense of starch)

Reference standard, A.N.R.C. casein

Assay period, 4 weeks

Calculation, PER × $\frac{2.5}{\text{PER for casein}}$

Table II. Effect of Strain of Rat on PER Obtained with Different Foods

Food	PER Values[a] Found with Strain A	Strain B Uncorrected	Strain B Corrected[b]	Strain C Uncorrected	Strain C Corrected[b]
Casein	2.59 (10)	3.35 (1)	...	3.18 (1)	...
Dried whole egg	3.44 (9)	4.00 (1)	2.99	4.00 (1)	3.14
Wheat flour–soybean flour mixture	2.09 (1)	2.64 (1)	2.00	2.56 (1)	2.01
Whole wheat flour	1.17 (1)	1.44 (1)	1.07	1.50 (1)	1.18

[a] Figures in parentheses indicate number of experiments in which 10 male weanling rats received each food at the 10% protein level for 4 weeks.

[b] Obtained by multiplying the determined PER value by the fraction 2.5/determined PER of reference standard casein.

as a result of variations in this factor. PER values for casein tended to decrease with age and with time on test. On the basis of these data, and other considerations, rats 20 to 23 days old were specified for use in a 4-week test.

The level of protein to be used in a rat assay is controversial. Barnes *et al.* (*5*) and Barnes and Bosshardt (*4*), confirming the earlier work of Osborne *et al.* (*25*), showed that the effect of the level of protein on PER values varied with the type of protein and they emphasized the importance of this factor. Most workers favor about 10% but Harris and Burress (*15*) recently suggested that a level of 15% is more realistic, as it is closer to the proportion of protein consumed on the average by Americans. Figure 2 illustrates data from an experiment in which the level of protein, sex, and length of the experimental period were studied with casein and a plant-protein mixture. Female rats tended to have lower PER values than males, but not consistently so. They tended to give maximum values at lower protein levels than did males. PER values found with both sexes dropped as the experiment progressed but the decline was greater with animals fed casein than with those fed the plant-protein mixture. Differences between casein and plant protein were greatest during the early stages of the experiment. At the 10% level, both proteins exhibited near maximum values while casein gave low values at 15% protein and the plant protein gave low values at 7%. Because sex, time on test, and protein level influenced PER values, these factors must be controlled if results are to be reproducible in different laboratories. Furthermore, if one level of protein is to be used, 10% seems to be the most generally satisfactory.

Some laboratories have reported markedly higher PER values for casein than others. This laboratory has exchanged casein with other laboratories, found essentially the same low values with the acquired sample of casein as with its own, and concluded that strain of rat may be the most important factor. Data on the response of three different strains are presented in Table II. Strain A was the original strain used in our laboratories. While differences were marked between this strain and rats from two other sources, the use of the correction for the value obtained with the casein standard largely eliminated the variation and placed the results in the same perspective.

Development of Simplified Chemical Score

While there appears to be general agreement at the present time that animal assay procedures are necessary for evaluating proteins, the use of simpler and more rapid techniques was also considered. These would be particularly valuable in development work and where facilities for animal assay are not available. They would also be useful as preliminary screening procedures.

Several microbiological methods had been suggested (*14*, *19*, *29*) for this purpose. Results obtained with modifications of these procedures using *S. faecalis* and *L. mesenteroides* were compared with PER values determined on the same samples of a variety of foods and were unreliable (*27*) as an indication of protein quality.

From these studies, however, it became evident that it might be possible to rate cereals according to their lysine content, and animal proteins according to their methionine (plus cystine) content. About this time, both Waddell (*30*) and Block and Weiss (*8*) indicated that in most commonly used foodstuffs the limiting amino acids were usually lysine, methionine, or the combination of methionine plus cystine. Because Block and Mitchell (*7*) showed that a close relationship existed between chemical score and nutritive value of protein as judged by PER values, the same relation was investigated, using what is

termed the simplified chemical score (S.C.S.), which is based on the determination of these three amino acids.

Table III illustrates the principle involved in the determination of S.C.S. The content of the three amino acids, lysine, cystine, and methionine, was determined and expressed in terms of the concentrations of these amino acids in whole egg. To reduce the variation inherent in the amino acid determination, an egg standard was used in all assays and results were expressed in terms of this standard. As recommended by Block and Mitchell, if lysine or methionine were the most limiting amino acid, it was the basis of the S.C.S. If the cystine content were the lowest of the three amino acids, the combination of methionine plus cystine content was used as the S.C.S., as methionine is convertible to cystine. All three amino acids were determined on 2-hour acid hydrolyzates of the food using *L. mesenteroides* in the procedure of Steele *et al.* (*28*) for methionine and lysine and the method of Horn and Blum (*18*) for cystine.

The simplified chemical score was calculated for a variety of foods and compared with PER values determined on the same samples by the procedure described earlier. Foods tested were separated into two categories: those which were limiting in lysine, or those limiting in methionine (+cystine). As shown in the top section of Figure 3, correlation was good between lysine concentration and PER in the first group and between methionine (+cystine) content and PER in the second group.

Although the relationships were different, a factor of 15 added to the SCS. of the latter group resulted in a good correlation of both sets of data with PER (Figure 3, middle). This adjustment indicated that it was possible to predict PER values reasonably well from SCS data knowing the limiting amino acid of the three tested.

The SCS was also applied to literature data. Block and Mitchell (*7*) listed amino acid and PER data for 18 foods which were deficient in either lysine or methionine (+cystine). SCS values were calculated and compared with their PER data (Figure 3, bottom). Although relatively few values were available, the data fell into two classes as before and the correlation in each group was reasonably good.

Data on the six other foods listed by Block and Mitchell are given in Table IV. Chemical scores have been calculated from their data indicating that these foods were limiting in threonine, isoleucine, or tryptophan. SCS was calculated on the assumption that they were limiting in lysine or methionine (+cystine). The agreement between the two sets of data is reasonably good and does not change the interpretation of the food as a source of good or poor quality protein. This would suggest that foods limiting in threonine, isoleucine, or tryptophan are also near limiting in lysine or methionine. Thus, even in those foods limiting in some amino acid other than the three used for SCS, this procedure may offer a useful indication of protein quality.

The method is rapid, reproducible, and particularly suited to control work. As there is good correlation with PER data, the criticisms mentioned by Grau and Carroll (*13*) of analytical difficulties, differences in absorption, and other effects do not appear to be too serious. As methods become more accurate it should be possible to dispense with the egg standard. While at the present time it is not proposed that the S.C.S. procedure should replace the animal assay, sources of variation in the latter enhance the attractiveness of amino acid analysis. In this connection it is interesting to note that Miller and Naismith (*22*) have pointed out that in many diets the cystine and methionine contents appear to be limiting rather than the lysine content and have based a method for protein quality on the total sulfur determination.

Protein Rating

Having decided upon the PER assay for evaluating protein quality, it was then necessary to set up criteria by which the value of various foods as protein sources might be described. By such criteria, the terms "high quality" or "excellent dietary source" would have a definite meaning. The food processor or manufacturer would know what experimental work would be necessary to establish claims regarding the protein content; the physician, nutritionist, and consumer would know that claims were uniformly and soundly based on acceptable experimental data.

Any claims for the protein content of a food must be made on the basis that, for the average individual, the food contributes a significant proportion of the total protein requirement when consumed in a usual or reasonable manner. This principle has been found most useful in Canada for governing claims which may be made for the vitamin content of natural foods—i.e., those to which no vitamins have been added. Under authority of the Food and Drugs Act and Regulations (*12*), a food may be described as a "good dietary source" or an "excellent dietary source" of any vitamin when the amount of that vitamin in a "reasonable daily intake" of the food furnishes more than certain specified amounts. It appeared reasonable to extend this principle to permit the description of a food as being an "excellent dietary source of protein" or a "good dietary source of protein."

Table III. Calculation of Simplified Chemical Score (SCS)

	Amino Acid Content, % of Egg			
Food	Lysine	Methionine	Methionine + cystine	S.C.S.
Whole egg	100	100	100	100
Beef	114	89	66	66
Peas	110	32	38	32
White bread	35	56	66	35

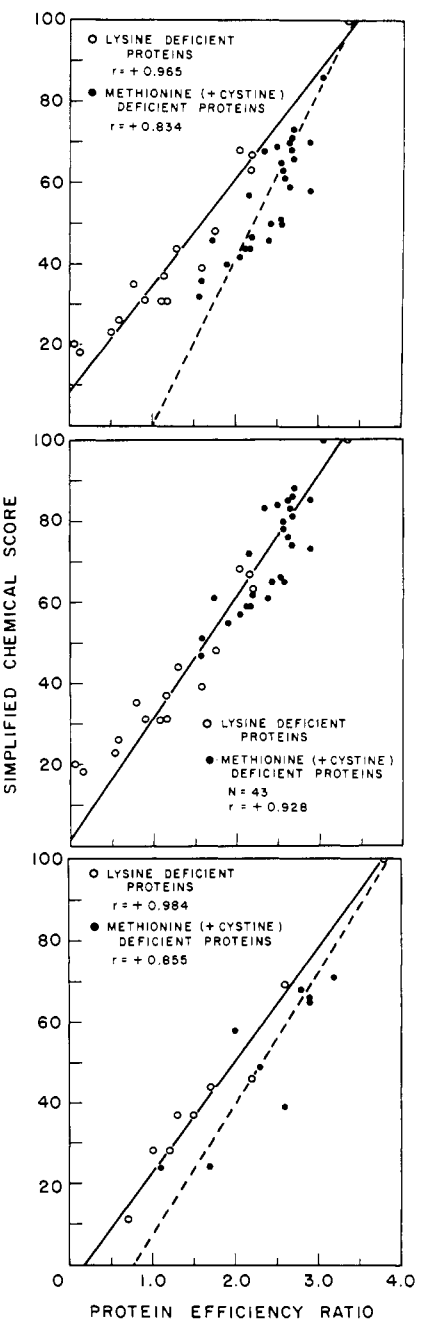

Figure 3. Correlation between SCS and PER values

Top. For 43 foods
Middle. For 43 foods after adjustment of methionine (+ cystine) scores
Bottom. For 18 foods [from data of Block and Mitchell (*7*)]

Table IV. Ratings by SCS and Chemical Score[a] of Foods Not Limiting in Lysine, Methionine, or Cystine

Food	Chemical Score: Actual limiting[b] amino acid	Chemical Score: Score	SCS: Apparent limiting[b] amino acid	SCS: Score
Egg albumin	Threonine	78	Lysine	90
Liver	Isoleucine	70	Methionine + cystine	71
Heart	Isoleucine	65	Methionine + cystine	68
Brain	Isoleucine	64	Methionine + cystine	74
Wheat germ	Isoleucine	38	Methionine + cystine	42
Gelatin	Tryptophan	0	Methionine + cystine	14

[a] Data taken from Block and Mitchell (*7*).
[b] Not necessarily the most limiting amino acid for growth of animals.

Table V. Protein Rating of Certain Foods

Food	Protein Content, %	Reasonable Daily Intake (Serving), Grams	Protein Intake, Grams	P.E.R.	Protein Rating[a]
		Literature Data (*7*)			
Cabbage	1.4	50	0.7	0.9	0.6
Wheat, whole	9.9	30	3.0	1.5	4.5
Oats, rolled	14.2	30	4.3	2.2	9.5
Bread, white	8.4	150	12.6	1.0	12.6
Soybean	34.9	30	10.5	2.3	24.1
Egg, whole	12.8	100 (2, eggs)	12.8	3.8	48.6
Beef	21.0	100	21.0	3.2	67.2
Milk, whole	3.5	708 (3 cups)	24.8	2.8	69.4
		Experimental Data			
White bread	8.4	150 (5 slices)	12.6	0.77	9.7
Whole wheat bread	10.5	150 (5 slices)	15.5	1.1	17.1
Gluten bread	16.9	150 (5 slices)	25.4	0.9	22.9
"Protein" bread	11.8	150 (2 slices)	17.7	1.3	23.0
Egg	12.8	100 (2)	12.8	3.5	44.8
Milk	3.5	708 (3 cups)	24.8	2.7	67.0
Oats	12.8	30	3.8	2.1	8.0
Oats plus milk	12.8 3.5	30 120	3.8 4.2	3.2	25.6
"Protein" cereal	20.0	30	6.0	0.03	0.2
"Protein" cereal plus milk	20.0 3.5	30 120	6.0 4.2	2.0	20.4

[a] These values do not take into account the known supplementary effect of animal proteins

Because protein requirements are met by combinations of amino acids, quantity as well as quality of protein must be considered. Three factors are involved: the amount of the food consumed in a reasonable daily intake, the per cent of protein in the food, and the biological value of the protein. As was pointed out by Hegsted (*17*) and others, if 20 grams of a protein with a biological value of 100 satisfied the protein requirement of an individual, it would take approximately 40 grams if the biological value is only 50. Within limits, therefore, protein requirements may be satisfied equally well by varying amounts of protein dependent on its biological value.

On this basis, and using the PER method as a measure of biological value, a protein rating was proposed which is the product of the PER of a protein multiplied by the grams of protein in a "reasonable daily intake" of the food. Table V lists representative foods with their protein content, protein efficiency ratio, and reasonable daily intake. All PER data in the upper part of the table are taken from Block and Mitchell (*7*). Reasonable daily intake is usually considered to be one serving of a food but may be more—for example, in the case of milk and bread. The protein ratings in Table V fall into two distinct categories. Meats, eggs, and milk have ratings varying from about 40 to 70, while cereals and vegetables have ratings of 12 or less. Soybeans are intermediate with a rating of about 24. Food with a rating above 40 is designated as an "excellent dietary source of protein." The protein contribution of an individual food with a rating below 20 is relatively insignificant and, therefore, it would seem misleading to attach any special significance to its protein content or to use it in any way as the basis of advertising claims. A food with a rating of 20 to 39 inclusive may be designated as a "good dietary source of protein." This category is one which could be reached by significant improvements in the quality and quantity of proteins in foods for which claims would not normally be permitted.

Platt and Miller (*26*) recently developed a somewhat similar system of rating diets using both quality and quantity of protein in the concept of "net dietary-protein value." There is no satisfactory way to allow for the supplementary effect of proteins at the present time although the work of Howard *et al.* (*20*), which appeared after this work was completed, offers definite possibilities in this regard.

To indicate how the technique may be used to classify foods, the protein ratings of some additional foods are also shown in Table V. These data were obtained by applying the PER assay procedure of Chapman *et al.* (*10*) to market samples. White and whole wheat bread are not "good dietary sources of protein." A new "protein" bread now appearing on the market in Canada and containing more and higher quality protein would be given a rating of 23 and hence might be referred to as a "good dietary source of protein." A sample of gluten bread also meets this requirement. The protein ratings found for white bread, eggs, and milk are close to the values calculated from the literature in the upper part of the table.

It has been argued that because cereals and milk are invariably consumed together, they should be evaluated as a combination rather than separately. If mixtures of 1 ounce of cereal with 4 ounces of milk are treated in this manner, it will be seen from Table V that some cereal plus milk combinations rate as good dietary sources of protein while others do not. The supplementary effect of milk tends to make the combinations more similar to each other than were the original cereals alone.

In proposing a single criterion to describe protein value, it was not possible to allow for the many points of view on this subject. Nevertheless, in view of the commercial interest in stressing the importance of protein in various foods and the claims being made at present, it was felt that some yardstick must be set up and a beginning made. It was hoped that the procedure recommended might not only serve a useful purpose but also encourage the development and adoption of better and more uniform criteria as more information is obtained. The protein rating procedure described has not been made the subject of regulations under the Food and Drugs Act but is being used administratively at the present time for the evaluation of the protein content of foods sold in Canada.

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PROTEIN QUALITY AND SUPPLEMENTATION

Effect of Amino Acid Supplements, Vitamin B_{12}, and Buffalo Fish on the Nutritive Value of Proteins in Sesame Seed and Meal

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A study of sesame seed and meal was undertaken, because of their importance to human nutrition and the animal feed industry. Addition of lysine, lysine and threonine, and buffalo fish benefited growth and the protein efficiency of sesame seed and meal. Sesame meal supplemented milled, white corn meal, enriched wheat flour, and white milled rice. Lysine and threonine influenced the biological value and net utilization of sesame seed. Vitamin B_{12} failed to supplement the lysine-threonine additions. Data are presented on amino acid, vitamin, and mineral content. The nutritive value of sesame seed and meal warrants their use in the enrichment of diets for certain population groups and for continued use in poultry and swine rations.

Sesame is one of the oldest staple, vegetable oil crops and a source of vegetable fats in India, China, Egypt, and Latin America. It was introduced in this country in the late 17th century in South Carolina and since 1953 has been grown on a commercial basis in Texas. Twelve million pounds a year are imported, and used for oleo, bakery products, candy, and cooking fat. The oil has the remarkable quality of remaining fresh and sweet for long periods of time. Foods, confections, oleomargarine, and bakery goods made with sesame oil remain free from rancidity up to 10 times longer than some of the better known vegetable oils. Detailed information about sesame meal, its production, trade, yields, breeding, harvesting, processing, and use are given in a recent publication by Altschul (*2*). Sesame seed is valuable to the diets of sections of the world's population and sesame meal is important as an ingredient of poultry rations (*1*).

This paper reports results of growth and metabolism experiments with young rats fed diets composed of fat-extracted sesame seed and meal with and without supplements of lysine, threonine, and vitamin B_{12}. Results are also presented of studies on the content of all members of the B-complex vitamins, amino acids (including nonessentials), calcium, phosphorus, and iron in sesame seed and meal. Included is a study of the supplementary value of the proteins of sesame seed as compared to those of milled white rice. The supplementary value of the proteins of sesame meal over those of milled white corn meal and of enriched milled wheat flour is presented, along with the supplementary value of the proteins of buffalo fish over those of sesame meal.

Experimental Procedure

Raw Material. Commercial samples of sesame seed and meal were used for the determination of vitamins, minerals, amino acids, and growth value of the proteins. The samples were fat-extracted with petroleum ether for use in rations. Growth value was determined in studies using albino rats as experimental animals fed fat-extracted sesame seed and meal rations containing 9% of protein. Fat-extracted sesame seed and meal furnished the only source of protein