METHODS OF MEASURING PROTEIN QUALITY: A REVIEW OF BIOASSAY PROCEDURES[1]

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The biological evaluation of proteins continues to be a subject of interest at scientific meetings. Various bioassays (biological value, net protein utilization, net protein ratio, relative protein value by slope assay procedure, relative nitrogen utilization, protein efficiency ratio, and a relative protein efficiency ratio) have been discussed and reviewed. This reviewer considers the relative nitrogen utilization, relative protein efficiency ratio, and protein efficiency ratio to be the most practical procedures for monitoring protein quality on a routine basis and for regulating nutritional labeling. None of the rat bioassays is really appropriate for testing protein quality of foods as consumed by humans, but, until more information is compiled from human investigations, will continue to be used for this purpose because of problems associated with human studies.

The biological evaluation of proteins has been of interest to scientists for many decades. Nutritional labeling regulations have stimulated further debates as to the best method for use in the evaluation of protein quality. Problems have arisen in trying to apply animal results to humans. This is further compounded in the U.S. because we have a surfeit of protein-containing foods available. The objective of this paper is to review some of the bioassay procedures that have been and are being used in various laboratories.

First, the nutritional evaluation of protein requires an estimate of protein content as well as an evaluation of the biological usefulness of the protein regardless of the body function. More specifically, the amino acids present in the food and their availability determines the biological usefulness of the protein for a specific body function. The amino acid needs for growth are different from those for maintenance of body tissue (1). Likewise, the growth of a fetus, the production of milk, tissue repair, *etc.*, have specific and different amino acid requirements (1).

The preceding comments serve to emphasize the magnitude of the problem of trying to take one animal bioassay and have it serve all the various body needs for protein. The problem is essentially impossible if the ultimate goal is to rank the protein-containing foods for humans from highest quality to the poorest, especially since man consumes several different proteins each day. The consumption of a variety of proteins may balance the deficiencies present in a single or several protein-containing foods, examples of which are the amino acids present in milk and corn, which supplement and complement each other (2).

The rat has been the most widely used laboratory model, and its amino acid needs for maintenance are different than those for growth (1,2,3). Also, the amino acid needs of humans vary, depending on the body's need for protein. With the advent of nutritional labeling regulations, the food industry and the compliance and regulatory agencies have a real need for a rapid and easy means

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of predicting protein quality. It is unfortunate that none of the methods in use today is adequate for predicting the biological value of a protein under all the actual consumption patterns in humans.

RAT BIOASSAYS FOR MEASURING PROTEIN QUALITY

Most of the biological methods for evaluating protein quality are predicated upon the feeding of a single source of protein because it is very difficult to separate the effects of each protein where more than one protein is consumed (4,5,6,7,8,9). Several investigators (3,8,9,10,11,12,13) have recently discussed the nutritional evaluation of proteins by various biological methods and the effects of protein quality and quantity on protein utilization.

Some of the factors that influence the results obtained with the various bioassays are shown below. The items listed will influence the values obtained with any bioassay, such as biological value (BV) by the Thomas Mitchell procedure (14), net protein utilization (NPU), as outlined by the British researchers (15), net protein ratio (NPR) (16), protein efficiency ratio (PER) (17), slope assay procedure (18), and the relative nitrogen utilization (RNU) procedure recently proposed by McLaughlan (19). Therefore, they must be carefully controlled in every study and standardization of each assay procedure is desirable.

Factors that influence the results obtained with various protein quality assay procedures:

- Age and sex of animal
- Weight of animal
- Protein quantity and quality
- Food intake
- Other Dietary components
  - Minerals
  - Fat
  - Carbohydrate
  - Moisture
- Husbandry
- Environmental conditions
  - Temperature
  - Humidity
  - Cage size
  - Light

Biological Value (BV)

Although the equation for the Thomas-Mitchell procedure (14) may appear simple from a mathematical point of view, it is an involved and cumbersome procedure that negates its use as a routine procedure for measuring protein quality in laboratory animals. Some of the major problems associated with use of the BV procedure are the extreme care which is necessary to effect complete collection of urine and feces; prevention of feed contamination in the urine and feces; the analysis of orts, urine, and feces for nitrogen; determination of metabolic fecal losses; and the measurement of endogenous urinary losses. Also, protein level in the diet and the age of the rat will influence the results (20,21,22).

The BV procedure measures the efficiency of utilization of the nitrogen absorbed and has been adequately reviewed by Campbell (5) and more recently discussed by McLaughlan (1972) (8,10). With some modifications, this nitrogen balance procedure is the method most generally used in human studies (23).

$$BV = \frac{\text{retained N}}{\text{absorbed N}} \times 100$$

$$= \frac{\text{N intake}-(\text{fecal N}-\text{metabolic fecal N})-(\text{urine N}-\text{endogenous urinary N})}{\text{N intake} - (\text{fecal N} - \text{metabolic fecal N})}$$

$$\times 100$$

Net Protein Utilization (NPU)

In 1953, Bender and Miller (15) described this procedure, which gives results similar to the Thomas-Mitchell nitrogen balance method, but it is considered to be somewhat simpler and easier to use by some investigators. However, the measurement of carcass nitrogen is a real stumbling block for this procedure. An alternate procedure is to measure body water. This is possible because of the constancy of the nitrogen/water ratio of the rat carcass (24).

$$NPU = \frac{\text{retained N}}{\text{food N}} \times 100$$

$$= \frac{\text{body N of test group}-\text{body N of nonprotein group}}{\text{N consumed by test group}} \times 100$$

Net Protein Ratio (NPR)

In 1957, Bender and Doell (16) described this procedure, which is simply the weight loss of a negative control group added to the weight gain of the test group, divided by the protein consumed by the latter. The NPR assay is similar to PER, but many investigators do not like to feed a nonprotein-containing diet to a group of rats every time they need to check a test protein.

$$NPR = \frac{\text{weight gain on test protein} + \text{weight loss of nonprotein group}}{\text{weight of test protein consumed}}$$

In assays with rats, NPR is preferred by this investigator to NPU because it is a simpler assay and for practical purposes gives the same answer.

Table I shows data taken from an FAO publication on BV, digestibility (D), NPU, and PER (25). The correlation coefficients for the BV, D, NPU, and PER bioassays have been computed and are shown in Table II. With this particular set of data, the correlation coefficient for NPU and PER is the highest (0.973) and the value for digestibility with PER is the lowest (0.479). Other data could be obtained that would show different correlation coefficients, but the main point is that each procedure is different and comparisons, as shown, are mainly academic

and tend to rank the protein foods in the same order in the rat.

Neither BV nor NPU is appropriate for routine bioassays for measuring protein quality because each is too time-consuming and neither is a simple procedure. Therefore, the balance of this paper will be concerned with a discussion of the pros and cons of PER, RNU, and relative protein value (RPV) procedures.

Slope Ratio Assay

Several years ago, Hegsted and coworkers (18,26) proposed the slope ratio technique for determining protein quality, in which gain is used as the response and nitrogen intake or protein intake as the measure of dose. Pellett (12) has reviewed this procedure. The assay utilizes lactalbumin as a reference standard. A relative growth index or a relative protein value can then be calculated for each protein source, based on lactalbumin as the reference standard.

TABLE I
Comparison of Four Bioassays for Measuring Protein Quality[a]

	BV[b]	D[c]	NPU[d]	PER[e]
Beans	58.0	72.8	38.4	1.48
Beef and veal	74.3	99.3	66.9	2.30
Casein	79.7	96.3	72.1	2.86
Cow's milk	84.5	96.9	81.6	3.09
Egg	93.7	97.0	93.5	3.92
Fish	76.0	85.0	79.5	3.55
Groundnuts	54.5	86.6	42.7	1.65
Peas	63.7	87.6	46.7	1.57
Rice	64.0	97.9	57.2	2.18
Sesame	52.0	81.7	53.4	1.77
Soybeans	72.8	90.5	61.4	2.32
Wheat	64.7	90.9	40.3	1.53

[a]Based on data in FAO, 1970 (25).
[b]BV = biological value.
[c]D = digestibility.
[d]NPU = net protein utilization.
[e]PER = protein efficiency ratio.

TABLE II
Comparison of Four Bioassays, Correlation Coefficients[a]

	r
BV:D	0.647
BV:NPU	0.899
BV:PER	0.885
NPU:PER	0.973
D:NPU	0.576
D:PER	0.479

[a]Based on data in FAO, 1970 (25). Protein sources are shown in Table I.

This procedure is a multidose assay which necessitates feeding several levels of protein; only values falling on the linear portion of a curve are used in computation of the slope assay value. The necessity for feeding several dietary protein levels is a major fault of the procedure, since much labor is involved. Also, the mixing of three to five diets increases the chance for error. Additionally, lysine-deficient proteins may not yield a valid slope ratio; in other words the linear portion of the curve is flatter (8). Finally, threonine-deficient proteins tend to yield too high a value—the slope is steeper[1].

McLaughlan and Keith[1] fed several diets considered to be marginally deficient in threonine to rats at protein levels ranging from 3–9% crude protein. They observed increased growth at low protein levels with threonine supplementation and little or no extra growth at high protein levels, which resulted in decreased slopes in the RPV assay. Thus, threonine supplementation apparently decreased RPV for threonine deficient diets (3–4% protein level), but PER and RNU were not affected. McLaughlan and Keith (27) concluded that the RPV assay may overestimate the protein quality of threonine deficient proteins.

A major difficulty with the RPV assay is the selection of the linear portion of the curve in some cases. In addition, the protein sources should have a common

[1]J. M. McLaughlan and M. O. Keith. Effect of threonine supplementation on the slope assay for protein quality (unpublished data).

TABLE III
Comparison of Slope Ratios Calculated Various Ways[a]

	Paired "t"					
	Slope[b]	RPV[b]	Slope[c]	RPV[c]	Slope[d]	RPV[d]
Lactalbumin	8.24		7.53		7.80	
Egg	9.25	1.12	10.95	1.45	9.10	1.17
Lactalbumin	7.64		7.53		7.80	
Casein	6.25	0.82	6.39	0.85	6.43	0.82
Lactalbumin	8.09		7.53		7.80	
Tuna	6.61	0.82	8.05	1.07	7.00	0.90
Lactalbumin	7.84		7.53		7.80	
Cottage cheese	6.31	0.80	6.69	0.89	5.13	0.66
Lactalbumin	7.92		7.53		7.80	
Promine F	4.64	0.59	5.45	0.72	5.68	0.73
Lactalbumin	8.01		7.53		7.80	
Peanut meal	4.12	0.51	5.37	0.71	5.52	0.71
Lactalbumin	7.33		7.53		7.80	
Wheat gluten	2.11	0.29	2.02	0.27	1.93	0.25

[a]Data from a Cooperative Agreement with C. E. Bodwell, USDA, Beltsville, Md.
[b]With a common intercept.
[c]Without a common intercept.
[d]Visual interpretation of slope, without a common intercept.

point of origin; recent discussions by Hegsted have centered on calculating the RPV without using a common intercept, although no official position has been taken.[2]

The results reported in Table III confirm the point just discussed—namely, that the point of origin of the slope is important. The data demonstrate very clearly that the RPV is influenced by the point of origin of the slope with or without a common intercept for the standard (lactalbumin) and the test proteins. The data with and without a common intercept for lactalbumin, egg, and tuna are graphically presented in Fig. 1, 2, 3, and 4. It should be noted that the fourth point on the curve for egg and tuna was not included in the slope calculation. It is readily apparent that the slopes are different when computed with and without a common intercept. Calculating the RPV with a common intercept has the practical effect of adding an additional data point. Without a common intercept, the RPVs for lactalbumin, egg, and tuna are 1.00, 1.45, and 1.07 (Table III), respectively, whereas, with a common intercept, the respective values are 1.00, 1.12, and 0.82. Some researchers, however, favor the RPV procedure since they feel it may more accurately characterize the usefulness of poorer proteins (18,26,27).

Protein Efficiency Ratio (PER)

The classic paper of Osborne, Mendel, and Ferry, in 1919 (17), recognized that growth rate was influenced by food intake and that it should be considered in any assay for measuring protein quality. Four weeks are required for this assay, as presently approved (28):

$$\text{PER} = \frac{\text{wt gain, g}}{\text{protein consumed, g}}$$

A serious criticism of the PER procedure is that it does not make an allowance for maintenance. In other words, a protein might meet the maintenance needs of an animal but not promote growth. Furthermore, it is difficult to measure the potential complementary effects of two or more proteins in a mixed feeding situation (6). This is a common criticism of all bioassay procedures. Also, the length of the assay and the specified dietary protein level have received some criticism. It may be possible to reduce the length of the assay by one-half without any loss in accuracy (7).

The data collected in our laboratory and reported 3 years ago (7) on various protein-containing foods showed that the protein quality, as measured by adjusted PER, varied from 0.324 to 3.29 at 2 weeks and 0.484 to 3.19 at 4 weeks (Table IV). The correlation coefficient for the adjusted PER values was 0.996. Hegarty (13) was recently critical of people not adhering to the 28-day assay, as the PER values are considerably higher at 2 *vs.* 4 weeks. However, if adjusted PERs are calculated from Hegarty's data, they also indicate that a 2-week assay would be adequate (Table V). The adjusted PER values at 2 weeks are 97 to 103% of the values for 4 weeks. The above data suggest that, under standardized conditions, the PER assay could be shortened to 2 weeks without any loss in accuracy. This would result in a savings of time and money for the PER assay.

[2]J. M. McLaughlan. Personal communication.

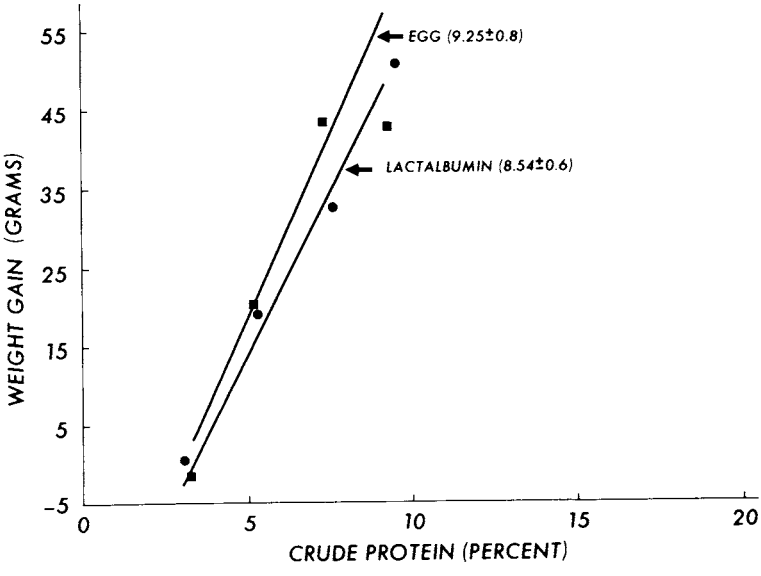

Fig. 1. Slopes for lactalbumin and egg proteins when calculated with a common intercept.

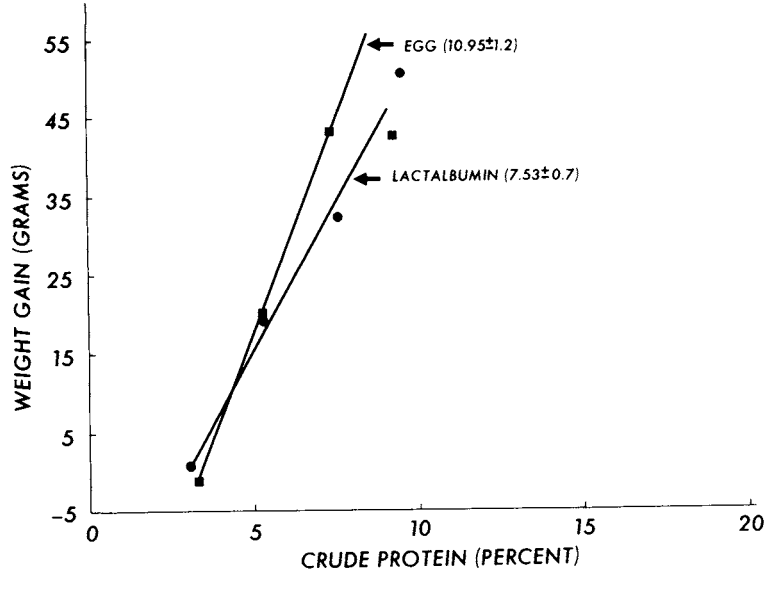

Fig. 2. Slopes for lactalbumin and egg proteins when calculated without a common intercept.

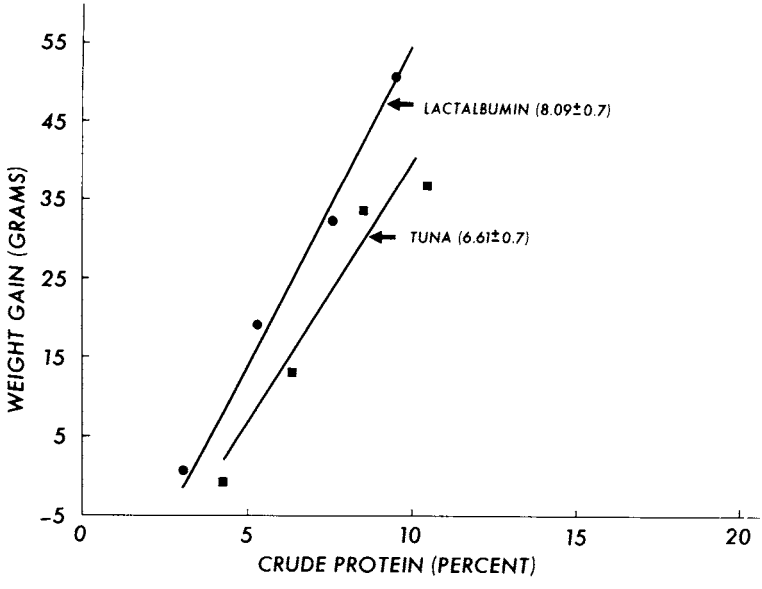

Fig. 3. Slopes for lactalbumin and tuna proteins when calculated with a common intercept.

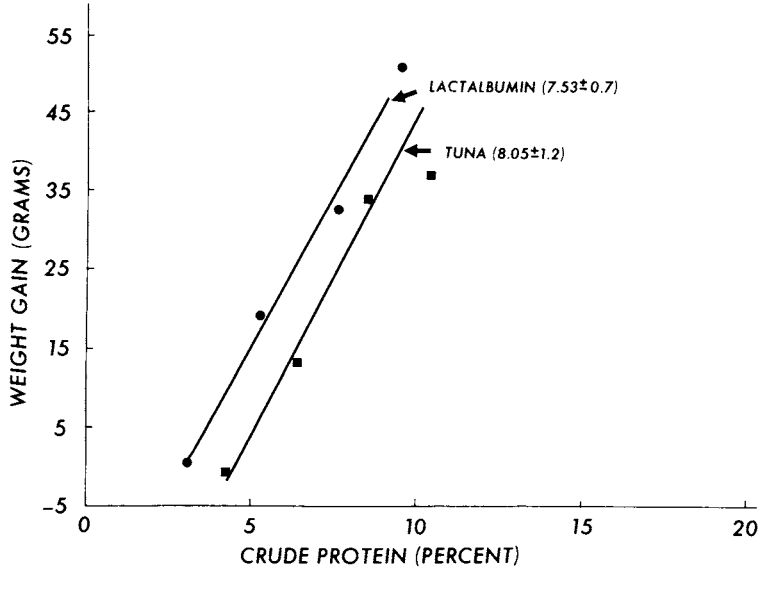

Fig. 4. Slopes for lactalbumin and tuna proteins when calculated without a common intercept.

Relative Nitrogen Utilization (RNU)

McLaughlan and Keith, in 1975 (29), proposed a modified PER procedure that takes maintenance into account. McLaughlan has referred to this procedure as a modified PER or as RNU when lactalbumin is used as the standard for comparing various test proteins (19). The assay is mathematically defined as follows:

$$NU = \frac{\text{B. W. change, g} \pm 0.1 \times (\text{In. B. W., g} + \text{final B. W., g})}{\text{N. I., g}}$$

$$RNU = \frac{\text{NU for test protein}}{\text{NU for lactalbumin}} \times 100$$

where NU = nitrogen utilization, B. W. = body weight, In. = initial, and N. I. = nitrogen intake.

TABLE IV
Comparison of 2- *vs.* 4-Week Protein Efficiency Ratios[a]

Protein Source	Number of Rats	PER			
		2 Weeks		4 Weeks	
		Unadjusted[b]	Adjusted[c]	Unadjusted[b]	Adjusted[c]
Egg	70	3.97	3.29	3.62	3.19
Nonfat dry milk	72	3.36	2.88	3.03	2.74
Casein	62	3.13	2.50	2.85	2.50
Ground beef	20	3.99	3.18	3.70	2.99
Beef liver	20	3.91	3.13	3.62	2.92
Cottage cheese	10	3.60	2.37	3.32	2.68
Soybeans	20	1.64	1.30	1.78	1.43
Cottonseed	10	2.59	2.34	2.48	2.23
Peanut meal	57	1.83	1.50	1.79	1.56
Wheat gluten	66	0.403	0.324	0.545	0.484

[a]Hackler, 1974 (7).
[b]Computation based on unadjusted values, r = 0.994, $\hat{y} = 0.839\times + 0.256$.
[c]Computation based on adjusted values, r = 0.996, $\hat{y} = 0.875\times + 0.244$.

TABLE V
Effect of Length of Assay Period on Adjusted PER[a]

	2 Weeks	4 Weeks
Raw hamburger	2.62	2.65
Cooked hamburger	2.73	2.64
Oats A	1.54	1.59
Oats B	1.73	1.78
Casein	2.50	2.50

[a]Adjusted PERs calculated from data of Hegarty, 1975 (13). Adjusted PER values were computed with casein equal to 2.50.

McLaughlan and Keith[1] state that the factor of 0.1 × (initial + final weight) is similar in magnitude to the weight loss of the nonprotein group in the NPR procedure. However, they believe it is an improvement since the NPR procedure tends to overestimate protein quality of lysine-deficient proteins when compared to the slope RPV. The data in Table VI illustrate the preceding point and also show that PER penalizes the lysine-deficient protein source.

The data in Table VII were obtained with rats. All of the protein sources, except peanut meal, were supplied by C. E. Bodwell, USDA, Beltsville, Md., in connection with a Cooperative Agreement on protein quality as measured by various rat assays and in humans. Each of the bioassays tends to rank the

[1]J. M. McLaughlan and M. O. Keith. Effect of threonine supplementation on the slope assay for protein quality (unpublished data).

TABLE VI
Comparison of Four Bioassays for Measuring Protein Quality[a]

	PER[b]	RNU[c]	RPV[d]	NPR[e]
Lactalbumin	100	100	100	100
Casein	72	79	92	82
Meat	68	73	74	77
Soy no. 1	70	68	67	75
Soy no. 2	62	72	72	72
Oat flour	55	64	65	67
White flour	23	41	32	46
Wheat gluten	5	30	20	37

[a]McLaughlan and Keith, 1975 (30).
[b]PER = protein efficiency ratio.
[c]RNU = relative nitrogen utilization.
[d]RPV = relative protein value by slope ratio assay.
[e]NPR = net protein ratio.

TABLE VII
Protein Quality as Measured by RPER, RNU, and RPV Using Lactalbumin as the Standard[a]

	Adj PER[b]	RPER[c]	RNU[d]	RPV[e]
Lactalbumin	2.80	1.00	100	1.00
Egg	2.95	1.05	107	1.12
Casein	2.50	.89	94	.82
Tuna	2.28	.81	75	.82
Cottage cheese	2.32	.83	87	.80
Promine F	1.39	.50	59	.59
Peanut meal[f]	0.99	.35	40	.51
Wheat gluten	0.30	.11	34	.29

[a]Hackler, unpublished data from USDA Cooperative Agreement.
[b]Adj PER = adjusted protein efficiency ratio.
[c]RPER = relative protein efficiency ratio.
[d]RNU = relative nitrogen utilization.
[e]RPV = relative protein value by slope ratio assay.
[f]Teklad Test Diets, 2826 Latham Drive, Madison, Wis.

protein-containing foods in the same order. Also, except for wheat gluten and peanut meal, there is very little difference between a relative protein efficiency ratio (RPER), RNU, and RPV. The RPER tends to downgrade or penalize the lysine-deficient protein, wheat gluten. This is expected since the lysine needs for growth are greater than those for maintenance in the rat.

CONCLUSIONS

It should be stressed that selection of the best bioassay procedure is an academic question. First, although rats may have many similarities to the protein needs of man, they do differ. The rate of growth, maintenance needs, and the manner of consuming a variety of foods containing various proteins by humans at each meal are very different from the procedures used in rat bioassays. Ideally, in bioassays, one should use as the test animal the animal for which the protein is intended.

What is needed is an ideal protein for growth, one for maintenance, *etc.*, that can be used as a standard in a laboratory animal that has the same or very similar needs for protein as man. Since this does not appear possible, we must make our selection based on other criteria, such as simplicity of the test, economics, labor, and reproducibility within and between laboratories.

The simplicity of the PER, RPER, and RNU procedures far outweighs any advantages reported for a multidose assay; these procedures are recommended for routine quality control of protein-containing foods by the food industry.

If the goal is to rank proteins, they can be ranked with casein or lactalbumin; however, it will be much easier to obtain casein than lactalbumin as a standard for use in rat assays, especially in the underdeveloped countries.

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Literature Cited

1. MITCHELL, H. H. Comparative nutrition of man and domestic animals. Vol. I. Academic Press: New York and London (1962).
2. MITCHELL, H. H. Comparative nutrition of man and domestic animals. Vol. II. Academic Press: New York and London (1962).
3. ANONYMOUS. Nutrient requirements of domestic animals. Publication 990. National Academy of Sciences—National Research Council: Washington, D.C. (1962).
4. ANONYMOUS. Evaluation of protein nutrition. Publication 711. National Academy of Sciences, National Research Council: Washington, D.C. (1959).
5. CAMPBELL, J. A. Methodology of protein evaluation. A critical appraisal of methods for evaluation of protein in foods. Publication 21. American University of Beirut: Beirut, Lebanon (1963).
6. HACKLER, L. R. Nutritional evaluation of protein quality in breakfast foods. Cereal Chem. 49: 677 (1972).
7. HACKLER, L. R. Evaluation of nutritional quality of proteins. AACC Annual Meeting, Montreal (1974).
8. McLAUGHLAN, J. M. Nutritional evaluation of proteins by biological methods. Cereal Sci. Today 17: 162 (1972).
9. PORTER, J. W. G., and ROLLS, B. A. Proteins in human nutrition. Academic Press: London and New York, 560 pp. (1973).

10. McLAUGHLAN, J. M. Effects of protein quality and quantity on protein utilization. In: Newer methods of nutritional biochemistry, Vol. 5, p. 33. Academic Press: New York (1972).
11. DREYER, J. J. Methods of protein evaluation with rats. II. In: Proteins in human nutrition, ed. by J. W. G. Porter and B. A. Rolls, p. 245. Academic Press: London and New York (1973).
12. PELLETT, P. L. Methods of protein evaluation with rats. I. In: Proteins in human nutrition, ed. by J. W. G. Porter and B. A. Rolls, p. 225. Academic Press: London and New York (1973).
13. HEGARTY, P. V. J. Some biological considerations in the nutritional evaluation of foods. Food Technol. 29(4): 52 (1975).
14. MITCHELL, H. H. A method of determining the biological value of protein. J. Biol. Chem. 58: 873 (1924).
15. BENDER, A. E., and MILLER, D. S. A new brief method of estimating net protein value. Biochem. J. 53: vii (1953).
16. BENDER, A. E., and DOELL, B. H. Biological evaluation of proteins. A new aspect. Brit. J. Nutr. 11: 140 (1957).
17. OSBORNE, T. B., MENDEL, L. B., and FERRY, E. L. A method of expressing numerically the growth-promoting value of proteins. J. Biol. Chem. 37: 223 (1919).
18. HEGSTED, D. M., and CHANG, Y. Protein utilization in growing rats. I. Relative growth index as a bioassay procedure. J. Nutr. 85: 159 (1965).
19. McLAUGHLAN, J. M. The relative nitrogen utilization method for evaluating protein quality. J. Ass. Offic. Anal. Chem. 59: 42 (1976).
20. HENRY, K. M., and KON, S. K. Effect of level of protein intake and age of rat on the biological value of proteins. Brit. J. Nutr. 11: 305 (1957).
21. FORBES, R. M., YOHE, M., and VAUGHAN, L. Level of protein in diet and its biological value. Fed. Proc. 15: 551 (1956).
22. FORBES, R. M., and YOHE, M. Net protein value of blood fibrin for the albino rat: Evaluation of nitrogen balance and carcass analysis methods. J. Nutr. 55: 493 (1955).
23. KIES, C., and FOX, H. M. Techniques in using normal human subjects in the protein bioassay of food products. In: Protein nutritional quality of foods and feeds. I. Assay Methods—biological, biochemical, and chemical, ed. by M. Friedman, p. 1. Marcel Dekker, Inc.: New York (1975).
24. BENDER, A. E., and MILLER, D. S. Constancy of the N/H_2O ratio of the rat and its use in the determination of the net protein value. Biochem. J. 53: vii (1953).
25. ANONYMOUS. Amino acid content of foods and biological data on proteins. FAO: Nutritional studies, No. 24. Food and Agriculture Organization of the United Nations: Rome (1970).
26. SAID, A. K., and HEGSTED, D. M. Evaluation of dietary protein quality in adult rats. J. Nutr. 99: 474 (1969).
27. HEGSTED, D. M., and JULIANO, B. O. Difficulties in assessing the nutritional quality of rice protein. J. Nutr. 104: 772 (1974).
28. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. Official methods of analysis (12th ed., sections 43.183–43.187). The Association: Washington, D.C. (1975).
29. McLAUGHLAN, J. M., and KEITH, M. O. Bioassays for protein quality. In: Protein nutritional quality of foods and feeds. I. Assay methods—biological, biochemical, and chemical, p. 79 (1975).

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