SHORTER PROTEIN BIOASSAYS

PAUL A. LACHANCE, RICARDO BRESSANI, and LUIZ G. ELIAS

□ DURING RECENT YEARS, the consumer and the food industry have become increasingly aware that certain foods are sources of protein, and that both the quantity and the quality are different among various protein sources. Food formulations complying with the Food and Drug Administration's nutrition labeling requirements must declare the protein quantity as number of grams per serving and the relative protein quality as percent of the U.S. Recommended Daily Allowances.

Recommending a bioassay method for purposes of monitoring raw materials and ingredients, processing conditions, final formulations, and products, as well as shelf life stability, is complicated because the quality factor measured by a given bioassay can be affected by many test variables (Miller and Lachance, 1977). Further, such a method should have precision and reproducibility as well as be of short duration, i.e. rapid, and low cost in terms of labor and facilities.

The nutritional quality of a protein is defined by the quantity, availability, and proportions of the available essential amino acids, and the presence, for optimum utilization, of sufficient non-essential amino acids. However, the concept of protein quality must be applicable to single proteins, as well as to mixtures of proteins. In the case of mixtures, however, the quality as measured biologically is not in every instance a simple algebraic average of the individual patterns; in several instances, a higher quality is biologically measured, believed to be the result of a mutual supplementary effect of the quantity of amino acids from the proteins as well as to the proportion or balance among essential amino acids (Bressani, 1977; Krofanyi, 1973).

The objective of the bioassay is to measure the efficiency of the biological utilization of dietary proteins as sources of the essential amino acids. A bioassay has two possible applications: The first is to rank protein foods according to their efficiency of utilization under a set of standard conditions; this indicates the nutritional "potential" of the protein. The second application is to measure the efficiency of utilization of proteins as sources of nitrogen and essential amino acids for meeting the amino acid requirements of man and animals; this measures "potential" but also measures "physiological performance." In a diet of varied protein foods, this application need not be applied to individual food products which are not meal replacements or the principal (i.e., exclusive) protein source of the diet.

Ranking of protein foods has been criticized because it appears to be unrelated to normal dietary conditions. However, this application is of value since it provides a measurement of the potential of a protein ingredient(s) and/or the conditions of processing, amino acid supplementation, and protein complementation, and has been used in the screening of genetically-improved food varieties.

AUTHOR Lachance is with the Dept. of Food Science, Rutgers University, P.O. Box 231, New Brunswick, NJ 08903.

AUTHORS Bressani and Elias are with the Div. of Agricultural and Food Sciences, Institute of Nutrition of Central America and Panama (INCAP), Carretera Roosevelt, Zona 11, Guatemala City, Guatemala, Central America.

AVAILABLE METHODS

Many methods have been proposed for evaluating the quality of dietary proteins (Bodwell, 1977). Most of them are based on the effects of dietary protein on the whole animal. Figure 1 (Allison, 1965) demonstrates the relationships between such tests. All mammalian methods are basically variants of the observation that the amount and quality of protein can result in a gain or loss of body substance. Such gain or loss can be identified by a change in body weight or by a change in a body component, which often is nitrogen content.

The methods also can be classified as to whether they are a one-point assay, or a multiple-point assay. The Protein Efficiency Ratio (PER) and the Net Protein Ratio (NPR) are based on weight changes, and are considered one-point assays. But in actuality, because it includes a group of rats fed on a nitrogen-free diet, the NPR should be considered a two-point assay. The methods based on body nitrogen change can be further divided into those based on a direct measurement, such as carcass nitrogen, and those based on an indirect measurement, such as nitrogen balance. Typical of the first is Net Protein Utilization (NPU) and of the second is Biological Value (BV), representing single-point assays. The Nitrogen Balance Index (NBI) is a multiple-point assay based on indirect measurements of body nitrogen change, and the Nitrogen Growth Index (NGI), or Slope Ratio Assay, is a multiple-point assay based on growth response to different levels of nitrogen intake.

The one-point assays, in our opinion, are useful for ranking proteins or measuring their "potential." The multiple-point assays are superior to the single-point assays for measuring the physiological efficiency of utilization, but the cost (duration) and amount of sample needed are invariably greater.

OTHER, SHORTER METHODS

Other biological methods of shorter duration than the PER that merit mention are as follows:

- **Animal Assay Methods Based on Protein Regeneration:** (1) measuring or estimating labile liver cytoplasm following fasting in rats (Campbell and Kosterlitz, 1948); (2) measuring the amount of protein required to maintain constant body weight in protein-depleted rats (Tomarelli and Bernhart, 1947); and (3) measuring the regeneration of body weight in protein-depleted rats (Cannon et al., 1944). The Rutgers collaborative study (Bureau of Biological Research, 1951) showed good correlation between the regeneration-of-body-weight method and all other protein evaluation methods used in that study.

The methods which measure specific blood components are based on the measurement of urea, ammonia, or specific amino acids in blood plasma after the consumption of a test dose of the protein under study. INCAP studies as well as that of others (Braham et al., 1977) show relatively good correlations to traditional techniques, but these blood component methods need much more developmental work.

- **Microbiological Assays:** (1) The use of *Tetrahymena pyriformis* W has been reviewed by Landers (1975). Problems relate to lack of knowledge of the nutritive requirements of the organism, and the lack of correlation with a wide range of PER values. The test requires a four-day

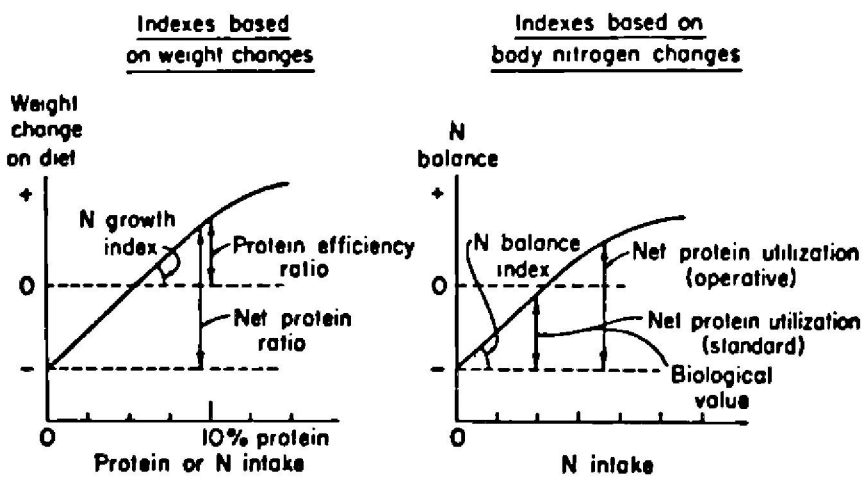

Fig. 1—**RELATIONSHIP** *between bioassay methods for measuring the nutritive value of proteins (source: Allison 1965)*

incubation. Landers' experimental data suggested that the bioassay of Stott et al. (1963) was not sufficiently "rugged" for general use. Modification of the method and sample preparation was critical to obtaining a linear relationship (r = 0.95) between PER for eight foods and Tetrahymena Relative Nutritive Values (RNV). Landers recommended more refinement before recommending the assay. Frank et al. (1975) believe the Tetrahymena method is especially useful for screening purposes, provided the test protein is solubilized (predigested 24 hr with trypsin and bromelain). Evancho et al. (1977) studied the protein quality (RNV) of 55 prepared foods as compared to PER results and obtained a correlation of r = 0.90. However, a particle (Coulter) counter with a computer discrimination capacity was required to provide quantitative information on cell size in relation to quality and quantity of protein tested. (2) Solberg (1976) has developed a one-day procedure using *Clostridium perfringens* 3624 to evaluate protein quality in terms of a Relative Slope Ratio; however, comparison with PER values for a wide variety of foods showed that the method could not be used to replace the PER procedures. However, the procedure seems feasible for detecting effects due to heating over time in a complex food. (3) *Streptococcus faecalis* (Teeri et al., 1956) and *Leuconostoc mesenteroides* P-60 (Horn and Warren, 1961) have been given limited scrutinity; however, it has not been shown that they could be used as a general procedure to evaluate the proteins in natural foods.

NPR AS A RAPID BIOASSAY

Net Protein Ratio (NPR) has been studied as a rapid bioassay method for a variety of foods:

• **Cereal Grains.** Eighteen samples of cereal grains which had been assayed with the standard PER method by Mertz et al. (1972) were assayed by INCAP at different levels of protein in the diet using the NGI (Slope Ratio Method) and the NPR method. The results (Table 1) suggest that the NPR method is more than adequate as a routine, simple, and rapid bioassay for the purpose of selecting cereal grain varieties of improved nutritional quality.

• **Legume Foods.** Similar studies have been conducted with 21 legume foods, including Phaseolus, Vigna, Cajanus, and Soya cultivars, with and without amino acid supplements. These samples were assayed by Elías and Bressani (1976) and Bressani and Elías (1976) for PER, NPU, and NPR (Table 1). The method which best served to screen for high-quality legume foods was the NPR.

• **High-Protein Foods.** The same comparative approach was used for seven high-protein foods being developed at INCAP (Table 1). The correlation coefficients are highly significant, indicating the close relationship which exists between methods, as shown in the original graphical representation by Allison (1965) of the various methods (Fig. 1).

Table 1—**REGRESSION EQUATIONS** *and correlation coefficients for various protein sources by various bioassay methods*

Regression equation	Correlation coefficient
18 cereal samples	
PER = −2 137 + 1.206(NPR)	r = 0 89
NGI = −0.210 + 1.085(NPR)	r = 0 98
NGI = 1.945 + 0 769(PER)	r = 0.91
21 legume samples	
NPU = 12.56 + 12.95(PER)	r = 0.87
NPU = −0.46 + 16.80(NPR)	r = 0.92
PER = −0.71 + 1.17(NPR)	r = 0.96
7 high-protein food products	
PER = −1.250 + 0 952(NPR)	r = 0.98
NGI = 0.462 + 0.929(NPR)	r = 0.98
NGI = 1.779 + 0.930(PER)	r = 0.95

Table 2—**TOTAL METABOLIC NITROGEN EXCRETION** *(a), nitrogen absorbed for nitrogen equilibrium (x), and protein quality by regression analysis (b)*

	a	x	b
	(mg/kg/day)		
Whole egg			
Short method	−53	50	1.07
	−59	88	0.67
Conventional method	−40	69	0.58
Young et al. (1973) method	−34	73	0.64
Inoue et al. (1973) method	−36	100	0.54
Whole milk			
Short method	−48	48	0 97
	−56	58	0.91
Conventional method	−56	64	0.88
High-protein cookies	−54	63	0.83
Tortilla/beans (73/27)	−63	68	0.95

Because of the particular characteristics of the NPR method, it evidently offers advantages as a rapid bioassay. Further, since NPR can be calculated by regression analyses of the effect of protein intake on weight gain, it is useful for evaluating proteins which do not promote growth and cannot be assayed by the PER. Based on the results obtained, the NPR method of Bender and Doell (1957) is therefore highly recommended for purposes of monitoring the protein quality of food products at this time.

A 10-DAY HUMAN BIOASSAY

It is our contention that foods which are marketed as meal replacements or as very significant sources of dietary protein but which are processed by formulation or fabrication technology should be assayed in humans at least once. To this end, we have developed a 10-day multiple-point Nitrogen Balance Index (NBI) assay in adult human subjects, which has given results comparable to Nitrogen Balance assays which take 2 to 3 times longer (Navarette et al., 1975).

In the short NBI in humans, the subjects are fed a protein-free diet, which will give a value equivalent to the non-protein-fed group in the NPR. After three days, the subjects are fed increasing levels of the protein under test, usually three levels changed every two days. The NBI is then calculated by regression analysis. Some results by this method are presented in Table 2. The results approximate those obtained for milk and eggs with the conventional nitrogen balance technique and literature findings. The high-protein-cookie data are for a commercial U.S. product. Other commercial products and mixtures have been tested with this short method and will be reported elsewhere.

RECOMMENDATIONS

The current state-of-the-art of protein bioassays would suggest that: (a) the food industry should collect NPR data as well as PER data (i.e., add a no-protein group and calculate at 10 days); (b) the official adoption of the NPR in lieu of the PER in federal regulations would be fostered by collaborative experience; (c) a human protein assay should be considered in the responsible marketing of meal-replace-

ment foods; and (d) continued research is needed to demonstrate a protein assay reflecting biological utilization in an analytical period of one day or less.

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