Rat Models in Protein Quality Evaluation

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ABSTRACT

The ideal methodology for establishing protein quality has not yet been elaborated. On the other hand, the proposed introduction on the market of novel protein sources urges the industry to ask for an in vitro test, characterized for being inexpensive fast, reliable, and acceptable at an official level. Obviously, the in vitro test must be confirmed by an appropriate in vivo test. In this connection the problems relative to the species on which to experiment – if it should or should not be the one to whom the protein food is directed – and the characteristics of the in vivo test are discussed. Although the rational appears to be to assay on man protein directed to him, the cumbersomeness of the procedure, technical difficulties, uncertainties in the interpretation of the results as well as ethical considerations for what children are concerned, don't favor experimentation on man. An extensive experimental study, aimed at testing various conventional and novel proteins, confirmed that the multiple doses test, as proposed by Samonds and Hegsted (RPV), has the highest discriminating capacity and is scientifically the most reliable. A research project to verify the correlation between the two methods for evaluating biological quality, namely RPV and the method of the in vitro ultrafiltered digest (EUD), is under investigation.

As is well known, the evaluation of protein quality has received recently a considerable revival of interest. In fact, the problem of the protein nutritive value, which only a few years ago appeared eclipsed as a reaction against the concept of "protein crisis," has come back to actuality for several reasons. First of all there is a need to test new protein sources, such as plant protein isolates and concentrates. Furthermore, the consideration that even procedures so far considered most satisfactory, such as NPU, overestimate proteins of poorer quality, most importantly for the nutrition of the third world (1). The disputes over the results of the most recent experiences and the stimulating reviews on the whole problem, which appeared particularly in the past two years, show that the ideal methodology has not yet been elaborated (2,3). Meanwhile, the food industry urges obtaining procedures characterized for being fast and more reliable than, for example, PER (4), This latter, although considered a poor assay, still remains the official method in the U.S. for testing protein quality and, thus, applies to labeling regulations.

Obviously, with proteins intended for direct human consumption, the rational would appear to evaluate their quality by direct experiments on humans. However, in reviewing the topic, Young, Rand, and Schrimshaw (5) point out the complexity of the problem. According to the method adopted, the main difficulties lie on the time and the sophisticated facilities required, on environmental influence, and, mainly, on practical and/or ethical considerations when realizing that the proteins, at the relatively low levels of intake required for detecting differences in protein quality, cannot be administered to growing children. However, to avoid misleading conclusions, even when experimenting on adults, Young et al. (5) suggest the experiments be conducted at multiple levels. But when taking into account individual variability and cost (6), this condition renders the investigation unfeasible.

On the other hand, as outlined by Pellet (3) even when using a procedure which meets most of the desired criteria, a single method cannot be expected to provide all the information required to assess the overall value of a given protein.

In real life conditions, health, nutritional status, age, and physiological conditions of the individual consuming the proteins together with the complete dietary composition, including the total energy value, can affect the final value of the protein to the consumer. Consequently, any method has to be utilized as a relative rather than absolute measure, possibly to be correlated to other methods complementing their significance.

Among rat models, the dose-response assay, in the different versions, offers at present the best solution for almost all the problems posed by single dose tests (1,7). Moreover, the protein value (PV) calculated as proposed by Samonds and Hegsted (1), in as much as it takes into consideration only the linear portion of the dose-response curve and omits the zero dose values, seems to eliminate the errors inherent in the adaptive responses of some amino acids at low level of protein intake, in particular when

TABLE I

Limiting Amino Acid and Chemical Score of the Seven Protein Sources

Protein source	Limiting amino acid	C.S.
Lactalbumin[a]	Cyst + met	0.781
Soy (supro 620)[b]	Cyst + met	0.428
Wafer (IBP)[c]	Cyst + met	0.593
Blood plasma (conc)[d]	Isoleucine	0.550
Faba minor beans[e] (raw conc)	Cyst + met	0.350
Gluten[f]	Lysine	0.203
Egg (defatted)[g,h]	(reference)	1.000

[a]Nestec (Lausanne).

[b]Ralston Purina.

[c]Wheat meal wafer stuffed with a mixture of soybean meal, skim milk and ultrafiltered milk serum protein concentrate.

[d]80% protein concentrate obtained by ultrafiltration and spray drying.

[e]65% Protein raw concentrate obtained by air classification.

[f]Piccioni (Brescia).

[g]Liophylized whole egg solvent defatted.

[h]d) and e) were protein sources prepared under the CNR "New protein sources" program.

TABLE II

Correlation Coefficients (r), between Protein Values (PV) Obtained with the Different Experimental Conditions

	70 g[a] (wt)	60 g (body N)	70 g (body water)
60 g[a] (wt)	0.982[b]	0.981[b]	---
70 g (body N)	0.957[b]	0.979[b]	0.976[b]
60 g (body water)	---	0.988[b]	0.985[b]

[a]60 and 70 g were the average initial body weights of the rats.
[b]$P < 0.001$.

TABLE III

Correlation Coefficients (r) between Different Rat Bioassays[a]

On rats 60 g initial body weight		
RPV_W[b]	NPR 0.944	NPR_{rel} 0.944
RPV_N[c]	NPU 0.968	NPU_{rel} 0.969
On rats 70 g initial body weight		
RPV_W[b]	NPR 0.960	NPR_{rel} 0.967
RPV_N[c]	NPU 0.942	NPU_{rel} 0.952

[a]$P < 0.001$ for all the correlation coefficients.
[b]Rat response Δ weight.
[c]Rat response body nitrogen.

lysine is limiting.

This last point was recently questioned by Mc Laughlan, who, on the basis of the results obtained with wheat flour supplemented with lysine and threonine, claims that the PV assay underestimates the protein quality of lysine-deficient proteins (8).

EXPERIMENTAL DATA

At the National Institute of Nutrition (Rome) a study has been undertaken to verify the applicability and suitability of the relative protein value methodology over some other tests, and in collaboration with the Institute of Food Science of the University of Perugia, to assess how values obtained with this methodology correlate with those obtained on the same samples with the in vitro ultrafiltered digest assay (EUD) (9). These researches are sponsored by the NRC of Italy under the finalized project "New Protein Sources and New Food Formulations."

For what concerns the first point, the topics that were investigated include, among others, influence of the age of the rats, influence of the parameters of response (body weight change, nitrogen, or water body content), correlation with the Net Protein Ratio and the Net Protein Utilization (10).

The experiments were conducted on two groups of Sprague-Dawley male weanling rats, weighing 60 and 70 g respectively, which were maintained at the different protein sources for two weeks. In preliminary experiments, a two week period gave a variability of the same magnitude of that of a three week period (7). The other conditions were those proposed by Samonds and Hegsted (1). The relative protein value (RPV) was calculated vs. egg protein.

Seven protein sources, characterized by a wide range of predicted biological value and different limiting amino acids, were utilized. In principle, the total metabolic response of the organism is proportional to the availability of the first limiting amino acids, irrespective of their nature. In practice, owing to the different behavior of the various amino acids, to obtain comparable results appropriate experimental conditions must be utilized.

The chemical score and the limiting amino acids of the protein sources are shown in Table I. As shown in Table II, the PV values, as obtained with the different experimental conditions, namely different initial body weight or different parameters of response, are highly correlated, with correlation coefficients near the unity. Thus, provided a suitable internal standard is established, growing rats of different initial weight and the three different response parameters (body weight changes, nitrogen, or water body content) can be indifferently used.

Utilizing values obtained with the dietary protein intake nearest to 10%, absolute and relative net protein ratio and absolute and relative NPU were also calculated. Even the correlation coefficients among these protein quality tests and RPV, both with body weight change or body nitrogen as response, were high and significant. (Table III).

However, when examining individual protein quality values, it can be observed that the two protein sources appear significantly better when utilizing single dose rather than multidose assays (Table IV).

Moreover, with the multidose test, the standard error was lower and thus the discriminating capacity increased. This means that the correlation coefficient, in particular if obtained from proteins falling in a very wide range of biological values, is not the proper test to evaluate the equivalence of the various protein quality tests in as much as it can mask the peculiar behavior of some protein sources of particular practical relevance.

Anyhow, it is not surprising that values obtained utilizing a growth index resulted in correlation and that the differences arising from the lack of linearity of the response from the zero dietary protein level to the highest were obscured.

The second point under investigation was the correlation between RPV and the EUD in vitro test. At present we have only preliminary results obtained on the same samples both with the RPV test (using body nitrogen as response) in Rome and with the EUD test in Perugia. These results are

TABLE IV

Comparison among Different Protein Quality Tests

Protein sources	PV_W[a]	NPR	RPV_W[a]	NPR_{rel}	RPV_N[b]	NPU_{rel}
Faba beans (raw conc)	1.43 (0.12)[c]	2.12 (0.22)	0.304 (0.028)	0.410 (0.049)	0.298 (0.025)	0.397 (0.048)
Gluten	1.29 (0.09)	2.51 (0.49)	0.275 (0.023)	0.486 (0.099)	0.244 (0.017)	0.325 (0.061)

[a]Rat response Δ weight.
[b]Rat response body nitrogen.
[c]Numbers in parentheses are SE; the standard error for the multidose assays 8.05%; the standard error for the single-dose assays 15.5%.

TABLE V

Comparison of Protein Quality as Measured by RPV[a] and EUD

	RPV[b]	EUD[b]
Poultry meat		
breast	0.762	72.6
back[c]	0.748	74.5
neck[c]	0.646	66.1
wing[c]	0.673	78.6
Lactalbumin (ICN)	0.901	86.7
Casein	0.720	73.9
Blood plasma (conc)	0.695	72.7
Faba beans (raw conc)	0.328	45.4
Sunflower (meal)	0.410	68.7
Sunflower (defatted meal)	0.410	70.1
Correlation coeff. (r)	0.808	
Regression equation y = 42.7 (7.58) + 45.1 (11.63)x		

[a]Rat response body nitrogen.

[b]Versus whole egg protein.

[c]Mechanically deboned.

shown in Table V together with the correlation coefficients and the correlation equation.

As it can be observed, the values for the animal protein sources fit quite well, while discrepancies emerge for what plant protein sources are concerned. On the whole, the correlation coefficients were satisfactory (0.808) but, owing to differences in the plant protein evaluation, the regression line intercepts the y axis much above the zero point.

Possibly, the discrepancies shown for plant protein sources, which are severely unbalanced, are not to be ascribed to the ultrafiltrate digest methodology per se, but rather to the scheme of calculation, based on the geometric mean of all the essential amino acids, which obscures the effect of the limiting amino acid (11). However, a definitive conclusion about the equivalence of the two methodologies will be drawn only when more data will be collected and carefully analyzed.

With regard to the problem of the best bioassay procedure, the results illustrated indicate that they can all be indifferently used when high quality proteins have to be tested, while the RPV test seems to be the method of choice for low protein quality, as plant proteins in general are.

Two more problems await for a solution. The first is the problem of the reference protein. All the data reported are relative to the egg protein, whose utilization for rats' growth was better than that of lactalbumin. Moreover, egg protein is the reference protein for the WHO/FAO standard.

However, in a recent paper (12) it was shown that the true safety nitrogen level for human maintenance has a protein quality at least 25% less than egg. If this is so, a more realistic reference protein should be proposed.

The second problem may be formulated as follows: when is it that two proteins must be considered as having different nutritive value? In our laboratory as in others (13), the standard error of RPV was around 8%. Together with the definition of the reference protein, this is of practical importance when recommendations for a minimum value for protein quality are to be made.

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The Nutritive Value of the Same Protein Preparations as Estimated by Human, Rat, and Chemical Assays

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ABSTRACT

Results are summarized from studies in which the protein nutritional values of thirteen protein sources were estimated by human, rat, or chemical assays. Generally, agreement was poor between nutritive value as estimated in adult men and as estimated by various rat assays or by chemical (amino acid) scores. Possible reasons for this lack of agreement are briefly discussed.

INTRODUCTION

Various animal and chemical assays have been developed for estimating the nutritional value of protein from different sources (1-7). These assays, however, are of little usefulness in human nutrition if they do not accurately predict protein nutritive value for humans. The few published comparisons of nutritive value as estimated by animal or chemical assays and nutritive value as estimated directly in humans with the same protein preparations were reviewed (8,9). In this paper, results from studies in which these comparisons have been made with two different groups of protein sources are summarized.

DESCRIPTION OF STUDIES

In the first group of six protein sources, nutritional value