APPLICATION OF ANIMAL DATA TO HUMAN PROTEIN NUTRITION: A REVIEW[1]

C. E. BODWELL[2]

Cereal Chem. 54(4): 958–983

ABSTRACT

The relation between animal and human assays for estimating the nutritional value of proteins is discussed with emphasis on assessing the merit of using data from animal assays for nutritional labeling purposes. It is concluded that data on estimated essential amino acid requirements are insufficiently precise to justify making conclusions about the relative requirements of rats and humans. The limited number of studies reported, in which assays, made with both rats and humans who have been given the same preparations of proteins, are reviewed. In these studies, agreement usually has been poor between the estimated nutritive values obtained by animal and human assays. Problems inherent in the application of the Protein Efficiency Ratio (PER) assay, for nutritional labeling, are briefly discussed. Various alternatives to animal assays are considered and the requirements of an ideal assay are noted. Finally, data are presented as a basis for questioning the practicality, in relation to nutritional labeling, of attempting to make precise quantitative estimates of nutritional value. It is stressed that considerable caution should be exercised to ensure that any assay used as a basis for nutritional labeling reflects, as differences in protein quality, only those differences which are real under practical conditions.

Although poor digestibility or impaired amino acid bioavailability may influence nutritional value, the single most limiting essential amino acid in a protein is the primary factor that determines the nutritional value of the protein for humans. Likewise, the values from any bioassay with the growing rat depend primarily upon the level of the single most limiting amino acid for the rat in the protein assayed. The relative requirements of rats and humans for each essential amino acid are, therefore, of critical importance relative to the use of the rat as an assay animal for predicting the nutritional value of proteins for humans. Since essential amino acid requirements for maintenance and growth differ (1,2,3,4), and since both the protein requirement and the utilization of protein for growth or maintenance are very different in the rat and the human at all stages of life (Table I), it could be anticipated that their requirements for essential amino acids would also differ greatly. The estimated requirements for total essential amino acids, expressed as mg/g protein, are indeed much higher for rats than for humans at comparable degrees of maturity (Table II). For the adult rat and human, the magnitude of these differences is decreased if estimated requirement levels are expressed in terms of total body protein synthesis (11). However, when the estimated requirement for each amino acid is expressed as a percentage of the total essential amino acid requirement, some marked differences between rats and humans are apparent (Table II) as well as some probable discrepancies in the estimates, *per se*.

When the values for adult rats proposed by Said and Hegsted (9) are compared

[1]Presented at a Symposium "Measuring Protein Quality for Human Nutrition" held at the 61st Annual Meeting, New Orleans, Oct. 5–8, 1976. Opinions expressed are those of the author and do not imply approval or disapproval by the U.S. Department of Agriculture.

[2]Research Chemist, Protein Nutrition Laboratory, Nutrition Institute, Beltsville Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705.

with estimates for the growing rat, on a percentage distribution basis, the lysine percentage of the total essential amino acid requirement is decreased (Table II). Other "shifts" are observed; not all of them are probable; *e.g.*, the percentage of the sulfur amino acids, threonine, and tryptophan are higher for the adult rats. Even more improbable shifts in the percentage distribution of specific amino acids are apparent in the values based on estimated requirements for humans. For example, it seems highly unlikely that the proportion of the total essential amino acid requirement provided by lysine increases from infancy to childhood and then decreases. Likewise, it is unlikely that the proportion of tryptophan and the aromatic amino acids is less in childhood than in infancy or adulthood.

These observations suggest that comparisons between the estimated requirements for individual essential amino acids for rats and humans cannot be made with any degree of confidence. In addition, the validity of such comparisons may be questioned since data from studies in which, by necessity, synthetic amino acid diets are fed, must be used to estimate requirement levels in both rats and humans. Such estimates may not be valid in terms of amino acids supplied by various proteins. This is suggested by the data shown in Table III in which Bressani (12) has estimated requirement levels from the dietary essential amino acid levels provided by mixtures of proteins giving maximal true complementation responses. Although the percentage distributions are generally similar to those listed in Table II, the total mg of essential amino acids/g protein is higher for humans than for rats, which is just the reverse of the same

TABLE I
Comparisons of Protein Intakes (g/kg body wt/day) and Estimates of Ratios of Nitrogen Requirements for Growth to Nitrogen Requirements for Maintenance for Rats and Humans

	Age		
Rats	Weanling (~21 days) g	Young adult (~60 days) g	Mature adult (males) (100–200 days) g
10% protein diet	22	7	3.8
20% protein diet	44	14	7.5
Ratio of nitrogen requirement for growth to nitrogen requirement for maintenance[a]	>7.0	~2.2	1.0–0.5
Humans[b]	Infants (0–1 year) g	Children (1–10 years) g	Adults (males) (>23 years) g
	2.0–2.2	1.2–1.8	0.8
Ratio of nitrogen requirement for growth to nitrogen requirement for maintenance[a]	<1.5–0.25	<0.2	<0.2

[a]After Narasinga Rao (5).
[b]Recommended dietary allowances (6).

TABLE II
Percentage Distribution of Essential Amino Acids in Estimates of Requirement Levels (mg/g of protein) for the Rat and Humans[a]

	Rat			Human[b]		
		Adult				
	Growing[c] %	A[d] %	B[e] %	Infant %	Child %	Adult %
Isoleucine	13.0	14.6	17.8	9.7	11.4	11.8
Leucine	16.6	13.0	20.0	22.5	17.2	16.5
Lysine	14.2	8.4	5.6	14.5	23.0	14.5
Methionine + cystine	11.8	14.6	15.6	8.1	10.4	15.8
Phenylalanine + tyrosine	17.0	16.9	8.9	17.6	10.4	16.5
Threonine	11.8	14.6	8.1	12.1	13.6	8.8
Tryptophan	2.6	3.5	3.3	2.4	1.4	4.3
Valine	13.0	14.6	17.8	13.1	12.6	11.8
Total mg essential amino acids per g protein	423	261	180	359	326	152

[a]Requirements for arginine and histidine for the rat and for histidine for humans not included in calculations.
[b]Source: FAO/WHO (3) assuming safe levels of protein intake of 2.0, 0.8, and 0.55 g protein/kg body wt/day for infants, children (10–12 years of age), and adults, respectively.
[c]Calculated from requirement levels for lysine given by Stockland *et al.* (7) and for all other amino acids by Rama Rao *et al.* (8), assuming a total protein requirement equivalent to 10% dietary protein. protein.
[d]Calculated from requirement levels given by Said and Hegsted (9), who assumed a protein requirement equivalent to 3.2% of the diet, by wt.
[e]Calculated from requirement levels given by Ashida and Yoshida (10), who assumed a protein requirement equivalent to 5% of the diet, by wt; the estimates of requirement levels by these authors are probably in error, since the assumed protein requirement is probably too high.

TABLE III
Percentage Distributions of Essential Amino Acids in Estimates of Requirement Levels (mg/g of protein) Calculated from Data Obtained on Mixtures of Proteins Resulting in Maximal Complementation Effects when Fed to Rats and Humans[a]

Amino Acid	Rats: Mixtures resulting in PER responses of 2.0–2.4	Rats: Mixtures resulting in PER responses of 2.6–2.9	Human Studies
Isoleucine	9.0	10.7	11.8
Leucine	14.4	17.4	16.7
Lysine	16.2	11.5	12.0
Methionine + cystine	10.8	6.5	8.2
Phenylalanine	16.2	11.1	10.6
Threonine	9.0	8.3	8.9
Tryptophan	2.7	2.5	2.8
Valine	12.6	11.6	13.1
Total mg essential amino acids per g of protein	467	346	504

[a]From data of Bressani (12).

comparison of estimated requirement levels based on data from the studies with synthetic amino acid diets (Table II).

Other problems related to essential amino acid utilization must also be considered in assessing the validity of using the rat as an assay animal to predict the nutritional value of proteins for humans. In the rat, deficiencies in different limiting amino acids are not necessarily reflected by equivalent responses. With diets completely devoid of different essential amino acids, Said and Hegsted (9) reported that the expected responses were observed only after feeding to adult rats the diets deficient in either threonine, isoleucine, or the sulfur amino acids. Likewise, Bender (13) observed Net Protein Utilization (NPU) values of 37, 0, and 0 for diets devoid of lysine, valine, and sulfur amino acids, respectively. For diets devoid of any one of the other essential amino acids, NPU values were less

TABLE IV
Biological Value of the Same Protein Preparations Estimated in Growing Rats, Adult Rats, and Adult Men[a]

Protein Source	Biological Value		
	Growing rats	Mature rats	Adult men
Egg albumin	97	94	91
Whole egg	87	82	94
Beef muscle	76	69	67
Casein	69	51	56
Peanut flour	54	46	56
Wheat gluten	40	65	42

[a]From Mitchell (18); most of the data on human subjects from Hawley *et al.* (19).

TABLE V
Net Protein Utilization (NPU) Values Determined in Children and Rats[a]

Protein Source	Children		Rats
	NPU	% Energy	NPU
	3–7 years		
Whole egg	87	2–3[b]	94 ± 4
Soybean	...	...	65 ± 7
Milk	75–78	2–7	...
Flour	54	6–7	...
Toasted grits	67–80	2–10	...
	8–12 years		
Corn	36	11–14	52 ± 6
Rice, polished	63	8–10	59 ± 4
Wheat, whole	49	11–14	48 ± 9

[a]Taken from FAO/WHO (3).
[b]Per cent of dietary energy supplied by protein.

than 20. For bioassays involving slope-ratio assay procedures, a deficiency in either lysine or threonine affects the values obtained in different ways than deficiencies in other essential amino acids (14). Thus, the absence or deficiency of

TABLE VI
Biological Value or Net Protein Utilization Value of Four Protein Preparations Estimated with Children (3–7 years) and Rats

Protein Source	Humans[a]			Rats[b]
	Protein intake level g/kg body wt/day	Biological Value	Net Protein Utilization	Biological value 10% protein diets
Reference cow's milk	0.52	90	81	80
	1.00	86	79	
	1.49	91	81	
	1.97	82	75	
Sesame flour	1.71	62	54	58
	3.23	59	53	
Peanut flour	1.55	61	57	48
	3.14	60	53	
	4.37	54	52	
Cottonseed flour	1.73	62	51	65
	3.51	53	47	
	5.15	42	39	

[a]From DeMaeyer and Vanderborght (20).
[b]From Bender (13,21).

TABLE VII
Nutritional Value of Milk or Casein and the Same Textured Soy Product Estimated with Children and Rats[a]

	Textured Soy Product	Milk	Casein
Children[b]			
mg nitrogen/kg body wt/day required for nitrogen equilibrium	138[c]	97[d]	...
Net Protein Utilization			
1.0 g protein/kg/day	60	74[d]	...
0.75 g protein/kg/day	66	...	...
Rats[e]			
Protein Efficiency Ratio	3.01, 3.30	...	3.38, 3.53
Net Protein Utilization	57	...	61

[a]From Bressani *et al.* (22), except as otherwise noted; soy product contained (by wt) 28.8% soy fibrils, 12.3% egg albumin, 11.8% wheat gluten and 9.6% toasted soybean meal.
[b]Eight children, 2–6 yrs of age.
[c]Based on data from feeding 1.0, 0.75, 0.5, and 0 g protein/kg body wt/day (each level for a period of 10 days).
[d]From Bressani *et al.* (22,23).
[e]Diets fed contained 10% protein.

different essential amino acids may also have different effects on the results of rat growth assays. Analogous effects in humans probably exist with proteins deficient in either lysine or tryptophan (15). Possible effects of deficiencies in other amino acids have not been studied. Such effects could be expected to occur, however, and they may be quantitatively and qualitatively different from the effects observed in the growing rat.

The level of protein intake may also affect the relative requirement for specific essential amino acids in both rats and humans. The differences in the estimated requirements for individual amino acids for adult rats, when the estimates were made at assumed protein requirement levels of 3.2 and 5% (Table II), demonstrate this effect in rats. In humans, Kies and Fox (16) observed that in subjects on nitrogen intake levels equivalent to 0.16 to 0.26 g of protein/kg body wt/day, methionine was the second limiting amino acid in corn (lysine being first limiting). With nitrogen intakes equivalent to 1 g protein/kg body wt/day, tryptophan was the second limiting amino acid.

COMPARATIVE DATA FROM ANIMAL AND HUMAN ASSAYS

On the basis of the above observations, it would seem unlikely that assays with growing rats could provide useful estimates of the nutritional value of proteins for humans. However, despite such an observation and despite the possibility that "the single figure obtained from rat assay has provided useful information is fortunate rather than scientifically based" (17), it is usually accepted that there is general agreement between protein nutritional value as estimated by animal assays and by human assays. Campbell and McLaughlan (1) thus concluded "until more is known of protein requirements and amino acid interrelationships, and providing the deficiencies of the methods are recognized, assays for protein quality using the growing rat at about 10% protein would still seem to have very

TABLE VIII
Biological Value and Net Protein Utilization Value of Five Protein Sources Estimated with Adult Male Subjects Consuming 0.4 g protein/kg Body wt/Day[A]

Protein Source	Biological Value		Net Protein Utilization	
	A[b]	B[c]	A[b]	B[c]
Spray-dried whole egg	61(100)	84(100)	57(100)	81(100)
Tuna	62(102)	84(101)	56(98)	79(98)
Cottage cheese	56(92)	77(92)	56(98)	77(95)
Soy isolate	53(87)	73(87)	51(89)	72(88)
Wheat gluten	34(56)	56(67)	34(60)	56(69)

[a]Unpublished data (Bodwell); values in parentheses are relative values, with values for egg = 100.
[b]Calculated by using values of 9.9 and 24.4 mg nitrogen/kg body wt/day obligatory fecal and urinary losses, respectively (nitrogen losses determined on three subjects participating in part or all of the protein evaluation periods); a value of 5 mg nitrogen/kg body wt/day assumed for integumental and miscellaneous losses.
[c]Calculated by using FAO/WHO (3) values of 12 and 37 mg nitrogen/kg body wt/day obligatory fecal and urinary losses, respectively; a value of 5 mg nitrogen/kg body wt/day assumed for integumental and miscellaneous losses.

important use in predicting the protein quality of foods for humans". Young and Scrimshaw (11) suggested that "the nitrogen-balance index approach represents the most appropriate research technique for comparing data derived in rats and in children. The available data support the use of the growing rat for purposes of estimating protein quality and indicate that the estimates have predictive quantitative significance for human nutrition."

Such generalizations are based on comparisons of data obtained for a given single source of protein in rat assays and limited data obtained for a different source of the same or similar protein in human studies. The number of investigations in which the same preparation of a specific protein source has been assayed with both rats and humans, under acceptable assay conditions, are very limited. These are included in the following discussion in an attempt to critically assess the value of rat assays for predicting protein nutritional value for humans.

Biological Value (BV) and Net Protein Utilization (NPU)

These two approaches for estimating nutritional value are considered together since, in studies with humans, Biological Value (BV) × digestibility = NPU. Typically, values for BV or NPU from animal and human studies such as those listed in Tables IV and V are used to indicate a reasonable relation, with either of

TABLE IX
Net Protein Utilization (NPU) Values for Wheat Gluten, Whole Wheat (Biscuits), and Spray-Dried Whole Egg Estimated with Young Adult Males Fed Different Levels of Protein

Protein Source	Reference	Intake g/kg body wt/day	NPU
Wheat gluten	Scrimshaw *et al.* (24)	0.27	84[a]
		0.73	34[a]
Wheat gluten	Inoue *et al.* (25)	0.1	139[b]
		0.2	101[b]
		0.4	53[b]
		0.6	41[b]
		1.0	26[b]
Whole wheat	Young *et al.* (26)	0.3	76[a]
		0.4	57[a]
		0.5	54[a]
		0.65	48[a]
Spray dried whole egg	Young *et al.* (27), Scrimshaw *et al.* (28)	0.2	115[a]
		0.3	99[a]
		0.4	69[a], 71[a]
		0.5	77[a]

[a]Authors assumed 37.2 and 8.8 mg/kg body wt/day obligatory urinary and fecal nitrogen losses, respectively; recalculated to include 5 mg/kg body wt/day integumental and miscellaneous nitrogen losses.
[b]Authors assumed 33.3 and 12.7 mg/kg body wt/day obligatory urinary and fecal losses; recalculated to include 5 mg/kg body wt/day integumental and miscellaneous nitrogen losses.

these assays, between estimates of nutritive value in rats and in humans. Relative to the BV data given in Table IV, this is clearly a false assumption since the data from the human studies were based on widely varying protein intake levels. The two higher quality proteins, egg albumen and powdered whole egg, were fed at very low levels (0.2–0.3 g/kg body wt/day) and the lower quality proteins at higher levels (0.4–0.6 g/kg body wt/day). In particular, the low intake levels of high-quality protein resulted in exaggerated estimates of nutritive value which thus agreed with the comparable values from the animal assay data. Since this is one of the rare cases in which the same protein preparations were used for studies with both rats and humans, it is unfortunate that the proteins were fed at variable intake levels in the human subjects.

NPU values estimated by human and animal assays, such as those in Table V, often have not been based on assay data obtained from the same lot or batch of a given protein source. For instance, animal assays may have been conducted on soy protein in England and the human assays on a different preparation of soy protein in India. Such data often agree in general, but the findings are not conclusive.

BV, estimated in both rats and children, on the same preparations of four protein sources, is listed in Table VI. Except for the values for peanut flour, agreement between the estimates of BV is good.

Data from studies in which Guatemalan children were subjects for evaluating a single protein source, a textured vegetable protein product, are shown in Table VII. For children, Bressani *et al.* (22) concluded that the soy product had about 80% of the nutritive value of cow's milk. The NPU values for the soy product were similar in both the rats and children.

BV and NPU values, for five protein sources, estimated with adult male subjects are shown in Table VIII. For the egg and wheat gluten proteins, the absolute values, assuming comparable levels of obligatory nitrogen losses, agree with values reported by others (Table IX) for similar proteins at equivalent intakes. Given this agreement (as well as the comments to be made below regarding the validity of assays conducted well below the maintenance level), the

TABLE X
Relative Biological Value and Net Protein Utilization of the Same Protein Sources Estimated with Humans and Rats[a]

Protein Source	Biological Value			Net Protein Utilization		
	Humans[b] A	Rats B	A−B	Humans A	Rats B	A−B
Spray-dried whole egg	100	100	...	100	100	...
Tuna	102	86	+16	98	89	+ 9
Cottage cheese	92	90	+ 2	98	91	+ 7
Soy isolate	87	66	+21	89	66	+23
Wheat gluten	56	65	− 9	60	66	− 6

[a]Unpublished data of Bodwell and Hackler; all values expressed as a percentage of values obtained with egg protein; adult male subjects consumed 0.4 g protein/kg body wt/day; rats fed 10% protein diets.

[b]Assumed losses of 9.9, 24.4, and 5 mg nitrogen/kg body wt/day for obligatory fecal, urinary, and integumental plus miscellaneous losses, respectively.

values in Table VIII, although obtained at a single protein intake level, are useful for making comparisons with values from animal assays. The latter, obtained at Cornell on the same preparations of each protein source (14), have been similarly expressed relative to the values for egg protein. BV and NPU values are shown in Table X. In relation to the estimates with the human subjects, the estimates with rats are markedly lower for the soy isolate and tuna and slightly higher for the wheat gluten protein.

Nitrogen Balance Index

Melnick and Cowgill (29) and Allison and Anderson (30) developed the concept of feeding proteins at varied intake levels in order to measure nitrogen

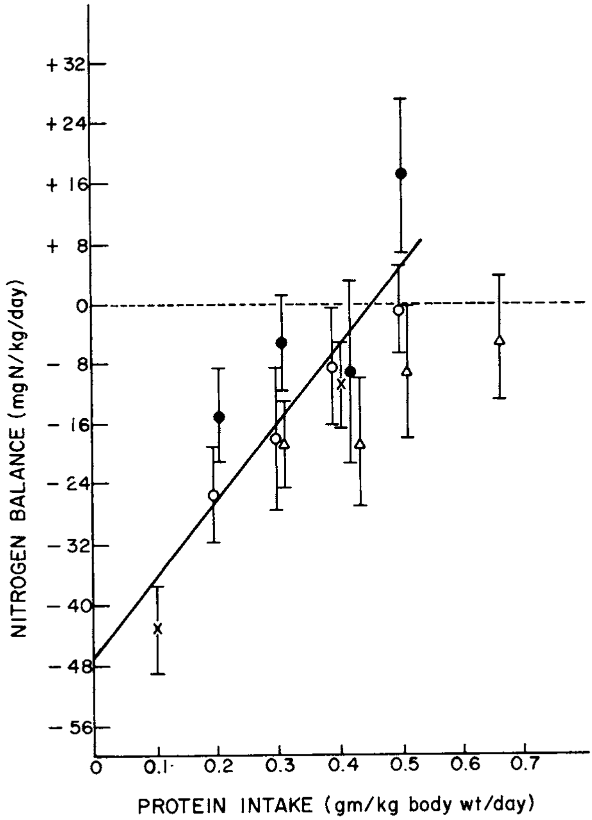

Fig. 1. Relation of nitrogen balance (assuming 5 mg/kg body wt/day integumental and miscellaneous losses) to protein intake level in young men; × = mean ±1 standard deviation for egg protein from Scrimshaw *et al.* (28,35); • = mean ±1 standard deviation for egg protein from Young *et al.* (27); o = mean ±1 standard deviation for canned strained beef and Δ = mean ±1 standard deviation for cooked stone-ground whole wheat from Young *et al.* (26). Line shown is for the regression equation (estimated N balance = 104 [protein intake] — 46.3) based on egg protein values (26). All proteins were fed at 0.1, 0.2, 0.3, 0.4, 0.5, or 0.65 g levels; some values have been offset for clarity.

balance as a function of nitrogen absorbed. The slope of the line relating nitrogen balance to nitrogen absorbed was termed the nitrogen balance index (31). This approach was subsequently applied in studies with humans by Bricker *et al.* (32,33) and, in a modified form, by Hegsted *et al.* (34). The term "nitrogen balance index" has also been used by Bressani (12) to denote the slope of the line, or regression coefficient, that relates nitrogen balance to nitrogen intake in plots of these parameters. An example of this type of plot is shown in Fig. 1.

The regression equations used to calculate the nitrogen balance index (nitrogen balance *vs.* nitrogen absorbed) can be used to predict the minimum level of nitrogen absorption required for nitrogen equilibrium. (An analogous calculation for predicting minimal nitrogen intake was discussed in another paper in these proceedings [36].) Mitchell (18) used this approach for evaluating the relation between nutritive value, as estimated by using the nitrogen balance index method, in humans and rats fed the same preparations of protein sources. When expressed as mg nitrogen/basal kcal (Table XI), agreement was poor between the values for rats and humans. Mitchell (18) suggested that the lack of agreement was due primarily to differences in the responses of humans and rats to deficiencies in either the sulfur amino acids or lysine.

The nitrogen balance index gave useful estimates of protein nutritive value in studies in which children recovering from protein malnutrition (38) and healthy children (39) were subjects. Viteri and Bressani (39) showed, however, that, for their children, the assay was valid only within a defined range of nitrogen absorption (corresponding to intakes of 0.3–1.25 g protein/kg body wt/day). Above or below this range, the relation between nitrogen balance and nitrogen absorbed was not linear. Within this range of nitrogen absorption, nitrogen balance was slightly above or slightly below the nitrogen equilibrium level.

Such assays may not be strictly valid when the subjects are normal adults and most of the protein intake levels are considerably below the maintenance level

TABLE XI
Requirements for Absorbed Nitrogen from Different Protein Sources for Nitrogen Equilibrium in Adult Rats and Humans[a]

Protein Source	Absorbed Nitrogen Required for Nitrogen Equilibrium: Adult rat	Adult human
	mg nitrogen/basal kcal	
Whole egg	2.63	2.08
Egg albumin	2.30	2.14
Milk	3.18	2.59
Soy flour	5.48	2.65
Beef muscle	3.11	2.93
Casein	4.24	3.31
Peanut flour	4.70	3.51
Wheat flour	3.32	4.61
Wheat gluten	3.20	4.67

[a]From Mitchell (18); data for humans from Hawley *et al.* (19), supplemented with unpublished data of J. R. Murlin, and from Bricker *et al.* (32); data for rats from Bricker and Mitchell (37) and Mitchell (18).

(*i.e.*, the subjects are markedly in negative nitrogen balance). At these levels, the responses reflect, in part, protein quality. However, the responses also reflect metabolic adaptations to submaintenance intakes of protein, and may vary according to the physiological state (age, sex, etc.) of the subjects. On the basis of limited data (15), it appears that the responses probably would vary with deficiencies in different essential amino acids. The unavoidable, but considerable, experimental variation in such studies (Fig. 1) precludes any critical evaluation of the influence of these factors on the validity of using this approach for estimating protein nutritional value. The most appropriate range of protein intake levels for assessing the nutritive value of most proteins in adults may be that range of intake levels which results in nitrogen balances from slightly above to slightly below the nitrogen equilibrium level. In this range of intakes, estimates of NPU or BV, for a specific protein, are similar. The range of intake levels would be relatively narrow for high-quality proteins and broader for lower quality proteins.

Regardless of any possible relation, or lack thereof, between nitrogen balance index values determined in rats and in humans, the required experimental procedures probably preclude the use of the index, *per se*, for routine rat assays on large numbers of protein sources.

Nitrogen Growth Index (NGI) and Relative Protein Value (RPV)

The Nitrogen Growth Index (NGI) is defined as the slope or regression coefficient of the line that relates growth to nitrogen intake level (40). This index, of course, is very similar to Relative Protein Value (RPV) since (NGI/values obtained for lactalbumin) × 100 would be equivalent to RPV expressed on a nitrogen intake basis. The regression coefficients of nitrogen balance with nitrogen absorbed, from studies with children, and of the NGI, from studies with rats, are compared in Fig. 2. Protein sources were not specified, but the agreement between the two estimates is good. Similar data, in tabular form, were given by Bressani (41) for six protein sources, four vegetable mixtures (INCAP 9, 14, 15, IRL; see below for definitions), a cassava-soy protein mixture, and skim milk. When those data are plotted in a manner similar to that of Fig. 2, the slope of the line relating the two regression coefficients differs markedly from the slope in Fig. 2. The slopes might have been similar if the data for both the children and the rats had been separately corrected by the use of reference proteins. On the basis of the data available, however, unequivocal conclusions cannot be made.

To this author's knowledge, no data have been reported, for the same protein preparations, relating RPV to estimates of nutritive value from human studies. Sammonds and Hegsted (42) reported RPV's of 57, 28, 57, and 54 for soy protein (young rats), wheat gluten (young rats), wheat gluten plus lysine (young rats), and wheat protein (gluten + bread; adult rats). The Cebus monkey utilizes these same proteins at levels equivalent to RPV values of 43 (young monkey), 16 (young monkey), 52 (young monkey), and 60 (adult monkey), respectively (42,43). The young monkey, however, utilizes lactalbumin at only 70% efficiency (compared to 100% by the young rat) and, thus, the comparable efficiencyof protein utilization would be considerably lower for the young monkey than for the rat (30 *vs.* 57 for soy protein; 11 *vs.* 28 for wheat gluten; 36 *vs* 57 for wheat gluten plus lysine).

Values for RPV and RPV-Modified from rat assays of the same preparations

TABLE XII
Relative Nutritive Values of the Same Protein Sources Estimated with Humans (Net Protein Utilization [NPU]) and Rats (Relative Protein Value [RPV]; Relative Protein Value-Modified]RPV-M], and Relative Nitrogen Utilization [RNU])[a]

	Humans	Rats					
Protein Source	**NPU[b] A**	**RPV B**	**A−B**	**RPV−M C**	**A−C**	**RNU D**	**A−D**
Spray-dried whole egg	100	100	...	100	...	100	...
Tuna	98	74	+24	73	+25	70	+28
Cottage cheese	98	61	+37	71	+27	81	+17
Soy isolate	89	50	+39	53	+36	55	+34
Wheat gluten	60	19	+41	26	+34	32	+28

[a]Unpublished data of Hackler and Bodwell; all values expressed as a percentage of values obtained with egg protein; adult male subjects consumed 0.4 g protein/kg body wt/day; see reference (14) for definition of Relative Protein Value-Modified and for experimental details of rat studies.
[b]Assumed losses of 9.9, 24.4, and 5 mg nitrogen/kg body wt/day for obligatory fecal, urinary, and integumental losses, respectively.

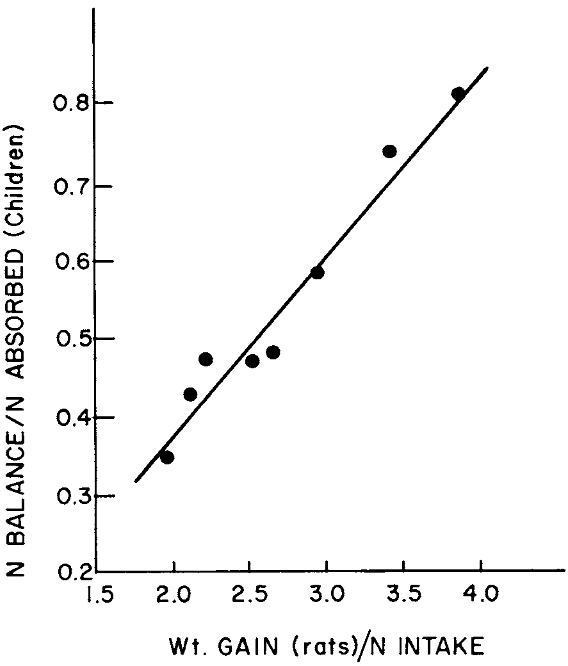

Fig. 2. Relationship between regression coefficients of nitrogen balance *vs.* nitrogen absorbed in children and wt gain *vs.* nitrogen intake (NGI) in rats; protein sources were not specified. From Bressani (41).

TABLE XIII
Nutritive Value of INCAP Vegetable Mixtures, Milk, or Casein Estimated in Children and Rats

	Children			Rats	
	No. of subjects	Protein intake levels g/kg body wt/day	% of Nitrogen intake retained	% Protein in diet	PER
Scrimshaw *et al.* (44)					
INCAP vegetable mixture 9B[a]	8	2.0	19.3	10.0	2.14
Scrimshaw *et al.* (45)					
INCAP vegetable mixture 8[a]	8	2.8	19.8	...	...
Milk	8	2.8	20.5	...	...
INCAP vegetable mixture 9A or 9B[a]	1–9	0.5–4	19.7[b]	...	...
Milk	1–9	0.5–4	17.7[b]	...	...
Bressani *et al.* (46)					
INCAP vegetable mixture 9[a]	...	...	...	10.3	2.03
INCAP vegetable mixture 9	...	...	...	10.6	2.04
Casein	...	...	...	10.0	2.29
Skim milk	...	...	...	10.3	2.88

[a]Vegetable mixture 9: 28% lime-treated corn meal, 28% lime-treated sorghum meal, 38% cottonseed flour, 3% leaf meal, and 3% torula yeast; vegetable mixture 9A: 29% cooked corn meal, 29% cooked sorghum meal, 38% cottonseed flour, and 3% torula yeast; vegetable mixture 9B: 29% uncooked corn meal, 29% uncooked sorghum meal, 38% cottonseed flour, and 3% torula yeast; vegetable mixture 8: 50% lime-treated corn, 35% soy flour, 9% cottonseed flour, 3% leaf meal, and 3% torula yeast.

[b]Average for range of protein intakes studied.

of proteins used for determining NPU values in our Beltsville subjects are given in Table XII, expressed relative to the values for the egg protein (see reference 14 for actual values). When compared to the similarly expressed NPU values for our human subjects, values for both RPV and RPV-Modified are markedly lower. The data are quantitatively insufficient for making any general conclusions. However, the data do not suggest a close relation between nutritive value as estimated by RPV or RPV-Modified assays in rats and as estimated by NPU for adult human subjects.

Relative Nitrogen Utilization (RNU)

The data shown in Table XII suggest that, on the basis of this single study, Relative Nitrogen Utilization (RNU) values also underestimate nutritive value for adult humans by a magnitude similar to Relative Nitrogen Value (RNV) or RNV-Modified.

Protein Efficiency Ratio (PER)

The only reports which have related estimates of nutritional value as determined in humans and as estimated by Protein Efficiency Ratio (PER) assays (both presumably on the same protein preparations) are those from the INCAP (Institute of Nutrition of Central America and Panama) studies in Guatemala. Data for INCAP vegetable mixtures 8, 9, 9A, and 9B are given in Table XIII. For the children, milk was used as a reference protein while, for INCAP mixture 9, casein and milk were used as reference proteins for the PER assays. The authors considered mixtures 9, 9A, and 9B to be of equivalent nutritive value for children. These mixtures, as well as mixture 8, were equal to milk in nutritive value for children. The PER values for mixture 9, however, were markedly lower than that for milk and about 10% lower than that for casein. For the rat studies in which mixture 8 was fed, dietary protein was 25% (47), and the values obtained are thus not useful for comparative purposes.

In more extensive studies, various mixtures were fed to children, each at several levels of protein intake; results are summarized in Table XIV. The regression coefficients for nitrogen retained *vs.* nitrogen absorbed (or nitrogen intake) are expressed as a percentage of the coefficients observed with whole egg as the protein source. In the study of Bressani *et al.* (38), valid comparisons cannot be made between the animal and human data because the levels of protein fed in the rat assays varied. In the study of Viteri and Bressani (39), a standardized PER assay method was used and casein was included as one of the reference proteins. The relation between PER values and nutritive value, as estimated in the children, was very poor (Fig. 3). In other studies (48,49), PER values were somewhat higher for mixtures 14 and 15 (Table XIV) than those observed in the study of Viteri and Bressani (39). However, the variable levels of dietary protein or the lack of a casein reference preclude direct comparisons.

In Fig. 3, the PER values obtained on the five protein sources studied at Beltsville and Cornell are also plotted *vs.* the human NPU values (Table VIII), expressed as a percentage of the NPU value obtained for egg protein. In this case, the PER values appear to reflect nutritive value as estimated in the human subjects. However, the variation in PER values (~1.5 to ~3.0) for the four higher quality proteins greatly exceeds the variation in the nutritive value as estimated in the human subjects (85–102% of that for egg protein).

TABLE XIV
Nutritional Value of the Same Protein Sources Estimated with Children and Rats

	Children			Rats	
Protein Source	**Regression coefficients for nitrogen retained *vs.* nitrogen absorbed % of value for egg**	**Regression coefficients for nitrogen retained *vs.* nitrogen intake % of value for egg**	**Nitrogen intake needed for nitrogen equilibrium: relative score % of value for egg**	**% Protein in diet**	**PER**
Bressani *et al.* (38)[a]					
Whole egg	100[b]	100[c]	...	12.8	2.90
Milk	108	93	...	12.7	2.56
INCAP vegetable mixture 9	78	48	...	13.8	1.93
INCAP vegetable mixture 14	98	93	...	14.2	2.24
INCAP vegetable mixture 15	83	81	...	14.6	2.20
Casein	...	...	...	11.2	2.88
Viteri and Bressani (39)[d]					
Whole egg "B"	100[e]	100[f]	100[g]	~10	2.5[h]
Whole milk	91	109	106	~10	2.2[h]
INCAP vegetable mixture 9	61	59	89	~10	1.7[h]
INCAP vegetable mixture 14	78	100	96	~10	2.0[h]
INCAP vegetable mixture 15	58	73	78	~10	1.9[h]
CSM	51	63	78	~10	2.0[h]
ARL	64	68	101	~10	2.3[h]
TRL	73	80	111	~10	2.1[h]
IRL	73	81	98	~10	2.1[h]

Bressani and Elias (48)					
INCAP vegetable mixture 14	...	...	...	9.7	2.79
INCAP vegetable mixture 14	...	...	...	10.1	2.56
Skim milk	...	...	...	12.0	2.73
Bressani *et al.* (49)					
INCAP vegetable mixture 15	...	...	...	10.8	2.22
Casein	...	...	...	11.9	2.52

[a]Four children/group (for the most part, different children were used in different groups), ages 2–4 yrs; 6 or 7 levels of protein fed (0.56–3.55 g/kg body wt/day). INCAP vegetable mixture 9: 3% torula yeast, 58% corn meal, and 38% cottonseed flour (by wt); mixture 14: 3% torula yeast, 58% corn meal, and 38% cottonseed flour (by wt); mixture 14: 3% torula yeast, 58% corn meal, and 38% soy flour; mixture 15: 3% torula yeast, 58% corn meal, 19% soy flour, and 19% cottonseed flour.
[b]Regression coefficient for whole egg = 0.64.
[c]Regression coefficient for whole egg = 0.59.
[d]Five preschool children used as subjects for all protein sources studied; protein intake levels were 1.5, 1.0, 0.75, and 0.5 g/kg body wt/day. CSM: 5% skim milk, 68% corn meal, and 25% soy flour (by wt); ARL: 10% skim milk, 28% wheat flour, 38% chick-pea flour, and 19% lentil flour; TRL: 12% skim milk, 37% wheat flour, 19% chick-pea flour, and 19% soy flour; IRL: 10% skim milk, 28% wheat flour, 28% chick-pea flour, and 24% split pea flour.
[e]Regression coefficient for egg = 0.80.
[f]Regression coefficient for egg = 0.59.
[g]Value for egg = 94 mg/kg body wt/day.
[h]Values recalculated assuming value for casein of 2.50.

General Appraisal

The above discussion indicates that few data are available from the evaluation of the same preparations of proteins in both rats and humans. When the same preparations have been evaluated, the results have not been encouraging. The suggestion of Hegsted (50) may be valid. He stated, "it is doubtful that the assays obtained with young rats are valid estimates of the utility of proteins for man, either adult or growing child, because at all stages of postnatal development a much larger proportion of dietary protein is utilized for maintenance than for growth".

APPLICATION OF ANIMAL ASSAY DATA TO NUTRITIONAL LABELING

The per cent of the U.S. Recommended Daily Allowance (U.S. RDA) provided by 20 g of protein (a reasonable serving), supplied by each of the five protein sources studied with human adults at Beltsville and with rats at Cornell, is given in Table XV. Except for soy isolate, the percentages of the U.S. RDA provided, based on the human or rat NPU values, agree closely. RPV, RPV-M, and RNU all underestimate the percentage of the U.S. RDA provided by 20 g of

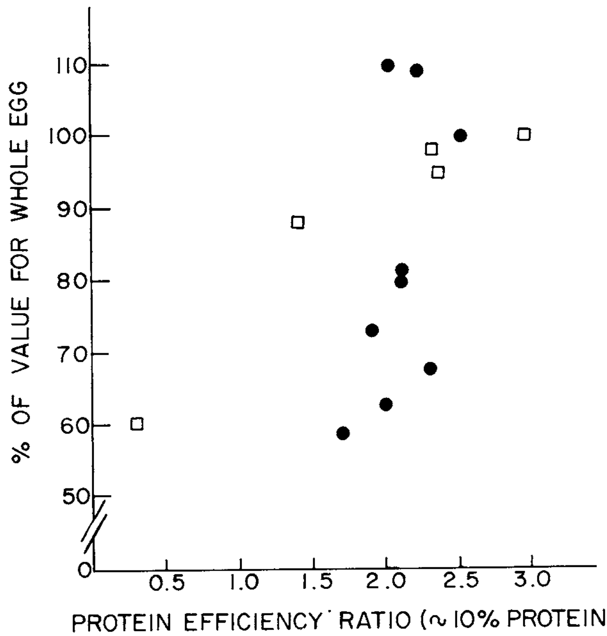

Fig. 3. Relation between Protein Efficiency Ratio (PER) values and estimates of nutritive value in humans: ● = values for children and corresponding PER values from Table XIV (Viteri and Bressani [39]); □ = values for adults (NPU values expressed as a percentage of value obtained for egg protein, see text) from Bodwell (unpublished data) and corresponding PER values from Hackler (14).

cottage cheese, soy isolate, or wheat gluten protein. Because of the parameters used, the PER values greatly overestimate the percentage provided by egg protein and underestimate that provided by wheat gluten.

In applying values from PER assays to nutritional labeling, the use of a breakpoint at a PER value of 2.50 (with a U.S. RDA of 45 g for proteins with a PER equal to, or greater than, 2.50 and a U.S. RDA of 65 g for those with a PER less than 2.50) cannot be commended. In the Beltsville study (Table VIII), tuna was equivalent to whole egg as a protein source for adults. Likewise, in the INCAP studies (Tables XIII and XIV), some of the vegetable mixtures were equivalent to milk in terms of estimated protein nutritive value for the children studied. According to the existing nutritional labeling parameters, because of their adjusted PER values of less than 2.50, these mixtures would have had to be provided at 144% of the level of egg, casein, or milk to supply an equivalent percentage of the U.S. RDA. From a nutritional viewpoint, this approach is not logical.

As previously discussed, it is doubtful that the level of the limiting amino acid (for the rat) in a protein source has much relevance to human nutrition. In proteins severely lacking in a single essential amino acid, obviously the results of rat growth assays are useful in detecting the deficiency. However, unless the protein source is severely damaged during processing, less complicated assays (*e.g.*, amino acid analyses) provide the same information. Furthermore, the usual rat assays provide no information that is useful in meeting the other criteria of an ideal assay as defined below.

ALTERNATIVES TO RAT ASSAYS

Given the inadequacies inherent in rat assays for the purpose of estimating protein nutritive value for humans, are there useful alternatives? A detailed consideration of this question is beyond the scope of this discussion, and only some brief comments will be made. Animal assays, in which various biochemical

TABLE XV
Per Cent of U.S. Recommended Daily Allowance of 65 g of Protein Provided by 20 g of Protein from Various Sources Calculated from Different Estimates of Nutritive Value[a]

Protein Source	% of 65 g RDA Provided by 20 g of Protein					
	Relative NPU[b] Human	Relative NPU[b] Rat	RPV	RPV Modified	RNU	PER[c]
Spray-dried whole egg	31	31	(31)	(31)	31	44
Tuna	30	27	(31)	25	22	31
Cottage cheese	29	28	27	25	25	31
Soy isolate, cooked	27	20	22	18	17	31
Wheat gluten	19	20	8	9	10	0

[a]Calculations based on unpublished data of Hackler and Bodwell.
[b]NPU values expressed as % of values for egg (egg = 100) used in calculations.
[c]Based on Protein Efficiency Ratios and nutritional labeling parameters (including U.S. Recommended Daily Allowance value of 45 g for the spray-dried egg protein).

parameters are measured as possible indices of nutritional value, have been reviewed (51). To this author's knowledge, no attempts have been made to relate these biochemical parameters, measured in animals, to the nutritive value of proteins for humans. The situation is similar with respect to assays with *tetrahymena pyriformis* (52,53). Studies in which plasma levels of various amino acids have been measured in humans for the purpose of determining the limiting amino acids or amino acid bioavailability, but not protein nutritional value, *per se,* have not been included in this review.

Assays with Human Subjects

The determination of nitrogen balance indexes in which human subjects are fed three or four levels of a specific protein source (see above) is impractical for routine use. However, Bressani (12) and Navarette *et al.* (54) suggested a shortened method in which human subjects are protein depleted for 3 days and then, on the following days, are fed levels of protein that are increased daily. Nitrogen balance data obtained for egg protein by the shortened method are given in Table XVI, together with comparable data from studies conducted in the conventional manner. These results are not conclusive. However, if 7- to 10-day studies were considered to be practical, this approach might be useful.

In an even shorter procedure, postprandial plasma urea nitrogen levels are measured in human subjects following ingestion of test loads of various proteins (51,55). Although this approach has some promise, other dietary components (*e.g.*, fat and carbohydrates) affect the postprandial response and the approach would require considerable refinement before it could be considered practical. The foregoing problems are obviated in an approach in which total urinary nitrogen excretion is measured after short-term (4–5 days) feeding of single-source proteins to human subjects (Fig. 4). Urinary sulfate excretion in human subjects consuming high levels of protein, proposed as a potential index of nutritive value (56), does not appear to be useful (57). Contrary to suggestions made on the basis of animal studies (58), the levels of glutamic acid and aspartic acid (plus their amides) do not appear to be related to protein nutritional value in humans (59). Other plasma or whole blood amino acids, measured in human subjects after 4–5 days of consuming a specific protein or protein mixture,

TABLE XVI
Comparison of N-Balance Indices for Egg Fed to Young Humans[a]

Protein Source	Method	Regression Equation[b]
Fresh egg	Short	$-59.45 + 0.678X$[a]
Egg powder	Short	$-46.08 + 0.481X$[a]
Fresh egg	Conventional	$-39.65 + 0.577X$[a]
Egg	Conventional	$-36.20 + 0.538X$[c]
Egg	Conventional	$-33.87 + 0.633X$[d]

[a]From Bressani (12).
[b]Regression equations of nitrogen balance *vs.* nitrogen absorbed, where X = nitrogen absorbed.
[c]Inoue *et al.* (25).
[d]Young *et al.* (27).

however, might be useful.

Human assays, *per se,* may not be practical even with approaches which require only 4- to 5-day feeding periods. Further, in all the human assays discussed, no information is gained about the usefulness of a specific protein source in supplying nonlimiting essential amino acids. It is also unlikely that such

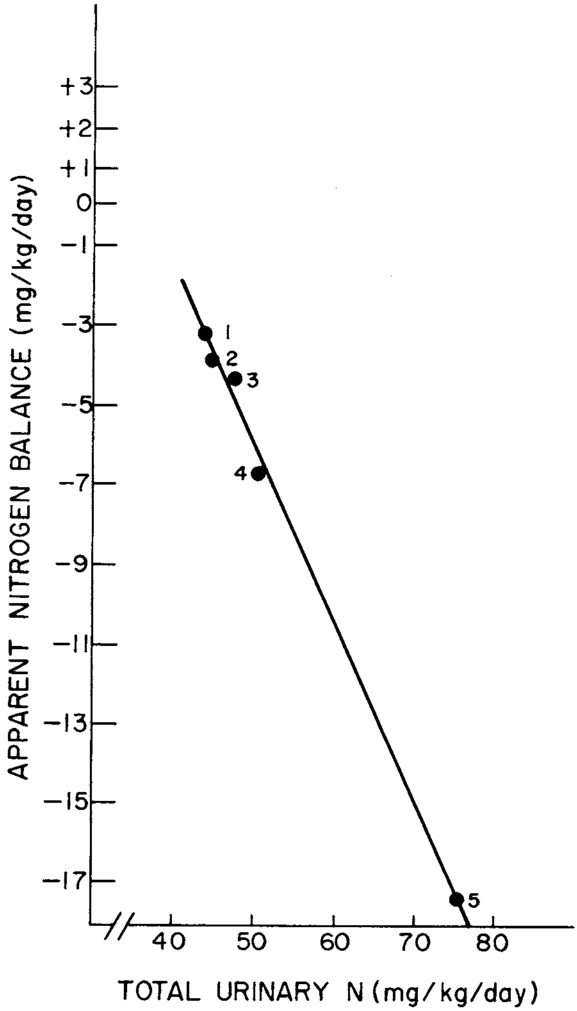

Fig. 4. Relation between total urinary nitrogen excretion, measured after short-term (4−5 days) feeding of single sources of protein, and apparent nitrogen balance determined in adult male subjects (Bodwell, unpublished data); protein sources: 1, spray-dried whole egg; 2, tuna; 3, cottage cheese; 4, soy isolate; and 5, wheat gluten.

assays would provide information about the nutritive value of a mixture of two or more proteins when each has been assayed separately.

Chemical Analyses

Assays involving *in vitro* enzymatic digestion followed by amino acid analyses have been suggested as potentially useful for estimating nutritive value (60,61,62,63). When the same preparations of various protein sources have been subjected to enzymatic digestion procedures, amino acid analyses, and rat bioassays, enzymatic digestion procedures do not appear to have any advantage beyond that provided by indexes or scores calculated from amino acid composition data (64).

Amino acid composition data alone, chemical scores, and amino acid indexes provide information about the value of a specific protein relative to its potential usefulness when mixed with other proteins. However, possible differences between the chemically determined levels of amino acids and the amounts of these amino acids which are biologically available are not usually reflected in amino acid composition data. This has been a serious defect in attempts to estimate nutritional value on the basis of amino acid analyses alone. This problem might be partially resolved by use of an *in vitro* method (65) that appears to be useful for predicting *in vivo* digestibility (Table XVII). If such *in vitro* digestibility estimates are shown to correlate with digestibility in humans, such an assay might reflect general changes in amino acid availability. If so, an *in vitro* estimate of digestibility plus amino acid composition data might be a useful approach for estimating the nutritive value of proteins for humans. Given modern computer analysis techniques, the amino acid composition data should be subjected to a more extensive analyses than mere calculation of chemical scores or the Essential Amino Acid Index. Possibly, the limiting amino acid, the level and distribution of all the essential amino acids, and the estimates of digestibility obtained by *in vitro* assay could all be incorporated in an analysis for predicting nutritive value for humans. Such an approach is being used by Nebraska workers to predict PER or RPV (66).

TABLE XVII
Comparisons of *In Vivo* (Rat Assays) and *In Vitro* (Multi-Enzyme, 10-Min Assay) Estimates of Digestibility[a]

Protein Source	*In Vivo* Digestibility	*In Vitro* Digestibility	Difference
Corn-milo grain	72.00	73.60	−1.60
High-protein wheat bran flour	77.52	76.86	0.66
General wheat flour	81.94	85.73	−3.79
Durrant wheat	82.80	85.37	−2.57
Wheat protein concentrate	89.90	90.44	−0.54
Soy concentrate	87.72	87.18	0.54
Yeast protein concentrate	86.52	83.20	3.32
Casein (NBC)	90.48	89.17	1.31
Casein (ANRC)	87.60	88.09	−0.49

[a]From Hsu *et al.* (65).

IDEAL ASSAY

For estimation of protein nutritive value for humans, an ideal assay would provide quantitative information about the nutritive value of a protein as a single source of protein for humans and its potential value when consumed with other proteins. In other words, the assay would provide quantitative information

TABLE XVIII
Variability in Estimates of Protein Nutritive Value Calculated from Data Obtained with Human Subjects

Parameter	Reference	Protein Source	Coefficient of Variation[a] %
Adults			
Net Protein Utilization	Young *et al.* (26)	Whole wheat	33(0.4), 15(0.5), 12(0.65)
		Beef	19(0.4), 7(0.5)
	Scrimshaw *et al.* (24)	Wheat gluten	11(0.27), 30(0.73)
Nitrogen intake required for zero balance	Calloway and Margen (67)	Egg	22(0.35–0.66)
	Inoue, *et al.* (68)	Egg	16(0.28–0.76), 9(0.28–0.76)
		Rice	16(0.28–0.76), 16(0.28–0.76)
Nitrogen balance/ nitrogen intake	Bricker, *et al.* (33)	Mixed	12(0.17, 0.34)
Children			
Nitrogen retained	Bressani *et al.* (38)	Milk	38(1.0–3.0)
		Whole egg	30(1.0–3.0)
		Vegetable mixture	21(1.0–3.0)
Net Protein Utilization	DeMaeyer and Vanderborght (20)	11 Different sources	14(0.5–5.0)
Nitrogen balance	Kurien *et al.* (69)	Rice, kaffir corn	26(1.6–1.7)

[a]Standard deviation/mean, expressed as a percentage; values given in parentheses are protein intake levels (g protein/kg body wt/day).

TABLE XIX
Application of Observed Variation in Estimates of Nutritional Value in Studies with Adults to Hypothetical Nutritional Ratings (NR)

	Nutritional Rating (NR)		
	0.40	**0.60**	**0.80**
NR ± 17%, range	0.33–0.47	0.50–0.70	0.66–0.94
NR ± 34%, range	0.26–0.54	0.40–0.80	0.53–1.07

about the amount and nutritional availability of the limiting amino acid and also about the total content and distribution of the nutritionally available nonlimiting essential amino acids in the protein. It would provide a basis for accurately predicting the nutritive value of a mixture of two or more protein sources which have each been assayed separately. Further, for nutritional labeling purposes, the ideal assay should also be simple in application and accurately reflect, as differences in protein quality, only those differences which are real under practical conditions.

If the above definition of an ideal assay is valid, it is readily apparent that neither rat assays nor any of the alternatives discussed above can possibly fulfill all of the stated requirements. From a practical viewpoint, however, this is probably not of serious consequence. Particularly in relation to nutritional labeling, efforts for quite precise quantitative estimates of protein quality may not be justified. This is especially true when the variability observed in estimates of nutritive value obtained from studies with human subjects is taken into account. Coefficients of variation, calculated from such studies, are given in Table XVIII. For the studies with adults, the mean coefficient of variation is 17%. When this level of variation is applied to hypothetical protein quality ratings (Table XIX), it is obvious that very precise quantitation of protein value may not be appropriate. Furthermore, the substantial differences that are observed between some individual protein sources may not always have practical meaning in view of the fact that most people consume, on a daily basis, a mixture of protein sources. A further factor, which has recently received renewed interest (68,70,71) is the influence of calorie intake levels on the apparent nutritive value of proteins. Inoue *et al.* (68) showed that a shift of about 10 kcal/kg body wt/day in energy intake level produces a marked change in estimated nutritive value.

The Recommended Dietary Allowances (6) include a large safety factor. Ideal proteins (*e.g.*, egg and milk) are assumed to be utilized at a 70% efficiency level, a factor of 30% is added to allow for variation in individual requirements and to allow for daily stress, and, finally, a further 33% is added to allow for lower efficiencies of utilization of poorer quality proteins compared to the ideal protein. If levels of protein are expressed in terms of the Recommended Dietary Allowances for nutritional labeling purposes, it can be argued that approximate estimates of nutritive value are sufficient. Such estimates, however, should be used in such a way as to avoid creating apparent differences in nutritional value when, in fact, such differences probably do not exist. Any assay used for nutritional labeling purposes would be inadequate if break-points, together with a system such as the 45–65 g U.S. RDA system, are incorporated in the application of the assay data.

Literature Cited

1. CAMPBELL, J. A., and McLAUGHLAN, J. M. Applicability of animal assays to humans. In: Proc. SOS/70, Third Int. Congr., Food Sci. Technol., Washington, D.C., 1970, p. 336. Inst. Food Technol.: Chicago (1971).
2. HEGSTED, D. M. Human amino acid requirements and protein quality. In: Proc. 1st Asian Congr. Nutr., India, 1971, ed. by P. G. Tulpule and K. S. Jaya Rao, p. 365. Nutr. Soc. India: Hyberabad (1972).
3. FAO/WHO (Food and Agriculture Organization/World Health Organization). Energy and Protein Requirements, Report of a joint FAO/WHO *ad hoc* expert committee, WHO Tech. report series 522. WHO: Geneva, Switzerland (1973).

4. WILLIAMS, H. H., HARPER, A. E., HEGSTED, D. M., ARROYAVE, G., and HOLT, L. E., Jr. Nitrogen and amino acid requirements. In: Improvement of protein nutriture, Food and Nutrition Board, p. 23. Nat. Res. Counc.-Nat. Acad. Sci.: Washington, D.C. (1974).
5. NARASINGA RAO, B. S. Evaluation of protein quality in infants and children. In: Proc. 9th Int. Congr. Nutr., Mexico, 1972, ed. by A. Chávez, H. Bourges, and S. Basta, Vol. 3, p. 338. Karger: Basel, Switzerland (1975).
6. FOOD AND NUTRITION BOARD. Recommended Dietary Allowances. Nat. Res. Counc.-Nat. Acad. Sci.: Washington, D.C. (1974).
7. STOCKLAND, W. L., MEADE, R. J., and MELLIERE, A. L. Lysine requirement of the growing rat: Plasma-free lysine as a response criterion. J. Nutr. 100: 925 (1970).
8. RAMA RAO, P. B., METTA, V. C., and JOHNSON, B. C. The amino acid composition and nutritive value of proteins. I. Essential amino acid requirements of the growing rat. J. Nutr. 69: 387 (1959).
9. SAID, A. K., and HEGSTED, D. M. Response of adult rats to low dietary levels of essential amino acids. J. Nutr. 100: 1363 (1970).
10. ASHIDA, K., and YOSHIDA, A. Nutritional specificities of essential amino acids and their relationship to evaluation of the nutritional quality of dietary proteins. In: Proc. 9th Int. Congr. Nutr., Mexico, 1972, ed. by A. Chavez, H. Bourges, and S. Basta, Vol. 3, p. 321. Karger: Basel, Switzerland (1975).
11. YOUNG, V. R., and SCRIMSHAW, N. S. Relation of animal to human assays of protein quality. In: Nutrients in processed foods—proteins, ed. by P. L. White and D. C. Fletcher, p. 85. Pub. Sci. Group, Inc.: Acton, Mass. (1974).
12. BRESSANI, R. Human assays and applications. In: Evaluation of proteins for humans, ed. by C. E. Bodwell, p. 81. Avi Pub. Co.: Westport, Conn. (1977).
13. BENDER, A. E. Determination of the nutritive value of proteins by chemical analyses. In: Progress in meeting protein needs of infants and children, p. 407. Pub. 843, Nat. Acad. Sci.-Nat. Res. Counc.: Washington, D.C. (1961).
14. HACKLER, L. R. Methods of measuring protein quality: A review of bioassay procedures. Cereal Chem. 54: 984 (1977).
15. HEGSTED, D. M. Variation in requirements of nutrients—amino acids. Fed. Proc. 22: 1424 (1962).
16. KIES, C., and FOX, H. M. Effect of level of total nitrogen intake on second limiting amino acid in corn for humans. J. Nutr. 100: 1275 (1970).
17. BENDER, A. E. Rat assays for protein quality—a reappraisal. In: Proc. 9th Int. Congr. Nutr., Mexico, 1972, ed. by A. Chavez, H. Bourges, and S. Basta, Vol. 3, p. 310. Karger: Basel, Switzerland (1975).
18. MITCHELL, H. H. Some species and age differences in amino acid requirements. In: Protein and amino acid nutrition, ed. by A. A. Albanese, p. 11. Academic Press: New York (1959).
19. HAWLEY, E. E., MURLIN, J. R., NASSET, E. S., and SZYMANSKI, T. A. Biological values of six partially purified proteins. J. Nutr. 36: 153 (1948).
20. DeMAEYER, E. M., and VANDERBORGHT, H. L. Determination of the nutritive value of different foods in the feeding of African children. In: Progress in meeting protein needs of infants and preschool children, p. 143. Pub. 843, Nat. Acad. Sci.-Nat. Res. Counc.: Washington, D.C. (1961).
21. BENDER, A. E. Nutrition and dietetic foods, p. 165. Chemical Pub. Co.: New York (1973).
22. BRESSANI, R., VITERI, F., ELIAS, G., DE ZAGHI, S., ALVARADO, J., and ODELL, A. D. Protein quality of a soybean protein textured food in experimental animals and children. J. Nutr. 93: 349 (1967).
23. BRESSANI, R., VITERI, F., and BEHAR, M. The protein value of several animal and vegetable proteins in children. Fed. Proc. 25: 299 (1966).
24. SCHRIMSHAW, N. S., TAYLOR, Y., and YOUNG, V. R. Lysine supplementation of wheat gluten at adequate and restricted energy intakes in young men. Amer. J. Clin. Nutr. 26: 965 (1973).
25. INOUE, G., FUJITA, Y., KISHI, K., YAMAMOTO, S., and NIIYAMA, Y. Nutritive values of egg protein and wheat gluten in young men. Nutr. Reports Int. 10: 201 (1974).
26. YOUNG, V. R., FAJARDO, L., MURRAY, E., RAND, W. M., and SCRIMSHAW, N. S. Protein requirements of man: Comparative nitrogen balance response within the submaintenance-to-maintenance range of intakes of wheat and beef proteins. J. Nutr. 105: 534 (1975).
27. YOUNG, V. R., TAYLOR, Y. S. M., RAND, W. M., and SCRIMSHAW, N. S. Protein

requirements of man: Efficiency of egg protein utilization at maintenance and submaintenance levels in young men. J. Nutr. 103: 1164 (1973).
28. SCRIMSHAW, N. S., YOUNG, V. R., SCHWARTZ, R., PICHE, M. L., and DAS, J. B. Minimum dietary essential amino acid-to-total nitrogen ratio for whole egg protein fed to young men. J. Nutr. 89: 9 (1966).
29. MELNICK, D., and COWGILL, G. R. The protein minima for nitrogen equilibrium with different proteins. J. Nutr. 13: 401 (1937).
30. ALLISON, J. B., and ANDERSON, J. A. The relation between absorbed nitrogen, nitrogen balance and biological value of proteins in adult dogs. J. Nutr. 29: 413 (1945).
31. ALLISON, J. B. Biological evaluation of proteins. Physiol. Rev. 35: 664 (1955).
32. BRICKER, M. L., MITCHELL, H. H., and KINSMAN, G. M. The protein requirements of adult human subjects in terms of the protein contained in individual foods and food combinations. J. Nutr. 30: 269 (1945).
33. BRICKER, M. L., SHIVELY, R. F., SMITH, J. M., MITCHELL, H. H., and HAMILTON, T. S. The protein requirements of college women on high cereal diets with observations on the adequacy of short balance periods. J. Nutr. 37: 163 (1949).
34. HEGSTED, D. M., TSONGAS, A. G., ABBOTT, D. B., and STARE, F. J. Protein requirements of adults. J. Lab. Clin. Med. 31: 261 (1946).
35. SCRIMSHAW, N. S., HUSSEIN, M. A., MURRAY, E., RAND, W. M., and YOUNG, V. R. Protein requirements of man: Variations in obligatory and fecal nitrogen losses in young men. J. Nutr. 102: 1595 (1972).
36. YOUNG, V. R., RAND, W. M., and SCRIMSHAW, N. S. Measuring protein quality in humans: A review and proposed method. Cereal Chem. 54: 929 (1977).
37. BRICKER, M. L., and MITCHELL, H. H. The protein requirements of the adult rat in terms of the protein contained in egg, milk, and soy flour. J. Nutr. 34: 491 (1947).
38. BRESSANI, R., VITERI, F., WILSON, D., and ALVARADO, J. The quality of various animal and vegetable proteins with a note on the endogenous and fecal nitrogen excretion of children. Arch. Latinoamer. Nutr. 22: 227 (1972).
39. VITERI, F. E., and BRESSANI, R. The quality of new sources of protein and their suitability for weanlings and young children. Bull. WHO 46: 827 (1972).
40. ALLISON, J. B. The efficiency of utilization of dietary protein. In: Protein and amino acid nutrition, ed. by A. A. Albanese, p. 97. Academic Press: New York (1959).
41. BRESSANI, R. Laboratory evaluation of protein-rich mixtures. In: Proc. 9th Int. Congr. Nutr., Mexico, 1972, ed. by A. Chavez, H. Bourges, and S. Basta, Vol. 4, p. 171. Karger: Basel, Switzerland (1975).
42. SAMMONDS, K. W., and HEGSTED, D. M. Animal bioassays: A critical evaluation with specific reference to assessing nutritive value for the human. In: Evaluation of proteins for humans, ed. by C. E. Bodwell, p. 68. Avi Pub. Co.: Westport, Conn. (1977).
43. SAMMONDS, K. W., and HEGSTED, D. M. Protein requirements of young cebus monkeys (Cebus albifrons and apella). Amer. J. Clin. Nutr. 26: 30 (1973).
44. SCRIMSHAW, N. S., BRESSANI, R., WILSON, D., and BEHAR, M. All-vegetable protein mixtures for human feeding. X. Effect of torula yeast on protein quality of INCAP vegetable mixture 9. Am. J. Clin. Nutr. 11: 537 (1962).
45. SCRIMSHAW, N. S., BEHAR, M., WILSON, D., VITERI, F., ARROYAVE, G., and BRESSANI, R. All-vegetable protein mixtures for human feeding. V. Clinical trials with INCAP mixtures 8 and 9 and with corn and beans. Amer. J. Clin. Nutr. 9: 196 (1961).
46. BRESSANI, R., ELÍAS, L. G., and SCRIMSHAW, N. S. All-vegetable protein mixtures for human feeding. VIII. Biological testing of INCAP vegetable mixture 9 in rats. J. Food Sci. 27: 203 (1962).
47. SQUIBB, R. L., WYLD, M. K., SCRIMSHAW, N. S., and BRESSANI, R. All-vegetable protein mixtures for human feeding. I. Use of rats and baby chicks for evaluating corn-based vegetable mixtures. J. Nutr. 69: 343 (1959).
48. BRESSANI, R., and ELÍAS, L. G. All-vegetable protein mixtures for human feeding. The development of INCAP vegetable mixture 14 based on soybean flour. J. Food Sci. 31: 626 (1966).
49. BRESSANI, R., ELIAS, L. G., BRAHAM, J. E., and ERALES, M. Vegetable protein mixtures for human consumption. The development and nutritive value of INCAP mixture 15, based on soybean and cottonseed protein concentrates. Arch. Latinoamer. Nutr. 17: 177 (1967).
50. HEGSTED, D. M. Nutritional research on the value of amino acid fortification—experimental studies in animals. In: Amino acid fortification of protein foods, ed. by N. S. Scrimshaw and

A. M. Altschul, p. 157. MIT Press: Cambridge, Mass. (1971).
51. BODWELL, C. E. Biochemical parameters as indices of protein nutritional value. In: Protein nutritional quality of foods and feeds. Part 1. Assay methods—biological, biochemical and chemical, ed. by M. Friedman, p. 261. Marcel Dekker: New York (1975).
52. LANDERS, R. E. Relationship between protein efficiency ratio of foods and relative nutritive value measured by *tetrahymena pyriformis w* bioassay techniques. In: Protein nutritional quality of foods and feeds. Part 1. Assay methods—biological, biochemical and chemical, ed. by M. Friedman, p. 185. Marcel Dekker: New York (1975).
53. FRANK, O., BAKER, H., HUTNER, S. H., RUSOFF, I. I., and MORCK, R. A. Evaluation of protein quality with the phagotrophic protozoan *tetrahymena*. In: Protein nutritional quality of foods and feeds. Part 1. Assay methods—biological, biochemical and chemical, ed. by M. Friedman, p. 203. Marcel Dekker: New York (1975).
54. NAVARETTE, D. A., ROQUE DE DAQUI, V., LACHANCE, P., and BRESSANI, R. A rapid method for protein quality evaluation in humans. Paper presented at the 35th Annu. Meet., Inst. Food Technol.: Chicago, Ill. (1975).
55. BODWELL, C. E. Biochemical indices in humans. In: Evaluation of proteins for humans, ed. by C. E. Bodwell, p. 119. Avi Pub. Co.: Westport, Conn. (1977).
56. FOOD AND NUTRITION BOARD. Evaluation of Protein Quality, Publ. 1100. Nat. Acad. Sci.-Nat. Res. Counc.: Washington, D.C. (1963).
57. BODWELL, C. E., BROOKS, B., WOMACK, M., and LAKSHMANAN, F. Urinary sulfate and creatinine excretion in humans at high intake levels of proteins varying in nutritive value. Fed. Proc. 35: 342 (1976).
58. STEPHENS, A. G., and EVANS, R. A. The distribution of free amino acids between plasma and blood cells of chicks fed on different proteins. Proc. Nutr. Soc. 31: 50A (1972).
59. SCHUSTER, E. M., BODWELL, C. E., and WOMACK, M. Plasma and whole blood amino acid levels in humans at high intake levels of proteins varying in nutritive value. Fed. Proc. 35: 521 (1976).
60. SHEFFNER, A. L. *In vitro* protein evaluation. In: Newer methods of nutritional biochemistry, ed. by A. A. Albanese, p. 125. Academic Press: New York (1967).
61. McLAUGHLAN, J. M., and CAMPBELL, J. A. Methodology of protein evaluation. In: Mammalian protein metabolism, ed. by H. N. Munro, Vol. III, p. 391. Academic Press: New York (1969).
62. MAURON, J. Nutritional evaluation of proteins by enzymatic methods. In: Improving plant proteins by nuclear techniques, p. 303. Int. At. Energy Ag.: Vienna, Austria (1970).
63. MAURON, J. The analyses of food proteins, amino acid composition and nutritive value. In: Proteins in human nutrition, ed. by J. W. G. Porter and B. A. Rolls, p. 139. Academic Press: New York (1973).
64. BODWELL, C. E. The use of enzymatic assays for the determination of nutritional quality. In: Evaluating the nutritional quality of mutants with altered grain protein characteristics. Int. At. Energy Ag.: Vienna, Austria (In press).
65. HSU, H. W., VAVAK, D. L., SATTERLEE, L. D., and MILLER, G. A. A multi-enzyme technique for estimating protein digestibility. J. Food Sci. (In press).
66. SATTERLEE, L. D. Personal communication (1976).
67. CALLOWAY, D. H., and MARGEN, S. Variation in endogenous nitrogen excretion and dietary nitrogen utilization as determinants of human protein requirement. J. Nutr. 101: 205 (1971).
68. INOUE, G., FUJITA, Y., and NIIYAMA, Y. Studies on protein requirements of young men fed egg protein and rice protein with excess and maintenance energy intakes. J. Nutr. 103: 1673 (1973).
69. KURIEN, P. P., NARAYANARAO, M., SWAMINATHAN, M., and SUBRAHMANYAN, V. The metabolism of nitrogen, calcium and phosphorous in undernourished children. 6. The effect of partial or complete replacement of rice in poor vegetarian diets by kaffir corn *(Sorghum vulgare)*. Brit. J. Nutr. 14: 339 (1960).
70. CALLOWAY, D. H. Nitrogen balance of men with marginal intakes of protein and energy. J. Nutr. 105: 914 (1975).
71. GARZA, C., SCRIMSHAW, N. S., and YOUNG, V. R. Human protein requirements: The effect of variations in energy intake within the maintenance range. Amer. J. Clin. Nutr. 29: 280 (1976).

[Received December 27, 1976. Accepted April 4, 1977]