Methods of Estimating Protein Quality

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It has long been known that proteins differ greatly in their nutritive value. This can be demonstrated grossly by any number of methods such as comparison of rates of growth, nitrogen retention, or other measures of physiological performance of animals or human subjects consuming diets containing approximately equal amounts of different proteins. It is also clear that these differences are in most instances related to the amino acid composition of the proteins since additions of essential amino acids to proteins often greatly improve their nutritive value.

For a number of years (1,2,3) it has been assumed that some of these measures of nutritional quality were sufficiently exact and appropriate to allow calculation of the protein requirement when proteins of differing quality were consumed if the requirement for one particular protein was known. Thus, the general procedure recommended has been to estimate protein requirements using a dietary protein that is maximally utilized. The appropriate values for other diets containing proteins of lower quality are then obtained by multiplying this value by correction factors based on the protein quality. For example, if the protein requirement for individuals of a certain size, age and sex is X when the dietary protein is of maximal quality, the requirement would be 2X when the dietary protein is only 50% utilized, 4X when the dietary protein is only 25% utilized, etc.

This method of calculating protein requirements clearly requires that the measure of nutritive quality, whatever it may be, must vary from a maximum of 100 to a minimum of zero in a linear fashion. Recent observations raise grave doubts as to the validity of these assumptions.

Biological Value (BV)

Biological value, as defined by Thomas (4) and Mitchell (5,6) has long been considered the method of choice for estimating the nutritive value of proteins. It has been defined as the "percentage of absorbed nitrogen retained in the body" and a complete evaluation of the dietary protein includes measurement of the Biological Value and the Digestibility. These values are obtained by measuring the fecal and urinary nitrogen when the test protein is fed and correcting for the amounts excreted when a nitrogen-free diet is fed. True digestibility is defined as the percentage of food nitrogen absorbed from the gut

$$\text{Digestibility} = \frac{I - (F - F_D)}{I} \times 100$$

and Biological Value as

$$BV = \frac{I - (F - F_o) - (U - U_o)}{I - (F - F_o)} \times 100$$

where

I = Nitrogen intake of test protein

F = Fecal nitrogen

F_o = Fecal nitrogen on nitrogen-free diet (Metabolic N)

U = Urinary nitrogen

U_o = Urinary nitrogen on nitrogen-free diet (Endogenous N)

In practice Mitchell (6) found that the endogenous N was very similar to that obtained when a small amount of very high quality protein was fed and preferred to feed limited amounts of egg protein rather than a nitrogen-free diet in order to prevent severe weight loss. The basic assumption made in the measurement of Biological Value is that the endogenous N and metabolic N are constant values and can be legitimately subtracted from the test values as shown in the equation. There is limited information to suggest that this may not always be true. For example, the excretion of urinary nitrogen in rats and dogs on a nitrogen-free diet may be lowered substantially by the administration of methionine (7,8) yielding a Biological Value of methionine alone much above 100%. This may not happen in man (9) but has not been thoroughly studied. Also, Mitchell et al. (10) found the Biological Value of gelatin to be 20%, i.e., 20% as satisfactory as the best quality proteins. Since animals will not survive on gelatin alone, this must be an overestimate of the real nutritive value. The discrepancy here appears to be similar to that observed by Bender (11) in NPU values for diets that provided low intakes of most of the essential amino acids.

The overall nutritive value of a protein (Net Protein Value) should be obtained from the Mitchell method as Biological Value x Digestibility and this should be identical with NPU as defined below.

Net Protein Utilization (NPU)

Like Biological Value, NPU estimates nitrogen retention but in this case by determining the difference between the body nitrogen content of animals fed no protein and those fed a test protein. This value divided by the amount of protein consumed is the NPU which is defined as the "percentage of the dietary protein retained". Miller (12) proposed a procedure which involved replicate groups of 4 weanling rats housed in group cages which were fed either the "protein-free" or the "test" diet for 10 days. These conditions were chosen empirically and the particular merits of these conditions remain to be demonstrated. Since in young animals there is a high correlation between body nitrogen and body water content (13-16), the substitution of body water measurements for body nitrogen measurements has been widely used. Indeed, measurement of body water may be more accurate than

measurement of body nitrogen because sampling errors are eliminated; also, it is much more convenient and less expensive.

Since both NPU and BV are based upon estimates of "retained nitrogen", they should measure the same thing except that in the calculation of NPU the denominator is the total protein eaten whereas in the calculation of BV it is the amount absorbed. BV would be expected to be higher than NPU by the amount of nitrogen lost owing to lack of digestibility (lack of absorption). In weanling rats, it is possible that total carcass analysis is a more accurate measure of "retained nitrogen" that can be obtained from nitrogen balance measurements although this has not been proven. It is certainly less tedious. Nitrogen balance measurements must be used in large animals and in studies on man.

Amino Acid Score

Block and Mitchell (17) originally proposed that since all amino acids must be present at the site of protein synthesis in adequate amounts if protein synthesis is to proceed, a comparable deficit of any amino acid would limit protein synthesis to the same degree. Thus, they suggested that if the composition of an "ideal protein" was known, i.e., a protein which contained every essential amino acid in sufficient amounts to meet requirements without any excess, then it should be possible to compute the nutritive value of a protein by calculating the deficit of each essential amino acid in the test protein from the amount in the "ideal protein". The "most limiting amino acid", the one in greatest deficit, would presumably determine the nutritive value.

In practice they suggested the protein in whole egg as the "ideal" since this was known to have a Biological Value closely approaching 100. They recognized that egg proteins might contain some amino acids in excess of requirements. If so, deficits of these in other proteins calculated by this procedure would be misleadingly high. That is, the calculated nutritive value would be lower than it actually was. However, Block and Mitchell (17) compared Biological Values which were thought to have been accurately estimated and with "amino acid deficits" calculated using egg protein as the standard found a rather high correlation (r = .86) suggesting the overall validity of this procedure (Fig. 1).

Amino Acid Scores have been widely used since that time. Generally they have been calculated as the "percentage of adequacy" rather than as deficits as suggested by Block and Mitchell (17). The FAO Committee of 1957 (1) recognizing again that egg proteins might contain various essential amino acids in excess of the amounts required proposed that Amino Acid Scores be calculated from an amino acid pattern that was based upon estimates of amino acid requirements in man. A similar approach was recommended by the Amino Acid Committee of the Food and Nutrition Board (13). However, the second Expert Group of FAO/WHO (2) concluded that the previously suggested pattern was not appropriate in certain respects and that there was not sufficient information to state that egg, cow's milk or human milk proteins differed in nutritional quality. They thus suggested that any of these patterns might be considered "ideal" for the calculation of Amino Acid Scores. Since these three proteins differ substantially in amino acid composition, this suggestion has led to confusion in the calculation of Amino Acid Scores. They also suggested that the ratio of essential amino acid nitrogen to total nitrogen (E/T) was related to, and might be a determinant of, protein quality. Since no method was proposed for combining this ratio with the Amino Acid Score, this has led to further confusion.

Critique

As has been stated, the use of estimates of protein quality to calculate the amount of protein needed to meet requirements when different diets are consumed requires that the estimate of quality vary in some known fashion, preferably in linear fashion, from zero to 100% utilization. Actually, when Block and Mitchell (17) first proposed the use of Amino Acid Scores (Fig. 1), they found that Biological Value did not follow the predicted relationship with Amino Acid Score. Rather, the regression line relating BV and Amino Acid Score indicated that proteins completely lacking an essential amino acid and which would therefore have an Amino Acid Score of zero would apparently yield a BV of approximately 25% This would mean that the requirement could be met with such proteins if they were fed at a level providing four times the estimated minimal protein requirement. This presumably cannot be true since it would imply that the protein needs could be met without a supply of all of the essential amino acids.

This apparent discrepancy between theoretical predictions and experimental data has been largely ignored. Indeed, the FAO Committee of 1955 simply assumed that the relationship must fit theoretical expectations. Figure 2 is taken from that publication. Obviously with the scatter of the data available on BVs and uncertainties as to the amino acid composition of the proteins actually tested for BV, the true relationship was difficult to ascertain. However, it now seems quite clear that the relationship proposed by Block and Mitchell is, in fact, substantially correct. The values presented in Table 1 are plotted in Fig. 3 to show the relationship between BV and Amino Acid Score. The regression line calculated indicates that a protein of zero score would be predicted to have a BV of 40%. If BV is to be accepted as the true measure of protein quality, then proteins of zero score should be capable of meeting protein needs if they are fed in amounts 2½ times greater than that required with egg protein.

Comparison of NPU and Amino Acid Score values taken from Table 1 shows essentially the same relationship (Fig. 4) although with somewhat less deviation from expectation. According to this plot, a protein of zero score yields an NPU of approximately 25%. Thus, if NPU be accepted as the true measure of protein quality, protein needs can be met by feeding proteins of zero score at 4 times the minimal requirement.

The weakness of collecting values from a widely scattered literature in which the accuracy of neither the biological determination nor the amino acid analysis is known is, of course, recognized. However, this does not negate the clear fact that Amino Acid Score does not measure the same thing as NPU and BV.

It can be pointed out, of course, that when one is concerned with diets in which protein quality is reasonably high - NPU, BV or Amino Acid Score above 60 or 70, for example - the error in the correction will be relatively small regardless of which measure of protein quality is used. However, it is with diets of poor quality that correction is of real practical importance and for these the significance of the various measures of protein quality is in doubt.

The reasons for the discrepancy between theoretical prediction and experimental fact are now beginning to become clear. In essence the results deny the supposed fact that equivalent deficiencies of any essential amino acid will produce the same limitation on protein synthesis. Whether measures of BV or NPU reflect Amino Acid Score depends upon which of the essential amino acids is limiting although there is

still disagreement on the details of the relationship. It is clear that proteins limiting in lysine yield much higher BVs and NPUs than would be predicted by the Amino Acid Score. Thus Bender (11) concluded that a lysine-free diet will yield an NPU of approximately 40 and Said and Hegsted (18) reached similar conclusions. Values for proteins limiting in lysine are most divergent from theoretical predictions and there is disagreement as to how far values for proteins limiting in other essential amino acids deviate. However, protein scores of zero rarely yield NPUs or BVs of zero. Since many of the natural proteins with low NPUs or BVs which have been studied are limiting in lysine, it is to be expected that the relationship such as shown in Figs. 1, 3, and 4 is probably influenced largely by such proteins.

As previously mentioned, the basic assumption underlying the thesis that Amino Acid Score and BV or NPU ought to measure the same thing is that protein synthesis should be limited to an equivalent degree by a comparable degree of deficiency of any essential amino acid and that protein synthesis should cease if the diet is devoid of any essential amino acid. Thus, a diet of zero score is expected to be equivalent to a protein-free diet. Since diets devoid of various essential amino acids do not produce comparable losses in body protein, and only in some instances are the losses comparable to those obtained with a nitrogen-free diet, this thesis is no longer entirely tenable. One can only assume that the body has varying degrees of ability to conserve different essential amino acids when they are in short supply. When body tissues are broken down during catabolism, certain of the amino acids are efficiently conserved and thus supplement the supply of amino acids from dietary sources. According to the results obtained by Said and Hegsted (18) with the adult rat, lysine is the most efficiently conserved of all essential amino acids and this is supported by considerable information in the literature. They found threonine, isoleucine, and total sulfur amino acids to be least efficiently conserved although this is not in entire agreement with Bender. Information on nitrogen balance in adult women (19, 20) supports the contention that the adult human being responds, at least in general terms, in a manner similar to the adult rat.

These departures from the theory upon which protein metabolism has been based for many years raise many questions for which adequate answers are not available. If the body has varying ability to conserve specific essential amino acids and the mechanisms controlling this are unknown, there is a question as to whether a general "ideal amino acid pattern" can be defined. The data accumulating with animals and with human subjects (22, 23) indicate that the amino acid requirements probably vary depending upon the protein status of the subject. They also point to substantial differences in the pattern of amino acids required for maintenance and for growth. With regard to growth, it should be emphasized that accretion of new body protein does require essential amino acids over and above the maintenance requirement. The results thus point toward a difference in the "ideal" pattern for growth and for maintenance. As might be expected in view of the above discussion indicating that lysine is rather efficiently conserved, the lysine requirement for the growth of the young rat appears to be substantially higher (relative to several other essential amino acids) than for maintenance. The conclusion to be drawn from this in terms of human nutrition is not very clear, however. The data available upon the amino acid requirements of human beings of different ages have generally been interpreted to mean that the relative proportions of essential amino acids required at different ages are rather similar, although it cannot be proven that they are the same (24). It must be emphasized that even in relatively young children the rate of growth compared to body size is very slow compared to the rates of growth of young rats and many other species. Thus, the major proportion of the dietary protein which is required is utilized for the maintenance of tissues already formed rather than for the formation of new tissue proteins. The question must, therefore,

be raised as to whether estimates of protein quality based upon rapidly growing young rats are an adequate estimate of the quality of proteins for human beings, even for rather young infants and children.

Mitchell (17, 25) concluded that the Biological Values obtained with various species (rats, dogs, pigs, and man) follow approximately the same relationship when compared to amino acid composition. Mitchell (26) believed that failure of much of the data obtained with man to correlate well with Amino Acid Score was probably due to "imperfections in technique, quite understandable in a field of research beset with so many difficulties". However, the combined data (25) from different species plotted against Amino Acid Score yielded a regression similar to that obtained by Block and Mitchell (17). Thus, the departure from theory appears not to be due to the fact that most of the data in the literature have been obtained with rats. Rather, it appears to be a general phenomenon in several species. Mitchell specified that BV must be measured at or below the maintenance requirement, and thus these conclusions do not necessarily bear upon the appropriateness of BVs for infants and children or, indeed, for other species when they are fed sufficient protein to allow for growth.

An additional technical point with regard to the determination of NPU and BV should be made. If the nitrogen retained is designated Y and the nitrogen eaten or absorbed is designated X, then the ratio Y:X which is NPU or BV is the slope of the regression line relating Y to X. Obviously, if NPU or BV are constant and characteristic of the protein being studied, the slope of the regression line is constant which is to say that there is a linear relationship between Y and X. It has been tacitly assumed, but little investigated, that this relationship is generally true for all proteins. As shown in Fig. 6, some proteins such as lactalbumin do approximately fulfil expectation. However, with most proteins and to varying degrees, the situation is more like that shown for gluten in the same figure. Extension of the linear portion of the regression line would indicate that animals fed no gluten should lose approximately 12 g of body water whereas, in fact, animals fed no protein lost approximately 25 g of body water. The true line must approximate that shown by the dashed curved line at the lower right hand portion of the figure, although it is difficult to define the curve exactly.

As has been indicated in the discussion above, proteins limiting in lysine (12,18,27) are apparently most deviant from expectation. The reason for the curvature in the line must be that whereas at high levels of gluten intake in Fig. 6 lysine is the limiting factor, at some low level of intake either total nitrogen or some other essential amino acid becomes limiting. In any event, the major point which must be recognized is that NPU or BV as usually determined is not a constant or characteristic of the protein.

In the scheme developed by Miller and Payne (28,29) to combine protein quality and amount of protein into a single value, called NDpCals %, they assumed first that NPU measured at low levels of intake would yield a value equivalent to the Amino Acid Score. It is apparent that this is far from true especially for proteins of rather poor quality. They also assumed that NPU measured at low intakes was constant but that NPU fell progressively at levels above the maintenance requirement. This also is an erroneous assumption as is indicated above. Indeed, variations in NPU measured with young rats as the intake is increased are primarily due to the nature of the response shown in Fig. 6, line B, rather than decreased efficiency of utilization at higher levels of intake as they assumed. Thus, attractive as this concept appeared to be originally, it does not adequately reflect the response of animals to proteins of differing value fed at various levels of intake. It

should also be pointed out that since the protein and amino acid needs of young rats are dominated by the requirements for growth, the application of such formulas to human diets is of very doubtful validity.

Other Methods of Estimating Protein Quality

Protein Efficiency Ratio (PER)

As has been indicated, qualitative differences in protein quality can be demonstrated by many methods. Protein Efficiency Ratio (PER) has been the method most widely used because of its simplicity. Osborne, Mendel and Ferry (30) observed that young rats fed certain proteins gained little weight and ate little protein whereas those which were fed better quality proteins gained more weight and consumed more protein. In an attempt to compensate for the difference in food intake, they calculated the gain in weight per gram of protein eaten and this has been called PER. It is known that the PER for any protein is dependent upon the amount of protein incorporated in the test diet. Standardized conditions have therefore been proposed (31). These include the use of 10 weanling rats per test group, diets containing 9.09% protein (N × 6.25), a test period of 4 weeks' duration, and that each experiment include a group which receives standardized casein. The PER is calculated as the average total weight gain divided by the average grams of protein consumed. Since PER in various laboratories was not constant for the same protein, it was recommended that a corrected value be calculated using an assumed PER of the standardized casein of 2.50 (Corrected PER = 2.50 × PER/PER of reference casein).

In spite of its simplicity PER has been severely criticized as a measure of protein quality (32,33,34). The most common criticisms have been that some dietary protein is required for the maintenance of the animal and this is not credited to the protein in the measurement of PER and that body composition may vary and not be an adequate measure of nitrogen retention. From the theoretical point of view the major criticisms of PER are that it is not a direct function of the nutritive value of the protein but is related to the weight gain, the amount of food consumed, the amount of protein in the diet, and the nutritive quality of the protein in the diet. The relationship between these is complex and undefined. PER also has the disadvantage that even under standardized conditions it is not reproducible in different laboratories (31). It is of interest that in the collaborative study (31) corrected PER values showed larger differences between laboratories than the uncorrected values indicating that this correction was not appropriate and of no advantage.

It is clear that PER is not proportional to the nutritive quality of the proteins tested and, for example, a protein which demonstrates a PER of 1.5 cannot necessarily be assumed to have 50% of the value of a protein showing a PER of 3.0. Thus, a statement that "the total protein (must have) ..... a Biological Value not less than 70% of casein" such as has been proposed (35) as a standard for Textured Protein Products is not a meaningful statement. A judgment often can be made with PER whether a protein is better or worse than another protein but it is not appropriate to express these differences as percentages since the differences are not proportional to nutritional quality.

Net Protein Ration (NPR)

A major criticism of the PER has been that it does not take into account the protein required for maintenance since only gain in weight is used in the calculation. Bender and Doell (36) suggested that this criticism could be avoided by the inclusion in each test of a group of animals fed a protein-free diet. Net Protein Ratio (NPR) was then calculated as the overall difference in gain (gain in weight of the test group plus loss in weight of the protein-free group) divided by the protein eaten. It is apparent that if body composition is constant, this procedure is identical to NPU except that it is expressed in arbitrary units which are less useful than the percentage of protein utilized. The weaknesses are, of course, identical with those discussed under NPU.

Relative Nutritive Value (RNV)

Hegsted et al. (34, 37, 38, 39) proposed a slope-ratio assay using rats in which the slope of the regression line relating body protein (or body water) of a standard protein (egg protein or lactalbumin) assumed to have maximal nutritive value was compared to that of the test protein. The tacit assumption made in the measurement of NPU or BV that these values are independent of the level of protein fed is thus tested in this procedure. As in the calculation of NPU and BV the original assumption was made that the regression line should bisect the Y axis at the point defined by the group fed the protein-free diet. As has already been discussed above, this often and perhaps, usually, does not happen. The regression lines above the maintenance level of intake are, however, linear over a substantial range of intakes with young growing rats (40) contrary to the conclusions of Miller and Payne (28). In young growing rats where maintenance requirements are relatively small compared to the growth requirements, this method is probably the most logically defensible of the assays available as an estimate of the protein quality for growth. The important question remains as to whether estimates of protein quality for growth in young rats are adequate estimates of quality for man including those of the young infant. Presumably, many proteins will be more efficiently utilized in human beings than they are for young growing rats.

Nitrogen Balance Index

Allison and Anderson (41) showed, as has been discussed above, that Biological Value is the slope of the regression line relating nitrogen balance and nitrogen intake and suggested that this might have certain advantages in practice over the usual method of determining BV. The concept of this index is rather similar to Relative Nutritive Value discussed above. Since it is becoming increasingly clear that nitrogen retention is not linearly related to nitrogen intake in the region of intake below maintenance, the validity of this index requires confirmation.

Tissue Regeneration

A variety of techniques involving the recovery of weight or of specific tissues after protein depletion have been proposed (42, 43, 44, 45). The specific merits of such assays as opposed to weight gain of young rats, for example, remain to be demonstrated.

Microbiological Assays

Many micro-organisms require the essential amino acids required by monogastric animals. If it were possible to find organisms which required not only the same pattern of amino acids but in the same relative amounts, their growth response when supplied with limited amounts of various proteins or protein hydrolysates would provide a simple and efficient assay of nutritive value. Considerable effort has been directed toward this (46, 47, 48, 49) and it is clear that the responses of some organisms resemble those observed with some of the rat assays described. The difficulties are clear, however, since the limitations in the animal assays mean that they provide an inadequate base for comparison with assays of this kind.

Plasma Amino Acids

As has been indicated in another section of this report, changes in plasma amino acid levels after the feeding of various proteins can under certain conditions yield estimates of the nutritional quality. It may be noted, however, that the range of each of the amino acids in the plasma in normal animals is relatively large. This variability imposes serious limitations upon the quantitative interpretation of any changes in the levels observed. Thus, while it may be possible to identify the limiting amino acid in certain proteins by this technique, the likelihood that good quantitative assays for nutritional quality can be developed using plasma amino acid levels is not promising.

Effects of Digestion and Availability of Amino Acids

If significant quantities of nitrogen or essential amino acids are not absorbed from the gut when a protein is fed, the nutritional quality of that protein will be impaired. There is much information to suggest that with many proteins digestibility is not a primary factor in determining nutritive value but there is insufficient detailed information on the subject. It may be noted that if a portein is 95% digested, i.e., only 5% is not absorbed, this would not be very significant if the portion not absorbed were of the same composition as the total protein. If, however, this relatively small fraction contained primarily one or two amino acids, the effect would be considerable.

Such information as is available upon the amino acid content of feces from animals fed various proteins does not indicate great selection in the digestive or absorptive processes. However, it has been shown that large amounts of amino acids are secreted into the gut (50, 51) and the actual source of the amino acids which eventually appear in the feces is unknown. Neither the destructive or synthetic capabilities of the gastrointestinal flora appear to have been investigated. Thus, while amounts of amino acids in the feces may be useful in comparative investigations where different proteins are studied, there is considerable doubt that the amounts of amino acids in the feces are an absolute measure of lack of digestion.

It should be noted that the formula on page 2 for the determination of "true digestibility" is based upon the assumption that F_o, the nitrogen in the feces when a protein-free diet is fed, is constant and independent of the diet. There is considerable evidence to show that this is not strictly true and that it varies with the kind and amount of food consumed (52, 53, 54). Although the "bulk" and

"roughage" in a diet have been often assumed to be major factors affecting fecal nitrogen excretion, direct studies (55, 56, 57) do not confirm these assumptions. Since a large proportion of the feces is composed of bacteria and relatively little is known of the factors which control the intestinal flora (54), the basic concepts underlying the assumptions made in the determination of digestibility may be questioned. One might suppose that when relatively pure proteins are compared, the remainder of the diet being held essentially constant, valid comparisons could be made. However, when crude food materials are tested there may be a multitude of factors which influence the amount and kind of fecal flora, the rate of transit through the gut, the excretions into the gut, etc., and it is doubtful that digestibility of proteins should be considered to be a function of the protein fed. Presumably true values for digestion of proteins might be obtained by the use of isotopically labelled food proteins. However, since the "amino acid pool" is rapidly labelled with dietary proteins which then appear in the gastrointestinal secretions, even this approach has serious limitations.

In processed foods, particularly those subjected to heat treatment, there may be reactions which result in the destruction of amino acids or the formation of compounds which are not easily digestible or utilized by the mammalian organism (58,59,60). These kinds of reactions apparently involve primarily lysine, methionine, arginine, histidine and tryptophan. "Unavailable lysine" has been particularly studied, especially by Carpenter and co-workers (61). They have used a method involving the reaction of the terminal amino group in lysine with 1-fluoro-2,4-dinotrobenzene. During heat treatment this amino group may react with carbohydrates or possibly other materials and is then unavailable to react with fluronitrobenzene. In some tests (62) the available lysine determined by the chemical method was shown to correlate reasonably well with available lysine determined by animal assay but in other studies (63) less encouraging results have been obtained. Comparable chemical methods for other amino acids are not available. Microbiologic assays (47,48) have also been used to assess the "availability" of various amino acids. The significance of all such methods clearly depend upon clear evidence that they do indeed reflect availability of the amino acids to animals. There is essentially no evidence available relating the results of these kinds of methods on "available amino acids" to human nutrition.

Various in vitro methods (64,65) have been proposed to evaluate "digestibility". While large differences in the rate and extent of release of amino acids from different proteins can be shown, the relationship between such findings and "true digestibility" in the gastrointestinal tract is relatively unexplored.

There is abundant literature (59,66,67) demonstrating poor digestibility and other untoward effects when raw legumes of various kinds are fed to animals. These products contain heat labile "trypsin inhibitors" and hemagglutinins. However, it is not certain that these factors explain all of the diverse effects observed such as hypertrophy of the pancreas and increased need for methionine. The significance of these effects in human nutrition is relatively unknown. Since legumes are usually cooked, they may be of minimal importance.

Conclusions

A critical examination of the various methods in use for the evaluation of protein quality reveals a less satisfactory situation than has generally been assumed. The failure of NPU and BV to show the expected relationship to Amino Acid Score is presumably due to difference in the rate at which different essential amino acid

deficiencies develop. The longer period required to produce severe lysine deficiency as compared to most other amino acid deficiencies, for example, yields higher NPUs or BVs than expected. Thus, Amino Acid Score may be the most logically satisfying method of evaluating protein quality. This obviously depends upon a correct evaluation of amino acid requirements. It will remain relatively undefensible, however, until improved or new biological methods are available to estimate protein quality.

Protein quality for growth with young animals such as the rat can be measured reasonably well. The major question to be answered is whether or not these measurements are sufficiently pertinent for a species, such as man, which grows very slowly. It appears likely that the quality of many proteins is relatively higher for adult or slowly growing species than for the young growing rat. This implies a difference in both the amount and proportion of the essential amino acids required for maintenance and growth.

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Table 1

QUALITY OF PROTEINS BY VARIOUS MEASURES*

Protein Source	BV	NPU	PER	Chemical Score
Buckwheat	77			51
Maize	59	51	1.12	41
Oats	65	66	2.19	57
Rice, polished	64	57	2.18	56
Rice, whole	73			57
Sorghum	73		1.78	31
Wheat, whole	65	40	1.53	43
Wheat, germ	74	67	2.53	54
Wheat, gluten	58	39		26
Wheat flour	52		0.60	28
White bread		37	0.89	
Potato	67			34
Beans (various)	58	40	1.48	34
Black gram	70		2.12	
Lima beans	66	52	1.53	41
Broadbeans	55	48		28
Chickpea	68		1.71	40
Cowpea	57	45		41
Groundnuts	55	43	1.65	55
Ground protein isolate	58		1.58	
Loblah bean	77	60		27
Lentils	45	30	0.93	31
Njugo bean	56			51
Peas	64	47	1.57	37
Pigeon pea	57	52	1.54	37
Soybeans	73	61	2.32	47
Soy milk			2.10	55
Velvet bean	40	27		
Coconut	69		2.14	55
Cottonseed meal	67	53	2.25	47
Linseed	71	56	2.11	59
Pecan	60			61
Sesame	62	53	1.77	42
Sunflower seed	70	58	2.10	61
Alfalfa	57			50
Turnip green	52			33

Lupine	83			25
Beef	74	67	2.30	69
Chicken	74			64
Egg	94	94	3.92	100
Fish	76	80	3.55	71
Crustaceans	81			66
Molluscs	81			71
Fishmeal	81	65	3.42	60
Casein	80	72	2.86	58
Cow's milk	85	82	3.09	60
Brewer's yeast	67	56	2.24	57

* Values selected from the FAO publication (68) for which several different measures are available.

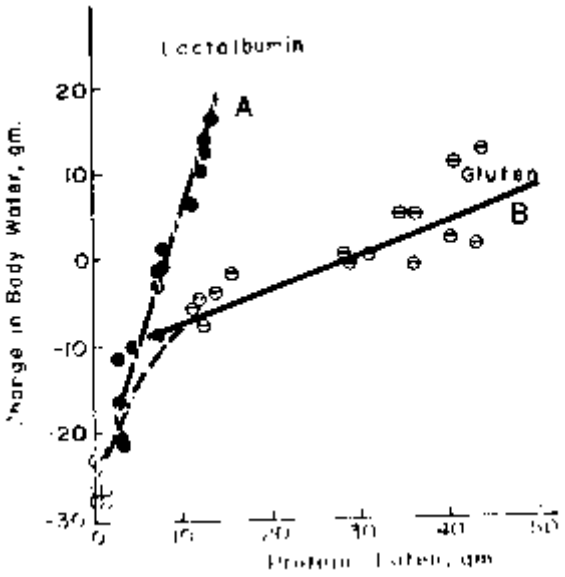

Fig. 1. Original figure from Block and Mitchell (17) relating "Amino Acid Deficit" to Biological Value. Note that 100% deficit would apparently yield a BV of about 30%.

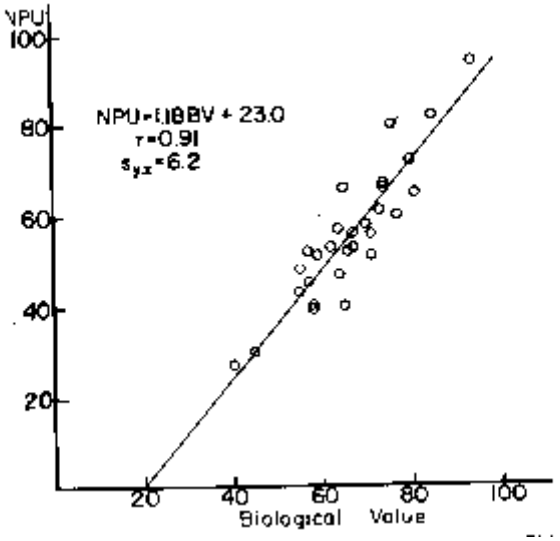

Fig. 2. Figure taken from the 1957 report of FAO on Protein Requirements (1). The Committee assumed that and Amino Acid Score of zero should yield a BV of zero.

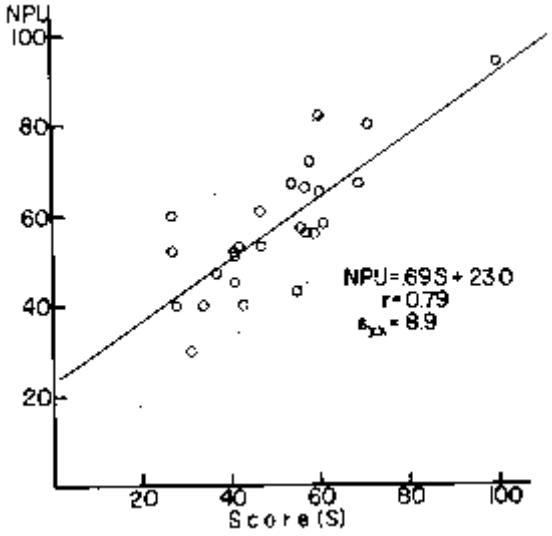

Fig. 3. Data taken from Table 1. Amino Acid Score of zero apparently yields a BV of about 40%. The dotted lines define 2 standard errors on either side of the regression line. The 3 points in the upper left hand portion falling outside the dotted lines were omitted in calculating the regression line.

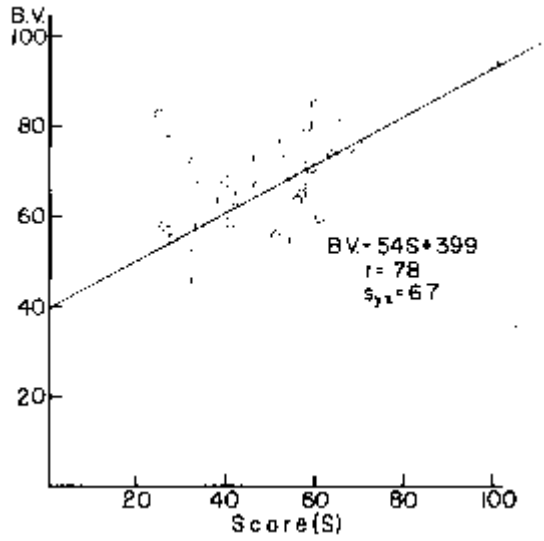

Fig. 4. Relationship between NPU and Amino Acid Score from data in Table 1. Dotted lines define 2 standard errors on either side of the line.

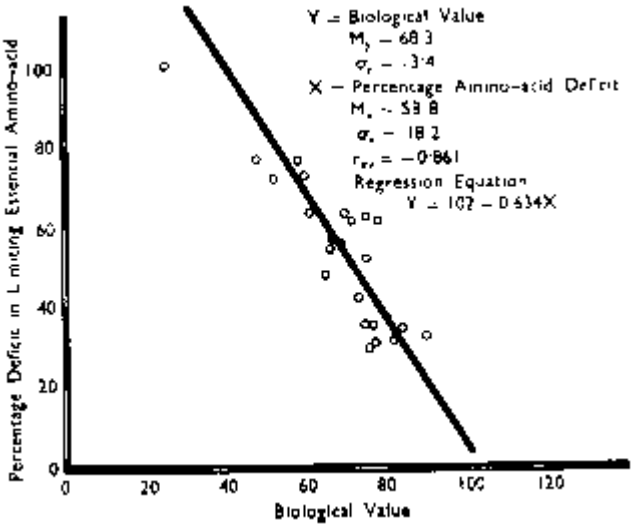

Fig. 5. Relationship between NPU and BV in data from Table 1.

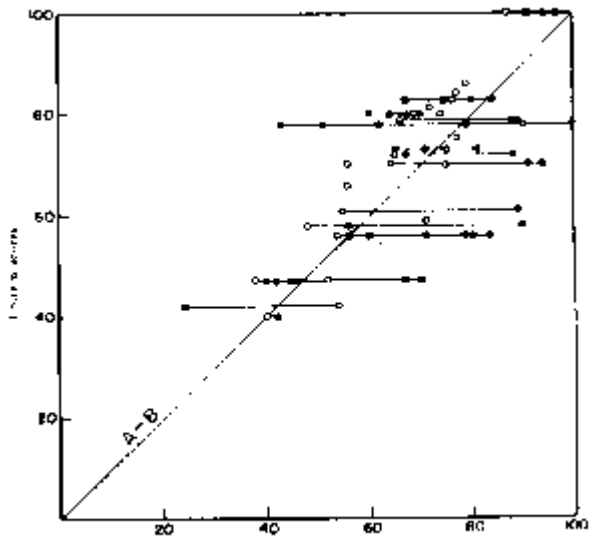

Fig. 6. Theoretical relationship between protein retained (Y) and protein eaten (X) is indicated by the line A obtained with lactalbumin. Since Y:X, the slope of the line, is NPU, the NPU is constant and a characteristic of the protein. The more usual relationship is demonstrated by line B, the data obtained with gluten. Y:X is obviously not constant at low levels of intake. Data from Said and Hegsted (18).