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1)**Biological Value of Food Proteins**

The biological value (BV) of a protein is an expression of a number of the nutritional characteristics of the food. These include (1) the digestibility, (2) the availability of the digested products, and (3) the presence and amounts of the various essential amino acids. The biological value can be calculated by determining the nitrogen of the food intake minus the urinary and fecal nitrogen excretions by the formula:

BV=Dietary N-(Urinary N+Fecal N)Dietary N-Fecal N×100.

The biological value of some proteins fed either to rats or humans is given in Table 7.2. The biological values reported in the literature for some of the proteins by different researchers vary in numerical value, suggesting that as yet, no definite assessment can be made. When 70% of the intake of nitrogen is retained (a biological value of 70) the protein will support growth if sufficient calories are available; with biological values of less than 70, questionable growth occurs.

Concurrently catabolism and anabolism of protein are occurring in the body; for anabolism to operate efficiently, all of the essential amino acids must be available in the sufficient quantity. If they are not, excess nitrogen is excreted in the urine due to the catabolic process. When this occurs, less absorbed food nitrogen is retained by the body and low biological value results from feeding this protein or protein mixture.

The biological value of soybean proteins is improved by heat treatment, probably due to methionine becoming more readily available to the organism. In the untreated soybeans the release of methionine seems to be delayed so that absorption occurs too late in the intestinal transit. For optimum utilization of protein all the essential amino acids must be liberated during digestion at rates allowing mutual supplementation.

2) **METHODS OF ASSESSMENT OF PROTEIN QUALITY**:

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.

i) Biological Value (BV)

ii) Net Protein Utilization (NPU)

iii) Amino Acid Score

iv) Relative Nutritive Value (RNV)

v) Nitrogen Balance Index

vi) Tissue Regeneration

**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

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

**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.