**1. BIOLOGICAL VALUE OF PROTEIN**

Biological value of protein is the percentage of a protein nitrogen that is absorbed and available for use by the body for growth and maintenance.

Proteins are functionally divided into complete, partially complete and incomplete proteins. A complete protein contains all essential amino acids in relatively the same amounts as human beings require to promote and maintain normal growth. (eg) Protein derived from animal foods. A partially complete protein contains sufficient amounts of amino acids to maintain life but fail to promote growth. (eg) Gliadin in wheat. Incomplete proteins are incapable of replacing or building new tissue and cannot support life or growth. (eg) Protein in Wheat germ.

The quality of a protein is determined by the kind and proportion of amino acid it contains. Proteins that contain all essential amino acids in proportions capable of promoting growth are described as complete protein, good quality protein, or proteins of high biological value. A good quality protein is digested and utilized well. Egg protein is a complete protein and is considered as a reference protein with the highest biological value. The quality of other proteins is determined based on their comparison with egg protein.

The eight essential amino acids (EAA) must be present in a protein in specific ratios. Egg protein has all eight in the correct proportions used most efficiently and completely by the body. Wheat germ is an incomplete protein because it is deficient in tryptophan. As a result of this deficiency only less of the total protein can be used.

The protein of animal foods like milk, meat, and fish generally compare well with egg in the essential amino acid composition and are categorized as good quality proteins. Plant proteins are of poor quality, since the essential amino acid composition is not well balanced. The amino acid, which is not present in sufficient amount in food protein, is called the limiting amino acid of that food. For (eg) Lysine in cereal protein, Tryptophan in Wheat germ.

**2. Methods of assessment of protein quality**

* Biological Value (BV)
* Net Protein Utilization (NPU)
* Amino Acid Score
* Protein Efficiency Ratio (PER)
* Net Protein Ration (NPR)
* Relative Nutritive Value (RNV)
* Nitrogen Balance Index

**Biological Value (BV)**

Biological value, as defined by Thomas and Mitchell 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

Digestibility = I -(F - Fo ) × 100

I

and Biological Value as

BV = I - (F - Fo )- (U - Uo ) × 100

I -(F - Fo )

where I = Nitrogen intake of test protein F = Fecal nitrogen Fo = Fecal nitrogen on nitrogen-free diet (Metabolic N) U = Urinary nitrogen Uo = Urinary nitrogen on nitrogen-free diet (Endogenous N)

In practice Mitchell 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 yielding a Biological Value of methionine alone much above 100%. This may not happen in man but has not been thoroughly studied. Also, Mitchell et al. 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 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.

**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 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, 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 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 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. 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. The FAO Committee of 1957 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. However, the second Expert Group of FAO/WHO 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.

**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 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. 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. 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. It is of interest that in the collaborative study 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 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 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 explained under NPU.

**Relative Nutritive Value (RNV)**

Hegsted et al. 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 explained, 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 contrary to the conclusions of Miller and Payne. 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 showed, as has been explained, 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. 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.