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**Biological value** (**BV**) is a measure of the proportion of absorbed protein from a food which becomes incorporated into the proteins of the organism's body. It captures how readily the digested protein can be used in protein synthesis in the cells of the organism. Proteins are the major source of nitrogen in food.

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.

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.

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).

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.

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.