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1. What do you understand by the term biological value of protein.

Biological value: Biological value of protein is defined as the percentage of absorbed nitrogen retained by the body and is calculated by:

$$BV = \frac{\text{nitrogen retained}}{\text{nitrogen absorbed}} \times 100$$

The amount of nitrogen in the diet eaten and in excreta of adult animals are measured and the percentage of nitrogen retained by animals from out of nitrogen absorbed from the diet is calculated. The value thus obtained is the biological value (BV) of the protein

2. List and explain the various methods of assessments of 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 ($N \times 6.25$), 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 $PER = 2.50 \times PER / PER \text{ of reference casein}$).

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.