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**1. WHAT DO YOU UNDERSTAND BY THE TERM ''BIOLOGICAL VALUE OF PROTEINS"**

**2. LIST AND EXPLAIN THE VARIOUS METHODS OF ASSESSMENT OF PROTEIN QUALITY.**

1. 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.

2.

**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.

**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.

**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.

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 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.