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Bioavailability: A Factor in Protein Quality

Constance Kies

While the amino acid proportionality pattern of a protein is probably the most important determinant of protein quality, bioavailability of these constituent amino acids consitutes the second most important variable. The degree to which the constituent amino acids of a food protein are actually available to the body is determined by such factors as protein configuration, amino acid bonding, other constituents of the diet, and the physiological condition of the gastrointestinal tract of the individual involved.

The most important determinant of the nutritional quality of a protein is its amino acid composition as compared to the amino acid requirements of the organism consuming it. If protein quality were, in fact, merely a function of amino acid proportionality patterns, scores based on chemical analyses of amino acid composition of food products would give easy, exact predictions of protein quality based on performance in living organisms (WHO/FAO, 1973; National Academy of Sciences, 1978; Block and Mitchell, 1946). Unfortunately, this is not the case (Holmes, 1965). Part of the problem is associated with the obtainment of fast, accurate information on the amino acid composition of the proteins in food products. In spite of remarkable advances in methodologies and instrumentation, analyses of the important essential amino acids methionine and tryptophan still present difficulties.

The other side of the ratio, quantitative requirements of the essential amino acids, presents even more difficulties, at least when the problem of protein quality in human nutrition is addressed. In spite of efforts of such pioneering scientists as Rose et al. (1955), Leverton (1959), Swendseid et al. (1956), Nakagawa et al. (1964), and Holt et al. (1960) as reviewed by Irwin and Hegsted (1971) on amino acid requirements of human men, women, and infants, questions exist not only on quantitative requirements but also even on the essentiality of such amino acids as histidine.

Even with these admitted difficulties, the correlation between prediction and performance is quite good, particularly at extreme ends of the curve. In other words, prediction for poor performance of proteins devoid or nearly devoid of an essential amino acid is excellent. Similarly, the prediction of good performance for proteins containing all essential amino acids according to idealized patterns is also excellent. However, fine-line predictions of intermediate quality are less accurate. Surprises are not uncommon. Improvement of amino acid proportionality patterns by fortification or by genetic selection as in development of high-lysine cereals does not always result in expected improvements in protein quality. Food processing results in changes in protein quality which cannot be explained by obvious alterations in amino acid constituents.

Deviations between prediction of protein quality based on amino acid content/amino acid requirement ratios and actual protein quality based on performance in living organisms assuming accurate determination of both of these factors would seem to be due to variations in the utilization of the amino acids comprising different proteins. More simply, the required essential amino acids may be there and may be there in ideal amounts, but the efficiency to which they may be used constitutes another whole series of problems. In part, many of these factors may be sub-

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classified under the general heading of bioavailability.

Bioavailability is a newly popularized catchterm for a long-known important factor in determining protein quality. However, a commonly accepted definition and application of the term by no means exists. For purposes of this paper, bioavailability will be defined as the degree to which amino acids or small peptides from a test protein consumed by a living organism ultimately are transported across the intestinal membrane and into the body. It thus includes digestibility of proteins, timing of digestibility of protein, and absorption mechanisms. It does not include efficiency of usage of the absorbed amino acids within the body proper, an important consideration but one more appropriately covered by the term bioutilization. Bioavailability of protein by the human will be stressed in this paper.

METHODS OF MEASURING BIOAVAILABILITY

In the human, the most common approach to measuring bioavailability is as part of the classical nitrogen balance procedure. In the approach, the test protein is fed as the nearly sole source of protein. The protein intake is expressed in terms of protein nitrogen. Nitrogen content in feces is also measured during a time sequence to equal the food residue excreted from the test protein. This is usually accomplished through use of feeding dye or opaque markers. Fecal nitrogen loss is also measured when the human subjects or test animals are fed a protein-free diet, a figure assumed to be equal to endogenous protein loss. The percent bioavailability is equal to the dietary nitrogen minus fecal nitrogen corrected for endogenous fecal nitrogen divided by the dietary nitrogen times 100. This formula is obviously identical with that often used to estimate protein digestibility. For both, the method is indirect; however, the assumption that fecal nitrogen largely represents unabsorbed nitrogen rather than protein which has not been broken down into absorbable units seems somewhat more acceptable.

Various in vivo methods to more directly measure protein bioavailability have been suggested. Measurement of changes in blood serum amino acid patterns at timed intervals following consumption of a test protein has been one approach. This approach has several disadvantages. In human subjects it is usually inconvenient to measure changes in portal blood amino acid patterns. Changes in venous blood in the general circulation do not really represent immediate changes in levels of absorbed amino acids. Changes in blood serum amino patterns are difficult to quantify. Amino acids are absorbed at different rates; hence, timing of the taking of blood samples is important. Of the total amino acid available for absorption in the intestinal lumen, only a small portion is from food. The major portion is from endogenous protein, as determined by Nasset (1965) and Nasset and Ju (1961). Thus, changes in blood serum amino acid patterns following ingestion of test proteins can be expected to be quite small.

Radiotracer studies also offer some promise in studying both the digestibility and absorbability phases of bioavailability. However, these approaches are currently difficult to do on a routine basis using human subjects because of ethical concerns of committees for human subject's rights.

In vitro assays of protein digestibility have recently received considerable research attention (Evancho et al., 1977; Saunders et al., 1973; Hsu et al., 1978). Valid in vitro methods of digestibility should give some indication of bioavailability of food proteins. Such enzymatic approaches as the pepsin digest residue method and pepsin pancreatin digest dialysate index method have been designed to better measure protein quality (Marable and Sanzone, 1980) but have been somewhat disappointing at this point. However, cost and time factors undoubtedly justify further research in this area.

BIOAVAILABILITY AND DIGESTION

Bioavailability of amino acids/proteins in part but not totally is a discussion of digestibility of protein. Through in-depth understanding of chemical digestion of proteins, it may be possible to develop in vitro methods of assaying protein digestion, which hopefully would be far easier, cheaper, and less time consuming than the in vivo methods currently employed. Furthermore, in-depth understanding of protein digestion may give information of use in improvement of the protein quality value of certain food products. But, while certain attributes, reactions, and factors may be absolutely true as demonstrated in the chemistry, physics, physiology, and nutrition laboratory, their importance to practical human nutrition may be practically nil. If regulatory agencies have, at times, been guilty of overstress, it may be that I as well as other research scientists may be partly at fault for having overstated the importance of our own field of research.

Chemical digestion of proteins has generated a considerable amount of research effort; hence, a considerable body of knowledge and a considerable amount of controversy exist. Protein digestion is in part influenced by protein configuration. One of the earliest classification systems of proteins was based on the solubility of proteins, characteristics which have an impact on the digestibility. As a result of various types of bonding, proteins form structures of essentially two shapes. These are fibrous proteins which tend to be insoluble, tough, and resistent to digestion such as collagen, keratins, and elastin and globular proteins which tend to be fairly soluble and have relatively high digestibility characteristics. These include albumins, globulins, and histines. However, such common globular proteins as albumins and globulins differ in their solubility and digestibility characteristics. Furthermore, proteins within food products are usually in combination with lipids, various metals, nucleic acids, or carbohydrates. These conjugated proteins can have quite different characteristics than the protein entity might suggest. As more information on chemical structure and physical characteristics is obtained, more precise systems of protein classification with relationship to function can be achieved (Hess and Rupley, 1971).

The chemical/physical reactions involved in protein digestion have been reviewed by Gilter (1964), Sleisinger (1979), and Munro and Crun (1980). Under normal circumstances, chemical digestion of protein originates in the stomach. Chief cells secrete the preenzyme pepsinogen, which is activated in the presence of HCl or pepsin itself to its active form, pepsin, by removal of a blocking peptide. Depending upon the system of classification, three to seven pepsins exist (Davenport, 1971). Pepsin rapidly breaks protein into 2–50 amino acid sized peptides by breaking the peptide bond on the amino side of aromatic amino acids (phenylalanine and tyrosine). In the stomachs of infants, the enzyme rennin acts on the milk protein casein in the presence of calcium ions to form calcium paracaseinate, a milk clot or curd which slows the passage of the protein in the intestinal tract, apparently allowing additional time for enzyme activity. While the existence of rennin in the calf has been well established, its existence in the human infant has been questioned. Another enzyme in gastric juice is gelatinase which acts on gelatin.

Efficiency of protein digestion in the stomach and thus to a degree the ultimate bioavailability of the constituent

amino acids is to a great extent determined by stomach pH. The variability in HCl secretion among different people may in part account for the great variability in efficiency of pepsin digestion from one individual to another.

The occurrence of fats, protein, carbohydrate, or HCl in the upper small intestine triggers the secretion of the hormone secretin into the small intestine which in turn is absorbed from the intestines into the blood stream and is carried to the pancreas where it stimulates the secretion of a pancreatic juice high in bicarbonate and low in enzyme content. Later, the hormone stimulates the pancreas to secrete a pancreatic juice of the opposite concentration, low in bicarbonate and high in enzyme. This change in content is seemingly mediated by another hormone, cholecystokinin (pancreozymin) (Adelson and Rothman, 1974). All the pancreatic enzymes involved are secreted in an inactive state.

Among the most widely studied is trypsin which is converted by the enzyme enterokinase from its inactive form, trypsinogen. Trypsin acts to break up peptide linkages on the carboxyl sides of arginine and lysine. Trypsin itself can activate trypsinogen. Trypsin also acts to convert all three forms of chymotrypsinogen to the active chymotrypsins A, B, and C. As does trypsin, all three of these enzymes act to break peptide linkages on the carboxyl side, but chymotrypsin A acts on linkages next to phenylalanine, tryptophan, and tyrosine, B on leucine, and C on methionine and glutamine. Trypsin also acts to convert the two procarboxypeptidases to carboxypeptidases A and B. Carboxypeptidase A breaks the peptide linkage on terminal amino acids that have a free carboxyl group, but also an aromatic or aliphatic side chain, while B splits off only basic amino acids. Other changes may be involved in the activation of procarboxypeptidase. Trypsin also is involved in a feedback mechanism which governs the amount of trypsinogen secreted (Ochoa-Solano and Gitler, 1968).

Enzymes involved in the digestion of proteins are also secreted in intestinal juice, for example, aminopeptidase which splits off terminal amino acids with a free amine group. Thus, proteins are ideally broken down to yield free amino acids or very short chain amino acid fragments for absorption. Intestinal enzyme digestion seemingly occurs within the brush border of the intestinal cell rather than in the intestinal lumen (Wiseman, 1974).

Absolute or apparent lack of digestive enzymes can result in decreased digestibility of a protein and hence a lowering of its bioavailability. What is "normal" and what is "abnormal" in relationship to bioavailability of protein by humans is not an absolute since the inability to utilize proteins may range from the very mild to the very severe. One example of an abnormal inability to utilize a cereal protein is Celiac's disease or gluten-sensitive enteropathy or nontropical sprue. Individuals with this condition characteristically develop a steatorrhea, weight loss, and malnutrition when fed products containing gliadin. When placed on diets eliminating wheat, oats, barley, and rye, dramatic relief of symptoms is usually seen. It was first thought that the condition was due to a relative or complete lack of an enzyme for breakdown of a specific amino acid bound fraction of gliadin. However, current theories to account for the damage of the intestinal mucosa which occurs in this condition are more complex (Katz and Falchuk, 1975).

BIOAVAILABILITY AND ABSORPTION

Bioavailability is not merely a matter of digestion. This implies that the greater the degree of digestibility the greater is the degree of bioavailability. This simply is not always the case. The considerable, pioneering research dealing with amino acid absorption has been reviewed by Wilson (1962). Timing of individual amino acids or combination of amino acids is of importance. Obviously, protein digested by microorganisms in the lower bowel is of little or no use since the sites of active amino acid absorption are long since past; hence, such digestion offers no contributions to protein quality. Several active transport systems have been defined for the absorption of groups of amino acids. Excessive levels of one amino acid having a particularly high transport affinity can inhibit others of the same transport group; in other somewhat similar situations, the end result is an enhancement of absorption of amino acids. Seemingly, amino acids first available are first absorbed in competition of groups of amino acids for the same shared transporting mechanisms. This may explain why amino acid supplementation of food products to improve protein amino acid proportionality have not resulted in the expected protein quality value improvements.

As previously stated, it is generally assumed that differences in nutritional value of food proteins having similar amino acid proportionality patterns are largely due to differences in digestibility of proteins. If this assumption is true, then mixtures of purified amino acids formulated according to the proportionality patterns of food proteins should have better nutritional value as judged by standard human bioassay tests than do the food proteins themselves. The reverse, in fact, has been found to be true. In evaluations involving both highly digestible proteins such as egg and less digestible protein sources such as corn, the food source of protein routinely gave better nitrogen balance test results than did the counterpart amino acid diets (Choo, 1960; Kies, 1960; Nasset, 1957; Rose et al., 1954). Explanations for these results included the following: (a) that the immediate availability of purified amino acids might lead to overall overtaxing of absorption mechanism systems or competition among particular amino acids might lead to selected competitive inhibition of amino acid absorption and hence to reduced efficiency of amino acid utilization; (b) that rapid absorption of amino acids over a short period of time rather than over a longer period of time might overtax the body's ability to efficiently use amino acids for protein synthesis purposes, thus leading to increased deamination of amino acids and utilization for energy purposes; (c) that experimental diets containing purified amino acids were not truly matched to those containing food proteins in contents of all trace nutrients and results reflected these deficits.

Less well known is the research involving comparisons of peptides and amino acids as reviewed by Mathews and Abibi (1976), Silk (1974), and Sleisinger and Kim (1974). Here again peptides were found to give better results than constituent amino acids. Most of this work has involved comparisons between dipeptides and purified amino acids. It has been postulated that two basic, separate mechanisms may be involved in peptide abosorption: (1) digestion of peptide enzymes in the brush border of the intestinal mucosa and subsequent absorption via usual amino acid absorption mechanisms and (2) absorption of peptides into intestinal mucosa cells and digestion of peptides intercellularly before release into the circulation. It is believed that this latter mechanism is both more efficient in (1) being less affected by competitive amino acid absorption inhibition and less affected by other aspects of intestinal intraluminal environment and (2) offering some kinetic advantages.

Comparisons among utilization of proteins, peptides, and amino acids are certainly of theoretical interest and also may have some practical impacts. One example is in the formulation of liquid diets for enteral (using the gastrointestinal tract) nutrition. These products are widely used in hospital situations to provide total or partial nutrition of patients with a wide variety of pathological characteristics. Examples based on intact proteins, polypeptides, and amino acids are currently being marketed.

The objective of a project in our laboratory was to compare the ability of several liquid formula diets for enteral nutrition based on protein, peptides, and amino acids to meet protein needs of assumed healthy human adults (Kies and Fox, 1980a,b). The purified amino acid based products could be thought of as predigested at the 100% level. Results indicated that the amino acid based products were more poorly utilized than were peptide- or protein-based products.

FACTORS INFLUENCING BIOAVAILABILITY

What factors determine bioavailability of proteins? First and foremost of these factors is who eats it. Different animal species differ in their ability to digest cereal proteins because of differences in their digestive enzyme systems and other physiological/biochemical differences within their gastrointestinal systems.

Even within the same species, considerable variation exists among members of that species. Functions involving the gastrointestinal tract seem to be particularly variable within the confines of what is generally considered to be normal. Bioavailability of a protein measured within the same individual can vary considerably. This is probably because even minor day-to-day physiological/psychological stress can cause profound changes in gastrointestinal tract activity which, in turn, can result in alterations in bioavailability measurements. In this day and age when it is not unusual to measure certain constituents of foods in parts per billion with a high level of reproducibility and accuracy, one simply cannot expect high reproducibility on protein bioavailability figures with humans.

Characteristics of proteins certainly account in part for their level of availability. Protein digestion is extremely complicated with stimulators, preenzymes, activators, specific peptide bonds, and feedback mechanisms. Furthermore, absorption of amino acids is complicated. Considerable research has been carried out on mechanisms of amino acid absorption, competition among amino acids for absorption sites, and, more recently, absorption of peptides. For efficient absorption, it would appear that not only must release of amino acids (or peptides) occur but also this must occur at the right time and in the most ideal mixture of other amino acids and peptides. Thus, it would appear that at least part of the explanation of the level of bioavailability of any specific protein must lie with its specific physical/chemical properties as determined by its amino acid content, amino acid sequence, and specific bonding. These characteristics have not been investigated extensively. If bioavailability of proteins is studied by feeding a product, it is difficult to differentiate between factors associated with the protein and those associated with other constituents of food products. If the protein or proteins of the food are isolated prior to feeding, it is difficult to determine how much the observed bioavailability was influenced by the processing techniques used in its isolation.

And processing techniques do influence the bioavailability of proteins—some positively and some negatively. Sometimes the same process can have positive effects on one characteristic and negative effects on another. An example of this is the milling of flour. Reduction of the grain size, breakage of the hard outer kernel, and possibly removal of the bran layer increase the surface area, making enzymatic digestion much more efficient. However, the heat generated in the milling process may adversely affect availability. Among the more common kinds of damage which may result in decreased bioavailability of proteins due to processing include the following as reviewed by Munro and Crim (1980). (1) Available lysine is lost due to milk heat treatment in the presence of reducing sugars (the free amino groups on the lysine side of the protein form an additive product with the reducing group of the sugar; this product undergoes rearrangement to form fructoselysine). (2) With severe heating, proteins become more resistant to digestion because of peptide bond formation occurring between the side chains of lysine and dicarboxylic acid. If sugar is present, more of these cross-linkages occur. (3) When protein is exposed to treatment with alkali, loss of cystine and lysine may occur. (4) Conditions of oxidation such as the use of SO_2 may give rise to loss of methionine.

Over the years, in my laboratory, a considerable number of nitrogen balance studies have been done on the protein value of cereal products using human subjects. Protein bioavailability values for these products have been calculated from that data. These studies were all similar in design. In all that I will be discussing, the food products were fed to provide 4.0 g of N/day. Other foods in the diet provided 0.8–1.0 g of N/day. Experimental periods were 5–10 days in length. Each study included at least 10 subjects. In most studies, the test products were fed during 3–4 periods to the same individual. Hence, each value to be reported represents a mean of at least 10 values and for most involves 40 or more measurements. Results of these studies are given in Table I (Kies and Fox, 1980a,b).

Other constituents of food products may act to make the protein in the cereal more or less available. Furthermore, processing may have an impact upon these components and thus secondarily have an effect upon protein bioavailability. For example, several of the trypsin inhibitors found in many plants are heat liable. Hence, heat treatment as a processing technique renders protein more, not less, bioavailable. Cereal brans are good providers of dietary fiber; however, research from our laboratory indicates that protein contained in the bran fraction of wheat is not available to the human, presumably because of the interference of dietary fiber.

In one study conducted in our laboratory, adult human subjects were fed supplements of 14.2 g/day cellulose, hemicellulose, or pectin to a low-fiber, low-protein diet. Both cellulose and hemicellulose adversely affected the nitrogen balances of subjects. Both the urinary nitrogen and fecal nitrogen of subjects were increased as a result of hemicellulose or cellulose supplementation. Fecal nitrogen losses of subjects fed no supplements, hemicellulose supplement, cellulose or pectin supplement were 0.86, 1.25, 1.03, and 0.97 g/day, respectively. Thus the bioavailability of protein was adversely affected by fiber supplementation (Kies and Fox, 1980a,b).

In another study using mice, rats, adult humans, and adolescent humans, comparative protein utilization from whole grain wheat flour and extracted wheat flour using five different wheat cultures was measured. The whole ground flours of several wheats had better amino acid proportionality patterns than did their extracted flour counterparts. However, the whole wheat flours failed in the human tests to give improved nitrogen balances. Fecal nitrogen losses were higher for these products, suggesting

Table I. Bioavailability of Protein from Cereal Products As Affected by Processing

product description		protein bioavailability, %
wheat		
bread		
cultivar 1:	whole ground	69
	extracted	80
cultivar 2:	whole ground	70
	extracted	74
cultivar 3:	whole ground	62
	extracted	72
cultivar 4:	whole ground	64
	extracted	68
cultivar 5:	whole ground	64
	extracted	69
breakfast cereals (dry)		
product 1		65
product 2		45
meat analogue		
blended wheat protein product		78
corn		
corn meal		
white degerminated corn meal, steamed		68
yellow degerminated corn meal, bread		67
yellow whole ground, bread		63
opaque-2 corn, whole ground		64
breakfast cereals (dry)		
product 1		50
product 2		50
rice		
polished rice		
polished rice, steamed		78
breakfast cereals (dry)		
product 1		58
product 2		65
oats		
cooked cereals		
oatmeal, regular		72
oatmeal, instant		63
breakfast cereals (dry)		
product 1		62
product 2		56

poor bioavailability of amino acids from the bran layers by humans. Rats and mice seemingly had a better capacity to utilize these proteins (Kies and Fox, 1980a,b).

In another series of studies, effects of graded levels of cellulose, hemicellulose, and wheat bran on protein utilization at two levels of protein intake using human bioassay procedures were done. Results indicated that increases in fiber intake adversely affected nitrogen balances and decreased apparent protein bioavailability at the low protein intake level. However, when protein was fed at higher, assumed adequate levels, nitrogen balances were not affected by fiber supplementation even though protein bioavailability was adversely affected (Kies and Fox, 1978). This stresses that, within limits, protein quality and amino acid bioavailability are of practical concern only at marginal or inadequate intakes of total protein.

SUMMARY AND CONCLUSIONS

Protein/amino acid availability, of which digestibility is a part, contributes to the determination of protein quality. Although chemical methods have been proposed and are currently being investigated, none has been well accepted. Thus, biological means still remain an important source of information in this area. Improvement in protein quality via improvement in digestibility and availability may constitute an important option for the food industry. However, increasing protein digestibility/availability under all circumstances will not improve protein quality. Furthermore, improvement of protein quality via this or other means will not necessarily have any practical impact on the human protein nutritional status.

Protein quality, in general, has been vastly overrated as an area of concern in human nutrition. At very low intakes of protein (perhaps, for the human adult, 20 g of protein/day or less), the dietary protein regardless of amino acid pattern or bioavailability of amino acids will be inadequate even though good quality proteins will usually support somewhat better nitrogen balances than will very poor quality proteins. At relatively moderate levels of protein intake (45–50 g/day for the human adult), most common food sources of protein will support adquate protein nutriture as indicated by distinctly positive nitrogen balances. While it is not surprising that this is true of a relatively good quality plant protein in terms of amino acid proportionality and bioavailability such as soya, that corn protein which has a fairly poor overall amino acid pattern and bioavailability can also completely meet human protein needs if fed in sufficient quantity is not generally known (Kies et al., 1965; Kies and Fox, 1971). Bioassays of protein quality in humans and rats are generally carried out on inadequate intake levels of protein so that differences or changes in protein quality can be measured. This may, in part, have led to an overemphasis of the importance of protein quality.

In conclusion, bioavailability, which is principally, but not entirely, a function of protein digestibility, ranks second to amino acid proportionality patterns in determining the quality of a food protein. However, the relative importance of protein quality except in extreme situations to overall protein nutritional status to most human populations is argumentative.

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Whole Body Protein and Amino Acid Metabolism: Relation to Protein Quality Evaluation in Human Nutrition

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Selected aspects of whole body protein and amino acid metabolism in human subjects are reviewed in relation to the assessment and significance of dietary protein quality. The limitations of N balance measurements for assessment of protein quality are emphasized. Examples of the use of amino acids labeled with the stable isotopes of carbon (^{13}C) and nitrogen (^{15}N) to examine the responses of protein and amino acid metabolism to dietary change are given. These studies suggest that the mechanisms responsible for adaptations of body N are intimately linked to the requirements for these nutrients. Hence, it is proposed that this observation be exploited to develop new and "dynamic" approaches for assessment of amino acid requirements and evaluation of protein quality in human subjects.

It is a privilege for us to contribute this paper in honor of Elmer V. McCollum, who, through his vision and research, has had such a lasting and positive impact on the advancement of nutritional science and its application to the solution of problems of human health.

The purpose of this paper is to explore selected aspects of human protein and amino acid metabolism, with the hope that this may lead to a better understanding of the metabolic consequences of altered nitrogen and essential amino acid intakes. An improved knowledge in this area is essential if the practical significance of dietary protein quality is to be defined in precise, quantitative terms. Furthermore, it is necessary to know how dietary-induced responses in nitrogen and amino acid metabolism in humans, at various stages of development and ages, compare with those observed in experimental animals. This is important if results obtained in assays of protein quality involving use of nonprimate and subhuman primate species are to find maximum application in resolving issues of direct concern in human nutrition. Finally, improved knowledge of human protein and amino acid metabolism should lead to more sensitive measures of protein nutriture and, in turn, determination of the adequacy of the dietary protein and amino acid intake.

Because there are a number of relevant and extensive reviews [e.g., Munro (1964), Allison (1964), McLaughlan and Campbell (1969), NAS/NRC (1974), and Waterlow et al. (1978a)], only a selected coverage of the topic will be undertaken here, with emphasis given to recent investigations that we and our colleagues have been carrying out in our laboratories. We will be concerned with the metabolic evaluation of protein adequency in humans, and this could be approached by using various measurements that indicate the status of specific aspects or phases of body amino acid and protein metabolism (Figure 1). However, our major focus will be on dynamic aspects of body protein and amino acid metabolism, studied with the aid of amino acids labeled with the stable isotopes of carbon (^{13}C) and nitrogen (^{15}N). These isotopes can be safely applied in humans of all ages, and, therefore, they provide an opportunity to examine the physiology of human protein metabolism throughout the life-cycle and the response of protein and amino acid metabolism to dietary change in healthy normal subjects.

NITROGEN BALANCE

It is worth emphasizing that much of the current knowlege of protein quality in human nutrition has been derived from studies based on use of the nitrogen (N) balance technique.

Following Schoenheimer's classic studies, involving the use of stable isotopes to explore the turnover of body constituents (Schoenheimer and Rittenberg, 1938; Schoenheimer, 1942), it is accepted that a major proportion of total body protein undergoes continuous synthesis and breakdown. Thus, the balance between the anabolic and catabolic phases of protein and amino acid metabolism determines cell and organ protein content and, in turn, the efficiency with which dietary nitrogen is retained. Hence, as depicted in Figure 2, both protein synthesis and breakdown are affected by various factors and their rates regulated through specific control mechanisms [e.g., Schimke (1970), Munro (1970), Goldberg and Dice (1974),

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