Amino Acid Content of Proteins Important for Nutritional Balance

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AMINO ACIDS AND PROTEINS

Their Place in Human Nutrition Problems

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During recent years medical and nutrition researches have defined the vital importance of adequate protein nutrition in promoting optimum growth, muscular development, and resistance to disease in children; in diminishing the incidence of toxemias and premature deliveries in pregnancy; and in maintaining the integrity and health of body tissues into old age. Protein-deficiency conditions occur among virtually entire populations in many parts of the world and among appreciable portions of the populations even in relatively well nourished countries like the United States. It has been shown that the nutritive value of proteins is primarily dependent on their amino acid balance. By proper combination of available proteins and by small additions of supplementary amino acids to staple foods, it now appears possible to effect the equivalent of a 50 to 100% increase in dietary protein without increasing the amount of food grown.

A DEFICIT OF FOOD PROTEIN today afflicts the great majority of the human race. Most seriously affected are the populations of the Far East and much of Africa, the peoples of the West Indies and Central America, and large groups in South America and Europe. However, even in the United States and other countries of relatively high average nutritional status, significant portions of the population are receiving too little protein or too poor a quality of protein for optimum health. Under these conditions, food protein must be looked upon as a limited resource requiring conservation and the most efficient possible use.

Because additional land can be brought under fruitful cultivation only with difficulty, while world population continues to increase at a rapid rate, the protein problem will become increasingly acute in the years ahead. However, knowledge of the function of protein in human health and disease has advanced tremendously in recent years. Extensive experiments with animals and humans by numerous investigators have unraveled the relations between the amino acid composition of proteins and their nutritive value. As a result, there is a growing realization of the potentialities inherent in the principle of amino acid supplementation for increasing the nutritive value of present protein resources, so that the devitalizing effects of chronic protein deficiency may be largely overcome. It is the object of this review to collect and correlate the evidence on the manifestations and prevalence of protein deficiency and on the means available for extending the effective protein supply without a corresponding increase in food production.

Protein and Health: Deficiency Manifestations

Relation of Protein to Other Nutrients; Types of Deficiency States

It is now accepted that most of the protein in living organisms exists as complexes and compounds of polypeptides, or amino acid polymers, with a variety of simpler materials, such as lipides (*25*, *26*), nucleic acids (nitrogenous base-sugar-phosphoric acid complexes) (*26*, *47*), porphyrins (*136*), calcium (*46*), iron (*88*), copper, and other metals (*28*). The enzymes, upon which the chemical reactions of the body depend, are of this nature, and their function for certain essential processes involves coenzymes of which the vitamins are an integral part (*28*).

The health disorders that follow deficient intakes of certain nonpolypeptide components of body tissue, especially the vitamins and minerals, are well known. However, the lay public is much less aware of the fact that deficiencies in intake of polypeptide raw materials can have quite as drastic effects on bodily well being. Either type of deficiency, because of the mutual interdependence of these nutrients in living tissue, characteristically causes specific or generalized failures in protein tissue anabolism, as well as other disturbances in the body's chemistry.

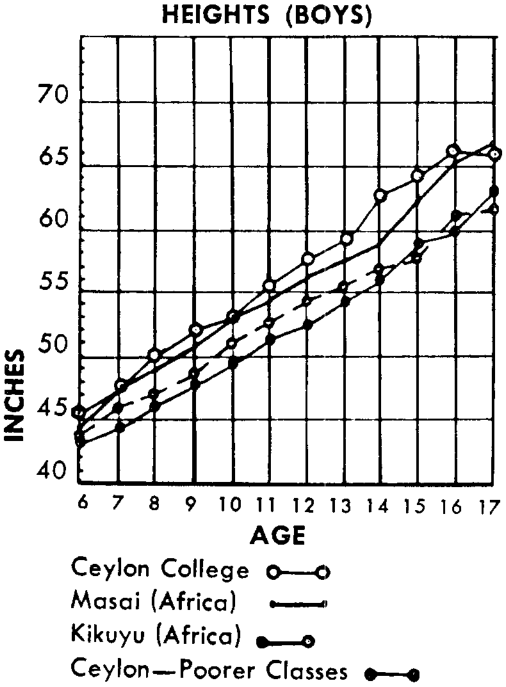
Polypeptide raw materials are provided by the protein components of the diet. In the process of digestion these proteins are hydrolyzed into their constituent amino acids, which are then absorbed and passed into the circulation. A temporary rise in concentration of amino acids in the blood can be detected after a meal rich in protein (*120*). Food proteins differ in their contents of required amino acids and therefore differ in nutritive value or quality. As Youmans has phrased it, high quality proteins are "more complete and richer in essential amino acids" than low quality proteins (*137*). In general, most proteins of animal origin are of high quality, while proteins from whole grains and most other vegetable sources are of lower nutritive value (*72*).

Miranda has proposed the term "hypoproteinosis" for the health disturbances caused by "a deficiency of protein food or of good quality protein, or a deficiency of essential amino acids" (*90*). He reserves the term "malnutrition" for vitamin and mineral deficiencies and states that the word "undernutrition" should be used to designate a deficiency in caloric intake. Manifestly, any two or all three of these deficiency states can occur simultaneously.

An adequate caloric intake is essential to efficient use of protein by the body. However, Allison has demonstrated with adult dogs on very low caloric intakes that if the dietary protein is provided from egg or milk, loss of tissue nitrogen is substantially less than if the animals receive wheat gluten, a low quality protein (*6*). Animals that had previously been markedly depleted in protein reserves actually showed temporary nitrogen storage at low caloric intakes if the protein was of high quality, but not if wheat gluten was supplied. This storage did not persist, of course, at these low caloric levels.

Protein and Growth From studies with animals, much is known about the relation between protein and growth, as well as adult size attained. That both quantity and quality of protein in the diet affect growth has been realized since the classic experiments of Osborne and Mendel. These workers, for example, showed that rats grew at satisfactory rates when 15% or more of casein was provided in the diet, but at subnormal rates when the casein level was 12% or less (*101*). With wheat gliadin, which is of low quality because it contains only 1.1% of the amino acid lysine as compared with 5 to 10% in high quality proteins (*11*), they obtained either no growth or a low rate of growth. Upon raising quality by lysine supplementation, they were able to induce successively greater growth rates by additions of 1, 1.5, and 2% lysine to the diet, the sum of gliadin and supplementary lysine levels being maintained at 18% of the ration (*102*). Hegsted and Worcester have summarized data showing that high quality proteins uniformly cause more rapid growth than low quality proteins (*53*).

Figure 1. Growth curves of Ceylonese, Masai, and Kikuyu boys



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The question arises as to the effect of retarded growth rate on ultimate adult size. Rosenberg and Rohdenburg found that rats raised on a white bread diet supplemented with minerals and vitamins, including vitamin B_{12}, reached an adult size only 75 to 80% as great as control rats grown on a mixed ration in which most of the protein was derived from milk, meat scrap, and soybean meal. When the bread diet was fortified with lysine to raise its protein quality, the animals reached the same size as the controls (*114*). Goettsch carried out somewhat similar experiments to compare the growth performance of control rats on a good diet with that of rats receiving a typical Puerto Rican diet of rice and beans. Animals that received the low quality protein diet throughout the growth period were inferior in size to those on the good diet. Furthermore, the stunting effect of the rice-beans diet early in life could not be fully overcome by transferring the animals to a good diet later in the growing period (*44*).

In the study of human growth, there is a dearth of experiments by which the effects of protein and other dietary essentials, as well as sanitation and heredity, may be separately evaluated. However, sufficient data exist to establish a strong probability that quantity and especially quality of protein in the diet exert a predominant influence on growth rate and adult size.

So-called racial differences in size are not apparent in the early months of life. Birth weight of infants born to races of small people does not differ significantly from that of infants born to larger races, as pointed out by Nicholls for the Ceylonese (*95*). Brou has shown that the growth curves of Congolese infants during the first 3 months of life correspond to those of infants in western nations. However, marked retardation of growth develops as the diet of these infants is changed from the high quality protein of mother's milk to the low quality proteins of manioc and other vegetables. Vitamin and mineral deficiencies may also play a part in this effect. Weight at maturity is substantially below that of the better nourished western peoples (*16*). Similar retardation of growth after the first several months of life is observed among native infants and children in the Kampala area of Uganda, where the deficiency disease known as kwashiorkor is widespread and low quality protein sources are the dietary staples (*15*). A growth pattern of the same type has been reported by Hou for Chinese refugee children (*3*, *58*).

In controlled experiments Albanese has shown that the low quality protein, wheat gluten, will not support normal growth in infants. However, if the gluten is supplemented with enough lysine to double the level of this amino acid naturally present, a normal growth rate is achieved (*4*).

Mack and coworkers made an intensive study of the relation of protein quality to nutritional status of two groups of institutionalized children over a 14-month period (*80*, *81*). The diets of the two groups were essentially equivalent in content of vitamins, minerals, and other nutrients, including quantity of protein. One group, however, received most of its protein from meat, poultry, and fish, while the other obtained most of its protein from low quality sources, principally legumes. Milk intake was the same for both groups. The children who received mainly high quality proteins surpassed the legume-fed children in growth and general pediatric rating. The high quality protein diet also produced higher bone density and hemoglobin values, indicating that an adequate intake of high quality protein is important in securing optimum mineral storage in the growing child.

It has been pointed out that adult heights and weights of peoples in the various countries of the world vary directly with the amount of protein in the diet (*4*). Table I has been presented by Albanese to indicate the correlation of height and weight, as well as life expectancy, with protein intake (*4*). He observes that sanitation, heredity, and other factors may be involved in the trends noted, as well as protein.

Heredity probably plays a much less important role in governing adult height among the various races than popularly supposed. Thus, the Scandinavians of today are generally considered tall by hereditary influence. Yet, according to Miranda, their ancestors of a century ago were on the average 10 cm. shorter (*90*). The Pygmies of equatorial Africa are reported by deCastro to lose their distinctive anthropological characteristics, including small stature, when

Table I. Effect of National Diets on Stature and Longevity

	Australia	*United States*	*China*	*India*
Protein N intake, grams/day	18.1	15.7	11.1	9.8
Animal protein, %	69	57	8.5	16
Average height, cm.	172	170	158	161
Average weight, kg.	77.2	70.0	54.3	50
Life expectancy, years	65	64	30	27

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transplanted to regions where they can obtain a better diet (*29*).

Nicholls has made an interesting comparison between the growth rates of poor and well-to-do boys in Ceylon and the boys of two African tribes. The poorer classes in Ceylon receive little high quality protein, while those who are well enough off to send their boys to college consume considerable quantities of good protein. The African tribes are the Masai and the Kikuyu (or Akikiyu), whose diets and physical status were studied by Orr and Gilks (*97*). The tribes are neighbors and of similar origin, but the former live chiefly on milk and meat, while the Kikuyu live largely on vegetable protein sources. Figure 1 is given by Nicholls, comparing the four groups (*95*).

These growth curves indicate that, as far as average group performance is concerned, genetic considerations are far less potent than nutrition in governing the rate and extent of human growth.

In view of the fact that most of the peoples of the world who dwell between the two tropics are of small stature, it has been suggested that high temperatures may be the growth-retarding influence. However, deCastro points out that these small peoples all live principally on the low quality proteins of cereals, tubers, and legumes. Those groups in the equatorial regions who obtain ample quantities of animal proteins in their diets, including the Masai, the Berber tribes of the Sahara, the Sudanese Negroes, herdsmen of the upper Nile, and inhabitants of the Punjab in India, are tall (*29*).

It is clear from these data and observations that humans attain optimum growth rates and adult size only when a substantial part of the protein consumed is of high quality. The quantity of protein in human diets also seems to play a part in controlling growth. Rapid growth and large adult size, while not necessarily desirable for their own sake, appear to be the almost inevitable outcome of diets which contain adequate amounts of good protein, provided that other dietary factors are not limiting.

Protein and Muscle Development

Creatinine excretion in the urine has been used as a clinical tool by Jeans and others to study adequacy of protein nutrition in children (*60*). Output of creatinine is directly proportional to the amount of muscle in the body (*59*). Jeans reports that the great majority of American children of preschool age are deficient in muscle mass, owing to inadequate protein intake, and ascribes the poor posture of most 4-year-olds to this condition. Frequently these children have average weight for their height and age, indicating deposition of fat rather than muscle. Muscle mass is readily increased to the optimum level by feeding the child more protein. In many cases, Jeans found, muscle mass can be increased by as much as 25% without increasing body weight, as muscle takes the place of fat.

Lynch and Snively, reporting on hypoproteinosis in American children, describe poor tissue turgor as a typical finding, which would appear to correspond to Jeans' observations (*77*). The condition is corrected by increasing the child's consumption of high quality proteins.

A relation between muscle development and the quality of protein supplied is indicated by Allison's observations on young dogs (*5*, *6*). He observed that "puppies fed wheat gluten increased their caloric intakes above those receiving whole egg. They became fat and soft, while those fed whole egg became lean and hard, but both sets of dogs weighed about the same after 70 days on the respective diets. Growth of the protein stores, however, was much greater in the dogs fed whole egg than in those receiving wheat gluten" (*5*).

Protein and the Blood

The proteins of blood plasma are known to be composed of albumins, globulins (alpha, beta, and gamma), and other fractions contributing to blood coagulation and blood typing. The anabolic processes whereby the albumins are formed occur in the liver, while those which produce the globulins appear to occur in the lymphoid tissue (*64*).

When protein is markedly deficient in the diet, production of plasma proteins declines and the weight of circulating blood protein per kilogram of body weight diminishes. If manifested as a subnormal concentration of total albumin-globulin in the blood, the condition is called hypoproteinemia. A decline in serum albumin is usually first observed, but reduction in globulin concentration may later occur (*137*). Frequently the net loss of plasma protein is masked by a decline in blood volume (*90*, *137*). Hypoproteinemia is regarded as clinical evidence of acute rather than moderate hypoproteinosis (*90*). Nutritional edema generally accompanies hypoproteinemia (*137*).

The hemoglobin of the red blood cells requires polypeptide (protein) raw materials as well as iron for its synthesis. Hemoglobin anabolism is known to suffer when the protein intake is inadequate (*137*). The Ortens found that a chronic anemia could be produced in rats by feeding them a diet low in protein (*98*). Orten, Bourque, and Orten demonstrated that young rats fed blood globin, which is deficient in the amino acid isoleucine, grew poorly and developed anemia (*99*). When the isoleucine deficiency was corrected, growth improved and the anemia was overcome.

Keys and coworkers observed that hemoglobin level is a better index of protein undernutrition in humans than plasma protein level (*66*). In protein-deficient children, Lynch and Snively found their anemia to be unresponsive to massive iron doses, but easily remedied by higher protein intakes or protein with small doses of iron (*77*).

Evidence obtained by the Ortens in rat experiments indicated that "those proteins which are qualitatively best constituted for supporting somatic growth are also most effective for hemoglobin formation" (*100*). Cannon recommends high quality proteins, together with adequacy in other dietary essentials and in caloric intake, for regeneration of plasma protein and hemoglobin in humans (*20*).

Protein and Resistance to Infections

Resistance to infections and immunity are largely dependent on the supply of phagocytes and antibodies in the blood (*19*). Production of both of these involves protein anabolic processes and requires polypeptide raw materials. The mesenchymal tissues of the body, including the spleen, bone marrow, lymph nodes and lymphoid tissue, and liver, produce the phagocytes and also store reserve supplies of phagocytes and their precursors (*19*). Both low quantity and low quality in the dietary protein have been shown to depress phagocytic activity in growing rats (*48*, *89*).

Studies of the nature of antibodies indicate that they are specific modifications, produced by the stimulus of antigens, of gamma-globulins originally synthesized in lymphoid tissue (*31*, *64*, *65*). An adequate supply of antibodies therefore depends on the efficiency of gamma-globulin anabolism, which in turn is largely dependent on an adequate supply of polypeptide raw materials (*19*).

In an important series of experiments Cannon and his coworkers have demonstrated that adult animals subjected to prolonged protein undernutrition exhibit a pronounced loss of ability to form antibodies and resist infection (*21, 24*). Normal capacity for antibody production, they found, can be quickly restored by feeding adequate amounts of high quality protein. In milder degrees of protein deficiency in the rat, other workers have been unable to demonstrate reduced resistance to natural infections (*87*).

Almquist, an authority on poultry nutrition, investigated levels of protein required for optimum growth of turkey poults and observed that the mortality rate declined as the protein level increased (*7*). High quality protein was used to raise the protein level. In another poultry study the resistance of chicks given measured doses of avian malaria was found to improve as protein content of the diet increased (*117*).

In human nutrition, Cannon has called attention to "the long known tendency of undernourished persons to acquire severe tuberculosis, rheumatic disease, and respiratory and enteric infections. . ." (*19*). Failure of plasma protein anabolism, whether due to hypoproteinosis, malnutrition, undernutrition, or a combination of these, leads to hypoproteinemia, and Cannon points out the high incidence and virulence of infections in severely hypoproteinemic hospital patients.

Johnston states that adolescent children displaying early tuberculous lesions usually give evidence of inadequate protein intake prior to coming under treatment (*62*). An increase in tuberculosis among Chinese Communist party workers and students has been noted by Adolph following popularization by the Red government, for ideological reasons, of the Chinese peasant diet, which supplies protein mostly from low quality (grain) sources (*2*). The Pottengers have noted the importance of adequate protein intakes in maintaining resistance to tuberculosis (*109*). Low quality and quantity of dietary protein appear to predispose toward rheumatic fever in susceptible children, while a good supply of high quality protein makes incidence of this disorder rare (*27, 32*). Brock and Autret quote Pales, who studied the diet and nutritional state of the Boyas of French Guinea in Africa, as expressing the view that the fish, mollusks, and crustaceans consumed by these people, dwelling in a marshy country, enable them "to resist malaria and to maintain, in spite of malaria, a good state of health" (*15, 104*).

Discussing hypoproteinosis in Mexican children, Miranda cites lowered resistance to infection as manifested "(1) by the frequency of infections in hypoproteinotic children; (2) by the special severity of these diseases, so as to be a cause of high mortality even in usually benign infections such as measles and whooping cough; and (3) by a special course of the infectious disease itself, as, for example, the absence in severe infections of fever and other body reactions such as leukocytosis and other immunity responses" (*90*).

Protein in Surgery Convalescence, and Susceptibility to Shock Elman states that protein deficiencies are often observed in clinical medicine and that hypoproteinemia is "frequent in both medical and surgical wards of any hospital" (*33*). Severe deficiency as manifested by hypoproteinemia may have a number of undesirable consequences in the patient. Proneness to infection has been discussed (*19*). Increased susceptibility to shock (*75, 111*) and delayed healing of wounds (*125*) are also observed. Lund and Levenson conclude that "for these reasons the protein-deficient individual is a poor operative risk" (*75*). Cannon stresses the importance of adequate intakes of high quality protein for conditioning the protein-deficient patient and for convalescence (*19*). Where food cannot be well tolerated, Elman recommends oral or parenteral administration of amino acids (*33*).

McLester has attributed the superior rate of recovery from injury shown by the American soldier in World War II to his liberal allowance of high protein foods (*82*).

Protein in Pregnancy and Lactation While the nutritional problems of the adult man are largely concerned with maintaining the body structure built up during the growth period, the adult woman is faced with more formidable demands. It has been estimated that between puberty and age 45 a woman bearing and nursing six children will have synthesized new tissue equivalent to twice her body weight (*94*). Even in the absence of pregnancy, it has been estimated that the replacement of menstrual losses up to age 45 will require the average woman to synthesize the equivalent of 100% of her body weight (*94*). It is significant that anemia, which reflects an inability of hemoglobin and red blood cell anabolism to keep up with catabolic losses, is known to occur much more frequently among women than men, with highest incidence during the years of child-bearing (*55*).

Typically, if the expectant mother does not ingest adequate protein, certain anabolic processes fail to keep up with her body needs. While birth weight of the baby is not usually greatly affected, production of plasma and red blood cell proteins falls below the maintenance needs of the mother, resulting in hypoproteinemia and anemia. Arnell, Goldman, and Bertucci state that in a study of 400 unselected pregnant women "a significant relationship was found to exist between the protein content of the diet and the serum protein concentration and hemoglobin content of the blood . . ." (*8*). The same physicians were able to secure complete recovery in 11 cases of massive nutritional edema, a concomitant of hypoproteinemia, by "a regimen the basic principle of which was reparation of the protein deficiency." Maternal morbidity and fetal mortality were observed to decrease as protein content of the diet increased (*8*).

Pre-eclamptic and other toxemic conditions and edema are observed to be more prevalent among pregnant women receiving inadequate protein (*18, 132*). Burke, Harding, and Stuart studied protein level in the maternal diet as related to pediatric rating of the newborn infant and found "that less than 75 grams of protein daily during the latter part of pregnancy results in an infant who will tend to be short, light in weight, and most likely to receive a low pediatric rating in other respects" (*17*). Sutherland has reported that in England during World War II a sharp drop occurred in the stillbirth rate in those counties where pregnant women were allotted extra amounts of high quality protein and supplementary vitamins (*124*). Leverton and McMillan made a study of the effect of supplementing the self-chosen diets of pregnant women with a 5-ounce serving of meat daily (*72*). The extra protein was supplied during the period from 4 months before delivery until 3 months after delivery. Higher hemoglobin and red blood cell values, less edema, and better success in lactation were observed in the women who received the meat supplement. Addition of vitamin B complex to the self-chosen diets of these women did not confer similar benefits.

At lower protein levels in the diets of pregnant women, low quality proteins characteristically furnish a major part of the total intake (*61*). Whitacre, Loeb, and Chin have called attention to the predominance of low quality proteins in the diets of eclamptic women in Peiping, China, and Memphis, Tenn., where high rates of eclampsia are observed (*131*). Duncan reports that eclampsia and other toxemias of pregnancy are extremely rare in a section of the Yukon where consumption of meat is heavy (*32*).

Tompkins has pointed out the importance, for freedom from prenatal complications, of an adequate nutritional status not only during pregnancy, but prior to its onset (*126*). Nutritional deficits in protein and vitamins are of common occurrence among pregnant women, he reports. In 654 unselected pregnancy cases in Philadelphia, 220 were found to be 5% or more underweight, and in this group the incidence of premature labor was twice as high as among those standard weight or above. A control group of 170 women receiving 65 grams of protein per day (10 grams more than the average daily intake of pregnant women sampled outside the research groups) showed a 4.12% incidence of toxemia, while a group of 160 women receiving 115 grams of protein per day plus additional vitamins had a toxemia rate of only 0.63%.

Mueller and Cox investigated milk output in lactating rats as protein (casein) level in the diet was increased. They found that milk production increased as protein level was raised from 5 to 30% of the diet, with greatest increase occurring between the 5 and 20% levels (*92*). Human mothers are observed to produce more milk on high protein diets than on high fat or high carbohydrate diets (*1*, *84*). Hoobler found that high quality proteins are more efficacious than low quality proteins for production of human milk (*57*).

Protein in Old Age

According to Stieglitz, "the elderly are prone to suffer from protein deficiency more than from any other form of deficiency." Mild protein deficiency may manifest itself chiefly in "a sense of habitual fatigue," while more extreme deficiencies may lead to atrophy of muscle and other tissue, anemia, and edema (*122*). Low quantity and a preponderance of low quality in the protein of diets selected by older women has been observed by Ohlson and coworkers (*96*). Kountz, Hofstatter, and Ackerman observed that many elderly patients could be benefited by diets providing 2 grams of protein per kilogram of body weight daily, which is twice the usual amount recommended for adults (*67*). With such a diet, some individuals showed nitrogen storage for as long as a year, indicating existence of a previous large deficit.

The frequent occurrence of negative calcium balance in the aged, considered in the light of experiments on relation of protein nutrition to calcium absorption, suggests that hypoproteinosis may be an etiologic factor in this condition. McCance, Widdowson, and Lehman found that adult humans on a high protein diet absorbed 15% of dietary calcium, while those on a low protein diet absorbed only 5% of the calcium ingested (*78*).

Protein and Anatomic Lesions of Malnutrition

Jolliffe, discussing the pathogenesis of nutritional deficiency diseases, states that nutritional inadequacy leads first to tissue depletion, then to biochemical and functional changes, and finally to anatomic lesions (*63*). The last of these represents an advanced stage of nutritional deficiency. It is significant that most of the anatomical changes of severe malnutrition represent failures of protein tissue anabolism. For example, in pellagra there is observed general wasting of the muscles and other body tissues, thinning of the skin with dermatitis and ulceration, and thinning of the mucous membranes of the alimentary tract (*95*). Other states of malnutrition are signalized by fissuring of the skin at the corners of the mouth (angular stomatitis), thinning and atrophy of the skin of the lips (cheilosis), and atrophy of the epithelium and even musculature of the tongue, with red or magenta coloring and ulceration (glossitis) (*43*, *63*, *95*). Kwashiorkor, characterized by Brock and Autret as a protein-deficiency disease, is marked by growth retardation, deficient muscular development, liver lesions, dermatosis, lesions of the mouth and gastrointestinal tract, and reduced production of pancreatic and duodenal enzymes (*15*).

A number of the lesions of malnutrition have been connected with deficiencies of the nonpolypeptide components of body tissue, chiefly the vitamins. Beri beri is accepted as due to thiamine deficiency (*95*). Niacin shortage is believed to play a major part in the precipitation of pellagra, although there is evidence that other dietary factors are also important in this disease (*43*). Stomatitis, cheilosis, and glossitis are generally attributed to riboflavin deficiency (*63*, *95*).

Because anabolic failures can be caused by an inadequate supply of polypeptide components, as well as by shortage of vitamins and minerals, it is important to examine the quantity and quality of protein available in those areas where malnutrition flourishes. The Gillmans, as a result of their study of this question, conclude that pellagra is associated with corn (maize) or corn and wheat as dietary staples, while other states of malnutrition are associated with rice, millet, manioc, and other vegetable products as the principal article of diet (*43*). Relatively low quantity and a preponderance of low quality in the protein intake are invariably observed in areas where malnutrition is prevalent. This factor must therefore be kept under consideration in studies of the etiology of the various lesions of malnutrition.

Recommended Protein Intakes

Quantity

For several reasons, it is difficult to establish minimum requirements for the intake of protein. One of the most important is that the amount required varies with the quality of the protein. High quality proteins quite literally "go further" in nutrition than low quality proteins. For example, in experiments with young adult women Bricker, Mitchell, and Kinsman found that, for a 70-kg. adult, 74.4 grams of white flour protein per day was required for nitrogen balance, while only 43.0 grams of milk protein was required (*14*).

Another factor that makes it difficult to establish minimum requirements is that the organism has considerable power to adapt itself to inadequate protein intakes. The immature animal, in the face of low quantity or quality intakes, reduces its growth rate (*53*, *101*, *102*). Adult females, receiving inadequate protein during pregnancy, will drain tissue reserves to produce an infant of normal or near-normal size (*8*, *17*). Even in the absence of the anabolic drives of growth and pregnancy, much of the maintenance need for nitrogen is directly related to such anabolic processes as synthesis of hair, digestive enzymes, intestinal mucus, and skin for desquamation. It is known that synthesis of such "expendable" proteins is markedly retarded in acute malnutrition or undernutrition (*15*, *43*, *95*), and conceivably the adult organism can make adjustments of the same nature in more moderate degree, thereby achieving nitrogen balance on protein intakes which are inadequate for optimum health but not poor enough to bring on the overt signs of faulty nutrition.

In 1920 Sherman analyzed the available evidence on protein required for maintenance of adult men and women and arrived at an average figure of 0.63 to 0.64 gram of protein per kilogram of body weight per day as the adult requirement (*118*). Allowing for individual differences, variations in protein quality, and other causes of fluctuations in requirements, he suggested a standard allowance for the adult of 1 gram of protein per kilogram of body weight per day. Sherman's study formed the basis for the recommended protein allowances for normal adults established by the Food and Nutrition Board of the National Research Council in 1941, which were reaffirmed in 1945 and 1948 (*36*). For an average 70-kg. man, the recommended allowance is 70 grams per day; for an average 56-kg. woman, 60 grams per day. These allowances are believed to carry "generous provision for differences in the proteins of normal diets as well as for individual variations of protein requirement among normal people" (*36*).

In the latter half of pregnancy the National Research Council recommends a protein intake of 85 grams per day for the average 56-kg. woman. In lactation, 100 grams per day is recommended. The quantity recommended in pregnancy does not appear much, if any, in excess of needs, as Burke, Harding, and Stuart found that infants born to mothers who received less than 75 grams of protein daily in the latter part of pregnancy tended to be of low pediatric rating (*17*).

For children, the National Research Council recommends intakes progressively declining from 4 to 3.5 grams per kilogram of body weight in infancy, from 3 to 2.5 grams in early childhood, and from 2 to 1.5 grams in late childhood and adolescence (*36*). Somewhat higher needs during adolescence than indicated by the last figure are implied by Johnston, who says of the adolescent child: "His protein needs . . . are higher than they will ever again be in life, and the attainment of an optimal storage of nitrogen in his tissues and of a normal metabolism will demand about three times as much as that required in adult life. It is true that when such demands are not met equilibrium is reached at a lower level, but only at the expense of a fall in basal metabolism. Beyond a certain point this will result in a failure to achieve sexual maturity" (*62*).

Measurement of Quality

A number of methods have been devised to measure the nutritive value of proteins. One of the most commonly used is the determination of the "protein efficiency ratio," a method introduced by Osborne, Mendel, and Ferry in 1919 (*103*). In this method a protein is fed to a weanling animal for a given period and the grams of body weight gained during that time, divided by the grams of protein consumed, gives the protein efficiency ratio. Block and Mitchell have compiled extensive lists of protein efficiency ratios after a review of the available data (*12*).

Other methods of protein evaluation include determination of "biological value," in terms of per cent of digestible nitrogen retained by an adult or growing animal; measurement of weight regain in animals previously subjected to protein depletion; and measurement of the regeneration of specific body proteins, such as those of the liver and blood, in protein-depleted animals. A review and critique of methods of protein evaluation were recently made by Frost (*38*).

With minor exceptions, the various methods of evaluating the quality of proteins classify them in the same relative order of nutritive value. Whether the quantum determined is total body tissue synthesized, specific body proteins synthesized, or percentage of total food protein assimilated into the body, all the methods have in common a measure of the relative value of food proteins for tissue anabolism in the body.

Recommended Proportions of High Quality Protein in Diet

The strong weight of present-day opinion among nutritionists and the medical profession is that, for optimum nutrition, half or more of total protein consumed should be of high quality. Thus, Turner states that "the current dietetic practice, that of providing one half of the protein from animal sources, will ensure a satisfactory factor of safety for the levels of protein intake recommended by the Food and Nutrition Board of the National Research Council for the adult maintenance diet. In therapeutic diets, and in diets in which growth is a factor, it is a common practice to provide one half to two thirds of the protein from animal sources" (*127*).

For children and adolescents, it is recommended by many authorities that two thirds to three quarters of total protein ingested should be of animal origin (*62*, *73*, *127*). Recommendations for pregnant and lactating women are that half to two thirds of protein should be from animal sources (*76*, *127*, *132*). Ample intakes of animal proteins are recommended by Stieglitz for the aged (*122*).

Prevalence of Hypoproteinosis

Areas of High Quality Protein Scarcity

Phillips, in a review of the possibilities of expanding livestock production throughout the world, has listed the estimated average intakes of vegetable and animal protein per capita in 58 countries (*106*). Using the arbitrary figure of 30 grams of animal protein per day as a desirable intake, he notes that this level is equaled or exceeded in only 18 of the 58 countries. Extreme shortages of high quality protein, sometimes in conjunction with a deficiency of total protein, are common in the Far East, Africa, Central America, the West Indies, and parts of South America and Europe. It is in these areas that the signs of hypoproteinosis abound, including retarded growth, high child mortality, inferior adult size and strength, and low resistance to tuberculosis and other infections (*29*, *90*, *95*). Here, too, the gross visible effects of faulty nutrition are frequently seen, including nutritional edema, kwashiorkor, pellagra, skin ulcers, glossitis, angular stomatitis, and cheilosis (*43*, *83*, *95*).

Numerically, the peoples of the world who receive inadequate protein nutrition far outweigh those who have adequate protein supplies.

Areas of Relatively Good Nutrition in the United States

Many degrees of protein deficiency lie between the state of optimum protein nutrition and the severest types of hypoproteinosis. In the better nourished countries of North America and Western Europe, as well as Australia and New Zealand, where high quality proteins are relatively abundant, severe hypoproteinosis is not commonly seen. However, there is ample evidence of mild-to-moderate hypoproteinosis in these nations, and among some population groups severe protein deficiency has been observed. The status of protein nutrition in the United States is discussed here to illustrate these points, because this country is recognized as one of the better nourished countries of the world. More data are available on the nutritional status of population groups in the United States than in most other countries that might be chosen for a study of this kind.

Employing as a standard the recommended protein allowances established by the National Research Council, the U. S. Bureau of Human Nutrition and Home Economics has shown that, on the average, 27% of families in a rural Georgia county and 13% in a rural Ohio county received insufficient protein (*130*). At low income levels and in large families in these counties, incidence of substandard intakes was much higher, ranging up to 75% of seven-member families in the Georgia county and 27% of six-member families in the Ohio county. A similar situation was found by the bureau in a study that covered both farm and nonfarm families in Mississippi (*129*). These investigations were carried out in 1945.

A survey by the bureau covering protein intakes of families in four large cities in 1948 showed an average of 11% consuming less than recommended allowances of protein, while at low income levels the percentage was as high as 34% (*128*). Table II, showing percentage of families at various income levels that failed to consume recommended amounts of protein, was prepared from the bureau's report on this survey.

Protein intakes somewhat below those recommended by the National Research Council would be of little or no concern if the protein consumed were mainly of high quality. However, it is the rule that at relatively low intakes the proportion supplied by low quality sources becomes larger. This is primarily the result of low family income or the necessity of stretching the income to cover the needs of large families. For example, in 1947 the Bureau of Human Nutrition and Home Economics studied the food expenditures of families in Cumberland, Md., and observed that "the larger the household size the smaller was the average expenditure per person for meat, poultry, eggs, and fats" (*107*). Even at relatively high income levels, however,

Table II. Less-Than-Recommended Protein Intakes among Urban U. S. Families in 1948

Annual Income, $	*Percentage of Families Not Receiving NRC Recommended Intake of Protein*
Under 1000	34
1000–2000	17
2000–3000	13
3000–4000	7
4000–5000	5
5000–7500	7
7500 and over	8

ignorance of nutrition principles or individual preferences for low quality protein foods may lead to inadequate protein nutrition, as found by Lynch and Snively for children (*77*) and by Mack for professional and businessmen and their wives (*79*).

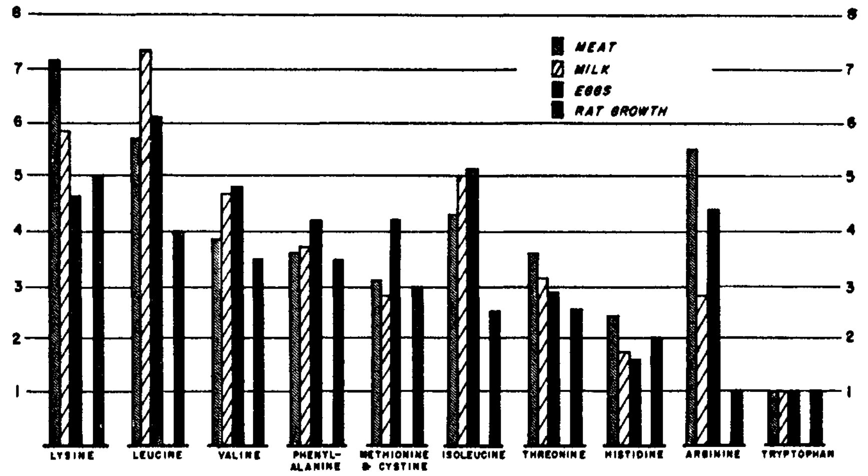
While dietary surveys afford presumptive evidence of protein undernutrition, confirmation is given by clinical and laboratory studies of nutritional status and also by competent medical opinion. Youmans and coworkers surveyed the protein nutrition of about 1200 persons in an average rural population in Tennessee (*138*). They report that "frequent and severe deficiencies in the intake of proteins, greater in the Negroes, the females, and certain age groups, were observed." Incidence of hypoproteinemia, which is an index of acute protein undernutrition, averaged 10%, with some groups, notably colored females 16 and over, showing 24 to 29% incidence.

The diets of pregnant women have been frequently surveyed, with the usual finding that 80 to 90% ingest less protein than the National Research Council's recommended intake (*8*, *17*, *61*, *133*). The frequent occurrence of protein deficiency states among elderly individuals has been noted by Stieglitz (*122*). Jeans' observation that the majority of American children have less than optimum muscle development, owing to inadequate protein intakes, has been mentioned (*60*). Lynch and Snively have reported on a mild form of protein deficiency observed in American children which they believe to be "one of the most prevalent clinically manifested deficiency diseases which affect children" (*77*). Anorexia, irritability, vomiting, poor muscular development, constipation, increased susceptibility to infections, a high rate of dental caries, and anemia are listed by them as common findings in hypoproteinotic children. Price and Hart describe a similar complex of symptoms frequently observed among young children in their pediatric practice (*110*). The low quality proteins supplied by cookies, crackers, grits, and other cereal products appear to feature prominently in the diets of these pale, flabby, undermuscled children (*77*, *110*).

In the years ahead, under the pressure of a rapidly expanding population, the protein deficiency problem in the United States is likely to become increasingly acute. DeGraff has pointed out that intake of high quality proteins is already on the decline, consumption of meat and poultry products per capita having dropped by 6% since 1947 and of milk products 7% since 1946 (*30*). In 1950 and 1951, he reports, livestock and poultry in the United States consumed more feed supplies than were raised, eating into reserves accumulated in the past. DeGraff concludes that marked increases in feed production and in out-

AMINO ACID PROPORTIONS IN HIGH QUALITY PROTEINS

AMINO ACID PROPORTIONS IN MEAT, MILK AND EGG PROTEIN COMPARED WITH PROPORTIONS UTILIZED IN RAT GROWTH.

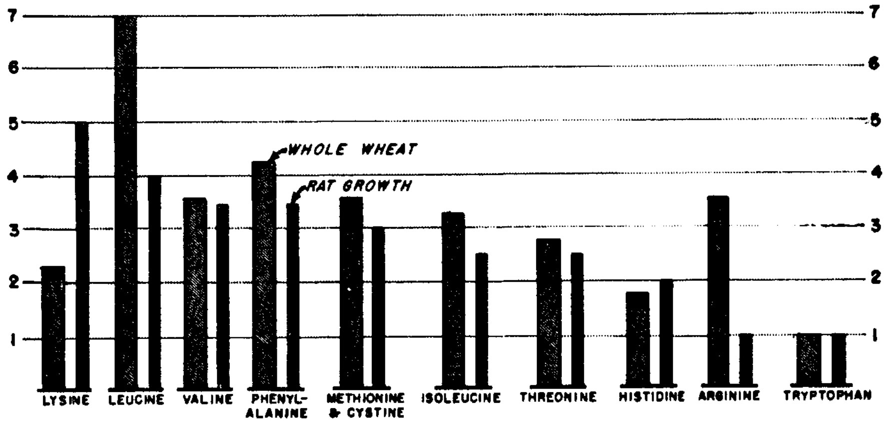


AMINO ACID PROPORTIONS IN PROTEIN CALCULATED ON BASIS THAT TRYPTOPHAN = 1.0

Figure 2

AMINO ACID PROPORTIONS IN WHOLE WHEAT PROTEIN

AMINO ACID PROPORTIONS IN WHOLE WHEAT PROTEIN COMPARED WITH PROPORTIONS UTILIZED IN RAT GROWTH.

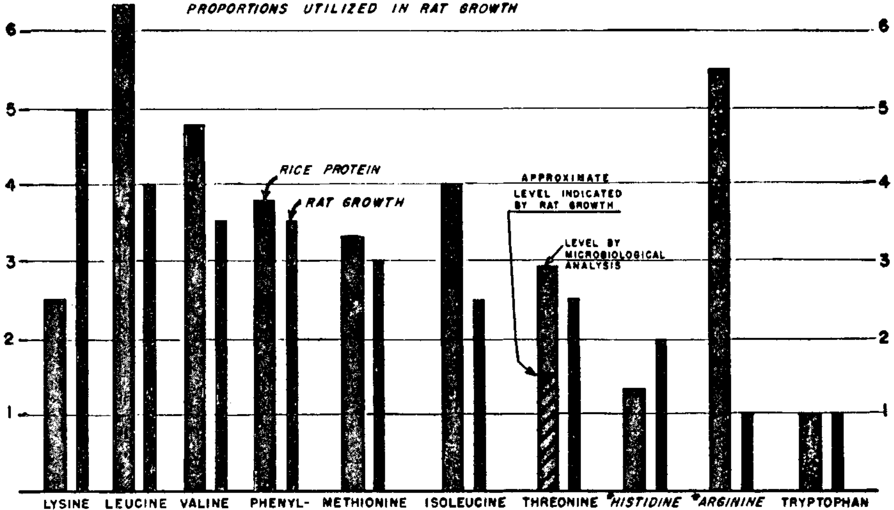


AMINO ACID PROPORTIONS IN PROTEIN CALCULATED ON BASIS THAT TRYPTOPHAN = 1.0

Figure 3

AMINO ACID PROPORTIONS IN WHITE RICE PROTEIN

AMINO ACID PROPORTIONS IN WHITE RICE PROTEIN COMPARED WITH PROPORTIONS UTILIZED IN RAT GROWTH



*Not Essential for Humans

AMINO ACID PROPORTIONS IN PROTEIN CALCULATED ON BASIS THAT TRYPTOPHAN = 1.0

Figure 4

put of meat, milk, and eggs per ton of feed will be necessary to maintain satisfactory nutrition for a United States population currently increasing at the rate of 2,500,000 persons annually.

Means for Extending Utilization of Protein Supplies

Combining Proportions in Utilization of Amino Acids for Tissue Anabolism The synthesis of protein tissue occurs continually at all stages in the life span, but the relation of amino acid intake to tissue anabolism has been most thoroughly studied in those states where the amount of synthesis can most readily be measured, as in growth of young animals and repletion of protein-depleted adults.

The first complete determination of the amounts of the various essential amino acids required for satisfactory mammalian growth was carried out by Rose (*113*). His results, obtained on the weanling rat, are shown in Table III. The proportionality relationships among the amino acid requirements, calculated from the Rose data by taking the tryptophan level as unity, are also shown.

The proportionality relationships among the essential amino acid requirements for rat growth are listed in Table III because of accumulating evidence that the proportions in which amino acids are ingested is of paramount importance in their efficient use for tissue anabolism. Cannon and coworkers, for example, have determined the amino acid requirements of protein-depleted adult rats for tissue regeneration, measured as milligrams of amino acid per kilogram of body weight per day (*121*). Converting these values to proportionalities, based on weight of tryptophan as unity, they find a marked similarity to the proportions required in growth. They explain the failures of a number of other investigators to obtain good rat growth on amino acid mixtures as due to imbalance in the amino acid proportions.

Work by Loosli and by Mertz, Beeson, and Jackson has shown that the amino acid requirements of growing pigs are qualitatively and probably also quantitatively about the same as those of the young rat (*10*, *74*, *86*). Beeson stresses the importance of "amino acid balance" in obtaining maximum utilization of protein for growth (*10*). Mertz, Beeson, and Jackson obtained excellent growth rates in young pigs by supplying them a mixture of the indispensable amino acids in essentially the same proportions as shown in Table III for the young rat, making allowance for the utilizability of unnatural isomers of certain of the amino acids employed in DL-form (*86*). Loosli points out that the lysine requirement of the pig varies with the level of protein in the diet, from a 0.6% requirement at 10% protein in the diet to a 1.2% requirement at 22% protein (*74*). However, if the lysine requirement is expressed as percentage of dietary protein, he notes that the lysine requirement is remarkably constant, varying through the narrow range of 5.7 to 5.5% of dietary protein as protein level goes from 10 to 22% of the diet.

Table III. Essential Amino Acid Requirements for Growth of Weanling Rat

Amino Acid	*% of Diet*	*Proportions*
Lysine	1.0	5
Leucine	0.8	4
Valine	0.7	3.5
Phenylalanine	0.7	3.5
Methionine	0.6	3
Isoleucine	0.5	2.5
Threonine	0.5	2.5
Histidine	0.4	2
Arginine	0.2	1
Tryptophan	0.2	1

Striking confirmation for the principle of combining proportions in the utilization of amino acids is found in the bread experiments of Rosenberg and Rohdenburg (*114*) and the rice experiments of Pecora and Hundley (*105*). The former workers were able to improve the protein value of white bread containing 3% nonfat milk solids by more than 100% by increasing the lysine content of the bread diet from 0.29 to 0.67%, but obtained no advantage by further additions. Pecora and Hundley, supplementing rice with lysine and threonine, obtained as good growth by adding 0.2% L-lysine as when they added 1.0% L-lysine, based on total ration (together with a threonine supplement in each case); total lysine in the diet at the lower addition level, including that present in the rice protein, was only 0.42%. In both the bread and rice experiments, if the tryptophan content is calculated from Block and Bolling's compiled data (*11*), it is evident that maximum growth response was achieved when sufficient lysine had been added to bring the lysine-tryptophan ratio to approximately 5. Additional supplementation elicited no further response.

That the proportions in which amino acids are utilized for growth by the rat may furnish a pattern typical of mammalian growth is suggested not only by research on swine, but also by investigations on the human infant. Thus, Albanese has determined lysine, isoleucine, and tryptophan requirements of the infant up to age 9 months and finds their relative proportions to be 5.6:3:1 (*4*). This is similar to the corresponding proportions for the young rat of 5:2.5:1.

Amino Acid Proportions in High and Low Quality Proteins The reason for differences in the nutritive value of food proteins becomes apparent when their amino acid proportions are compared with the proportions in which the amino acids are utilized for mammalian growth. This is shown in the following figures, making use of a bar chart technique. Each shaded bar represents a proportionality factor for an essential amino acid in a staple food protein, calculated from analytical data compiled by Block and Bolling (*11*). The black bars represent the proportions in which the essential amino acids are utilized by the growing rat, based on weight of tryptophan as unity, as calculated from Rose's data (*113*). The proportion of nonessential amino acids is not indicated in these figures, as experience indicates that consideration of the essential amino acids alone will suffice for an approximate comparison of the nutritive value of proteins. The methionine and cystine contents of the food protein are lumped together for comparison with the methionine need of the rat, as cystine will fill part of the methionine requirement (*52*, *56*, *134*).

Figure 2 shows the amino acid proportions in three typical high quality proteins: those of meat, milk, and eggs. The proportions supplied by the proteins match fairly closely the need pattern of the animal.

Figure 3 gives the amino acid proportions in whole wheat protein. Here the proportions supplied by the food are in good correspondence with the needs for tissue synthesis, except for the proportion of lysine. The lysine content limits the protein efficiency of wheat to about half that of high quality proteins. The chart indicates that if the lysine content were approximately doubled, a given amount of wheat protein could build twice as much tissue in the growing animal. Amino acid proportions shown here for wheat are typical of several grains, including oats, barley, rye, and millet.

The amino acid proportions in rice protein are shown in Figure 4. Analytical data indicate that lysine is the only important amino acid deficiency in rice, but the rat experiments of Pecora and Hundley show that threonine is also short, suggesting that a portion of the content of this amino acid in rice protein cannot be released by the rat's digestive enzymes (*105*).

Figure 5 shows the amino acid proportions in whole corn (maize). Here a departure is made from the practice of using tryptophan content as unity in calculating the proportions. Empirically, it is observed in preparing charts of this kind that if the proportion of tryptophan is low, its use as denominator results in the appearance of multiple large positive deviations from the growth requirement pattern. This is the situation with corn. If the tryptophan content is relatively high, multiple negative deviations are observed. In the case of corn protein, a chart in which most of the amino acid proportionality columns are in fair balance with the requirement pattern can be obtained by setting the threonine proportion equal to 2.5 and adjusting the other column heights in proportion. The amino acid imbalance of corn protein is thereby revealed in terms of severe lysine and tryptophan shortages.

Legumes are an important staple in many parts of the world. Figure 6 gives the amino acid proportions in the protein of peas and beans, based on Block and Bolling's data. Important shortages in tryptophan and methionine are indicated, with possibly a small deficiency in lysine. Heat-treated soybean meal shows good acid balance except for a slight deficiency of methionine (Figure 7).

Root products, such as potatoes, yams, and cassava, are important sources of protein in many regions. The amino acid proportions in white potato protein are given in Figure 8. Good amino acid balance is indicated, except for a marked shortage of methionine.

The nutritive value of the total protein furnished by a meal of several mixed foods can be assessed in the same way, by calculating the total amounts of each amino acid and comparing their proportions with the growth requirement pattern. Similarly, the nutritive value of the protein of a single food made of several ingredients can be indicated in chart form, as shown in Figure 9 for white bread containing protein contributed by flour, 3% nonfat milk solids, and baker's yeast. The lysine shortage is apparent.

In using charts of this kind for assessing the nutritive value of proteins for humans, it should be noted that arginine and histidine appear dispensable for man (*4*, *112*). The charts should be regarded as merely indicative, in view of the limited accuracy of existing data on protein composition and mammalian amino acid requirements. Final proof always lies in actual feeding trials.

Amino Acid Requirements for Adult Maintenance

The minimum requirements of each of the eight amino acids essential for maintenance of the adult young man have been determined by Rose (*112*). The values are tabulated in Table IV.

Table IV. Minimum Essential Amino Acid Requirements of the Young Male Human Adult

Amino Acid	*Grams/Day*
Lysine	0.80
Leucine	1.10
Valine	0.80
Phenylalanine	1.10
Methionine	1.10
Isoleucine	0.70
Threonine	0.50
Tryptophan	0.25

Rose has stated that these data were obtained with healthy young men and are not to be applied to other population groups, such as children, pregnant women, adults depleted by illness or poor diet, or the aged. Nevertheless, they furnish a standard by which dietaries may be judged for their adequacy in content of essential amino acids for the adult male human.

A number of investigators have shed light on the relation of amino acid composition of individual food proteins to the effectiveness with which they may be utilized for tissue anabolism in the adult human. For example, Mitchell and coworkers studied the efficiency with which various proteins and mixtures of proteins were utilized by adult young women (*14*). They found that for an adult subject weighing 70 kg., 74.4 grams of white flour protein was required for "true maintenance and adult growth," which includes maintenance of existing tissues, storage of nitrogen in hair, skin growth, losses from the skin, and tissue changes incident to adult aging. White flour protein contains 1.9% of lysine, which is its limiting amino acid (*11*). It is practically 100% digestible. Theoretically, therefore, 42 grams of white flour protein would have provided the adult minimum requirement of lysine, as well as adequate amounts of the other amino acids. Actually, a 59-kg. subject was in negative balance on a daily intake of 6.819 grams of flour nitrogen or 42.6 grams of flour protein per day and did not go into positive balance until a lysine supplement was added. On milk protein, Mitchell and his associates found that 43.0 grams was sufficient for a 70-kg. subject. An intermediate case was provided by a mixed diet containing meat, egg, milk, soy, oat, and wheat protein, in which 49.5 grams per day proved to be the requirement. From this study, it appears that the higher the average quality of protein in the diet, the less is needed to provide for maintenance and "growth" functions in the adult.

Murlin and his associates have established the superior biological value for adult humans of high quality proteins like egg, beef, and casein over the low quality proteins of peanut and wheat gluten (*51*, *93*). As biological value determinations measure the percentage of protein retained, it appears from these studies also that high quality proteins are more efficiently utilized for those anabolic functions which are necessary for adult maintenance.

Studies have recently been made of the relation between nitrogen retention and the dietary levels of the essential amino acids for mature (*39*) and aging women (*85*). In these investigations no satisfactory correlation could be developed between nitrogen retention and the dietary level of any one amino acid, including methionine, which appeared to be limiting. On the other hand, an examination of the data indicates a generally good correlation between nitrogen balance or storage and the intake of high quality proteins.

It appears from these data that the amino acids required for maintenance of the human adult are most efficiently assimilated if they are supplied by the food in the proportions characteristic of high quality proteins. These are approximately the same as the proportions in which the amino acids are best utilized for growth and repletion.

Simultaneous Availability of Essential Amino Acids

In animal feeding experiments with amino acid mixtures, Geiger, Cannon, and others have demonstrated that tissue anabolism occurs only when all the essential amino acids are supplied simultaneously (*23*, *40*, *116*). A time interval any greater than 1 hour between the ingestion of an incomplete amino acid mixture and its supplementation with the missing amino acids resulted in failure of tissue synthesis (*23*). Similarly, Harte, Travers, and Sarich found that rats grew more poorly when they received alternate feedings of wheat gluten and milk protein hydrolyzate than when they received the two amino acid sources simultaneously (*50*).

In the same way, mixtures of proteins which to some degree supplement one another's amino acid deficiencies give better growth than the same proteins supplied at separate feedings. This was demonstrated by Henry and Kon for the combinations of milk and potato and of bread and cheese (*54*), and by Geiger for several other proteins (*41*).

These results demonstrate that not only should all the essential amino acids be provided simultaneously, but they must also be simultaneously present in desirable proportions if they are to be efficiently utilized.

Distribution of High Quality Protein among Daily Meals

Leverton and Gram, working with young adult women, found that subjects who received a moderate amount of high quality protein divided among three daily meals were in positive nitrogen balance, while those who received the same amount of high quality protein at only two of the three meals were in negative balance (*71*). Total daily protein intake was the same for all subjects. In their studies on nitrogen retention by aging women, Mertz and coworkers found indications "that irregular ingestion of high quality protein at each of the three meals may contribute toward the high incidence of negative retention of nitrogen. . ." (*85*). These data are suggestive of a principle

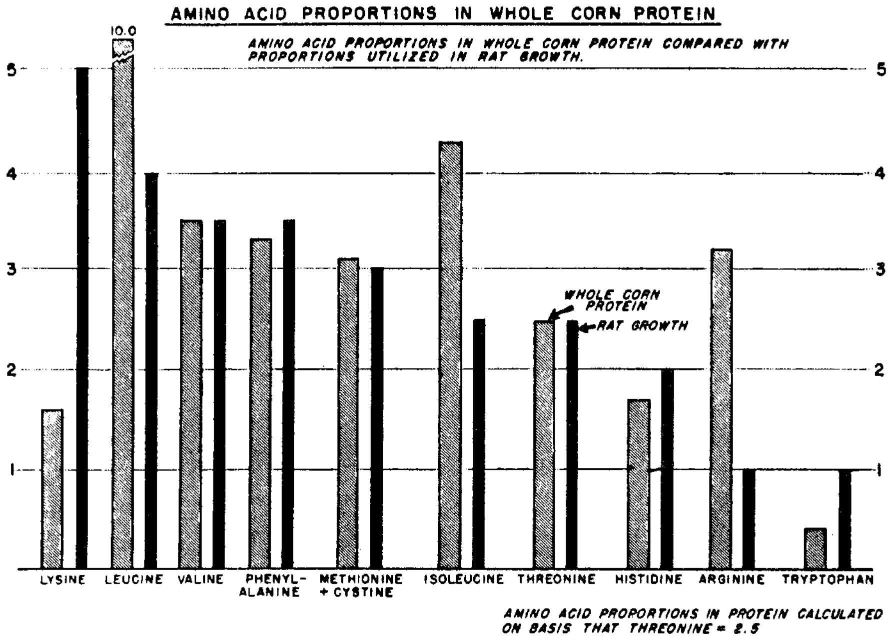


Figure 5

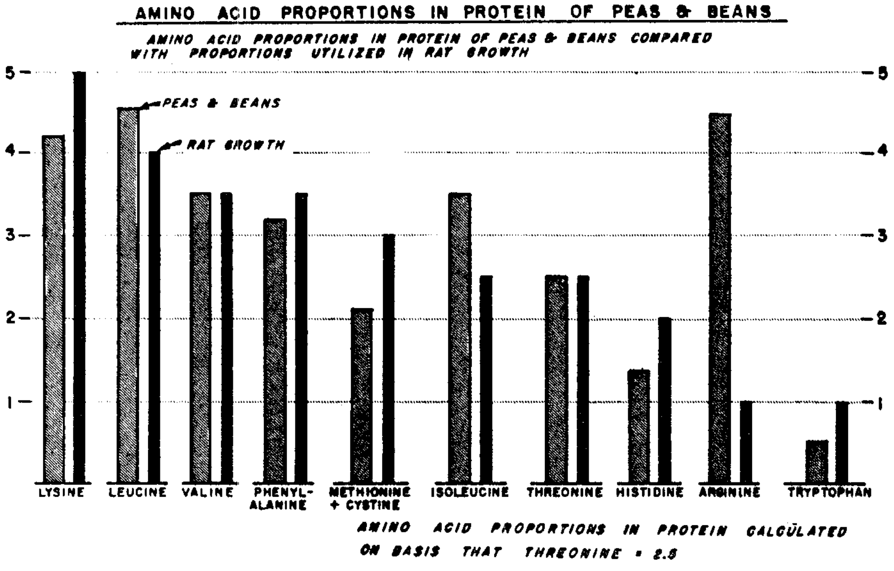


Figure 6

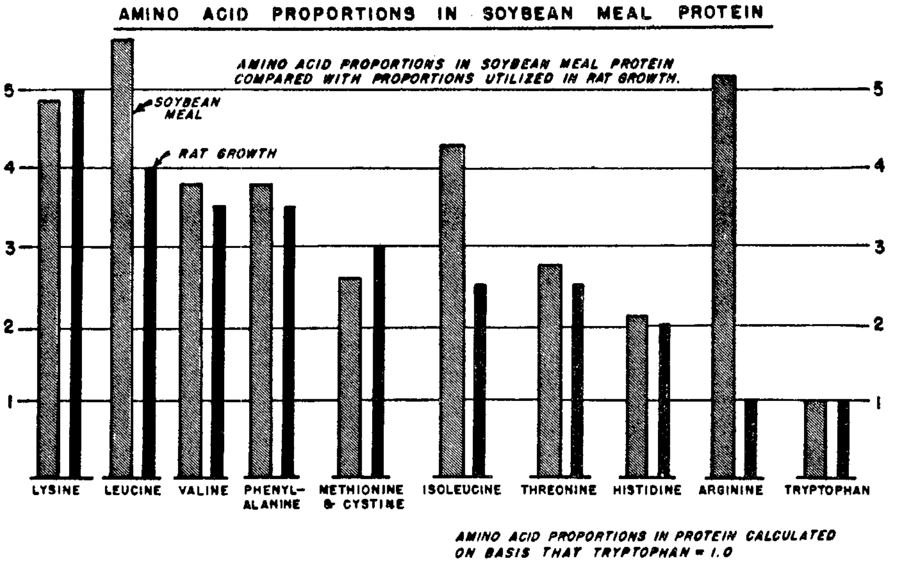


Figure 7

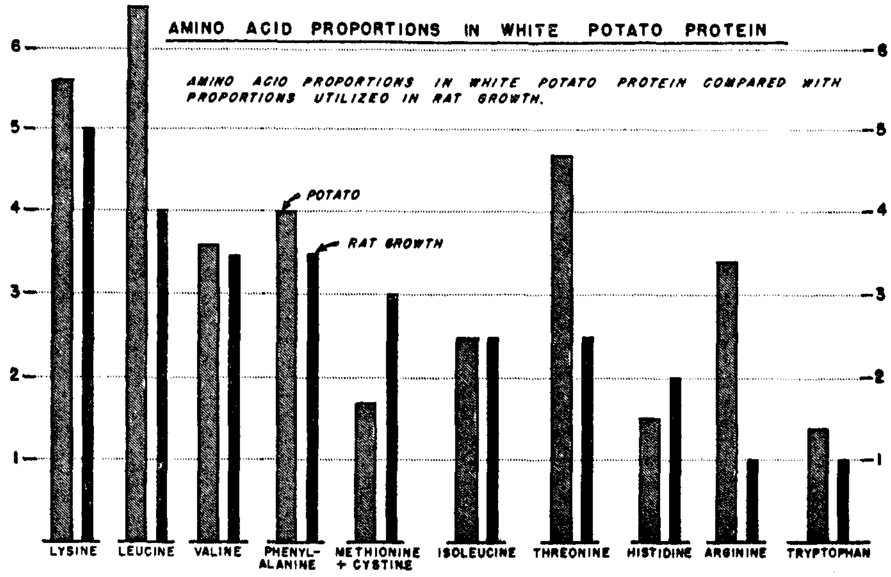
that optimum utilization of dietary protein may be secured by a more even distribution of high quality protein among the daily meals.

Mechanism of Protein Tissue Anabolism

It is a striking fact of protein nutrition that the growth rate performance obtained with high quality proteins cannot be duplicated with low quality proteins by simply increasing the amount of protein fed—that is, if protein A has half the protein efficiency of protein B, it does not follow that the two proteins will give the same performance if the intake of protein A is doubled. Bosshardt and coworkers, in developing a technique for measurement of protein quality by growth of the weanling mouse, found that growth rate on wheat gluten increased as the percentage of gluten in the diet was increased up to about the 25% level. However, even at 25 to 34% gluten levels in the ration, the weight gains did not equal those obtained with 11 to 12% levels of egg and milk protein (*13*). Morgan has reported that at no level of feeding did peanut meal or wheat gluten produce the growth in young dogs that is obtainable with high quality proteins (*97*). Allison has noted that growth as measured by weight gain, rather than nitrogen storage, may cause spuriously high ratings for the growth-promoting value of low quality proteins, as consumption of a poor protein may be attended by deposition of more body fat than consumption of a high quality protein (*5, 6*).

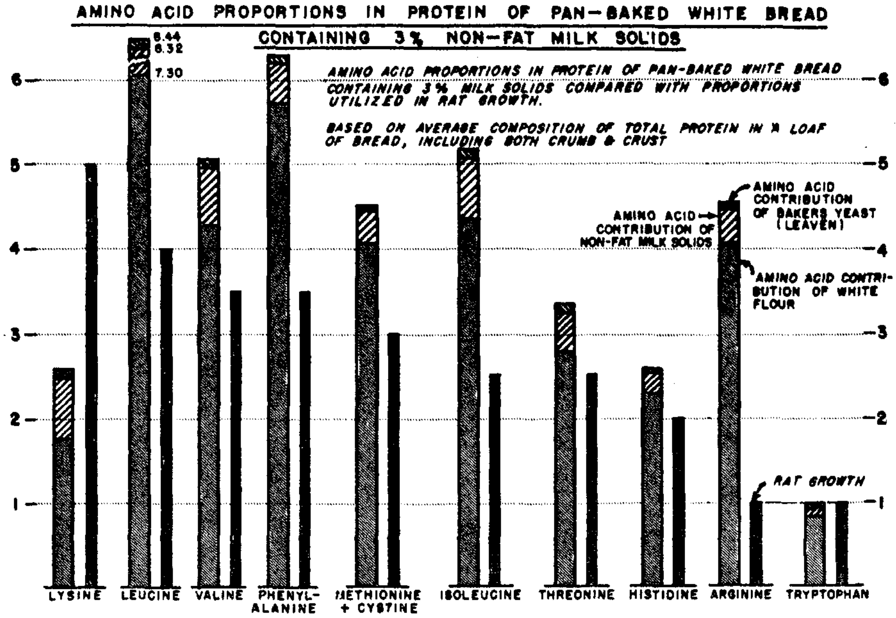
Grau, in studying the lysine requirement of the chick, utilized the lysine-deficient protein source, sesame meal, to provide the dietary protein. He detertermined that growth rate reached a maximum when the protein level in the diet reached 20%, with sufficient lysine added to make the total content of this amino acid 0.86% of the ration. The maximum growth rate obtained was 7% of body weight per day. When he raised the level of sesame protein to 30% of the ration, the diet without supplementation was calculated to provide 0.84% of lysine, practically equivalent to the supplemented level employed at the 20% protein level. Obviously, all the other essential amino acids were in sufficient supply at the higher protein level. Yet growth rate fell to 4.7% of body weight per day, until 0.3% of lysine was added, sufficient to restore the proportion of lysine approximately to the optimum level, when the 7% growth rate was again attained (*45*).

Any hypothesis presented for the mechanism of protein tissue anabolism must take into account this growth-inhibiting effect of amino acid imbalance, as well as the critical importance of simultaneous availability of the amino acids. It must also consider the fact that amino acids absorbed from the intestines during digestion of protein foods are utilized rapidly, causing only a temporary and moderate rise in the free amino acid content of the blood (*35*). On the other hand, Geiger has shown that the capacity of the body to utilize amino acids for tissue anabolism can be greatly exceeded if a superabundant supply of amino acids is provided in a short interval. That amount by which the utilization capacity was exceeded was permanently lost for purposes of tissue synthesis (*42*).



AMINO ACID PROPORTIONS IN PROTEIN CALCULATED ON BASIS THAT ISOLEUCINE = 2.5

Figure 8



AMINO ACID PROPORTIONS IN PROTEIN CALCULATED ON BASIS THAT TRYPTOPHAN = 1.0

Figure 9

Nucleoproteins in the nucleus and cytoplasm of the body cells are concerned in the synthesis of tissue protein. In Greenstein's words: "Whenever growth occurs within a living medium, nucleoproteins are found in high concentration, whether in the metaphase chromosome, the normal gland cell, the embryonic cell, the avian neoplasm, or the plant or animal lesion" (*47*). Greenstein suggests that the nucleoproteins act either as a model or a directing control for the synthesis of protein. It seems likely that synthesis of tissue proteins is a process that occurs at specific centers of anabolic activity within the cell (*26*).

With the above considerations in mind, the following hypothesis is presented as a qualitative explanation of the observed data on protein anabolism. It is assumed that amino acids are absorbed from the digestive tract in essentially the proportions in which the food protein gives them up in digestion and that they are then transported into the body cells in the same proportions. Here it is supposed that they are nonselectively adsorbed at the boundaries of anabolic centers. The adsorptive capacity of these boundaries is assumed to be limited, and any excess supply of amino acids must therefore be lost for purposes of tissue synthesis, owing to their removal from the field of action by excretion or irreversible metabolic processes.

Under these conditions the rate of protein synthesis will depend on the concentrations and proportions of the amino acids at the boundary of the anabolic center. At any given concentration, the rate of synthesis will be at a maximum if the proportions adsorbed match the proportions in which they are utilized in the center. If optimum proportions are maintained, rate of tissue synthesis will increase with the concentration of amino acids in the boundary, up to a limit fixed by the maximum rate at which anabolism can operate. The fact that maximum growth rates in young animals can be obtained with 11 to 12% of high quality proteins in the ration, while growth rate on low quality proteins increases up to a 20 to 25% level in the diet, suggests that adsorptive capacity of the boundary is highly relative to the needs of the anabolic center (*13*, *86*).

If the proportions of amino acids in the boundary do not match the utilization pattern of anabolism, rate of synthesis is reduced. The rate will be increased as the concentration of the unbalanced amino acid mixture in the boundary is increased, but its limit will be reached when the boundary is saturated. This accounts for the observations that low quality proteins cannot be made to give the growth performance of high quality proteins even at high intakes. It also provides an explanation for Grau's observation that sesame protein at the 30% level in chick diets still required lysine supplementation, even though the unsupplemented protein provided approximately the same amount of lysine as was found to give optimum growth at a 20% protein level (*45*).

Presumably the number of active anabolic centers declines as growth rate slows. In adulthood their number is in equilibrium with the need to replace catabolic losses, although exceptional anabolic activity, comparable to that observed in growth, may be shown following a period of severe protein depletion, as demonstrated by Cannon and coworkers (*22*). The average proportions in which the amino acids are used by all the anabolic centers of the body probably correspond closely to the proportions in which the amino acids are utilized in growth by the young animal. The efficacy of high quality proteins in adult nutrition, as demonstrated by Mitchell, Murlin, and others, suggests that the proportions in which the amino acids are required by anabolic centers in the adult are about the same as those determined for the immature animal.

Amino Acid Imbalance and Malnutrition

The physical status of malnourished individuals reflects failures of tissue anabolism and the areas of malnutrition coincide with the areas where low quality proteins predominate in the diet.

In areas where pellagra, a particularly severe type of malnutrition, is endemic, corn (maize) provides a large part of the protein consumed (*43*). High intake of corn protein will not prevent development of pellagra. For example, Aykroyd and his associates found a serious pellagra problem in certain districts of Romania where protein intake averaged over 100 grams per day (*9*). Nicholls has reported that African railroad workers, given a diet of 2 pounds of maize meal per day, exhibited signs of severe malnutrition within a few weeks, including emaciation, sore mouth, and intractable diarrhea, although the skin lesions of pellagra did not develop (*95*). The amount of protein in 2 pounds of corn meal should supply at least twice the minimum adult requirement of both lysine and tryptophan, the limiting amino acids in corn (*11*).

Inspection of Figure 5 reveals the poor amino acid balance of corn protein, marked by unusually low proportions of both lysine and tryptophan. Marked retardation of anabolic processes could be the only outcome of diets deriving their protein mainly from this source, particularly when the total daily protein intake is also low.

Although extensive supplementation with lysine and tryptophan would be necessary to bring the amino acid proportions of corn protein into conformance with anabolic needs, there is evidence that relatively slight alterations in either the protein or the body's anabolic-catabolic balance will suffice to prevent the skin lesions characteristic of pellagra. The Gillmans, for example, observed that the skin and liver lesions of their adult patients disappeared on bed rest, even if an exclusive corn diet was maintained (*43*). This may be interpreted as due to a reduction in catabolic losses, enabling even the slow rate of anabolism permitted by corn protein to keep up with needs. The dermatosis of pellagra is rare in Mexico, where corn meal is treated with lime water before use. Laguna and Carpenter found that lime-treated corn gives better growth in rats than untreated corn, and that the effect is on the nonstarch portion of the corn (*70*). Liberation of niacin from an alkali-sensitive precursor is suggested by these investigators as an explanation, but the possibility of a slight favorable alteration in the amino acid balance would seem worthy of study.

The curative action of niacin upon pellagra can be interpreted in similar fashion, on the assumption that niacin spares tryptophan for its essential function in tissue protein construction. In the absence of sufficient niacin in the diet, some of the tryptophan may become unavailable for tissue synthesis through diversion to synthesis of this vitamin in the body (*4*, *119*). It is of interest to note the apparent anomaly observed by Elvehjem and coworkers, that animals on 9% casein diets which contained no niacin grew better if the ration contained 40% cornstarch than if it contained 40% corn grits, despite the tryptophan contributed by the grits (*69*). The anomaly disappears, as Elvehjem and associates later pointed out, if one takes into account the amino acid imbalance introduced by addition of corn gluten (*68*).

Extension of Effective Protein Supply through Food Combinations

A strong warning has been sounded by Hart against the condemnation of a food "because its proteins, when fed alone, are not of high biologic value" (*49*). By suitably combining different foods at the same meal, it is often possible to achieve a satisfactory over-all amino acid balance and therefore to secure efficient utilization of the total protein supplied. For example, equal parts of milk and cereal protein will provide a mixture in which the lysine-tryptophan ratio is close to 4, which is sufficient for good protein efficiency, though below the value of 5 required for optimum rate of tissue anabolism. Similarly, good amino acid balance can be obtained by combining grain proteins with approximately equal weights of protein from fish, meat, cheese, or eggs.

Peas and beans, relatively deficient in methionine and tryptophan but fairly well supplied with lysine, may be advantageously combined with wheat and rice, which are low in lysine but well balanced with respect to methionine and tryptophan (see Figures 3, 4, and 6). The value of peas and beans in combination with corn would seem doubtful unless legume varieties containing a satisfactory proportion of tryptophan are employed.

Potato protein, which is relatively short in methionine but well supplied with lysine (Figure 8), should make a desirable partner for grains. Because of its relatively high proportion of tryptophan, it should be especially good with corn. Of course, the low protein content of potatoes means that the bulk of this food would have to be large in relation to the grains in the diet. If cassava or manioc has an amino acid balance similar to the potato, grain supplements should be effective in improving the nutritional status of populations which live largely on this root. Pieraerts has noted decreased incidence of kwashiorkor in a tribe of the Belgian Congo during seasons of the year when maize and millet are available for incorporation in the manioc bread consumed as the principal food (*108*).

Extension of Effective Protein Supply by Amino Acid Supplementation

Several important factors militate against a solution of the protein problem of human nutrition by food combinations alone. In large areas of the world there are insufficient supplies of foods that might be combined with the chief dietary staples to improve amino acid balance. Even in countries with greater protein resources, hypoproteinosis and waste of protein are the frequent result of ignorance, low income status, or the strength of individual dietary preferences. In the United States, a rapid population increase is placing visible strain on protein resources by reducing the per capita supply of high quality proteins that can be applied for improvement of over-all amino acid balance (*30*).

Where amino acid balance cannot be, or in practice is not, achieved, it is possible to improve nutrition and reduce protein waste by supplementing staple foods with amino acids. A number of typical examples are given in Table V, based on animal feeding studies with rations supplemented with vitamins and minerals.

As an increase in the protein efficiency ratio means an increase in the amount of body tissue that can be synthesized from a given weight of protein, it is obvious from Table V that amino acid supplementation is potentially capable of providing a large increase in the supply of utilizable protein. The high effectiveness of low addition levels is due to the fact that remedy of deficiencies permits utilization of all the other essential amino acids which are present in relative excess in the unsupplemented food.

The gains realizable by amino acid fortification may be illustrated by a specific example. Bread consumption per individual in the United States has been estimated by the Food and Drug Administration at 5.5 ounces per day, or approximately 120 pound loaves a year (*34*). At 39 grams of protein per loaf, this amount of bread would make an annual contribution of 4680 grams of protein to the average individual's diet. In weight, this is equal to the protein contained in approximately 54 pounds of fresh lean beef, 140 quarts of milk, or 660 eggs (*115*), but in protein efficiency it is equivalent to only about half these values.

Table V. Extension of Protein Value of Staple Foods by Amino Acid Supplementation

Food	Amino Acid Supplement, % of Food	Improvement in Protein Efficiency Ratio over Unsupplemented Food, %	Reference
White flour (enriched)	0.6% L-lysine[a]	111	Sure (*123*)
White bread (3% non-fat milk solids)	0.2% L-lysine[b]	70–100	Rosenberg and Rohdenburg (*114*)
Corn meal	0.2% L-lysine and 0.1% L-tryptophan[a]	73.5	Sure (*123*)
White rice	0.2% L-lysine and 0.12% L-threonine	130	Pecora and Hundley (*105*)
Cooked soybeans[c]	1.27% DL-methionine[d]	74	Hayward and Hafner (*52*)
Autoclaved Alaska field peas[e]	1.3% DL-methionine[f]	300	Woods, Beeson, and Bolin (*135*)

[a] Calculated from Sure's data (*123*).

[b] Based on flour content of bread (*114*).

[c] In cornstarch-soybean ration containing 23.3% soybeans (*52*).

[d] Corresponds to 0.3% DL-methionine in total ration (*52*).

[e] In cane sugar–field pea (*Pisum sativum*) diet containing 46.3% field peas (*135*).

[f] Corresponds to 0.6% DL-methionine in total ration (*135*).

The utilizability of bread protein is much improved when it is consumed with adequate amounts of high quality proteins, but in many poorly balanced diets this situation does not prevail. In such diets the fortification of bread with lysine is potentially capable, according to the studies of Rosenberg and Rohdenburg, of doubling the value of bread protein for tissue synthesis (*114*). Thus, in diets where intake of high quality proteins is low or poorly distributed among meals, lysine fortification of bread could add, at the average consumption of bread, the equivalent in protein value of as much as 27 pounds of meat, 70 quarts of milk, or 330 eggs per person per year. Individuals living on low incomes and therefore consuming larger-than-average amounts of bread and other low-cost cereal foods could benefit to an even greater extent.

In large areas of the world where low quality proteins predominate in the diet, it should be possible to effect the equivalent of a 50 to 100% increase in protein supplies by amino acid fortification. To accomplish the same result by agricultural means would at best require drastic alterations in farming practices and dietary habits, and in many regions it would be impossible.

The economics of amino acid fortification appear favorable. There is no apparent reason why the four amino acids most needed in human dietaries—lysine, methionine, threonine, and tryptophan—could not be made available at, say, 1 to 3 cents per gram if manufactured by modern mass production methods. As individual consumption would be at the rate of only a few tenths of a gram to 1 gram or so of amino acid supplement per day, the cost would appear modest in comparison with the nutritional benefits to be achieved.

Caution must be observed in improving the amino acid balance of human diets in which the supply of other dietary essentials is marginal. Shortages of the vitamin and mineral components of body tissue will limit tissue synthesis, even if all needed amino acids are provided abundantly and in good proportions. Improved protein nutrition may magnify the effects of vitamin and mineral deficiencies during growth, when demands for all the essential nutrients are high. Thus, in the Steenbock rachitogenic diet, which lacks vitamin D and is composed chiefly of yellow corn meal and wheat gluten, rickets can be more speedily precipitated if the diet is supplemented with lysine to improve growth rate (*37*).

Summary

The quantity and quality of protein supplied by the diet are of vital importance to health at every portion of the life span. Wherever total quantity or average quality of the protein consumed falls significantly below accepted standards for good nutrition, the signs and symptoms of protein deficiency (hypoproteinosis) appear, involving various degrees of retardation or failure of tissue synthesis.

Chronic states of moderate-to-severe hypoproteinosis are characteristic of those world areas where dietary protein is chiefly of low quality, as in the Far East, Africa, the West Indies, Central America, and large parts of South America and Europe.

Mild-to-moderate hypoproteinosis, usually precipitated by physiological stress in conjunction with inadequate intakes of high quality proteins, is frequently observed in the United States, as shown by clinical observations of conditions known to be caused principally or in large part by poor protein nutrition, including deficient muscle development and retarded growth rates in many children, low resistance to tuberculosis and rheumatic fever in some population groups, anemia among women of childbearing age and children, the common occurrence of low plasma protein levels, toxemias and premature labors among pregnant women, and states of nitrogen depletion among many elderly individuals that are reversible by protein therapy. Dietary surveys indicate that the protein nutrition of a substantial fraction of the American people is at a marginal level for good health.

Nutrition research has shown that amino acids are utilized in definite combining proportions for tissue anabolism. Those proteins which contain amino acids in approximately the same proportions in which they are utilized in the body—e.g., the high quality proteins—are most effectively assimilated by both adults and children. For most efficient utilization of amino acids from the diet, they must reach the anabolic centers of the body cells simultaneously and in proper proportions.

The evidence indicates that the efficiency of protein utilization may be improved to an important degree by the following means:

Use of combination of food proteins whose amino acid proportions exert a supplementary effect upon one another.

More even distribution of high quality protein among the daily meals.

Use of specific amino acid supplements to low quality proteins to achieve amino acid proportions that conform to anabolic needs.

The four essential amino acids most needed for improvement of human dietaries are lysine, methionine, threonine, and tryptophan. By addition of these amino acids many important low-cost foods could be converted to high quality protein sources. In areas where bread, white flour, and other foods derived from wheat are important staples, substantial over-all improvement in protein nutrition could be realized by fortification with lysine alone. Diets consisting largely of rice would require supplementation with lysine and threonine, while those largely composed of corn (maize) could be improved by supplementation with lysine and tryptophan. Where root or legume proteins are leading staples, fortification with methionine or combinations of methionine with lysine and tryptophan could accomplish similar results. It appears probable that in many instances the effective supply of dietary protein could be increased by 50 to 100% through amino acid supplementation.

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