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NUTRITION[1]

BY NEVIN S. SCRIMSHAW, GUILLERMO ARROYAVE, AND RICARDO BRESSANI
Institute of Nutrition of Central America and Panama (INCAP), Guatemala

The last few years have seen a growing recognition of the seriousness of protein malnutrition in a large part of the world and a steady increase in the understanding of the scientific bases for the evaluation of protein quality. An indication of this is that investigation into those aspects dealing directly or indirectly with protein metabolism has become a very significant part of the total research in the field of nutritional biochemistry. In view of the large number of articles in this field and the complete coverage given in last year's review to work on lipides, which is another biochemical field of major current nutrition interest, no attempt has been made to deal with articles unrelated to the main topic of protein nutrition. A special effort has been made, however, to give consideration to the reports of workers in technically underdeveloped countries who are dealing with protein malnutrition as an urgent national problem. Unfortunately, many interesting papers on even this more limited subject could not be mentioned in the space allotted.

PROTEIN REQUIREMENTS

Although the F.A.O./W.H.O./Josiah Macy Foundation Conference on "Human Protein Requirements and Their Fulfillment in Practice" (1) revealed the sparseness of quantitative knowledge on this subject, it also marshaled evidence to suggest that current *National Research Council* allowances are higher than necessary. The subsequent report of the F.A.O. Study Group on "Protein Requirements" (2) proposed new figures which vary with the rate of growth from 2.00 gm. per kg. body weight for the infant to 0.35 gm. per kg. body weight for the adult with an increase during adolescence. A safety margin of 50 per cent above these figures was recommended to allow for individual variation. These figures are expressed in terms of protein of high biological value; the greatly increased requirements when the protein is of poor quality are emphasized and detailed instructions for adjusting these recommendations to various types of diets are given in this very stimulating and useful report.

Evidence for lowering recommended allowances for protein has come from various sources. Rose & Wixom (3) concluded that when the eight essential amino acids were administered at the "safe" levels of intake (4), nitrogen equilibrium could be maintained with the addition of nonessential amino acid nitrogen in the form of glycine to provide a total daily intake of only 3.5 gm. of total nitrogen. Calculated as nitrogen $\times 6.25$, this is only about 0.32 gm. of protein per kg. body weight for the lightest subject tested and 0.25 for the heaviest one. In 171 apparently well-nourished young women, 17 to 27 years of age, consuming self-selected diets in the United

[1] Based primarily on articles published in 1956 and the first eight months of 1957.

States, the average daily intake was 0.84 ± 0.19 gm. of protein per kg. body weight according to five-day balance studies by Scoular *et al.* (5). Barness *et al.* (6) studied nine healthy infants under one month of age fed isocaloric and isonitrogenous formulas of cow's milk and breast milk for alternate periods. Two of these infants remained in nitrogen balance and grew satisfactorily on 0.8 gm. protein per kg. body weight per day on either formula.

Persons consuming poor vegetarian diets can attain nitrogen balance at low intake levels, but at the cost of adequate nitrogen stores or poor growth or both. For example, Murthy *et al.* (7) have reported that, even on a rice diet, five girls aged seven to nine were in nitrogen balance on a total calorie intake of 1010 and a protein intake of 1.2 gm. per kg. body weight per day. Sur *et al.* (8) studied 12 children, eight to eleven years of age, maintained on either a rice diet or a rice-peanut curd diet providing mean daily protein intakes of approximately 1.1 and 1.5 gm. per kg. body weight respectively. Despite the low protein intake, only one of the children (on the rice diet) showed a negative nitrogen balance. Subrahmanyan *et al.* (9) also report adults in nitrogen balance on 1.2 gm. of protein per kg. body weight when eating a vegetarian diet with an apparent protein digestibility of only 50 per cent.

Recent reports on the poorer nitrogen utilization during caloric restriction are based on work with rats (10 to 13), mice (14) and poultry (15, 16). Any decrease in the apparent digestible energy of the diet is compensated for by increased food consumption (13, 17). Sibbald *et al.* (17) found that the ratio of apparent digestible energy to apparent digestible nitrogen of the food controlled the percentage of nitrogen retained. The optimum ratios appeared to be 250 to 300 calories of apparent digestible energy per gm. of apparent digestible nitrogen. In a more recent article, the same authors (18) confirmed these findings and showed that they apply also to a protein-free amino acid diet.

Evidence that dietary fat has a specific protein sparing action independent of its caloric value, comes from work with rats [Cohn & Joseph (19); Schreiber & Elvehjem (20)] and dogs [Ontko, Wuthier & Phillips (21)]. Evidence to the contrary, however, is exemplified by the recent work of Metta & Mitchell (22) who found that, when fed in isocaloric amounts, dietary fat and carbohydrate have essentially the same effect on protein utilization.

Heinicke, Harper & Elvehjem (23), working with guinea pigs, found no differences in protein requirements between diets containing dextrin or sucrose. Womak & Marshall (24), however, report that in experiments on rats with low levels of amino acid intake both sucrose and fructose resulted in greater negative nitrogen balance than starches or corn dextrin. The data of Cornely, Barness & György (25) also suggested that nitrogen retention was greater in lactose-fed babies than in those for whom dextri-maltose was the carbohydrate source. That the effect of certain carbohydrates on the over-all nitrogen need of the animal could be due to interference with digestibility has been shown by Yoshida & Morimoto (26) who found marked decreases in casein digestibility in rats when raw (but not steamed) potato starch was included in the diet. The effect of the type of carbohydrate on amino acid requirements has been well reviewed by Harper & Elvehjem (27).

It has long been recognized that urea can fill part of the nitrogen needs of ruminants, but it is now clear that this substance can also be used by rats [Rose & Dekker (28)], hamsters [Matsumoto (29)], human adults (3) and even premature infants [Holt & Snyderman (30)]. It is noteworthy that breast milk contains approximately 20 per cent of its nitrogen in the form of urea and other nonprotein compounds, and cow's milk only about 5 per cent. Despite this the nitrogen retention calculated as percentage of total nitrogen intake has been shown to be slightly (but not significantly) higher in breast milk (6). Although Finlayson & Baumann (31) report that both diammonium citrate and urea exert a growth depressing action in rats in either spaced or *ad libitum* feeding, the levels employed were extremely high.

Since the nitrogen balance method is used by most investigators studying protein and amino acid requirements in humans, the critique of this technique by Lowe & Pessin (32) is useful. In addition, Allison & Wannemacher (33) stressed that the requirement for maintaining adequate nitrogen reserves is greater than that for nitrogen balance. Meyer (34) showed that each added increment of cellulose increased the metabolic fecal nitrogen, although there was no influence of fiber on urinary endogenous nitrogen in growing rats. This emphasized again the importance of maintaining constant the fiber content of experimental diets in balance studies of the digestibility and biological value of proteins.

Biological Value of Foodstuffs and Diets

Assay methods.—More attention than ever has been centered on evaluating the biological value of plant and animal proteins, although the methods used have for the most part been well established. Bender (35) has shown that the Protein Efficiency Ratio as determined in rats varies directly with food intake and does not represent true nitrogen utilization since total body weight used for the calculation of the Protein Efficiency Ratio is affected by other factors such as fat deposition. Bender & Doell (36) have advocated a shorter method of assessing protein quality by feeding rats at a 10 per cent level of test protein and another group of litter mates a protein-free ration. The algebraic difference between the gains in weight of the two groups divided by the weight of protein eaten at the end of ten days is called the Net Protein Ratio. The Net Protein Ratio can be converted to a percentage scale by multiplying by the factor 16, to give the Protein Retention Efficiency.

Miller & Bender (37), Bender & Doell (38) and Dreyer (39) described the calculation of the Net Protein Utilization by the formula $(B_F - B_K + I_K) \div I_F$, where B_F is the carcass nitrogen of protein-fed rats, B_K the carcass nitrogen of rats on a protein-free diet, I_F total dietary nitrogen and I_K nitrogen from other than the test protein. They believe the Net Protein Utilization to be a more precise estimate of the proportion of dietary nitrogen retained by the carcass of the animal. Net Protein Utilization, as determined by carcass analysis, correlates highly with the Protein Retention Efficiency, but the latter has the advantage of combining both maintenance and growth requirements for protein.

Chick assays have been used for many years for the evaluation of the biological value of proteins, but Beaty *et al.* (40) have advocated the use of chicks which have been protein-depleted to about 75 per cent of their initial weight. They assay the protein under test at dietary levels of 6.5, 9 and 12 per cent protein. The protein depletion-repletion technique is a sensitive measure of protein quality in baby pigs according to Peo *et al.* (41). The same authors (42) emphasize that as levels of dietary protein increase, the differences in protein quality of the test foods become less critical. Meade (43) and Meade & Teter (44) have also stressed that the relative proportion of amino acids is more important when the total amount of dietary protein is inadequate.

These observations, which are of great practical importance, are well supported by a study by DeMaeyer & Vanderborght (45) which shows that the biological value of soya fed to children, as determined by nitrogen retention, is higher at high levels of intake than at low. This is also suggested by Sure (46) who has studied the nutritive value of the proteins in various foods at increasing protein intakes and found 15 per cent to be the most efficient level for poultry and livestock feeding. However, human nutrition is concerned with the end result as well as efficiency, and increasing the amount fed of a protein of intermediate value may be as nutritionally effective as improving its quality.

Allison (47) has continued to call attention to the fact that nitrogen balance is only the sum of the over-all nitrogen metabolism and does not measure the gain or loss of protein from specific organs or tissues. Recent work by Vijayaraghavan (48) gives further evidence that growth and nitrogen balance are not the only criteria by which the nutritive value of protein should be judged. In his study casein, egg, meat, wheat gluten, and rice protein varied widely in their efficiency for blood formation in rats. Perdue, Lambert & Frost (49) show human and dog blood to be poor sources of essential amino acid nitrogen for protein-depleted rats. This emphasizes the well-known fact that special tissues may have amino acid requirements different from those for growth and maintenance.

Sheffner *et al.* (50, 51) have used the microbiological determination of the pattern of amino acids released *in vitro* by pepsin to reveal differences between proteins which were not apparent from their total content of each essential amino acid. They have proposed a new index, the Pepsin Digest Residue, which, when divided by the digestibility, accurately predicted the biological value of such diverse products as egg, soya, casein, yeast and white flour. Additional microbiological methods for measuring the utilization of intact protein have been advocated by Mertz *et al.* (52) using *Pseudomonas* *aeruginosa*, by Fernell & Rosen (53, 54) employing *Tetrahymena pyriformis* and by Teeri, Virchow & Loughlin (55) using enzymatic hydrolysis of the sample prior to assay with *Streptococcus fecalis*.

Amino acid reference pattern.—The review of Flodin (56) documents well the principle that the amino acid pattern found in animal proteins provides superior efficiency of protein utilization for periods of high anabolic activity and for the maintenance of labile protein stores. It also closely parallels the pattern of amino acids found best for rat growth and for maintenance of nitrogen balance in adults. For example, Albanese *et al.* (57) have provided further evidence that the utilization of dietary proteins increases as their proportion of lysine and tryptophan approaches that of muscle tissue. For evaluating protein-containing foods, they propose the calculation of a Protein Utilization Index which is equivalent to the body weight gain×the nitrogen retention in mg. per kg. body weight÷100.

These considerations led the F.A.O. Study Group (2) to designate a theoretical reference protein, the amino acid pattern of which could be used for calculating a "protein score" based on the per cent deficiency of the most limiting amino acid. The F.A.O. reference amino acid pattern in gm. of amino acid per gm. of nitrogen is as follows: isoleucine, 270; leucine, 306; lysine, 270; methionine and cystine, 90; phenylalanine and tyrosine, 180; threonine, 180; tryptophan, 90; and valine, 360. Preliminary evidence obtained by the authors (58) suggests that the pattern may be too low in lysine and too high in methionine. The principle of such a reference pattern is very useful, and the necessary research to perfect it should have a high priority.

Amino acid availability.—Deshpande *et al.* (59) have taken advantage of the low isoleucine content of blood meal to demonstrate the availability of isoleucine in eight different proteins used to supplement it. They concluded that isoleucine is available for the rat only to 30 per cent in zein, 60 to 70 per cent in casein and gelatin, and over 90 per cent in beef fibrin, egg albumin, and Drackett (soybean) protein. Gupta & Elvehjem (60), using rats, found that the biological availability of tryptophan from beef and pork protein, egg albumin, beef fibrin, purified soybean protein, and milk powder varied between 80 and 100 per cent. Stevens & Henderson (61) obtained similar results for casein. Kratzer & Green (62) found that the availability of lysine in spray-dried soluble blood meal for the chick was only 75 per cent of that found by microbiological assay after acid hydrolysis. Vat-dried blood meal had available only 60 per cent of the lysine determined microbiologically. The many studies of amino acid destruction with food processing and storage are beyond the scope of this review.

Improvement of Proteins

Vegetable mixtures and mixed diets.—An excellent report from the Indian Council of Medical Research (63) reviewed work in China and India on the preparation, testing and use of milk substitutes for areas where milk production is inadequate. The report concluded that milks made from soybeans, peanuts, cashew nuts, coconuts, almonds, or legumes can be helpful in supplementing poor cereal diets. In the Belgian Congo a milk, prepared from peanuts, given to 96 lactating women and 135 infants by Holemans *et al.* (64) proved to be a good supplement as judged by biochemical and clinical indices.

A report from Russia (65) described several mixtures of cereals and legumes which proved to have a high nutritive value, the best of which was better than casein in rat growth tests, and contained 60 per cent buckwheat, 20 soya and 16 rice. Scrimshaw *et al.* (58) have described a 25 per cent protein mixture for human feeding which contains 50 per cent dried corn "masa" (from lime-treated corn), 35 per cent sesame oil meal, 9 per cent cottonseed meal, 3 per cent kikuyu leaf meal and 3 per cent torula yeast. This mixture was as effective as cow's milk when tested isonitrogenously and isocalorically in rats, dogs, and well children.

Ladell & Phillips (66), in human nitrogen retention studies, found that, provided there is a certain minimum amount of animal protein (at least 14 gm.), one part of animal protein may be replaced by 2.2 parts of peanut protein. Desikachar, Sankaran & Subrahmanyan (67) and Phansalkar & Patwardhan (68) have used rats to demonstrate the improvement in nutritive value obtained in cereal proteins by adding legumes. The paper of Kamath & Sohonie (69) was one of the latest to show that the amino acid content of green leaves, while inferior to casein, is a potentially useful supplement to cereal diets. Other potential sources of vegetable protein which rat growth trials have shown capable of supplementing cereal diets are the green algae, *Scenedesmus obliquiuss* and *Chlorella pyrenoidosa* [Hundley, Ing & Krauss (70)], the South American Quinoa, *Chenopodium quinoa* [Quirós-Pérez & Elvehjem (71)] and Buckwheat, *Fagopyrum tartaricum* [Sure (72)].

The many recent studies on the nutritive value of fish and fish products cannot be referred to individually, but reports have also appeared on the use of fish products as supplements to cereals. Costamaillere & Ballester (73) showed that fish flour was as effective as whole milk powder in improving the rate of growth of rats on a diet patterned after that consumed by low economic groups in Chile. Cravioto *et al.* (74) found that five of seven fish flours added at a level of 10 per cent of a "tortilla" diet, improved the biological value of the corn protein as indicated by rat growth experiment. The two fish flours which were ineffective had been deodorized by industrial processing. Sure (75) and Carpenter *et al.* (76) have found that fish flours make effective supplements to cereal diets.

Corn.—In view of the poor biological value of the protein of corn, it is surprising that the only published reports of improving the biological value of predominantly corn diets by use of complementary vegetable proteins were those of Scrimshaw *et al.* (58) previously referred to, and Mangay, Pearson & Darby (77) who showed that millet, *Setaria italica*, will correct the niacin deficiency induced in rats by a 9 per cent casein and 40 per cent corn diet. Mangay *et al.* (77) also showed that 1 per cent lysine added to a diet of 40 per cent maize and 40 per cent millet improved growth considerably, but produced no growth response when added to a diet of 80 per cent maize and 10 per cent millet unless niacin or tryptophan or both were also added. It is thus clear that millet improves the tryptophan but not the lysine deficiency of corn. Scrimshaw *et al.* (58) have shown some improvement in nitrogen retention of young children when tryptophan was added to a corn "masa" plus gluten mixture but a much greater increase when both tryptophan and lysine were supplied to the levels of the F.A.O. amino acid reference pattern. For rat growth, Hogan *et al.* (78) have reported lysine to be the most limiting amino acid in whole corn, with tryptophan second. Mosqueda-Suarez (79) found that the addition of 0.2 per cent tryptophan to a degermed corn product increased the rate of weight gain of rats 214 per cent, addition of lysine alone, 59 per cent and supplementation with both amino acids 414 per cent.

Wheat.—Many studies have shown that supplementing wheat products with lysine results in an improvement of their protein quality. Among the most recent are those of Hutchinson, Moran & Pace (80, 81); Sure (82); Deshpande, Harper & Elvehjem (83); and Rosenberg (84). Deshpande *et al.* (83) showed that lysine prevented fatty livers which developed on a low-protein diet (5.4 per cent) in which wheat was the principal protein source.

It has also been shown that milk and meat can supplement a wheat flour basal diet and make lysine supplementation unnecessary [Westerman, Hays & Schoneweis (85); Westerman, Kannarr & Rohrbough (86)], although Jahnke & Schuck (87) demonstrated that lysine addition is beneficial even when the wheat flour was already enriched with three per cent nonfat milk solids. Sarett (88) studied a mixture of 70 per cent of a cereal food (Pablum, Mead Johnson & Co.) and 30 per cent milk powder and found that as measured by rat growth it was not improved by lysine supplementation, a result which was to be expected since the basal diet contained adequate lysine to satisfy the rat requirement.

Other vegetable protein sources.—The supplementation of other vegetable proteins with synthetic amino acids has also received attention. The work on beneficial effects of the supplementation of most animal feeds with methionine or lysine or both has been well reviewed by Rosenberg (84). Cowlishaw *et al.* (89, 90) found that leaf protein concentrate for children was improved by lysine but not by methionine addition. Kik (91, 92) has demonstrated that the proteins of commercial rice bran and rice polishings, as the sole source of protein in rats, can be improved by the addition of 0.2 per cent L-lysine and 0.2 per cent DL-threonine. The addition by Sure (93) of 0.4 per cent L-lysine, 0.5 per cent DL-threonine and 0.5 per cent DL-methionine to the proteins in milled barley resulted in 151 per cent increase in growth and 224 per cent increase in the Protein Efficiency Ratio. Supplementation of peanut flour with 0.5 per cent each of DL-methionine and DL-threonine improved weight gain 60.6 per cent and Protein Efficiency Ratio 61.5 per cent.

Obviously, foods are not improved by amino acid addition unless a limiting deficiency is corrected. For example, lysine addition failed to improve soybean protein [Block *et al.* (94)]. That more than one essential amino acid may be necessary to bring about satisfactory improvement in a cereal protein is illustrated by the studies of Sure (95) on rice and wheat, and Hundley *et al.* (96), Kik (91, 92), and Deshpande *et al.* (97) on the supplementation of rice.

Amino Acid Requirements

In man.—The F.A.O. Committee on protein requirements (2) took the important step of considering amino acid as well as protein requirements for man. Basic to the discussion was a series of papers by Rose and co-workers, published from 1947–55, describing the minimal amounts of individual essential amino acids required to maintain "a distinct positive balance, as measured by the average of a period of several days." These reports were well summarized in an article by Elvehjem (98) and in a comprehensive and critical review by Rose (4).

Available to the F.A.O. Committee, although still unpublished at that time, were a similar series of studies by Leverton *et al.* carried out on young women. In these studies the requirement range reported for threonine was found to be 103 to 305 mg. per day (99); for valine 465 to 650 mg. (100); for tryptophan 82 to 157 mg. (101) and for leucine 170 to 620 mg. (102). Phenylalanine was studied with diets furnishing 900 mg. of tyrosine daily, and, under these circumstances, 120 to 200 mg. were required (103). Swendseid, Williams & Dunn (104) in similar studies on young women found a range of 350 to 550 mg. for methionine plus cystine, and Swendseid & Dunn (105) have reported a range of 250 to 450 mg. for the isoleucine requirement.

All of these figures tend to be lower than those found previously for men, but as Rose points out (4), they are based on a different criterion for nitrogen equilibrium, namely "The zone in which the difference between the intake and excretion does not exceed 5 per cent." Thus, eight of the sixteen subjects reported to be in nitrogen equilibrium by Leverton *et al.* (99) in the threonine study would have been considered in negative balance by Rose. Another difference, possibly contributing to the lower values for women, was the practice of adjusting the calories to prevent gain or loss of body weight instead of keeping them constant throughout an experiment as done by Rose.

Jones, Baumann & Reynolds (106) have studied the lysine requirement of women and reported it to vary from 400 to 500 mg. per day. These investigations used diets providing all or almost all of the dietary nitrogen as synthetic amino acids and urea or ammonium citrate. Doubt remains as to the errors introduced by determining amino acid requirements on diets containing only minimal quantities of other essential amino acids. Nasset (107) showed clearly that for rats a mixture based on the mean minimum requirements of the nine essential amino acids is grossly inferior to either egg protein or an amino acid mixture which simulates egg protein. In the first of a projected series of papers on the amino acid requirements of both men and women, Clark *et al.* (108) used cereals and other foods to provide about half of the total nitrogen. They report that ten men and women maintained nitrogen equilibrium at 500 to 900 mg. of lysine. No sex differences were apparent. Since both of these studies employed criteria for nitrogen equilibrium similar to those of Rose, it was reassuring that their results agreed well whether the diet was entirely or only partially synthetic.

As pointed out in the previous section on protein requirements, it must not be assumed that measuring the requirements for nitrogen balance necessarily gives the requirements for optimum nutrition. Schultze (109) found mixtures of amino acids that supported "normal" reproductive and lactation performances in rats for as long as four successive generations and still were not completely adequate for optimum pre- and postweaning weight gain of the young nor for the prevention of fatty livers in the mothers during lactation. The work of Allison *et al.* (110) illustrates the point that the nutritional demands of tissues of an animal may sometimes be revealed more adequately by studying the effects of various stresses upon nutrient requirements.

Snyderman *et al.* (111) have restudied the lysine requirement of six normal infants using a synthetic diet made up of 18 L-amino acids in the same proportion found in breast milk; all six required less than 90 mg. of lysine per kg. body weight per day. The information available on phenylalanine and threonine requirements in children is well summarized by Holt & Snyderman (30).

In rodents.—Additional studies of amino acid requirements of rats have also appeared. Forbes, Vaughan & Norton (112) have employed a measure which they refer to as "minimum nitrogen loss" (exogenous urinary nitrogen ÷nitrogen intake)×100. The least amount of isoleucine required for minimum wastage of dietary nitrogen in growing rats fed synthetic diets was 2.6 per cent of the conventional protein equivalent of the diets regardless of its total nitrogen content. The requirement of the adult rat for histidine was reinvestigated by Moore & Wilson (113) and this amino acid found to be essential for normal nitrogen retention. The discrepancy between this conclusion and most earlier reports may be due to differences in the composition of the experimental diet. Hartsook & Mitchell (114) have demonstrated that the percentage of methionine plus cystine required in the diet of growing rats decreases in an exponential manner with age, while it increases as percentage of the protein requirement from 7 to 8 per cent, probably due to the continued synthesis of keratin for hair, despite the decreasing rate of body growth with age. Womack, Snyder & Rose (115) showed that D-valine is relatively ineffective for promoting rat growth even at the 2 per cent level and still less utilized when L-leucine in the diet is replaced by twice the amount of DL-leucine. Apparently, D-valine is difficult for the organism to use under any circumstance and more so when other D-amino acids are fed.

The second in a series of papers by Heinicke, Harper & Elvehjem (116) has also appeared showing that guinea pigs have a particularly high need for arginine and methionine which decreases as they mature, paralleling the decrease in growth rate.

In the chick.—Baldini & Rosenberg (117) have found the methionine requirement of the chick to increase as the productive energy level of the diet increases. Relating the requirement of a nutrient to the productive energy level of the diet is applicable to most of the nutrient requirements for the chick. For example, Griminger, Scott & Forbes (118) have shown that as the dietary bulk increases the chick requirement for tryptophan and arginine, as percentage of the diet, decreases. Williams & Grau (119) have similarly observed that when 12 per cent or more of cellu-flour replaced glucose, thus reducing the digestible energy concentration of the diet, feed consumption and growth increased in all diets deficient in lysine. When sufficient energy is available, the requirement of the broiler for methionine increases as the protein content of the diet increases [Rosenberg & Baldini (120)]. Griminger, Scott & Forbes (121) have shown that the tryptophan requirement of the growing chick increases with the protein level, although at a slower rate than the latter.

Fisher (122) has shown that, in the presence of sufficient tyrosine, 0.5 per cent of L-phenylalanine was required for chick growth and maximum feed utilization. Since previous studies have established a requirement of 0.9 per cent of the DL-form, it appears that chicks, like rats, do not utilize D-phenylalanine efficiently. The L-tyrosine requirement in the presence of 0.46 per cent L-phenylalanine has been reported by Fisher, Johnson & Leveille (123) to lie between 0.3 and 0.5 per cent.

Amino Acid Interrelationships

The authors regret the impossibility of including, within the space of this review, the many valuable studies of the metabolic relationships between amino acids and other metabolites. It is essential, however, to refer to the important recent studies of amino acid interrelationships.

Since Elvehjem has been directly involved in much of this work, his review articles are particularly informative (124, 125, 126). He distinguishes between amino acid imbalances, antagonisms between two amino acids and toxic effects of excessive amino acid levels. Benton *et al.* (127) have shown antagonism between isoleucine and valine, between phenylalanine and isoleucine, and between phenylalanine and valine in rats fed a 9 per cent casein diet supplemented with various amino acids. In a subsequent article these authors (128) showed that threonine prevents the growth depression caused by 3 per cent DL-phenylalanine or 3 per cent tyrosine under the above conditions.

Rerat, Bouffault & Jacquot (129) have shown that lysine can produce growth retardation in rats by either a deficiency or an excess in a diet based on corn or wheat glutens. Similar systematic studies are needed for each of the other essential amino acids. Wu & Lewis (130) found that a deficiency of lysine or of lysine and tryptophan greatly enhances the excretion of homogentisic acid following the injection of DL-phenylalanine. This effect is abolished by supplementing the gliadin diet with lysine but only partially abolished by supplementing zein with lysine and tryptophan.

The work of Sauberlich (131), while entitled "Amino Acid Imbalance as Related to Methionine, Isoleucine, Threonine and Tryptophan," only shows again that the growth of rats is decreased and food consumption per gm. of gain in body weight is increased when any of these amino acids is deficient and that the restoration of the deficient amino acid corrects these changes.

Gessert & Phillips (132) have reported that either methionine or lysine alone added to a mixed diet for growing dogs caused a growth depression which could be overcome by adding both together. The observation is of qualitative interest even though the use of literature values for the amino acid content of the diet ingredients precludes quantitative conclusions.

Protein and Amino Acid Metabolism

Protein turnover.—A number of studies not directly related to problems of deficiency have contributed to knowledge of protein and amino acid metabolism. The urinary excretion of I^{131} after administration of labeled albumin has provided a direct measure of albumin degradation. In four of a group of five human adults in good nutritional state studied by Fremont-Smith & Iber (133) a sudden increase in nitrogen intake from 0.5 to 3.0 gm. per kilo body weight was associated with a slight but definite increase in the albumin degradation rate which persisted even after nitrogen equilibrium was restored. $Nitrogen^{15}$ tagged yeast protein has been used by Sharp *et al.* (134) to show that neither achlorhydria nor age in adults depresses the capacity to absorb labeled protein during high protein intake. Sharp *et al.* (135), using the same technique, found the average retention of two younger subjects (age 24) to be greater than that of four older ones (age 51 to 66), the values being 57.6 per cent and 49.1 per cent, respectively. The physiological half life of N^{15} from yeast was 61 days in the younger subjects and 86 days in the older. The average total daily requirement for nitrogen from the "pool" (both fed and recycled nitrogen) was found to be 0.23 gm. per kg. body weight.

Protein Deficiency in Experimental Animals

Studies of protein depletion in animals are relatively few compared to the large number of papers which have appeared in the past two years on human protein malnutrition. Stanier (136) and Widdowson & McCance (137) have investigated the composition of muscle, liver, skin, and carcass of adult rats after approximately two months on a diet very low in protein (*a*) and a diet very high in protein (*b*) but limited to keep animals at the same weight as with the low protein diet. Diet *a* was similar to that responsible for kwashiorkor in children and diet *b* similar to that causing marasmus; changes in liver composition in the rats were very similar to those reported for each of these two clinical conditions. Iacobellis, Muntwyler & Dodgen (138) found that either protein or potassium depletion resulted in increased lysine and arginine and lowered aspartic and glutamic acids in skeletal muscle, diaphragm, and kidney. Since simultaneous protein and potassium depletion caused no alteration in amino acid pattern, attention was directed to the probable importance of maintaining a correct protein to potassium ratio in the diet therapy of human malnutrition.

Dogs were depleted of body proteins by repeated phlebotomy superimposed on a low protein diet in work reported by Hahn, Baugh & Meng (139). Despite relatively little change in total plasma protein levels, a marked decrease in albumin and increase in alpha, beta, and gamma globulins was observed. Of particular importance is the observation that anaphylactic reactions following immunization to horse serum were diminished or absent in the depleted animals. Of similar interest are papers which suggest mechanisms whereby protein deficiency may reduce resistance to *β-haemolytic streptococci* in rabbits (140), *Salmonella typhi* in rats (141) and tuberculosis in hamsters (142).

Srinivasan & Patwardhan (143) have shown clearly that the growing rat reacts to induced protein deficiency more rapidly and suffers greater histological and biochemical damage than adult animals. Liver xanthine oxidase in mice was shown by Mangoni, Pennetti & Spadoni (144) to decrease with decreasing protein intake within 21 days at dietary levels of 8, 18, and 30 per cent protein. The decrease was accentuated in those groups given 24 mg. of xanthine per 100 gm. body weight. Of fundamental interest is the investigation by Ross & Batt of the relationship between the activity of four hepatic enzymes and diet (145) and age (146). A high casein diet altered the pattern of enzyme activity from that of young to that of old animals and a low casein diet had the opposite effect.

The demonstration by Magee & Hong (147) that 1 per cent of a test amino acid added instead of additional casein to a 7 per cent casein diet can alter pancreatic enzyme activity is pertinent to kwashiorkor studies. Methionine increased lipase and protease activities, phenylalanine or isoleucine increased protease activity alone and no amino acid supplement tested increased amylase. Magee & Anderson (148) found that valine stimulated lipolytic and proteolytic enzyme activity in the pancreas of rats on a similar diet.

While the period was relatively barren of studies of the relationship between protein deficiency and endocrine changes, Gopalan (149) working with monkeys fed diets containing either 20 or 3 per cent protein, has added to his series of excellent contributions on this subject.

Protein Deficiency in Man

During the review period, at least fifty papers have appeared contributing to the understanding and solution of the problem of severe protein malnutrition in children, now well known by the name kwashiorkor. Most aspects of the problem have been reviewed in a series of papers by the senior author and his colleagues. These deal with characteristics (150), response to protein therapy (151), and epidemiology and prevention (152). This information has also been compiled in a monograph (153). Waterlow & Vergara (154) in a publication on protein malnutrition in Brazil and Gopalan & Ramalingaswami (155) in a review of kwashiorkor in India also give authoritative summaries of recent knowledge. An article by Waterlow & Scrimshaw (156) documents the international agreement on the basic features of kwashiorkor and leaves little doubt that they are essentially the same wherever the syndrome is encountered.

In studies of the liver in kwashiorkor, Waterlow (157) has found that, although fatty infiltration and loss of liver protein are both severe in kwashiorkor, the degree of these changes is not a reliable measure of the severity of the case. He also noted that the total nitrogen lost from muscle is much greater than from the liver. Waterlow & Weisz (158) showed that the average loss of liver protein and ribonucleic acid is 40 per cent in Jamaican cases calculated on the assumption that the amount of deoxyribonucleic acid per cell and per liver remains constant even during severe malnutrition. Both Waterlow & Patrick (159) and Burch *et al.* (160) have studied the activity of a number of enzymes in liver biopsy specimens from children with kwashiorkor. Cytochrome-*c* reductase (159, 160) cytochrome oxidase (159), lactic dehydrogenase (159), malic dehydrogenase (159, 160), succinic dehydrogenase (160), glycolic acid oxidase (160), and transaminase (159, 160) were not significantly reduced when calculated per unit of protein. Xanthine oxidase and D-amino acid oxidase (160), however, were highly significantly decreased on both a weight basis and per unit of protein.

Bras, Waterlow & DePass (161) report pancreatic pathology in "malnourished" infants and children and also in marasmic children. Both Waterlow *et al.* (162) and Senecal & Dupin (163) emphasize the necessity of distinguishing between kwashiorkor and marasmus ("Inanition") and point out that in the latter the liver is not fatty. Senecal & Dupin (163) re-emphasize that the fatty liver of kwashiorkor occurs because of an excess of calories relative to protein whether the total calorie intake is high or low.

Badr-El-Din & Aboul-Wafa in Egypt (164) confirmed previous findings of a marked reduction or absence of trypsin activity and the lesser reduction of amylase activity in infants with kwashiorkor. Their observation that trypsin activity is also reduced in marasmus, however, contradicts another recent report (165). Mehta, Venkatachalam & Gopalan (166) showed that children with Nutritional Oedema Syndrome (kwashiorkor) receiving diets containing negligible amounts of fat, excrete more fat in the feces than they consume.

The blood serum biochemical findings in kwashiorkor, which have been summarized in the papers cited previously (150, 153, 154, 155) include serum protein changes; lowered levels of fat soluble vitamins; decreased blood serum activity of cholinesterase, lipase, amylase and alkaline phosphatase;

and lowered blood serum cholesterol and total lipides. Ramanathan (167) calls attention to the lowered blood urea, nonprotein nitrogen, total cholesterol and cholesterol esters as well as total nitrogen, but found the gamma globulin fraction high. The serum electrophoretic patterns reported by Bollo & Montero (168) in Chile and Senecal *et al.* (169) in French West Africa are in agreement with the many previous studies of electrophoretic patterns in this syndrome which show albumin both absolutely and relatively decreased and gamma and alpha globulin fractions relatively increased.

Four technically superior papers on intracellular composition and homeostatic mechanism of infants with severe chronic malnutrition have recently been published by Gómez *et al.* (170 to 173). By analysis of blood serum, muscle, and skin it was found that, independent of decreases or increases in its volume, the extracellular fluid was hypotonic. The intracellular fluid volume was increased except in patients who were classified as "atrophy" (marasmus). Among other electrolyte changes, the potassium content of muscle tissue was found reduced to a larger degree in the patients presenting edema. Unfortunately, the cases varied very greatly in their relative proportions of marasmus and kwashiorkor, and many of them presented secondary complications. Politzer & Wayburne (174) have similarly found no correlation between the degree of edema and either serum Na^+ or K^+ levels, nor was the severity of diarrhea correlated with serum Na^+. The serum K^+ levels were reduced only in the subjects suffering from moderate to severe diarrhea.

In five cases of kwashiorkor studied by Ramachandran, Venkatachalam & Gopalan (175), the 24-hour urinary excretion of 17-ketosteroids was much lower than in 12 well-nourished children, but returned quickly to normal with the administration of a high protein diet. Venkatachalam, Srikantia & Gopalan (176) also studied urea space and nitrogen balance during the recovery of eight undernourished adults and found that changes in body weight gave a misleading picture of the actual gain in tissue during recovery due to variations in the water body content.

Cheung *et al.* (177) in three children with kwashiorkor have used column chromatography to study the excretion of amino acids. The distinctive findings were a high ratio of isoleucine to leucine due to an increased excretion of isoleucine and a high phenylalanine to tyrosine ratio due to the increased excretion of phenylalanine. A strikingly low output of threonine was seen in two of the patients. Additional cases must be studied under more standardized conditions before these results can be generalized. Although data of Garrow (178) on the increased S^{35} uptake in two Jamaican children with marasmus (one with a large groin abscess) and one with mild atypical kwashiorkor are too preliminary for conclusions regarding either condition, he also shows that dogs depleted of 0, 17.7, 21.1 and 37.7 per cent of body proteins incorporate progressively greater amounts of test doses of S^{35} with increasing protein depletion.

The amino acid composition of hair of African children suffering from kwashiorkor has been found by Close (179) to be low in total cystine content, but a Central American kwashiorkor case did not show this reduction. The severe retardation in maturation of the bones of the hand in kwashiorkor has been described by Jones & Dean (180), who also find decalcification and many transverse lines indicating past disturbances in growth.

Due largely to work of Brock and his group in South Africa, the doubts as to the primary importance of protein deficiency in producing the basic features of kwashiorkor appear to have been resolved. After the demonstration that "initiation of cure" could be induced not only with skim milk but also with vitamin-free casein plus water, glucose, and salts [Brock *et al.* (181)], studies were begun of the effectiveness of synthetic amino acid combinations for this purpose. Hansen, Howe & Brock (182) first reported that cure could also be initiated by a mixture of 18 synthetic amino acids simulating casein, plus glucose, water and salts with or without vitamin mixtures. Initiation of cure could also be obtained, although less satisfactorily with a mixture of 11 amino acids. It has been made clear that vegetable protein formulas which are as satisfactory as milk for the treatment of kwashiorkor can be developed [Scrimshaw, *et al.* (58); Venkatachalam *et al.* (183); Dean (184) and Thompson (185)]. Close (186) has studied the breast milk of two women in the Belgian Congo whose infants developed kwashiorkor while still nursing and concludes that the milk was insufficient in quantity but not different in its amino acid composition from that of other African mothers.

Balance studies carried out on infants suffering from kwashiorkor at the time of admission and at intervals during treatment are also revealing as to the nature of the syndrome. Senecal *et al.* (187) and Robinson *et al.* (188) have found the intestinal absorption of infants with kwashiorkor receiving different amounts of milk protein to be good and the percentage of retention to remain high for several weeks despite high intakes. Gómez *et al.* (189) also demonstrate strongly positive balances throughout treatment and recovery on *ad libitum* diets of milk, black beans and corn tortillas. In a study of the effect of lysine supplementation of milk, Gómez *et al.* (190) did not call attention to the difference in response between three children with edema (apparently kwashiorkor) and two without edema (apparently marasmus). In the edematous cases nitrogen retention was high and gradually decreased as nitrogen stores were repleted, and no effect of stepwise increases in lysine addition was evident. In the children with no edema, initial retention was low, indicating that relative nitrogen stores were not greatly depleted, but gradually increased as the greatly reduced muscle mass began to fill out. For this reason, no conclusion is justified in those latter cases as to the effect of the gradually increasing lysine supplements.

The need to distinguish between the conditions of kwashiorkor and marasmus has been emphasized by Matsaniotis (165) who found no difference in either the rate of amino acid absorption or intestinal proteolytic activity in 12 "undernourished" children compared with 15 well-nourished children. He considered these results expected since undernourished infants "although exhibiting a chronic reduction of their protein store, do not suffer from acute protein depletion." The differences between marasmus and kwashiorkor have also been discussed by Béhar *et al.* (191).

McCarthy (192) believes that the predominance of starchy foods and the correspondingly low protein content of the diets of Polynesians are responsible for the relatively high percentage of liver enlargement in children and cirrhosis in older adults. Fierro del Río, *et al.* (193) showed that in patients with severe malnutrition the infusion of 1 liter of 0.9 per cent NaCl solution caused a marked decrease of total circulating proteins due to a reduction in all fractions as compared with an increase in well-nourished subjects for all except gamma globulin. A paper by Aschkenasy (194) contains a comprehensive review of the blood disorders associated with protein deficiency.

Studies of Specific Amino Acid Deficiencies

In addition to the many studies of the consequences of protein deficiency discussed in the previous section, a growing number of papers investigating the effects of single amino acid deprivations have appeared. Vohra & Kratzer have shown that lysine deficiency produces hair lighter and finer in texture than normal in rats (195) and feather depigmentation in turkey poults (196). This has been confirmed for chicks of four breeds by Klain *et al.* (197). Apparently, lysine deficiency also results in a decreased skeletal deposition of Ca^{45} due at least in part to retarded bone growth [Likins, Bavetta & Posner (198)].

Pair-fed rats given a diet marginally deficient in lysine (0.25, 0.50, and 1.00 per cent) show a lower activity of xanthine oxidase at the 0.25 per cent level. In the group fed the diet with 0.50 per cent of lysine, there was a preferential synthesis of xanthine oxidase over liver protein [Bavetta & Narrod (199)]. Both *ad libitum* and force feeding techniques were used by Van Pilsum, Speyer & Samuels (200) to investigate the effects of diets deficient in tryptophan, isoleucine, or phenylalanine upon the activities of a number of tissue enzymes in rats. The absence of any of these amino acids caused a decrease of liver arginase and aconitase and kidney D-amino acid oxidase parallel to protein loss of the organ, while catalase and xanthine oxidase decreased irrespective of the total protein of the liver.

In the fifth of a series of papers on the histopathology of amino acid deficiency, Scott (201) has studied the effect of the complete lack of isoleucine in rats. The multiple alterations observed in the pituitary, testes, and secondary sex glands were closely similar to those previously observed as a consequence of threonine, histidine, phenylalanine, and tryptophan deficiencies. The changes apparently represent the interference of single amino acid deficiencies with protein metabolism rather than specific effects attributable to the lack of the amino acid itself.

Liver necrosis in rats is easily produced on a diet of either torula or brewer's yeast fed at the 9 per cent level but can be prevented by adding 0.5 per cent of either DL-methionine or L-cystine [Goyco (202)]. Interestingly enough, one-third brewer's and two-thirds torula yeast, fed together at an 18 per cent protein level, did not have this effect and instead resulted in full liver protein regeneration. Again, it appears that giving enough of a protein of poor biological value will overcome, under certain conditions, the effect of an amino acid deficiency apparent at lower levels of total protein intake.

Hallanger & Schultze (203) show that rats can develop a severe fatty liver during lactation when fed a supposedly complete essential amino acid mixture simulating casein and adequate for growth. This is another indication of the possibility that a ration may be adequate for growth and maintenance but still inadequate to prevent pathological changes in tissues under stress conditions. Harper & Benton (204) found fatty infiltration in rat liver greater on a diet containing 9 per cent casein plus 12 per cent gelatin than on one containing 18 per cent or more casein. This appeared due to the low content of threonine and the high content of arginine and glycine in gelatin. Arata *et al.* (205) concluded that both a defect in diphosphopyridine nucleotide production and improper metabolism of endogenous diphosphopyridine nucleotide in liver are major factors in the accumulation of liver fat which results from partial threonine deficiency in the rat.

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