Malnutrition and Amino Acid Metabolism

Alan A. Jackson and R. F. Grimble

Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO9 3TU, England

Severe childhood malnutrition embraces a spectrum of clinical presentations and pathologic disorders. Across the entire spectrum, there is clear evidence of major changes in the state of amino acid and whole body protein metabolism, from the skeletal wasting and stunting of marasmus to the varied multisystem changes characterized by the kwashiorkor syndrome. For this reason, amino acid and protein metabolism have commonly been held to lie at the center of the metabolic complexities of severe undernutrition. Indeed, the use of the name protein-energy malnutrition (PEM) implies an etiologic role for protein, although this may not be fully justified. When Cecily Williams (1) first described kwashiorkor in 1933, she suggested a primary protein deficiency as the cause of the disease, rather than an infection or vitamin deficiency. Subsequent research has shown that interactions with deficiencies of specific micronutrients and infection probably play an important part in the development of the disease and may account for the wide pattern of clinical presentation. Certainly, any model that seeks to explain the cause of the disease has to account for a bewildering spectrum of pathophysiologic changes (2).

In 1963, Holt and co-workers (3) explored the possibility that kwashiorkor was the result of a specific deficiency of one or a small number of essential amino acids. The rationale for their study was that the deficient amino acid "would be revealed by the pattern of free amino acids in the blood plasma." There was a striking similarity in the fasting amino acid profile in the plasma of 64 children with kwashiorkor of varying severity from nine countries around the world, despite diverse sources of dietary protein. The conclusion reached by Holt was similar to that of Williams 18 years earlier, that "the limiting nitrogenous component in a kwashiorkor-producing diet . . . is not an essential amino acid but nitrogen, either essential or non-essential." The pattern of the amino acid concentrations revealed a reduction of all the essential, indispensable amino acids, with some of the nonessential, dispensable amino acids having reduced or variable concentrations. Of the indispensable amino acids, valine, leucine, and isoleucine showed the largest reduction, while phenylalanine and lysine were less affected. Of the dispensable amino acids, tyrosine, arginine, and citrulline were reduced in concentration, while glycine, serine, proline, and histidine were found to be raised, lowered, or normal.
The battle to explain and understand the distorted pattern of amino acids seen in kwashiorkor was joined in many parts of the world. It provided a stimulus for the development of biochemical methods for use in the detection of subclinical forms of PEM. Different groups have adopted different approaches to the problem, but most have been stimulated by the idea articulated by Waterlow et al. (4), that death from severe malnutrition is a consequence of the failure of a protein-dependent function(s).

Perhaps the single most important difficulty inhibiting progress in this area of work has been the absence of a suitable animal model for the disease state. This reflects an imperfect appreciation of the fundamental pathophysiologic changes taking place, which makes for considerable difficulty in trying to explain and understand the observed changes in the established human disease (5). The only clinical materials that are readily available for analysis are plasma and urine. The concentration of amino acids in the plasma is the result of many processes and is subject to a variety of influences (Fig. 1), but at any point in time it has to be the result of the rates at which amino acids are flowing into and being removed from the amino acid pool. Therefore, although the hypothesis advanced by Holt considers one inflow to the pool, the diet, as being a major determinant of the concentration in the pool, amino acids are also added to and removed from the pool by the processes of protein synthesis and protein degradation within the body. These processes, in turn, are affected by a range of hormonal influences generated by nutrient and stress signals. The processes involved in the loss of amino acids from the pool following the conversion of the carbon skeleton to energy substrate, and the amino group to excretion products, are themselves under hormonal control. To an extent, the amino acids may be interconvertible. All the enzymes required for the transformations, oxidation, synthesis, and degradation are themselves proteins, most with requirements for cofactors, and all are open to nutritional influence.

![Fig. 1. The Holt et al. (3) model of amino acid metabolism only considers the influence of a single variable—dietary intake. A more complete model would require consideration of a range of influences that are likely to influence amino acid metabolism.](image-url)
DIETARY EVIDENCE

The implication in much of the literature is that kwashiorkor is more common in areas where dietary protein tends to be deficient (6,7). This apparent association has been elevated to a causal relationship and is the main basis for implicating protein deficiency in the etiology of the disease. Therefore, one of the first points to clarify is the extent to which the evidence supports the thesis of dietary protein inadequacy. Landman and Jackson (8) collated data from dietary surveys carried out in seven countries over the course of 30 years in communities where kwashiorkor was a major problem. In general the mean energy intakes were inadequate, 22 to 48% of the recommended daily allowance (RDA) (9), except in healthy Jamaican children and Indian toddlers. By contrast, mean protein intakes frequently met or exceeded the RDA. When mean protein intakes were inadequate or marginal, mean energy intakes were so low that the protein would have been utilized inefficiently. For practical and ethical reasons, these data rarely represent the actual intake of children in the process of developing kwashiorkor. However, extensive clinical experience has shown that on a dietary protein intake of only 0.6 g/kg-day, about 50% of the RDA, children with kwashiorkor lose their edema (10). Therefore, a protein intake of 0.6 g/kg-day is sufficient to "maintain the edema-free state," and to promote correction of pathologic processes seen in kwashiorkor. This figure can be used as a minimal intake necessary to prevent the development of the kwashiorkor syndrome. From this perspective, the protein content of all the diets studied is abundant. Overall, the data support the contention that "there is no situation in which the child is adequate with regard to calories and deficient with regard to protein alone. . . . The major bottleneck is calories and not protein" (11).

Nevertheless, this conclusion presumes that the requirements for protein are not different from those of a normal, healthy population; that there are no abnormal or unusual losses; and that intercurrent infections, an everyday reality in populations at risk from severe undernutrition, play no part at all (12).

BODY COMPOSITION

Although all tissues are affected by an inadequate dietary intake, the weight lost during the development of malnutrition is not evenly shared by all tissues (13). There is always a significant reduction in lean tissue mass, although there is a relative sparing of visceral tissues, with more obvious and extensive loss of muscle (14). Even within the visceral tissues, some functions are spared more obviously at the expense of others. Hypoalbuminemia, a consequence of decreased albumin synthesis by the liver, is representative of the situation that occurs with other export proteins that carry out carrier functions in the circulation, e.g., transferrin and retinol binding protein (15,16). The development of fatty liver is in part a consequence of inadequate synthesis of the carrier apolipoprotein (17). The evidence suggests that the decrease in specific protein synthesis may represent a redistribution of
It is thought that a large part of the effect of infection on amino acid and protein metabolism is mediated through the effects of cytokines released from leukocytes. Cytokines induce an orchestrated sequence of metabolic changes, which involve a range of tissues and functions.

Amino acids to the synthesis of acute phase reactants. The specific mechanisms that control this redistribution of amino acids are not altogether clear. One important factor is almost certainly infection. In response to an infection, macrophages elicit cytokines, such as interleukin-1 and tumor necrosis factor, which have the ability to inhibit the synthesis of albumin in the liver and to elevate the production of acute phase proteins (Fig. 2) (18). However, other causes cannot be excluded; for example, potassium deficiency is invariably present in severe undernutrition, as either a balanced deficiency or a specific depletion (13). It is not possible to retain intracellular potassium in the face of a marked potassium deficit, although it has been suggested that loss of potassium from muscle represents a mechanism for retaining basic amino acids.

HORMONES

The concentration of amino acids in plasma is the result of metabolism in visceral and peripheral tissues, under the influence of a range of hormones. Insulin, thyroid hormones, growth hormone, somatomedin, glucagon, and glucocorticoids have all been shown to exert an influence by affecting uptake into protein in many sites and dispersal into degradative pathways in the liver. A hormone may act directly on a process but may also influence the sensitivity of target tissues to other hormones. For example, growth hormone and glucocorticoids may produce insulin insensitivity. Even within a carefully controlled laboratory experiment, it is difficult to predict the effect that a particular hormone might have on any aspect of protein metabolism. Therefore, with the limited control possible in metabolic or field studies on children exposed to varying degrees of infection and malnutrition, interpretations of the specific effect of individual hormones must be guarded.

In an elegant series of animal studies, Millward et al. (19) examined the metabolic effects of hormones on protein synthesis in skeletal muscle (Table 1). They
TABLE 1. Effect of hormones on protein synthesis and degradation in skeletal muscle

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Protein synthesis</th>
<th>Protein degradation</th>
<th>Net effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>↑</td>
<td>↓</td>
<td>Anabolic</td>
</tr>
<tr>
<td>Growth hormone and somatomedin</td>
<td>↑</td>
<td>No effect</td>
<td>Anabolic</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;: normal levels</td>
<td>↑↑</td>
<td>↑</td>
<td>Anabolic</td>
</tr>
<tr>
<td>hyperthyroid levels</td>
<td>↑↑</td>
<td>↑↑</td>
<td>Catabolic</td>
</tr>
<tr>
<td>Glucocorticoids: fed</td>
<td>↓</td>
<td>↑</td>
<td>Catabolic</td>
</tr>
<tr>
<td>fasted</td>
<td></td>
<td></td>
<td>Catabolic</td>
</tr>
</tbody>
</table>

In malnutrition: Insulin ↓↑, Growth hormone ↑, somatomedin ↓, T<sub>3</sub> ↓, glucocorticoids ↑↓. ↑↑, increase; ↓↓, decrease.

From ref. 19.

concluded that both insulin and T3 were independently important for maintaining protein synthesis. Either hormone could maintain synthesis when the other was present in low concentrations. They confirmed the suppressive effect of glucocorticoids on synthesis and demonstrated that they could override the stimulatory effect of insulin. Thus, insulin and thyroid hormones would promote net uptake of amino acids by muscle, and glucocorticoids would cause outflow from muscle. Somatomedin, the effector of growth hormone, would stimulate muscle protein synthesis.

Many studies have shown that the concentrations and production rates of hormones are changed in malnourished children. Thyroid hormone, glucagon, and somatomedin are depressed, while growth hormone is elevated. Insulin and glucocorticoids have been found in normal, reduced, or elevated concentrations, with a significant reduction in the affinity of insulin for its receptor (20–27).

Lunn et al. (28) attempted to obtain a prospective picture of changes in insulin, cortisol, and growth hormone as children moved from a marginally nourished to a malnourished state. They concluded that an early rise in insulin concentration fell to lower levels once a malnourished state had become established, at which time growth hormone concentration rose. Cortisol concentrations were normal in marginally malnourished children but became elevated as malnutrition developed. Thus, in marginally nourished children, the outflow of amino acids from muscle for gluconeogenesis might be suppressed, with a reversal of this condition in the malnourished state, particularly with a decrease in thyroid hormone and elevated cortisol. A comparative study on the hormonal profile of children in Uganda and the Gambia illustrates the importance of the relative balance between hormones in their effect on muscle protein (29). Marginally malnourished children in the Gambia had a higher ratio of cortisol to insulin, with greater depletion of muscle than marginally nourished Ugandan children. Further studies on the Gambian children showed a negative correlation between growth and the cortisol/insulin ratio (30).

The influence of the intensity of muscle-protein turnover on the amino acid concentration in plasma is illustrated by studies in goitrous patients (31), which showed
a marked fall in the concentration of all essential amino acids with the exception of methionine, and slightly less intense reductions in the nonessentials. A reduction in the excretion of 3-methyl-histidine was interpreted as a diminished rate of muscle protein degradation.

METABOLIC ADAPTATION

Whatever other features may be present as a component of severe undernutrition, a negative energy balance with the concomitant loss of tissue, and hence weight, is invariable. As a consequence, the adaptive responses of the body are brought into play, although they may be overshadowed by other responses, such as that to infection. Classic balance studies can yield information on the overall status of nitrogen metabolism in the body, and in general, these show very efficient utilization of dietary nitrogen, with no evidence of poor absorption. There is appropriate retention of nitrogen, so that balance is defended down to very low levels of intake (32-34). In some studies, there is evidence of a protein-losing enteropathy, but the extent to which this might contribute to a negative nitrogen balance is poorly documented.

The evidence suggests that a significant proportion of basal energy expenditure supports the physiologic processes associated with protein turnover. The physiologic cost to the body of protein turnover is estimated to be 7.3 kJ/g protein, which may represent as much as 30% of resting energy expenditure in the adult (35). During malnutrition, protein synthesis is significantly reduced to 4 g/kg·day, compared with 6.3 g/kg·day after recovery (36), a theoretical saving of 16.8 kJ/kg·day. In both malnourished and recovered children, the dietary protein intake only represents a relatively small proportion of overall protein turnover, with protein synthesis being 90% of the total. Malnutrition is characterized by intermittent periods of growth faltering and catch-up. During catch-up growth, there is an increase in the intensity of protein synthesis to 9.7 g/kg·day, with only 3% of flux coming from the dietary intake (36). In children of normal weight, the response to infection is a marked increase in the rates of both protein synthesis and breakdown, and as breakdown exceeds synthesis, there is a negative nitrogen balance. In malnourished children, there may be a doubling of synthesis and breakdown in the face of infection, bringing turnover to the level seen in a normal, uninfected child. However, the rates of synthesis and breakdown remain similar, with the consequence that nitrogen balance is maintained (Fig. 3) (37). It has been suggested that one of the important functions of protein turnover is to give the organism a capability to respond to change. A reduced turnover would, therefore, imply a measure of compromise in the ability of the organism to withstand environmental variation.

It is not clear to what extent the body is able to accommodate changes in energy intake without sacrificing function. The evidence shows that with progressive reductions in energy intake from 100, to 90, 80, and 70 kcal/kg·day, young children defend nitrogen balance very effectively, and measurements of protein turnover made over 18 hr show little change in the overall rate of protein degradation, with a pro-
MALNUTRITION AND AMINO ACID METABOLISM

**FIG. 3.** Changes in protein turnover can be seen as a part of the response of normal and malnourished children to infection. The changes in protein synthesis and degradation are modified by nutritional state (37).

**FIG. 4.** There is an adaptive response seen in young children to defend nitrogen balance as the energy metabolizable intake is reduced progressively from 100 to 70 kcal/kg-day (33,34).

Progressive fall in protein synthesis (Fig. 4) (33,34). This remarkable ability to preserve nitrogen balance was associated with a significant fall in stool frequency and hence fecal nitrogen at the lowest levels of energy intake (34). In the circumstances in which metabolic studies are conducted, the absorption of nitrogen is of the order of 90% or more, with little variation in fecal nitrogen (32). However, there is evidence that in children with diarrheal disease, there is a significant protein-losing enteropathy, which should give rise to a more variable fecal nitrogen, due to increased endogenous losses rather than to any change in adsorption.

**DIARRHEAL DISEASE**

Diarrheal disease and varying degrees of malabsorption are an almost invariable accompaniment of severe undernutrition. Although it is impossible to determine the extent to which these represent cause and effect, it is quite clear that the loss of specific nutrients in the stool can contribute to a compromise of function. Diarrhea has been associated with small intestinal overgrowth, which gives rise to bile salt deconjugation and bile salt malabsorption (38,39). There is a marked reduction in the size of the bile salt pool in severe malnutrition, which is even more marked in the pres-
ence of diarrhea (40). Bile salts are conjugates of either taurine or glycine. Hence, bile salt malabsorption will give rise to a drain on the pool of glycine-serine and sulfur amino acids (12). This specific fecal loss, which is associated with severe gastrointestinal dysfunction, may lead to increased fecal losses of total nitrogen, fat, fat-soluble vitamins, and trace elements.

The metabolic activity of the lower bowel probably plays a far more important role in the intermediary metabolism of amino acids than has been appreciated. The normal response to a relative reduction in nitrogen intake is a fall in the activity of the urea cycle enzymes, with a shift of amino acids from catabolic to anabolic pathways (41). Under normal circumstance, 70% of the urea produced in the body is excreted in the urine, with 30% being recycled through the lower bowel. The urea nitrogen that is recycled through the lower bowel is a small part, 15 to 20%, of a much larger enterohepatic pool of metabolic nitrogen, with a flux equivalent to 1.3 g protein per kg-day in the adult (42). On a low-protein diet, only 30% of the urea produced is excreted in urine, with the greater proportion being retained within the metabolically active pool (43). This function is closely associated with the metabolic needs of the gastrointestinal microflora, which are responsive to the dietary intake of both protein and fiber (Jackson et al., unpublished data) (Fig. 5). The evidence is clear that the microflora can make both nonessential and essential amino acids available to the host, and the implication is that this might be particularly important on low protein intakes (44). The recent evidence that shows that protein infused into the colon can be utilized by the growing infant is important evidence in favor of these arguments (45).

Evidence suggests that the metabolic activity of the lower bowel may be of particular importance in the metabolic response/adaptation to undernutrition, reflecting an interaction with dietary nutrients. It is well recognized by pediatricians that many young children develop a sympathetic "diarrhea" as a nonspecific response to a variety of infective insults. Infective diarrhea produced by specific pathogens represents an enormous load of morbidity and mortality throughout the world. There is evidence to show that deficiencies of specific nutrients, such as zinc, can of themselves produce diarrhea (46). Therefore, the demonstration of a metabolic interaction of nutritional significance between the host and his gastrointestinal microflora

![FIG. 5. One part of the adaptation to a reduced protein intake is a decrease in urea excretion, with a greater proportion of the urea that is produced being salvaged in the bowel. In young children growing rapidly, the proportion of urea produced that is available for anabolic pathways is affected by the source of energy (diets rich in either fat or carbohydrate) and the level of protein, protein energy ratio (PER) of ~10% and ~8%.](image-url)
becomes of increasing importance. We are only just beginning to appreciate the extent to which the lower bowel may be important for general homeostasis. As our knowledge of this area of metabolic control increases over the next decade, it is likely that we shall develop a much clearer appreciation of the metabolic interaction between energy and protein in health and disease.

**REQUIREMENTS FOR SPECIFIC AMINO ACIDS**

Since the early definition of Rose (47), amino acids have been categorized in two groups: those that have to be provided in the diet to maintain normal weight gain and nitrogen balance (essential or indispensable amino acids) and a group that can be made in sufficient amounts in the body to satisfy normal metabolic requirements (nonessential or dispensable amino acids). As understanding grows, this simple distinction is becoming increasingly difficult to maintain (44,48,49).

**Leucine, Isoleucine, and Valine**

The specific way in which the branched chain amino acids are handled has been studied extensively. Dietary branched chain amino acids are taken up by liver only to a limited extent and pass directly to the periphery where they are taken up by muscle tissue in particular. Leucine has been credited with exerting a specific control on protein turnover in muscle. The extent to which this may be effective or may be modulated in malnutrition is not known. Given the reduction in turnover, a change in leucine kinetics is to be expected, but no data are available for severe malnutrition. The branched chain amino acids are deaminated in the periphery, the amino group being carried to the liver as either alanine or glutamine. The carbon skeleton may be oxidized locally or at a more distant site such as liver. The plasma concentration of branched chain amino acids may play an important role in the rate at which tryptophan and phenylalanine, large neutral amino acids that are precursors for neurotransmitters, are taken up by the brain.

**Lysine**

The specific metabolism of lysine in the human is poorly understood. There is a relatively large free lysine pool. There is evidence for a disturbance in the catabolic pathways in some malnourished children. The presence of a blue spot on amino-grams of urine was identified as an unusual breakdown product of lysine, which suggested a metabolic block on the catabolic pathway (50). Post-translational modification of lysine residues takes place to form trimethyl-lysine. Protein degradation makes trimethyl-lysine available for the endogenous synthesis of carnitine, required for the oxidation of free fatty acids. There is evidence of carnitine deficiency in mal-
nutrition (51). There are specific interactions of lysine with copper and ascorbic acid in desmosine formation.

Phenylalanine and Tyrosine

The increased phenylalanine/tyrosine ratio implies a metabolic block at the level of phenylalanine hydroxylase, a step that requires a normal iron status. Tyrosine is a precursor for neurotransmitters, epinephrine, norepinephrine, dopamine, and thyroid hormones. It is not known to what extent the limited availability of tyrosine might affect metabolic activity of these compounds.

Tryptophan

Tryptophan, not measured on a routine aminogram, is important as a precursor for the neurotransmitter serotonin. This amino acid was originally implicated as a specific cause of malnutrition, because of its metabolic conversion to niacin and its presumed causal role in the pellagra/pellagroid skin rash. Tryptophan is carried in blood bound to plasma albumin, from which it is readily displaced by free fatty acids. Therefore, it is difficult to identify an active plasma pool. It competes for transport into brain with other large neutral amino acids and has been associated at some time with the neurologic, affective changes seen in malnutrition and with changes in appetite.

Threonine, Glycine, and Serine

Serine and glycine can be derived from threonine. Glycine and serine are interconvertible, and the interconversion is absolutely necessary for the remethylation of homocysteine to methionine to make the methyl group of folic acid available. Threonine, serine, and glycine comprise a large component of the amino acid residues in a number of extracellular proteins, proteoglycans, glycoproteins, collagen, elastin, and others. There is an absolute requirement for glycine in a range of metabolic processes other than protein synthesis, e.g., nucleotides, porphyrins, creatine, glutathione, bile salts. All these compounds are functionally end products of glycine metabolism and, therefore, represent a net drain on the glycine pool.

Glycine and serine are conditionally essential, especially during periods of stress or rapid growth (52). There is direct evidence to show that the endogenous synthesis of glycine is limited in normal man (53), and the relative availability of glycine can be determined indirectly by measuring the urinary excretion of 5-oxoproline (52). The excretion of 5-oxoproline is increased in malnutrition. The carbon skeleton of glycine, glyoxilic acid, is toxic and not normally found free in the body. In the most
severely malnourished children, there may be a significant increase in plasma glyoxylate concentrations (12).

Methionine, Cysteine, and Taurine

Cysteine is often not determined on a routine aminogram. Sulfur amino acids are often the first limiting amino acids on many diets. Irreversible loss from the body takes place in a number of forms such as proteins in desquamated skin or shed hair and through the bowel or urine as bile salts or in a conjugated form with a range of endogenous metabolites or xenobiotics. Methionine is required for the normal metabolism of methyl groups, for example, in the production of choline (an important component of the lipoproteins in membranes), in the synthesis of nucleotides, carnitine, and creatinine. Cysteine may be derived from methionine and serine and has particular functional significance because of the reactivity of the sulfhydryl group as, for example, in the structure and function of proteins, be they enzymes, carrier proteins, or receptor molecules. Moreover, cysteine is a constituent of the tripeptide glutathione, which participates in the control of many cellular functions: the maintenance of the oxido-reductive state, protection from oxidative stress, and the excretion of xenobiotics.

Glutathione acts as an intracellular storage form of highly reactive cysteine. Fatty liver is associated with a limited availability of methyl groups, a low level of cysteine, and free radical—induced cell damage. The specific pathway to taurine synthesis is not clear. Taurine has a large muscle pool, and it may be a conditionally essential amino acid in the infant (54). The only specific function identified is in bile salt formation. However, it is also considered to have membrane-stabilizing function in excitable tissue and a central function in the control of body temperature.

Alanine, Glutamate, Aspartate, Proline, and Glutamine

Alanine is the main common final pathway for carrying amino groups to the liver for gluconeogenesis, a function maintained in severe malnutrition (55), and for secretion as urea. Glutamine has an important role as ammoniagenic precursor in kidney, and a renal acidifying defect may be associated with phosphate deficiency. There is an absolute requirement for glutamine for cellular multiplication, and hence, there is a specific requirement by rapidly dividing cells, notably in the gastrointestinal tract and in immunologically active tissues (56,57). The specific pathways for the formation of glutamine in the periphery are not clear, but muscle and brain are important in making glutamine available to visceral tissues. Whole body glutamine flux is little altered from normal by acute or chronic acidosis and in the fed or fasted state (Jahoor, Golden, and Jackson, unpublished observations). However, infection is thought to bring about a massive efflux of glutamine from muscle,
which may contribute to the increased demands of the immune system for hyperplasia and gluconeogenesis. It has been proposed, therefore, that a specific relationship may exist between muscle wasting and the requirements for glutamine to maintain visceral function.

**Histidine and Arginine**

These two amino acids are conditionally essential in the infant. There is a large muscle pool of histidine that may be depleted in severe wasting. A specific deficiency has been associated with impaired hemoglobin formation. There is no direct evidence in favor of a limited availability of arginine from the urea cycle; however, there are well-recognized lysine/arginine antagonisms.

**Carnitine and Creatine**

The formation of carnitine and creatine represents a net drain on the amino acid pool of significant proportions, and both function as essential components in normal energy exchange. Creatine is synthesized in kidney and liver from arginine, glycine, and methionine. There is a marked reduction in the pool size in malnutrition (14). Carnitine is synthesized from lysine and methionine, and there is evidence that its availability is limiting in malnutrition (51).

**Glutathione**

Glutathione is a tripeptide of the three nonessential amino acids, glutamic acid, cysteine, and glycine. There is a significant reduction in the concentration of glutathione in the blood of children with nutritional edema (58). The reduction is most severe in the sickest children, and the levels approach normal with clinical improvement. In the rat, a reduced dietary cysteine is associated with a low hepatic glutathione and fatty liver (59). In malnourished children, there is increased excretion of glutathione conjugates such as mercapturic acid (60).

**INFECTION AND PLASMA AMINO ACIDS**

High levels of infection and infestation are characteristics of environments where malnutrition is prevalent. Infection and infestation bring about an activation of the immune system during which macrophage populations around the body are stimulated to produce a range of cytokines. The circulating levels of cytokines may be reduced in malnutrition. Cytokines, of which interleukin 1 (IL-1) and tumor necrosis factor (TNF)/cachectin are examples, bring about an enhancement of the immune
response (61,62). In animal models, this involves a radical shift in the intensity and direction of protein metabolism, resulting in negative nitrogen balance (Fig. 2). Muscle and skin protein becomes depleted, and the amino acids released as a consequence are used by the liver for the synthesis of acute phase proteins, and by the immune system as substrates for gluconeogenesis. The profile of the secretory proteins produced by the liver changes. Serum albumin synthesis is curtailed, and there is increased synthesis of acute phase proteins such as C-reactive protein, fibrinogen, and the zinc-binding protein metallothionein (63).

There appears to be a particular need for enhanced gluconeogenesis during infections. It is not clear whether this is a specific need to support glycolysis or the activity of the hexose monophosphate shunt or simply a response to anorexia.

The pattern of amino acids in the plasma of subjects with malnutrition reflects the superimposed effects of infection and loss of appetite. It may be possible, therefore, to differentiate the relative contribution of each influence to the pattern observed (Table 2). Studies carried out by Wannemacher et al. (64,65) on well nourished, infected subjects and by Young and Scrimshaw (66) on healthy subjects fed protein-deficient diets, together with various studies in animals, help in this respect. Wannemacher found that in healthy adults during experimentally induced sandfly and typhoid fever, the plasma concentrations of phenylalanine and tryptophan became raised, while valine, isoleucine, leucine, glycine, and alanine were reduced. In contrast, simple starvation of healthy subjects brought about a response in which plasma alanine was reduced, while the concentrations of valine, isoleucine, leucine, and glycine became elevated, phenylalanine was unaffected, and tryptophan fell. Thus, there is a contrast in the pattern of change with infection compared with a reduced food intake for the plasma concentrations of phenylalanine, tryptophan, valine, leucine, isoleucine, and glycine. When protein-deficient diets were fed to healthy young subjects, there was an elevation in glycine, alanine, and serine and a reduction in valine, isoleucine, and tyrosine, while phenylalanine was unaffected. Thus, infection and a protein-deficient diet affect the branched chain amino acids in the same way but have opposite effects on glycine and alanine. Whereas a protein-

<table>
<thead>
<tr>
<th>TABLE 2. Effect of starvation, a protein-free diet, infection, or severe malnutrition on the plasma concentration of selected amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Valine</td>
</tr>
<tr>
<td>Leucine</td>
</tr>
<tr>
<td>Isoleucine</td>
</tr>
<tr>
<td>Phenylalanine</td>
</tr>
<tr>
<td>Alanine</td>
</tr>
<tr>
<td>Glycine</td>
</tr>
</tbody>
</table>

↑, increase; ↓, decrease.

From refs. 64–66.
deficient diet has no effect on phenylalanine, infection brought about an increase. However, both infection and protein deficiency resulted in a reduced concentration of tyrosine. Experiments carried out with animals reinforce these conclusions to some extent. Following enteric viral infections in well-nourished pigs, there were decreases in the branched chain amino acids, as well as glycine, alanine, and tyrosine. However, neither phenylalanine nor tryptophan became elevated. Protein-deficient pigs responded to the infection in the same manner. Since protein deficiency alone had caused elevations of glycine and alanine, the changes brought about by infection were of greater magnitude than in the well-nourished animals (67).

What interpretations can be given to the relative pattern of response in the plasma amino acids to infection, starvation, and protein deficiency? Alanine and glutamine make up 60% of the amino acids released from skeletal muscle during infection. The glutamine, in addition to being a possible substrate for macrophage metabolism, is also a substrate for alanine production by the intestine (57). Long et al. (68) have shown that alanine is a major precursor for the increased glucose formation that occurs in the liver during sepsis. In experimental situations where low-protein diets are fed to humans or animals, gluconeogenesis is curtailed, resulting in an elevation in plasma alanine concentrations. If, however, infection and its accompanying anorexia are superimposed on these dietary situations, then a large fall in concentration would be expected due to enhanced gluconeogenesis. It has been suggested that the decreased concentration of branched chain amino acids in plasma is a consequence of the increased requirement for alanine synthesis in muscle. Wannemacher (64) concluded from studies in humans and rats that decreased output from muscle occurred because of the conversion of branched chain amino acids within the intracellular pool into alanine and glutamine, the keto acids so formed being utilized as metabolic fuel within the muscle. Thus, although muscle protein breakdown is enhanced by infection and reflected in increased output of phenylalanine and tryptophan, there is no equivalent increase in the outflow of branched chain amino acids.

The reductions brought about by feeding healthy experimental animals and human subjects an inadequate protein diet are due to a quite different effect. In this situation, branched chain amino acids released from muscle are reduced due to decreased muscle protein loss. Once the rate of output from muscle is exceeded by the demands for protein synthesis in other tissues, such as the liver, then the concentration in the blood will fall. This is illustrated in studies by Grimble and Whitehead (69,70) on children recovering from malnutrition and pigs fed a range of protein intakes. At intakes where growth ceases and serum albumin concentrations are affected, blood valine concentrations start to fall. The reduced concentrations of branched chain amino acids in the blood of children who have been exposed to multiple infections and have been consuming an inadequate diet in terms of protein and energy content might be due in varying degrees to either of these metabolic scenarios.

An examination of data from a study of severely malnourished Peruvian children
conducted by Baertl et al. (71) supports this concept. In this study, information was collected on the presence or absence of infection, anthropometry, and clinical signs associated with malnutrition, in addition to measuring the serum amino acids. The data show the ratio of valine to glycine in plasma. If valine is affected by both malnutrition and infection, with glycine being affected by infection alone, then a higher ratio might be expected in children suffering from malnutrition and infection. When a comparison is made among children with a length age greater than 6 months, the 12 subjects who were uninfected had a ratio of 0.22 ±0.16, whereas those who were infected had a ratio of 0.33 ± 0.16, \( p<0.05 \) (Fig. 6). Care has to be taken in the interpretation of such data, given the great difficulty in identifying infection during malnutrition. In this series, there was a strong correlation between the plasma concentration of most amino acids and serum albumin, with the exception of taurine and glycine. While anorexia might play some part in the low level of amino acids observed in the circulation of infected, malnourished subjects, it would not seem to be a determinant of low glycine levels, since starvation per se raises glycine concentrations. The low levels may be accounted for by the metabolic conditions created by infection and by the multiple metabolic pathways in which glycine participates.

CONCLUSION

The early workers in the field of severe childhood malnutrition were convinced that protein deficiency, or derangements in protein metabolism, represented one of the most important features of the kwashiorkor syndrome. It is difficult to find conclusive evidence to support the concept of a simple dietary deficiency of protein, especially as, under normal circumstances, the body is very efficient at retaining nitrogen when necessary (72). Therefore, this concept has fallen into disrepute.

However, there are a number of considerations that might cause us to reflect on this perspective. First, malnourished children are exposed to a heavy load of infection, which on the one hand, alters the demand for specific nutrients, and on the
other, produces unbalanced losses from the body (12). In this situation, a diet that may have been adequate to satisfy normal requirements is very likely to become inadequate for one or more nutrients in the presence of an increased demand. Second, diarrheal disease disturbs the delicate ecological balance between the host and his gastrointestinal microflora. A disturbance of this kind is likely to lead to a loss of specific nutrients, as well as to an alteration in the metabolic exchange across the lower bowel. Third, our ideas about the relationships between individual amino acids, our concepts of essentiality, and our appreciation of the specificity of the metabolic flow of individual amino acids have developed rapidly over the past decade. In each of these areas, the advances in understanding have been associated with the development of powerful new tools for investigations in vivo.

It is self-evident that there are major changes in protein and amino acid metabolism in malnourished individuals. There is a need to characterize these changes more specifically.

REFERENCES


64. Wannemacher RW. Key roles of various individual amino acids in host responses to infection. *Am J Clin Nutr* 1977;30:1269–79.


69. Grimble RF, Whitehead RG. Fasting serum amino acid patterns in kwashiorkor and after administration of different levels of protein. *Lancer* 1970;i:918–21.


**DISCUSSION**

*Dr. Worrier:* Do selenium and zinc deficiency affect the activity of superoxide dismutase or a glutathione peroxidase?

*Dr. Jackson:* Golden and Ramdath (1) have measured the level of ferritin on admission and
related it to the activity of red cell glutathione peroxidase (GPx), an index of selenium status. They found that the association of a high ferritin with low GPx was predictive of those children who were going to die. They have suggested that increased free iron may increase the risk of free radical–induced damage in a situation where the mechanisms to protect against such damage are impaired. This is such an important defense system that if one is short of a single nutrient or component, there are backup systems to give an alternative source of protection. In order to get expression of disease, one might need a major failure in more than one system at the same time. Depending on the particular pattern of deficiency, one might expect to get different patterns of disease.

Studies in Chile (2,3) have demonstrated that supplementation of all malnourished children with appropriate quantities of zinc and copper leads to a better quality of rehabilitation.

**Dr. Haschke:** Protein-energy malnutrition (PEM) is common in children with cancer. In a prospective study, we looked at serum albumin, transferrin and retinol-binding protein in 25 children, 12 with solid tumors and 13 with leukemia. Measurements were taken before and 2 weeks after initiation of chemo- and nutritional therapy, which were begun when at least three of the four proteins were below our age-specific reference values (Table 1). Oral and/or parenteral nutritional therapy resulted in significant increases in albumin, prealbumin, and retinol-binding protein.

**Dr. Jackson:** Many of the measurements made in clinical practice are of concentrations. We may make presumptions and extrapolate these to rates of production or removal. Although this may be justified, there are many situations when it is impossible to know whether a decreased concentration of albumin, for example, is a consequence of a reduced rate of synthesis or an increased rate of degradation or loss. The approach to management might be critically determined by which option is chosen.

In general terms, our knowledge of the specific metabolism of individual amino acids is very poor. Most of the tracer studies have used carbon labels, assuming that this would reflect the handling of the amino group. But this is not so. Nitrogen is, in fact, handled by the body in a very specific way, being channeled through its own metabolic pathways.

**Dr. M. Mehta:** Our studies indicate that glycine plays an important role in rehydration of patients with acute diarrhea. It helps in the reabsorption of electrolytes and water in the intestinal lumen as well as in weight gain. This gives further credence to the prevention of diarrhea with a rice-based oral rehydration solution (ORS).

**Dr. Jackson:** In the early days of parenteral nutrition, glycine nitrogen was added as a filler to amino acid solutions and given to patients in quite large quantities. Production of glycine

<table>
<thead>
<tr>
<th></th>
<th>Albumin (g/dl⁻¹)</th>
<th>Pre-A (mg/dl⁻¹)</th>
<th>transferrin (mg/dl⁻¹)</th>
<th>RBP (mg/dl⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before x (SD)</td>
<td>2.6 (1.0)</td>
<td>10.0 (3.2)</td>
<td>162 (71)</td>
<td>1.8 (0.9)</td>
</tr>
<tr>
<td>After</td>
<td>4.0 (1.1)</td>
<td>26.1 (9.7)</td>
<td>220 (58)</td>
<td>4.2 (1.4)</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.05</td>
<td>&lt; 0.01</td>
<td>NS</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Pre-A, pre-albumin; RBP, retinal-binding protein; NS, not significant.
by the body is tightly controlled; excessive glycine can have deleterious effects. This may be an indication of the role that this group of amino acids plays in the body. Although they can be produced by the body, there is an upper limit to the production. Without further information, I would be cautious about saying that we need more.

The problem in management is to appreciate the relatively narrow range of therapeutic effectiveness, particularly in malnourished children, where the degradative pathways are compromised. One can easily move from a position of deficiency to one of excess. Caution, therefore, is needed when considering supplementation.

Dr. Guesry: Why have you included taurine in the list of essential amino acids? It is not incorporated with protein synthesis and there is no evidence of taurine deficiency in normal infants. In addition, all rehabilitation programs for malnourished children carried out with cow's milk not containing taurine have been successful.

Dr. Jackson: My reason for listing taurine as one of the conditionally essential amino acids is that as far as we know, taurine is synthesized from cysteine. The de novo synthesis of cysteine is through the trans-sulfuration pathway, requiring methionine and serine as precursors. Glycine and serine are metabolically interchangeable. Therefore, it seems that the carbon chain and the amino group of glycine, cysteine, and taurine are all derived from serine. So, if I make the statement that glycine and serine are conditionally essential amino acids, then I feel obligated to consider that the derivatives may also be conditionally essential.

Dr. Suskind: Could you comment on the impact of amino acid metabolism on neurotransmitter synthesis in the malnourished state and the effect of malnutrition in generally lowering essential amino acids? Dr. Robert Olson, several years ago, looked at the rate of protein synthesis in malnourished children. He proposed that there was a hierarchy of visceral protein synthesis and that those proteins having the smallest pool size and the fastest turnover rate were the most sensitive markers of visceral protein synthesis. He referred to such proteins as retinol-binding protein and pre-albumin as being sensitive markers.

Dr. Jackson: There have been many suggestions that the availability of specific amino acids may modulate either the synthesis or function of neurotransmitters. I am not aware if there is direct evidence, although one is particularly concerned about the role played by tryptophan. One difficulty in studying the metabolism of tryptophan is to define the active circulating pool, since it is bound to plasma albumin and can be displaced by free fatty acids, for example.

Regarding the sensitivity of proteins in relation to the rate at which they turn over, if there is a general depression of protein turnover, it will become obvious in the fast-turning-over proteins first. However, the control in individual proteins is more specific, and there is seldom a general depression of turnover.

A clear definition of the metabolic state is important in understanding the processes that are taking place. I suggested that classifications might be based on the extent to which the energy requirements of an individual were being satisfied by dietary intake. The extent to which nutrient supplements can be utilized by the body is determined, in part, by the energy available to the body. If the requirement for energy is not satisfied, energy is made available through net catabolism. In the process of catabolism, nutrients are wasted. If, however, the requirements for energy are covered, then the next requirement is to replete specific nutrients and to satisfy other metabolic requirements. Therefore, in considering whether to supplement with amino acids or other specific nutrients, one has to consider whether the individual has already satisfied his energy needs. I do not think it is possible effectively to retain supplements if the energy requirements of the body are not being satisfied.

Dr. Suskind: It is essential to satisfy both protein and energy needs. In Chiang Mai, we
tried to ascertain the optimal nutritional support for malnourished children by putting them on varying protein and calorie ratios. The children received on a per kg basis 100 calories/1 gram of protein, 100 calories/4 grams of protein, 175 calories/1 gram of protein, or 175 calories/4 grams of protein. At 175 calories and 1 gram of protein there was a decrease in visceral protein synthesis. This study emphasized a need to be concerned about the protein as well as the energy needs of malnourished children during rehabilitation.

**Dr. Jackson:** I am not clear about the overall metabolic state of the children you described. Because maintenance requirements are about 100 kcal/kg·day, a child given 150 kcal/kg·day must handle an additional load of 50 kcal/kg·day. This may be deposited as tissue, but with the existence of specific deficiencies, there is a limit to which this is possible.

When I described the acute management of severely ill children, it was to suggest giving energy levels sufficient to cover energy expenditure. Protein needs can be met by giving the maintenance requirements of 0.6 g/kg·day until specific nutrient deficiencies are repaired. Until repair, it is not possible to synthesize balanced new tissue, and, more important, there will be continued anorexia. This loss of appetite appears to be an effective defense against inappropriate nutrient intake in relation to the body's demands.

The problem that occurs in infection, particularly with diarrheal disease, is that there are unbalanced losses of nutrients. In consequence, the usual dietary intake may no longer be appropriate to provide the particular balance of nutrients required to replenish the specific losses. An excellent example is the increased loss of potassium from diarrheal disease, which may lead to a significant depletion of total body potassium, perhaps one of the more important factors in the genesis of nutritional edema. Although normal potassium intake is sufficient for a child in normal health, it is insufficient to replete these unusual losses. Generous supplements of potassium are, therefore, critical to successful management. This highlights the need to be careful, when discussing supplementation, of being clear about the metabolic background against which the supplementation is to be provided.

One of the most important considerations for successful management is the ability to control carefully the energy intake to a level that simply covers the need for maintenance. Reports in the literature describe the adverse effects of excessive energy intake during the early period of rehabilitation, reinforcing the idea of the sensitivity of the adapted body to the level of energy intake.

**Dr. Suskind:** Our study found that these malnourished children, within 7 days, were doing very well on 175 calories/4 grams of protein/kg·day. When a second group was placed on 175 calories/1 gram of protein/kg·day, plus other nutrients, there was no anorexia. In fact, the children took the formula as easily as those on 175 calories and 4 grams of protein/kg·day.

I do concur, however, that we must be concerned about the infant's being able to differentiate an appropriate from an inappropriate intake ratio.

**Dr. Jackson:** I agree. We must begin to recognize responses such as anorexia as being protective and to learn to be very cautious about overriding such protective mechanisms.

**Dr. Guesry:** Intensive discussions are taking place regarding the special need for branched chain amino acids (BCAA) in stressed patients. Dr. Keusch has suggested that stress may be an important factor in PEM. Do you think, therefore, that malnourished children would have special requirements for BCAA?

**Dr. Jackson:** Although there is considerable literature on BCAA and the specific role they may play in catabolic states, the conclusions are not clear. One of the most recent suggestions has been that the BCAA act as important precursors for glutamine synthesis in muscle. Glutamine may have a particular role in activation of the immune system and protection of the integrity of the gastrointestinal tract.
Dr. Truswell: About 5 years ago, Dr. Jackson (5) wrote in the Lancet that carbohydrate was used by the colonic bacteria for their energy, and fat was not available as an energy source. However, in one slide, you showed that at a single protein level, there was actually better utilization of fat as compared with carbohydrate.

Dr. Jackson: I might have misled you by the rather brief description I gave of the diets. There were complex carbohydrates in all the diets received by the children. The diets were enriched in energy with the addition of either arachis oil or cornstarch. It is generally considered that fats act as a poor substrate for the colonic microflora because of the predominantly anaerobic environment.

REFERENCES