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# AN UPDATE OF CONCEPTS OF ESSENTIAL AMINO ACIDS

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## INTRODUCTION

The essentiality of amino acids was recognized early in this century, but it was not until Rose and his associates discovered threonine in the 1930s that diets containing only purified amino acids could be prepared to identify necessary amino acids and determine quantitative requirements for them (98). The concept that specific amino acids are essential if required in the diet for growth and nitrogen balance has been the touchstone for determining amino acid requirements within and across species. The author endorses continued use of these criteria, but they should be placed in perspective and evidence about amino acid needs based upon these and other measures should be continually



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evaluated. It has become apparent that the classic criteria of amino acid essentiality (indispensability) or nonessentiality (dispensability) have important limitations as nutrition knowledge advances and its application expands. The data that support these statements have been derived in studies principally with arginine and histidine, and are a major component of this review.

## GENERAL OVERVIEW OF ESSENTIAL AMINO ACIDS

### *Dispensable Versus Indispensable Amino Acids*



It is often not appreciated that the distinction between indispensable and dispensable amino acids is strictly related to the diet. Once amino acids have been consumed in adequate amounts, their interrelationships are not between the indispensable or dispensable but between and among pairs regardless of whether or not they are dietary essentials. The distinction also appears to have little meaning metabolically, except that one group is synthesized by mammals and the other is not. For ruminants, the distinction also has dubious value because of the extensive degradation and resynthesis of amino acids by the rumenal microflora (22, 72). It must be kept in mind that about 20–22 alpha amino acids are constituents of body proteins that are essential for life. The so-called nonessential amino acids also have importance for normal growth (50, 51). When rats are fed all of their dietary N as indispensable amino acids, their growth rate is severely depressed compared to the rate when the contribution to dietary N from dispensable and indispensable amino acids is more equally divided (see Table 1), and the growth response also depends upon total protein content of the diet (119). Such data agree with studies in chicks (3, 118). The indispensable amino acids other than lysine and threonine can be replaced by their alpha keto analogs (9) with efficiencies that are species- and amino acid-dependent (14, 28). This makes lysine and threonine the only truly indispensable amino acids. As our understanding of amino acid metabolism advances, it is clear that amino acids not included in the indispensable category

**Table 1** Weight gain of weanling rats fed indispensable and dispensable amino acids in different ratios for 17 days with different percentages of total dietary nitrogen[a]

| Indispensable (%) | Dispensable (%) | Weight gain | | |
|---|---|---|---|---|
| | | 1.2% N | 1.8% N | 2.4% N |
| 100 | — | 33 | 37 | 21 |
| 80 | 20 | 52 | 69 | 57 |
| 50 | 50 | 64 | 71 | 67 |

[a]Modified from Stucki and Harper (119).

may become essential under particular circumstances (65). It has been suggested that nutrients that are dispensable in normal animals be classified as conditionally indispensable or essential when metabolic impairment or physiological state causes the need for them to exceed the synthetic capacity of the target organism (24). Adoption of such a practice may prove cumbersome if not confusing; however, the idea emphasizes that those who discuss essentiality and nonessentiality must continue to define the conditions under consideration. Readers desiring more extensive analyses of this terminology are directed to the publications of Harper (50, 51).



## *Nitrogen and Amino Acid Requirements*

No subject in nutrition has been studied more extensively than the protein requirements of food-producing animals and man. Two approaches are generally followed. The factorial method sums the obligatory fecal, urinary, and skin losses on a protein-free diet plus the amounts needed for new tissue or product formation. The second method is based upon measurements of the minimum nitrogen (N) intake to maintain N equilibrium for a particular set of conditions. Both attempt to estimate the minimum intake of N that will equal losses. Expressed another way, both sum the requirements for individual essential amino acids plus nonessential N. Both often receive a degree of respect for precision that is not fully justified (44, 89, 109). Although much has been learned about the sources of error with these methods, the range of values derived for normal healthy animals continues to be significant. Hegsted (53, 54) has discussed balance studies and because of the variability in results has been moved to comment that there has been little apparent improvement in the N balance technique for studies in man during the nearly 100 years that it has been used (54). The balance method reveals little about the cellular processes of intermediary metabolism. The data from balance studies are often difficult to interpret when intakes of protein are high. On the other hand, when N intake is low, enzyme systems adapt and specific amino acids are conserved (54). Energy intake also strongly influences N balances, so data obtained under conditions of adequate energy intake are of questionable value for malnourished populations whose metabolic processes adapt and interact in ways that are not understood. It is not known whether the large range in values obtained, particularly for human subjects, results from inherent differences between individuals, their nutritional histories, or errors in the balance techniques themselves. Regardless of these shortcomings, the balance technique remains the most popular and is the standard of reference for determining protein requirements and quality for adults of most species including man. Determining N and other nutrient requirements of the ill and the aged are areas where data are urgently needed and where balance techniques are especially

difficult to apply. Recruitment of experimental subjects from these two very important segments of our society is a major impediment in conducting studies. There is great need for simple, reproducible, inexpensive indicators that can assess protein quality and amino acid nutrition quickly in a wide variety of clinical and nonclinical settings. Such measures would hasten the advancement of nutritional knowledge and increase its application in health care. Excellent reviews of N balance techniques and other methods of protein evaluation are covered elsewhere (13, 39, 44, 53, 54, 109, 129). Traditional as well as newer methods for evaluating protein quality have been tabulated and referenced well by Bender (10).



## *Comparative Study of Metabolism of Nitrogenous Compounds*

Data obtained from a given species, usually the rat, are in a general way related to protein and amino acid requirements of other species. However, the significance of the data cannot often be well defined. In the limited comparative studies in which the same proteins were studied in human subjects and in rats, consistent relationships between men and rats have not been found (13). Nevertheless, one of the striking features about the comparative metabolism of nitrogenous compounds is the similarity between animals that appear quite distant on the phylogenetic scale (21). The mechanisms of protein synthesis also appear to differ only in minor aspects throughout the animal kingdom (86). Further, there appears to be great similarity in content of essential amino acids in tissues whether one examines food-producing ruminants or simple-stomached mammals including man and poultry (114). It has been suggested that dietary amino acid requirements could be more reliably compared based on the amino acids absorbed from the small intestine. Smith (114) points out that it is quite plausible to make comparative assessments of the requirements for deposition in tissues, secretions, or products and to assume that they are related to the small intestine or the diet. However, for reliability, such measurements would require an understanding of amino acid transport and metabolism between the site of absorption in the alimentary tract and the final destination in tissues. An understanding would also be required of the metabolic fate of the large endogenous contribution to the intestinal content of amino acids from secretions and mucosal cells; in man, this exceeds the dietary supply (85). Should it be established that the apparent differences in composition of organs or histologically similar tissues are mainly due to analytical methods, it is likely that a standard animal can be conceived to predict amino acid needs and to guide research in areas where data are not available from direct observations (114). Students contemplating such research will find recent reviews informative (10, 21, 114).

## RECENT INFORMATION ON HISTIDINE REQUIREMENTS

Histidine has been established as a dietary essential for human infants and for lower animals (38, 64, 88, 115). It is generally designated as nonessential for adult humans (57), although the National Research Council (NRC) gave it an indefinite classification for children and adults in 1980 (89). Investigators in the last 10–15 years have suggested that it is essential for human adults with chronic disease (11, 45). Other recent studies and the lack of convincing evidence for endogenous synthesis argue that histidine is essential for children and normal human adults, and should receive additional study. Ackroyd & Hopkins (1) first reported that histidine is essential for growing rats, and their report was later confirmed by Rose & Cox (99).

The classic studies by Rose showed that eight amino acids were essential for N balance in adult human beings (98). Based upon qualitative experiments in young men, histidine and arginine were not needed for N balance (100, 102). When Rose and coworkers were determining the quantitative amino acid requirements of human adults they fed low-histidine diets for 3–4 weeks (107). Subsequently they fed men diets containing negligible concentrations of histidine, and in both instances observed positive nitrogen balance without visible adverse side effects for as long as 2.4 months (104–106).

Although histidine is considered essential for both growing and adult rats (57), the early evidence concerning its indispensability for adult rats was contradictory. However, there is general agreement with Nasset & Gatewood (88), who proposed that the histidine requirement of adult rats is very low, that histidine deficiency is manifested very slowly, and that it cannot be reliably demonstrated in short-term balance studies. In support of this postulate, prolonged depletion of histidine in adult rats led to negative N balance and decreased hemoglobin concentrations. They further suggested that histidine requirements for young men were met by hemoglobin breakdown in Rose's studies.

Kofranyi et al (66) reported a rise in serum enzymes indicative of liver damage in men fed a diet low in histidine and arginine for 12–16 days, and they concluded that at least one of the two amino acids was essential. Anderson and coworkers (6, 7) found that diets excluding histidine but containing the eight other essential amino acids, proportioned as in casein and combined with glycine, glutamate, and diammonium citrate, lowered N retention and caused a slower rate of plasma amino acid clearance and a decline in plasma histidine and proline in young men. Subsequently, Kopple & Swendseid (67) observed progressively negative N balance, decreased serum albumin, decreased plasma and muscle histidine, hematological changes, and subjective clinical symptoms

in normal men fed low-histidine diets for an average of 31 days. Weller et al (132) found that adult men failed to attain N balance when fed amino acid mixtures based on Rose's requirements. Since this deficit was not corrected by doubling total N or by increasing the total essential amino acids by one third, they concluded that an essential component of the diet was lacking and that it was probably histidine.

Wixom et al (134) conducted a study in a 48-year-old normal human male from their research team. During the overall study period of 72 days, the subject received intravenous alimentation for 48 days after two periods of oral intake. Deletion of histidine from the intravenous solution for 27 days brought N balance close to zero without significant changes in urea, uric acid, creatinine, or proteins of short biological half life in the serum. When histidine was omitted from the infusions, plasma and urinary histidine stabilized at low concentrations. The authors concluded that differences following gradual changes may have been greater had the administration of histidine-free infusions been extended for a longer time. A companion paper (120) described in the same subject a mild hemolytic anemia which began with the initiation of central venous alimentation but not with the beginning of histidine deletion. The anemia seemed to correspond in time to the intravenous infusion, which was free of fatty acids. Anderson et al (5) and Kopple & Swendseid (67) also observed a gradual decline in hemoglobin or hematocrit in 56- and 35-day studies of normal young men receiving low-histidine intakes. The results of the intravenous study agreed with the decline in plasma histidine in normal subjects studied by Kopple & Swendseid (67).

If the constancy of plasma amino acid concentration is a valid indicator that an amino acid is dispensable, that criterion has not been fulfilled for histidine by the intravenous study or others (6, 7, 67, 134). Since the plasma and histidine concentrations were relatively constant for the last 20 days of the 27-day histidine-free intravenous infusion (134), the low steady-state concentration may have been related to the slow release of histidine from the tissues (57, 67, 88), a decreased rate of histidine degradation, and/or a rate of histidine biosynthesis adequate to maintain plasma concentrations but at subnormal levels. Such possibilities are consistent with the incorporation of $^{15}NH_4Cl$ into histidine by the subject that had been intravenously fed (110).

Early in the 1970s it was reported that histidine is indispensable for human adults with chronic renal failure (11, 45). Kopple & Swendseid (67) reassessed this possibility by comparing histidine deletion in four normal and three chronically uremic adult males. When an L-amino acid diet containing 60 mg of histidine was fed, N balances generally became negative after 15–30 days, and serum albumin decreased in six of the subjects. Plasma and muscle histidine fell an average of 82 and 62% respectively, while hematocrit declined and serum iron rose. There were symptoms of fatigue, with anorexia and

nausea. Four subjects complained of slight memory loss. Five developed dry scaly skin with mild erythema. With administration of the histidine repletion diet, plasma and muscle histidine rose, serum iron fell abruptly, reticulocytosis ensued, the hematocrit rose, N balance became positive in six subjects, and serum albumin rose in five. The clinical symptoms and skin lesions disappeared. The authors concluded that their evidence indicated the indispensability of histidine for normal and chronically uremic men and that dietary histidine is needed for normal erythropoiesis. The skin eruption observed in several of the subjects and in infants fed low-histidine diets (115) has not been described by others in relation to histidine or other amino acids (104–106, 134).

In subsequent studies, Kopple & Swendseid (68) found that changes in post-absorptive plasma or 24-hour urinary histidine excretion correlated with histidine intake but failed to predict dietary histidine requirements reliably in normal or uremic human adults. Plasma histidine fell progressively to abnormally low levels when histidine intakes were reduced, and intakes of 2 mg/kg body weight/day were associated with lower N balances. It was concluded that studies for determining histidine requirements for normal or uremic human subjects should exceed 71 days. There was also a lack of correlation between 3-methylhistidine excretion and histidine intake, suggesting that muscle degradation varies with histidine intake. Evidence obtained in adult female dogs (25) is in general agreement with these data for human adults. Groups of dogs that were tube-fed isocaloric isonitrogenous amino acid diets that were free of histidine or contained 67 mg of histidine/kg body weight/day showed no differences after 5–6 days, but at almost 60 days dogs fed no histidine showed significant declines in plasma and muscle histidine, muscle carnosine, body weight, hematocrit, and serum albumin.

Although studies with lower animals (63, 81, 96, 117, 138) and man (95, 121, 122, 137) show that plasma concentrations of a particular amino acid may vary with dietary or intravenous supply, Kopple & Swendseid (68) found in short-term feeding that the break point in plasma histidine concentrations in man was not consistently associated with a particular dietary intake and that the response may be influenced by previous histidine intake. From their more recent studies with $^{14}$C-labeled histidine, Kopple and coworkers reported that histidine degradation in normal and uremic men fell markedly on a histidine-deficient diet (59). This adaptive response has also been seen in rats for histidine (60) and other amino acids (17, 47, 136).

Histidine present in muscle carnosine (β-alanyl-L-histidine) serves as a reservoir of histidine during histidine deficiency. Robbins et al (96) found that weight gain in chicks became maximal at lower histidine intakes than required for maximum muscle carnosine concentration or for a rise in plasma histidine. Thus, plasma histidine overestimated the histidine requirement for maximum protein synthesis and weight gain. Other adaptive responses to low histidine

intakes that have been reported include decreased oxidation and degradation of histidine (59, 60), reduced synthesis of hemoglobin (67, 88), and increased utilization of muscle carnosine (36).

Although there is evidence suggesting that small quantities of histidine are synthesized by mammalian tissues, the evidence for significant endogenous synthesis is not available. Fürst (43) reported incorporation of $^{15}N$ into histidine by healthy adult men, but the site of incorporation in the histidine molecule was not reported. Such information is essential because Schoenheimer et al (108) found that there is transamination at the alpha carbon. Sheng et al (110) reported incorporation of $^{15}N$ into the imidiazole ring of histidine in the parenterally fed adult (134), but participation of the intestinal microflora was not excluded. Plant and associates (91) found no incorporation into histidine of $^{14}C$ from [$^{14}C$]formate in normal and formate-deficient rats, which strongly suggests that histidine must come from the diet. Levy & Coon (69) reported $^{14}C$ incorporation by liver slices into a compound they identified as histidine, but apparently no follow-up studies have been reported.

Fortunately, meats and most vegetables, which are the main sources of proteins in the diet even in developing nations, contain significant amounts of histidine. Only a few selected foods, if eaten as the only source of protein, would lead to histidine deficiency even in infants. A diet of varied foods supplying 2 g protein/kg body weight/day would supply the recommended dietary histidine allowance for infants of 33 mg/kg/body weight/day (89). Since requirements for growing children and adults are lower than for infants, a histidine deficiency should be expected only when the intake of total protein is suboptimal. Despite these assurances, recent metabolic evidence argues that a dietary supply of histidine is required for infants, children, and adults, and continues to support the notion that histidine is not endogenously synthesized.

## RECENT INFORMATION ON ARGININE

Dietary arginine is required for either optimal growth or N balance in young cats (4), rats (15, 80), guinea pigs (55), dogs (31), rabbits (2), swine (73), and mink (71). However, it has been generally believed that adult rats (20, 135), adults dogs (103), children, human adults (87, 101), and pregnant or non-pregnant swine (37) synthesize adequate amounts for maintenance and some N storage. The evidence from these studies is consistent with the widely held view that arginine is dispensable for adult mammals. Recent evidence raises doubts about the validity of this belief with regard to adult dogs (19). It has also been generally believed that dietary arginine is required by birds and other species because they lack a urea cycle. However, recent experimental evidence contradicts such generalities by demonstrating that there are wide differences between species and that near-adults of some ureotelic mammals may die with severe ammonia intoxication shortly after a meal lacking arginine (33, 82).

Arginine deficiency appears unique in this respect. No other dietary deficiency has been found to cause such drastic effects as rapidly as removal of arginine from the diet of susceptible animals. Animals conditioned to a low-protein diet may show severely depressed feed intake and perhaps weight loss within a few hours if they are fed a diet deficient in one of the amino acids considered essential by classic criteria. However, acute life-threatening responses seen with the arginine-free diet have seldom been reported. Development of clinical signs of deficiency of other generally recognized essential nutrients requires days or weeks.

Near-mature cats (82) fed an arginine-free diet have been shown to experience severe hyperammonemia, convulsions, and death within 2–3 hours. Cats also develop significant hyperglycemia seen in other species with experimental (92) or congenital hyperammonemia (112). The clinical signs are similar to those reported for simple-stomached or ruminant species given toxic dosages of ammonium salts, urease urea, or amino acids (127, 128).

The studies in cats, as in other species, showed that the severity of intoxication also depends upon the previous dietary history. The postprandial profile of plasma amino acids in the arginine-free (−Arg) cats was similar to that found during hepatic encephalopathy in dogs and human patients, suggesting that cats may offer a useful model for studying hepatoencephalopathy associated with liver insufficiency (40, 41).

The susceptibility of cats to a lack of dietary arginine may represent a peculiarity of carnivorous species. Recently it has been observed that ferrets develop acute signs of ammonia intoxication when fed −Arg diets similar to those producing ammonia intoxication in cats (33). Immature and mature dogs also show tremors, vomiting, profuse salivation, and hyperglycemia after receiving, by stomach tube, an agar-gelled L-amino acid diet lacking arginine (19, 49). The dogs also show profound citric and orotic aciduria, which are characteristic of arginine deficiency in other animals, but other species show minimal signs of intoxication (80, 116). It appears that dogs allowed to consume arginine-deficient meals ad libitum adjust their intake to avoid these severe consequences. However, with the completely −Arg diet, total feed intake was severely depressed, the dogs lost weight, and within 24 hours their urinary orotic acid excretion rose 50–100 times. With 0.28% dietary arginine, mature dogs showed no significant changes in ammonia, urea, or orotate excretion. Forced feeding of the −Arg diet also caused significant elevations of urinary citrate. Consistent with the report of Rose & Rice (103), the dogs showed no differences in N balance with or without dietary arginine.

Some of the apparent disagreement regarding arginine requirements for growth of rats and other animals arising from more recent data probably stems from differences in basal diets. Recent experiments have had the benefit of newer nutritional knowledge in formulating diets that more nearly meet dietary requirements and sustain greater growth. For instance, Rose and associates,

nearly 40 years ago, found the growth of control rats fed arginine (+Arg) averaged 1.65 and 1.29 g/day compared to 1.35 and 1.03 g/day for the −Arg animals (15). Their diets contained 30% lipids compared to 10% fed by Milner et al (80), who reported growth rates of 3.6 and 1.35 g/day respectively for +Arg and −Arg L-amino acid diets fed to weanling rats. The total dietary N content is also important in these comparisons because high protein intake increases the urea cycle utilization of arginine, which in turn reduces the quantity available for growth (131).

## *Arginine, Protein Repletion, Insulin, and Glucose Tolerance*

Arginine influences the release and metabolism of metabolic hormones. Paradoxically, revival of animals comatose from ammonia intoxication occurs after injection of arginine (48) and has been reported after overdoses of insulin (97). Hyperammonemic (92) rats are refractory to exogenous insulin, their endogenous insulin concentrations are elevated, and their release of insulin after arginine injection is depressed (84). Similar responses are found when there is an arginine deficiency. When mature rats were protein-depleted by a 0.5% lactalbumen diet to a 40% body weight loss over 14 weeks, and then assigned to an L-amino acid repletion diet containing 0, 0.25, 0.5, 0.75, or 1.0% dietary arginine, their repletion rates differed significantly, as shown in Table 2 (61,

**Table 2** Weight gain, feed intake, urinary orotate and liver lipids of mature rats repleted with different dietary intakes of arginine in an L-amino acid diet[a]

| | Dietary arginine (%) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 0.25 | 0.5 | 0.75 | 1.0 | 1.5 |
| Weight gain, g/15 days | 42 | 85 | 106 | 94 | 94 | 124 |
| Feed intake, g/15 days | 301 | 290 | 319 | 236 | 311 | 264 |
| Gain/feed ratio | 0.14 | 0.30 | 0.33 | 0.40 | 0.31 | 0.47 |
| Urinary orotate, mg/15 days | 56 | 34 | 25 | 10 | 0.8 | 2.2 |
| Liver weight, g/100 g body weight | 4.3 | 4.7 | 3.8 | 3.5 | 3.7 | 2.2 |
| Liver lipids, mg/100 mg tissue | 11 | 12 | 6 | 4 | 4 | |
| Plasma | | | | | | |
| Insulin, ng/ml | 2.1 | | 2.8 | 2.4 | 2.4 | |
| Glucagon, pg/ml | 212 | | 148 | 132 | 132 | |
| Glucose disappearance[b] | 0.045 | | 0.058 | 0.125 | 0.125 | |
| Insulinogenic index[c] | 3.5 | | 8.2 | 10.3 | 10.3 | |

[a]From Kari et al (61) and Mulloy et al (83); where possible, means of data from both studies have been calculated.

[b]Values are K coefficient for glucose disappearance.

[c] $\frac{\Delta \text{ insulin}}{\Delta \text{ glucose}} \times 10^3$

83). Without arginine, weight gain and efficiency of feed utilization were significantly lower without significant differences in feed intake. After intravenous glucose injection, plasma insulin for the 1.0% arginine group reached a peak in 5 min and declined steadily to nearly basal levels at 45 min. Plasma insulin concentrations for animals repleted with 0 and 0.5% arginine were lower than for 1% arginine at 5 min, but remained above basal concentrations throughout the experiment. There was a concurrent slower rate of glucose disappearance, indicating refractoriness to endogenous insulin. With 1.0% arginine, the coefficient for glucose disappearance and the ratios of plasma insulin to glucose were significantly greater. Although pharmacological injections of arginine cause insulin release (18, 70), these studies show that dietary arginine influences this process at physiological concentrations (61, 83). The evidence suggests that arginine is preferentially used for ammonia detoxification and that a low arginine intake decreases the amount available for its yet-to-be-defined role in insulin metabolism. It is also of interest that plasma glucagon concentrations were related to arginine intake (Table 2).

Studies with ruminants and other species (23, 27) show that plasma glucose and other metabolites of intermediary metabolism rise with hyperammonemia. In ruminants, amino acids and proteins undergo rapid degradation to yield ammonia in the rumen. Arginine is rapidly degraded, while lysine, an arginine antagonist (90, 124), is among the most resistant of the amino acids to microbial degradation (22, 72). These processes may, under some circumstances, create a functional deficiency of arginine and depress the capacity for ammonia detoxification and urea synthesis. Whether ruminants increase their urinary orotate excretion during such imbalances like other species (127) remains to be established.

## *Arginine and Reye's Syndrome*

Reye's Syndrome occurs in children and occasionally in adults (32). It is an acute multisystem disorder often preceded by a viral illness, vomiting, and rapid progress to delirium, coma, and seizures. Biochemical abnormalities include hyperammonemia, elevation of liver transaminases, prolonged prothrombin time, hypoglycemia, and elevated plasma amino and fatty acids. One of the most common causes of encephalopathy in children, it is associated with high mortality, and there is no specific treatment. The severity of the hyperammonemia is highly correlated with mortality (42). There appears to be generalized mitochondrial dysfunction, perhaps due to hyperammonemia secondary to inhibition of ureagenesis by elevated levels of organic acids. Fat accumulates in the liver and other organs, and ultrastructural changes in liver mitochondria have been reported (32).

Investigators have speculated that Reye's Syndrome results from interactions between a virus and other environmental agents. In recent years there has

been increasing concern about salicylates because correlations between serum salicylate concentrations and the severity of Reye's Syndrome have been reported (32). Ferrets, which are highly susceptible to experimental virus infections, were recently used in experiments employing acetylsalicylic acid (aspirin), influenza B virus infection, and −Arg diets (33). Within two to three hours after consuming an −Arg diet, ferrets showed seizures, convulsions, 15- to 20-fold increases in blood ammonia concentrations, and moderate increases in serum enzymes indicative of liver damage. Most of the animals infected with influenza B virus, given aspirin, and fed the −Arg diet died. There was a rise in serum ornithine transcarbamylase (OCT), indicating destruction of mitochondria and leakage of the enzymes of the urea cycle into the circulation. As in patients with Reye's Syndrome, the ferrets showed decreases in liver OCT and increases in serum OCT activity which were significant. Since the effects of the three pathogenic agents were additive only in raising liver lipids, and aspirin and influenza alone or in combination produced significant signs only in the ferrets that were also fed the −Arg diet, ammonia toxicity appeared to have been required to have produced adverse effects. It is known that aspirin uncouples oxidative phosphorylation, inhibits ATP/ADP exchange in isolated liver mitochondria (8, 16), and hinders citrulline synthesis in liver slices (46). Since the severity of Reye's Syndrome in children is correlated with serum ammonia concentrations, and arginine deficiency causes hyperammonemia in certain circumstances, nutritional studies with ferrets should yield valuable data concerning this and other hyperammonemic disorders (34).



## *Arginine and Recovery from Trauma*

Trauma initiates a series of metabolic events that include a significant increase in the urinary N loss (30). Fisher and associates (94, 113) supplemented high-casein diets with arginine and glycine, which significantly increased N retention after experimental trauma in rats. The authors postulate that additional arginine facilitated ammonia detoxification and tissue synthesis, while glycine facilitated conjugative detoxifying reactions during the metabolic stress of trauma.

## *Arginine and Hereditary Urea Cycle Deficiencies*

Hereditary disorders of the urea cycle are generally accompanied by hyperammonemic and abnormal plasma and urinary concentrations of metabolites due to depressed activity of urea cycle enzymes. Walser's comprehensive review (130) includes the rationale for different approaches to therapy. Arginine supplementation has proven beneficial in a limited number of cases with argininosuccinase lyase deficiency characterized by argininosuccinic aciduria, presumably because an additional supply of arginine facilitated greater

ornithine formation. Walser also utilizes data from various sources to present an analysis of arginine needs for creatine formation. He points out that a 70-kg adult consuming 50 g of protein will barely consume sufficient arginine for this purpose, let alone to meet other needs (130).

### *Arginine and Gyrate Atrophy*

Gyrate atrophy of the choroid and retina is a rare autosomal recessive disease characterized by hyperornithemia, hyperornithurea, night blindness, and diminished peripheral vision in the first and second decades of life, followed by progression to total blindness. Lymphocytes (125) and fibroblasts (62, 111, 123) of patients with the disease lack the mitochondrial pyridoxine-dependent enzyme, ornithine-δ-aminotransferase (OAT), which catalyzes the interconversion of ornithine and glutamate semialdehyde. Although the pathophysiologic mechanisms are not known, low-protein, arginine-deficient diets (9, 12, 126) and high doses of pyridoxine have lowered plasma ornithine in some cases (12, 126). Diets based on L-amino acids have been required to achieve the necessary restrictions in arginine. It is not known whether correcting the hyperornithemia would modify the long-term course of the disease. The drop in plasma ornithine indicates that arginine is the only significant source of ornithine in individuals lacking OAT and that alternate pathways for ornithine formation do not exist in man and, possibly, other mammals. The work of Dunn & Jones (35) showing that rat intestinal homogenates can convert glutamate to ornithine, presumably via OAT catalysis, has led to the suggestion that animals for which dietary arginine is essential lack the ability to synthesize adequate ornithine through the OAT reaction (126).

## OROTIC ACID EXCRETION

Abnormally high urinary orotic acid excretion occurs during enzyme deficiency states, drug therapy with allopurinol or 5-azauridine, pregnancy, feeding of diets deficient in urea cycle amino acids, and ammonia intoxication caused by administration of excessive doses of amino acids, ammonium salts, urease, or urea (52). It is also associated with solutions used for intravenous alimentation that cause hyperammonemia due to insufficient arginine (56, 133). The quantity of urinary orotic acid excreted in growing animals is inversely related to their dietary supply of arginine (31, 76, 78, 116). Since most dietary proteins contain negligible ornithine and citrulline, arginine is the dietary amino acid of importance (Table 3). Rats show elevations of urinary orotic acid within 24 hours (75) after ingesting L-amino acid diets lacking the urea cycle amino acids or when their diet is high in lysine with respect to arginine (124). The quantity of orotic acid excreted rises as N intake increases at a particular level of

arginine intake (78). Arginine-deficient diets also markedly increase urinary orotic acid excretion by growing mice, guinea pigs, hamsters, rabbits, and dogs (74), but increase excretion by growing kittens and pigs less so (29, 116). Growing animals need more arginine than mature animals and show greater urinary orotic acid excretion when fed arginine-deficient diets (75). Liver slices from rats adapted to low-arginine diets synthesize more orotate than controls. However, their nonhepatic tissues show insignificant changes in orotate synthesis (52). Arginine supplementation of casein diets fed to pregnant rats reduces their orotate excretion and increases the birth weight and weaning weight of their pups. Women in the third trimester of pregnancy may also excrete enhanced quantities of orotic acid, but it is unknown whether that is related to arginine intake (79).

Since ruminant mammary glands extract large amounts of arginine from the blood, the availability of arginine for ammonia detoxification during high milk production deserves investigation (26). Although ruminant milk is high in orotate, a large fraction appears to arise via pathways unassociated with ammonia detoxification (58). The rationale for using orotic acid as a measure of urea cycle capacity and ammonia intoxication has been reviewed (127).



**Table 3** Blood $NH_3$-N, blood urea, weight change, feed consumption, urinary citric acid and urinary orotic acid summarized for weanling rats fed an amino acid diet devoid of the indicated amino acid[a]

| Treatment | 4-day feed intake (g) | 3-day weight change (g) | Peripheral blood | | Urine | |
|---|---|---|---|---|---|---|
| | | | $NH_3$-N (μg/ml) | Urea (mg/100 ml) | Citrate (μg/24 hr) | Orotate (μg/24 hr) |
| Control | 37 | +12 | 2 | 8 | 262 | 46 |
| -Arg | 29 | + 7 | 2 | 29 | 5739 | 3982 |
| -Lys | 29 | + 3 | 2 | 14 | 1213 | 58 |
| -Try | 22 | 0 | 2 | 14 | 140 | 105 |
| -Phe | 21 | − 3 | 2 | 16 | 154 | 27 |
| -Val | 18 | − 3 | 2 | 16 | 184 | 30 |
| -Thr | 17 | − 9 | 3 | 58 | 206 | 33 |
| -His | 14 | −10 | 2 | 53 | 221 | 31 |
| -Met | 15 | − 7 | 2 | 62 | 232 | 36 |
| -Iso | 14 | −11 | 2 | 64 | 167 | 27 |
| -Leu | 15 | −10 | 2 | 61 | 164 | 32 |
| -Arg, Pro, Glu, Asp, Asn | 27 | + 6 | 3 | 34 | 8690 | 2840 |
| -Arg + Gly | 28 | + 4 | — | 46 | 4368 | 2753 |
| -Arg + Cit | 43 | +13 | — | 28 | 2708 | 64 |
| -Arg + Orn | 32 | + 5 | — | 54 | 261 | 49 |

[a]Data summarized from Prior et al (93) and Milner & Visek (77). Arginine was replaced on an equal nitrogenous basis by glycine unless otherwise stated.

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